A Family of Fuchs Endothelial Corneal Dystrophy and Anterior Polar Cataract with An Analysis of Whole Exome Sequencing

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Research article

Keywords: Fuchs endothelial corneal dystrophy, anterior polar cataract, whole exome sequencing

Posted Date: February 14th, 2020

DOI: https://doi.org/10.21203/rs.2.23532/v1

Version of Record: A version of this preprint was published at Ophthalmic Genetics on May 3rd, 2020. See the published version at https://doi.org/10.1080/13816810.2020.1759109.

Abstract

Background Our aim was to introduce a family affected by this rare phenotype, and perform the whole exome sequencing (WES) to explore the potential candidate genes causing the disorders.

Methods A five-generation family including 5 patients affected by FECD with APC, and 9 patients suffered from only FECD was recruited from the First Affiliated Hospital of Harbin Medical University. All participants received ophthalmic examinations. 8 family members were selected to perform WES with a bioinformatics analysis.

Results Patients in this family had FECD as the common feature. The proband (a 65-year-old female) was affected by FECD and APC in both eyes, with epithelial bullae in the left eye of the. Slit-lamp, specular and confocal microscope and OCT images showed guttae more serious in the central cornea than the peripheral area, confirming the diagnosis of FECD. In this family, most corneal guttae were bilateral with almost equal degree of progression in Descemet membrane, APC was found around the age of 10, perhaps even earlier. Bioinformatics analysis showed that 83 potential candidate genes in total may cause this condition in this family. No known causative gene of this phenotype or FECD was found by WES.

Conclusion We introduced a family of FECD with APC, with no known causative gene found by WES, inferring that there may be a novel gene-locus associated with this condition. Further study will be engaged on candidate gene screening and variants validation with corresponding functional aspects to clarify the relationship between genotype and phenotype.

Background

Fuchs endothelial corneal dystrophy (FECD, OMIM: 136800) is the most common form of corneal dystrophy, first documented by Ernst Fuchs in 1910[1]. FECD is a bilateral, slowly progressive corneal disease. It is characterized by deterioration of endothelial cells and development of guttae excrescences of Descemet's membrane [1–4], which may eventually lead to corneal edema and reduced vision [4, 5]. The pathophysiology of FECD involves several proposed mechanisms involving channelopathies, oxidative stress, apoptosis, and the epithelial-mesenchymal transition. The underlying pathophysiology remains unknown[6, 7].The prevalence of FECD in people over 50 years old is 4%-9%, varing by regions[5, 6, 8]. In contrast to Caucasians with much higher FECD prevalence, fewer cases of FECD occur in the Asian population[7].

Age and gender are important factors influencing the development of FECD. People over 40 and female have a higher risk, with a female-to-male ratio of 2.5-3:1 [1, 5, 6]. FECD displays in an autosomal dominant inheritance with incomplete penetrance, about 50% of the patients have a positive family history[6, 9]. Clinically, FECD can be divided into early-onset FECD and late-onset FECD. Early-onset FECD, which began in the first decade of life, shows similar progress to classic phenotypes. The initial clinical manifestation

of late-onset form of FECD (corneal guttae) usually occurs in the fourth decade of life[1, 6, 10]. Usually, patients do not need intervention until the sixth or seventh decades[1, 6].

FECD is genetically complex and several genetic variations are known to be related to it [5]. Mutations in COL8A2 (OMIM: 120252) is associated with the rare early-onset FECD. Different variations in TCF4, (OMIM: 602228), ZEB1, (OMIM: 189909), SLC4A11 (OMIM: 610206), AGBL1 (OMIM: 615496), LOXHD1 (OMIM: 613072)[1, 4, 5] are associated with more common late-onset FECD. Genes, such as KANK4 (OMIM: 614612), ATP1B1; (OMIM: 182330), LAMC1 (OMIM: 150290), DMPK (OMIM: 605377) were recently identified through a GWAS, linkage analysis, or candidate gene studies as new risk factors for FECD [5–7, 11]. Other late FECD loci were found on chromosomes 13, 18, 5 and 9[12].

Corneal guttea with anterior polar cataract is a rare phenotype which is inherited in an autosomal dominant pattern ,initially proposed by Ichikawa and Hiraga in 1951[12, 13]. Chen P et al. have reported that mutations in TMCO3 gene were related to this rare phenotype in 2016[12]. In view of FECD may occur independently or in association with other ocular or systemic abnormalities[6, 7, 12], it remains to be clarified whether the phenotype of cornea guttata with anterior polar cataract is caused by FECD gene alleles or closely linked modifiers. With the breakthrough of next-generation sequencing(NGS) technology, whole exome sequencing (WES) analysis has been applied in the detection of variants in exons (protein-coding regions) and splicing sites in the human genome[14]. It is estimated that the exome contains about 85% mutations, which has a great influence on the disease-related traits[15, 16]. In order to find likely causal variant, WES analyses were applied in a family affected by FECD with anterior polar cataract in Hei Longjiang Province, China.

Methods

Subjects

All study subjects were recruited from the First Affiliated Hospital of Harbin Medical University. A five-generation family with 33 members (14 affected; Fig. 1) was enrolled. All participants received detailed examinations by ophthalmologists, including vision, slit lamp microscope, intraocular pressure measurement. Specular microscope, OCT examination, and confocal microscope were performed on selected subjects. There was no other systematic abnormal family history in this family. This study is based on the declaration of Helsinki and approved by the ethics committee of the First Affiliated Hospital of Harbin Medical University. All participants (or their guardians) received written informed consent.

Genomic Dna Preparation

Peripheral blood from family members was collected. 5 ml peripheral blood was drawn from elbow vein of each subjects and was preserved at − 80 °C prior to use. Genomic DNA was extracted from peripheral leukocytes using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's

protocol. A NanoDrop ND-2000 spectrophotometer was used to DNA quantification (analyzed by agarose gel electrophoresis). DNA was stored at − 20 °C for subsequent analysis.

Whole exome sequencing and library construction

Exome sequencing was applied on eight patients (II-7, II-9, II-I0, III-7, III-8, III-11 III-12, and IV-4) at CapitalBio Technology Co. Ltd., Beijing, China. Approximate 1 μg of genomic DNA sample was sheared into fragments of 300–500 bp in length. The sheared fragments were blunt-end repaired and a single adenine base was added to the 3' ends using Klenow exonuclease. Illumina adapters were ligated to the repaired ends and DNA fragments were PCR amplified for 8 cycles to each sample. Agilent SureSelect Human All Exon V6 kit (Agilent Technologies, Canada) was used for whole exome capture and library construction. Captured libraries were then sequenced on the Illumina HiSeq X-Ten PE150 (Illumina Inc,USA).

Variant analysis

Sequencing data were analyzed with NextGene V2.3.4 software (Softgenetics, State College, PA), and the raw reads were then aligned to hg38 genome using Burrows-Wheeler Aligner (BWA) bwa-0.7.15[17], followed by variant calling using ANNOVAR[18] to annotate the detected SNV and indels. Nonpathogenic polymorphisms were filtered comparing with the 1000 Genomes Project database, ESP-6500, the Exome Aggregation Consortium database (ExAC), and the Single Nucleotide Polymorphism database (dbSNP). Variants were assessed for the potential deleteriousness as determined by 12 in silico prediction scores included in dbNSFP[19].

Results

Clinical Findings

Patients of corneal guttae with anterior polar cataract

Proband II-7 is a 65-year-old female complained of binocular red eyes with pain, and vision decline in left eye for 2 years, with visual acuity of 0.12 Oculus Dexter (OD) and CF/20 cm Oculus Sinister (OS). She has poor eyesight since childhood. Slit-lamp examination revealed guttae and pigment in both posterior corneas with anterior polar cataract in both eyes. Epithelial bullae in the left eye. The changes in central cornea is more serious than the peripheral area (Fig. 2A).FECD grade was assessed according to Krachmer et al[6, 9]. Specular microscope demonstrated that a large number of vacuolated pathological black areas can be seen in corneal endothelium. The corneal endothelial cells were uneven in morphology, enlarged and pleomorphic, and the density of corneal endothelial cells was significantly

reduced (Fig. 2B). OCT showed the corneal epithelium of the left eye was edematous, and the corneal endothelium of both eyes was not smooth (Fig. 3A-B). Confocal microscope showed a lot of high reflective, confluent guttae between corneal endothelial cells, and the structure of endothelial cells was unclear. (Fig. 3C-D). Her daughter III-8 is a 40-year-old female with guttata and anterior polar cataract phenotype in both eyes who complained of vision decline for 30 years, with visual acuity of 0.25 OD and 0.3 OS. The proband's granddaughter IV-4 is a 16-year-old female who found a little white dot in the center of the pupil in both eyes around the age of 10, with visual acuity of 0.4 OD and 0.3 OS. She was also found corneal guttae in both eyes. The clinical features were similar with the proband's son III-9, and granddaughter IV-5. APC was also found at around 10 years old. The eyesight of proband's mother I-1 has been poor while the proband's father had good eyesight during the lifetime according to the families, indicating that at least FECD may be inherited by I-1.

Patients of corneal guttae with no anterior polar cataract

The proband's sister II-9 and her daughter III-11 and son III-12 had corneal guttae phenotype with no anterior polar cataract (Fig. 4), the same as II-11, II-12, II-13, and III-5. (See Supplementary Table).

Patients in this family had FECD as the common feature. Most corneal guttae were bilateral with almost equal degree of progression in Descemet membrane, and the central corneal injury was more serious than the peripheral endothelial injury. The degree of injury varies from person to person. Only the sub-pedigree of the proband had anterior polar cataract at the same time. Anterior polar cataract was found around the age of 10, perhaps even earlier in this family. There was no family history of other systemic abnormalities. No obvious abnormality was found in ophthalmic examination of other members. Fundus examination showed no abnormalities in all subjects.

Heredity Findings

To identify the causative mutation underlying this phenotype, we initially performed whole exome sequencing on 8 subjects (II-7, II-9, II-l0, III-7, III-8, III-11 III-12, and IV-4) in this family with high quality (mean coverage: 148.56×; regions with > 30 × coverage: 97.84%). According to the genetic pattern map of the family, it can be inferred that the phenotype may be autosomal dominant inheritance. The family can be further divided into two sub-pedigrees, subpedigree1 and subpediree2 (Fig. 5). The samples actually sequenced in the family subpedigree1 are II-7, III-8, IV-4 with both FECD and APC, and their normal control III-7. The samples actually sequenced in the family subpedigree 2 are II-9, III-11, III-12 with only FECD and their normal control II-10.

Since FECD may occur independently or in association with other ocular or systemic abnormalities[6, 7, 12], and only sub-pedigree 1 had two abnormalities, while FECD only occurred in the rest of their whole family. Patients in this family had FECD as the common feature. Besides, there was no APC with normal cornea subject in this family. It is speculated that the mutation of genes sharing by both sub-pedigree 1 and 2

may cause FECD and the formation of APC at the same time. According to disease type and sub-pedigree classification, the genetic model analysis was carried out according to the Table 1. Statistical results of autosomal dominant genetic pattern variations were shown in Table 2. The selected SNVs and small indels were used to extract the genes of the mutation that affect the amino acid sequence, including stoploss, stopgain, non-synonymous, non-frameshift substitution, non-frameshift insertion, non-frameshift deletion, frameshift insertion, frameshift deletion) or splice site variants, which were shown in Table 3. The Venn Diagram was drawn in (Fig. 6) for the genes of all the variations with functional influence screened under three dominant modes.

Table 1
Several situations of genetic model analysis.

No.	Pedigree related	Description
01	subPedigree2	Sub-Pedigree with only FECD
02	subPedigree1	Sub-Pedigree with both FECD and APC
03	SubPedigree1 + subPedigree2	Sub-Pedigree with FECD and sub-Pedigree with both FECD and APC were analyzed together (but only FECD was considered)
(1)The first column is the coding of three cases of genetic pattern analysis		
(2)The second column is the subpedigree involved in the corresponding genetic model analysis		
(3)The third column is the brief introduction of corresponding genetic model analysis		
No.: Number, FECD: Fuchs endothelial corneal dystrophy, APC: anterior polar cataract.		

Table 2
Statistics of the variation types of autosomal dominant genetic pattern.

Type	SNV	InDel	Total
01	6,615	651	7266
02	4,593	519	5112
03	565	60	625
(1)Type: No. 01、02、03			
(2)SNV: The SNV number in accordance with the autosomal dominant genetic pattern.			
(3)InDel: Indel number in accordance with autosomal dominant inheritance pattern.			
(4)Total: The total number of variants in accordance with autosomal dominant genetic pattern.			

Table 3
Statistics of variation site types affecting amino acid sequence

Type	SNV	InDel	Total	Gene
01	1,287	71	1358	1001
02	932	43	975	705
03	97	4	101	85
(1)Type: No. 01、02、03				
(2)SNV: The number of SNVs that conform to the autosomal dominant genetic pattern and have influence on amino acid sequence				
(3)InDel: Indel number in accordance with autosomal dominant inheritance pattern and having influence on amino acid sequence				
(4)Total: The total number of mutation that conform to the autosomal dominant genetic pattern and have influence on amino acid sequence				

Based on the above analysis, it was speculated that this rare phenotype was caused by the common variation shared by two sub-pedigrees. The intersection of 01, 02 and 03 was the key genes, which had 83 genes in total in this family. WES data were shown on Supplementary data. However, No TMCO3(Chen P et al.), or other known genes causing FECD were found according to the results, indicating there may be a novel gene or locus causing this rare phenotype.

Discussion

The phenotype of corneal guttae with anterior polar cataract (OMIM: 121390) is rare. Dohlman described a Swedish family in which 15 members had this rare phenotype in 1951[13]. He demonstrated that corneal changes were limited to the posterior cornea, i.e. endothelium and Descemet membrane, and no change in other layers of the cornea. In his study, the central cornea is more affected than the periphery in all patients, which was consistent with our patient's phenotype since in II-7 (OS), III-11(OS), guttae concentrated in the central area, which was too serious to be caputured by specular microscope. Dohlman also reported that at least one symptom was observed in the other 3 cases. It appeared that some relatives of the deceased may had at least polar cataracts. Guttae cornea did not seem to exist at birth, but later appeared and progressed slowly, resulting in a "beaten metal" appearance in the reillumination, and the changes of cornea were mostly bilateral with almost the same progress in each eye, but the severity of changes varied with patients. Polar cataracts were not always evident at birth. It occured most often between the ages of 3 and 10 becoming motionless after puberty[13], which was matching with the features of II-7, III-8, IV-4, III-9,IV-5 in our study, who found a little white dot in both eyes since childhood, at the age around ten. Besides, with the increase of age, the more serious the corneal injury, the more likely it is to cause corneal edema, leading to vision loss, which was similar with the proband II-7 in our study. However, there was no patient with single anterior polar cataract in our study. In

young people, polar cataract is the main cause of visual impairment, but with the increase of age, corneal exacerbation is the main cause of visual impairment[13]. This was also identified with our family characteristics. Dohlaman inferred that the two ocular abnormalities were caused by the interference of the posterior surface of the cornea and the anterior part of the lens due to the formation of the anterior chamber during the eighth week of embryonic development[13].

Traboulsi and Weinberg observed 12 members who were affected by this rare phenotype in an American family. The eyesight of all affected subjects were excellent. They were descended from a family who immigrated from Ireland to the United States in the 17th century. They settled in Ireland from Scandinavia in the 13th century [20]. Chen et al. performed Genome-wide linkage and exome sequencing analysis showed a possible association between variation in the TMCO3 gene and the rare phenotype of cornea guttata with anterior polar cataracts[12].

Whole exome sequencing (WES), making sequencing of all protein-coding regions(exome) in the human genome rapidly became the most widely used targeted enrichment method, especially for Mendelian diseases, compared with the selection of genes followed by Sanger sequencing in the past decades[12, 14]. This approach allowed the detection of both exons (coding) and splice-site variants, while requiring about 2% of the sequencing "load" compared to whole genome sequencing (WGS). Unbiased analysis of all genes removes the need for time-consuming selection of candidate genes before sequencing. It has been estimated that the exome contains about 85% of mutations with a great influence on the disease-related traits[14]. In view of the obvious ophthalmic changes in our family, we performed WES in this family to explore the pathogenic genes.

In the current study, we described a Chinese family with this rare phenotype. The common characteristic of patients was FECD in this family. Due to FECD may occur independently or in association with other ocular or systemic abnormalities[6, 7, 12], and there was no single phenotype of anterior polar cataract, the main consideration should be that the variations in candidate genes shared by all patients can cause both FECD and APC at the same time. There were 5 individuals who had both abnormalities, while only corneal changes in the other 7 patients (another two FECD patients were deceased). Since IV-4 and IV-5 has been diagnosed with FECD at 16 and 18 years old, or maybe the age of the disease was even earlier, indicating that corneal changes were more likely to be early-onset FECD(3 to 40 years) [7], which was influenced by genetic factors, and was usually a familial autosomal dominant disease, first reported by Magovern et al in 1979[21]. Affected children developed corneal guttae were as young as 3 years old. In contrast to the rough and clear guttae of late-onset FECD, the early-onset FECD was characterized by small and patchy guttae in the reillumination. Guttae in early-onset FECD appear in the center of endothelial cells whereas big guttae in late-onset FECD positioned at edges of endothelial cells by specular microscope, which was corresponding to the slit lamp and specular images of our patients. Gottsch et al. suggested that the average onset age of familial FECD patients without COL8A2 mutation is 50 years old. The disease develops from early to late stage in 25 years, and its incidence is similar to that of the more common late-onset FECD, except that it appeared earlier and had obvious clinical

symptoms at the age of 30–40[21–23]. The pathogenic mutation of COL8A2(MIM 120252) gene which encodes for the a-2 chain of collagen VIII at chromosome 1p34.3 – p32.3 (FECD 1) is related to the early form of FECD pathology, since this mutation affects the structure of the Descemet membrane[1, 4, 6, 7]. Mutations(L450W, Q455K) positioned in the triple helical domain of α2 alter the structure and composition of Descemet's membrane, leading to the early onset type of FECD[22, 24, 25]. To date, there has been no further report on the relationship between genotype and phenotype in early-onset FECD except for mutations in COL8A2.

For mutations in late-onset FECD, TCF4 (MIM 602272)gene is the most common cause of FECD[4]. (CTG repeats) in intron 3 of the TCF4 gene encoding E2-2 protein are associated with FECD in many different populations, which is the most commonly identifed genetic contributor to FECD. Baratz et al. first found there was a strong correlation between TCF4 intron polymorphic marker rs613872 and late-onset FECD after processing a genome-wide association study (GWAS) [5, 8]. Severity of disease seems to be correlated with repeat length in Caucasian populations, whereas no link could be detected in a Japanese cohort[26–28]. Males were also found to have a higher risk of developing FECD based on the presence of CTG repeats, suggesting that the interaction of this locus with gender could be important [5]. It can be seen from our family that the onset age of FECD is early (16 years old or maybe earlier), with more female patients than male patients, which were not very similar to the characteristics caused by TCF4 gene. In addition, we cannot detect GTC duplication using WES. There were other causative genes related to late-onset FECD. SLC4A11 (MIM 610206) gene encodes an ion channel that promotes the absorption of water in the endothelial layer, and is an important medium for corneal deturgescence. Mutations in this gene can cause corneal edema and are associated with FECD[6, 10, 29–31]. Similarly, mutations in the ZEB1 (TCF8, MIM 189909) gene encoding the transcription factor zinc finger E-box binding domain 1 are correlated with late-onset FECD[4, 6, 32, 33]. AGBL1(MIM 615496) gene encodes deglutamylase enzyme ATP / GTP binding protein like 1, and gene mutation is related to FECD. The missense mutation of LOXHD1 gene is related to progressive hearing loss and corneal endothelial cell dysfunction in FECD [4–7, 10, 34]. However, we haven't found TMCO3 gene, or any known causative gene in FECD as a candidate gene in our analysis at present, indicating there may be a novel variant or gene-locus associated with this rare phenotype. This disorder may also have genetic heterogeneity like FECD. Therefore, the next step is using Sanger sequencing to assess whether any of the selected variants cosegregated with the disease phenotype in this family followed by variant exclusion and prioritization. However, if Sanger sequencing do not achieve genotype-phenotype cosegregation in this family, suggesting that the pathogenic variants may exist in the noncoding sequence of the genome, which needs to be explored via whole genome sequencing(WGS).

Conclusion

we introduced a family of FECD with APC, with an analysis of WES was performed to explore the cause and mechanism of the rare phenotype. No known causative gene was found to cause the rare phenotype in our analysis via WES, inferring there may be a novel variant or gene-locus with two possibilities: the

novel variation is either in 83 screening genes, or may exist in the non-coding sequence of the genome, which needs further study by WGS. Further study will be engaged on candidate gene screening and variants validation with corresponding functional aspects to clarify the relationship between genotype and phenotype.

Declarations

Abbreviations

FECD, Fuchs endothelial corneal dystrophy;

APC, Anterior polar cataract;

WES, Whole exome sequencing.

Ethical approval and consent to participate

This study has been approved by the Ethics Committee of the First Affiliated Hospital of Harbin Medical University. All of the participants and the parents or guardians of the study participants that were minors provided informed written consent that was endorsed by the First Affiliated Hospital of Harbin Medical University.

Consent for publication

The written informed consent for publication of identifying patient/clinical data and identifiable images was obtained from the participants and the parents or guardians of the study participants that were minors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author up on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by National Natural Science Foundation of China (NO. 81970776 and 81671844), the role of the fundings are in the collection, analysis, and interpretation of data and in writing the manuscript.

Authors' contributions

XJiang and HZ contributed to analysis and interpretation of data, and drafted the work; XJiang, XJin□NZ and HZ contributed participated in the research planning, provided clinical material, revised the manuscript and approved it to be published. All authors have read and approved the manuscript for publication.

Acknowledgments

Not applicable.

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Figures

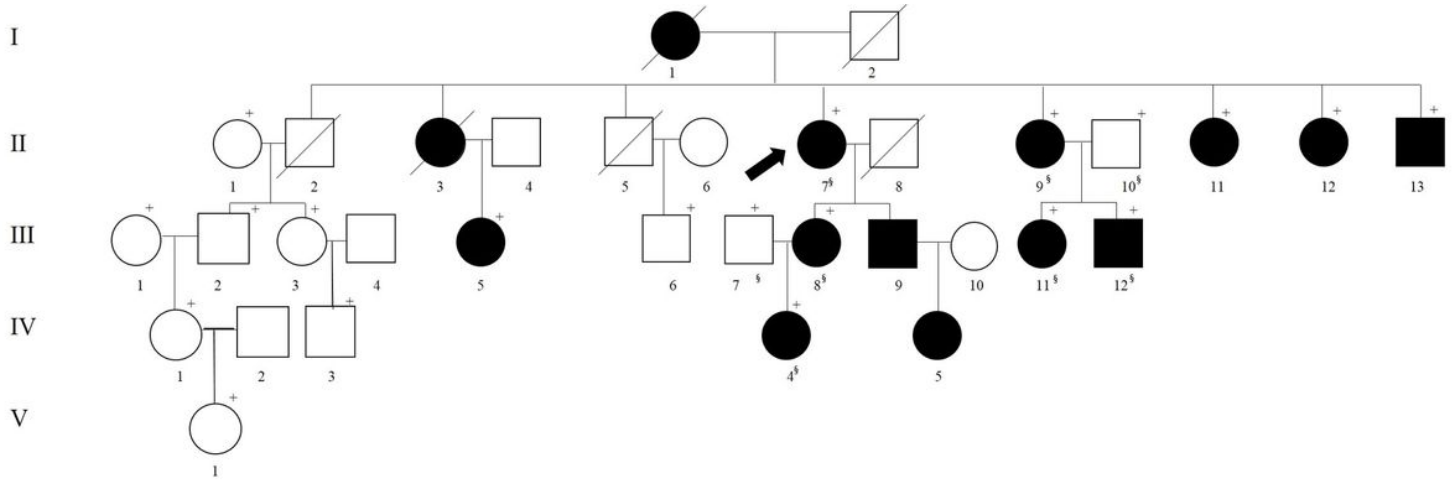

Figure 1

Solid squares and solid circles represent male and female affected individuals, respectively. The deceased is represented by a slash (/). '+' indicates DNA available for this study, and '§' indicates samples used for whole exome sequencing. the arrow points to the proband.

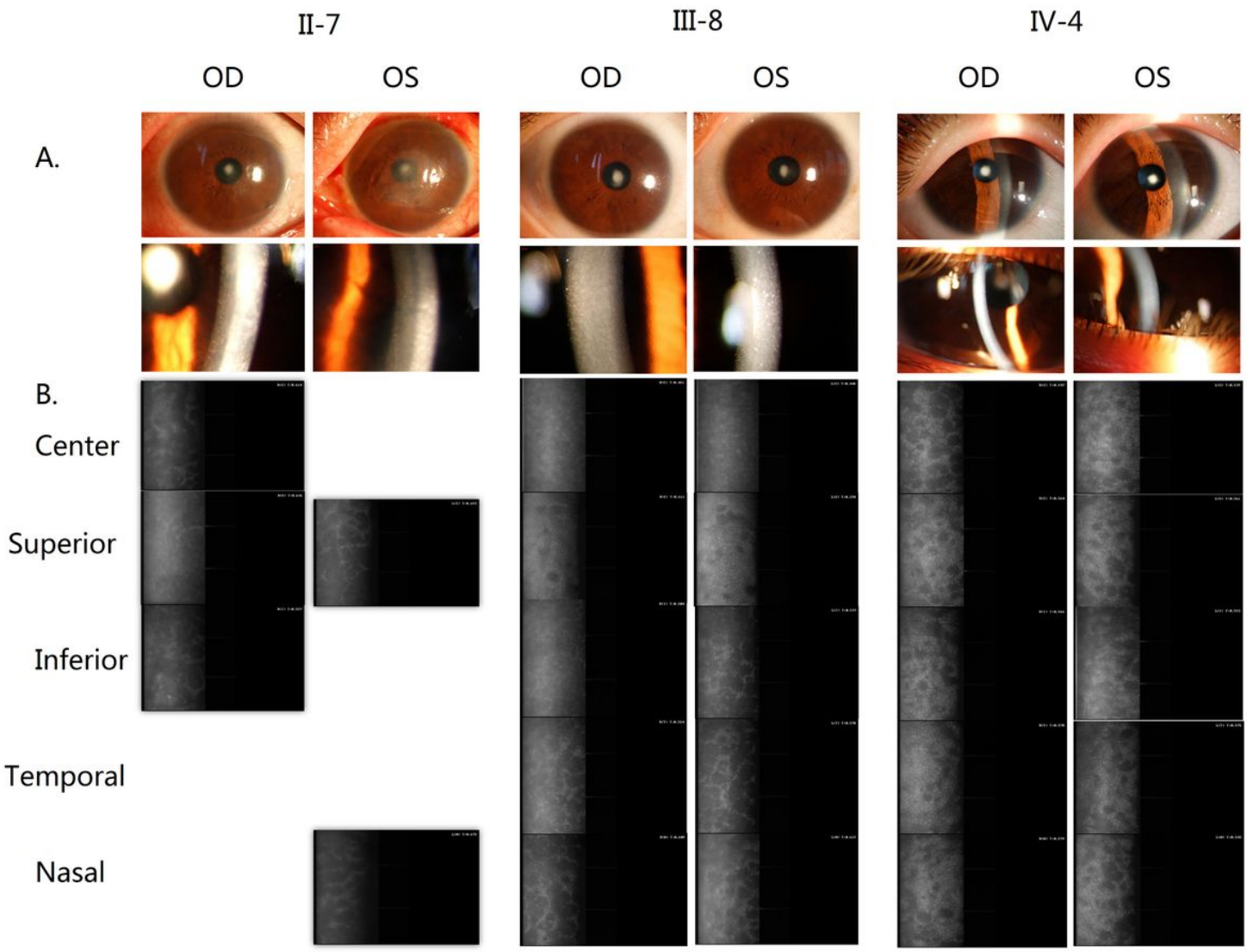

Figure 2

Slit-lamp photography (A) and specular microscope (B) of patients who had both corneal abnormalities and anterior polar cataract were shown. OD: Oculus Dexter OS: Oculus Sinister.Specular microscope images were taken at five points: Central, upper, lower, nasal, temporal.

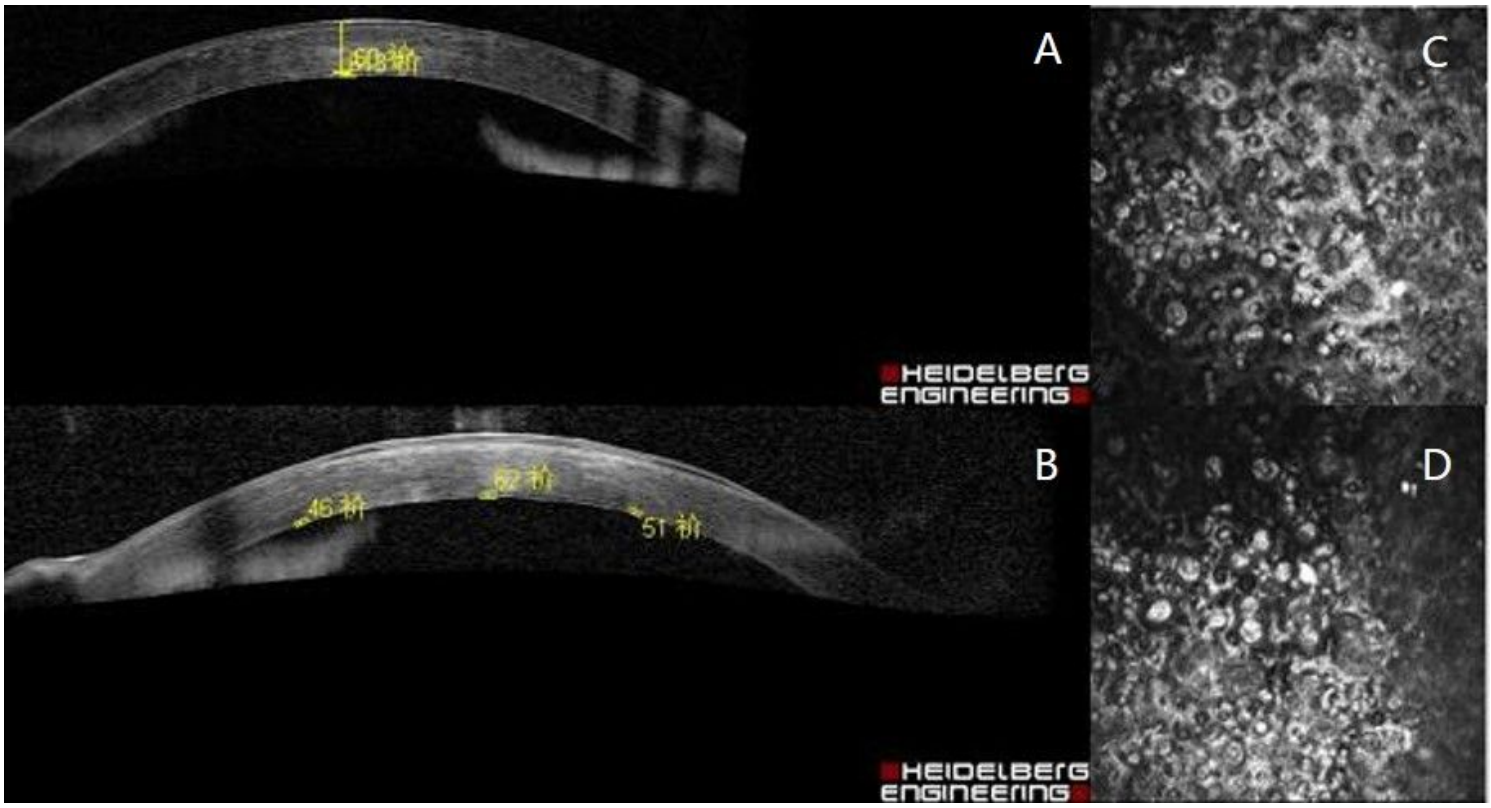

Figure 3

OCT and confocal images of the proband were shown. The corneal epithelium of the left eye was edematous, and the corneal endothelium of both eyes was not smooth. A: Oculus Dexter. B: Oculus Sinister. Confocal images showed that there was a large number of confluent guttae and no endothelial cell structure in the advanced stage of FECD. C: Oculus Dexter. D: Oculus Sinister.

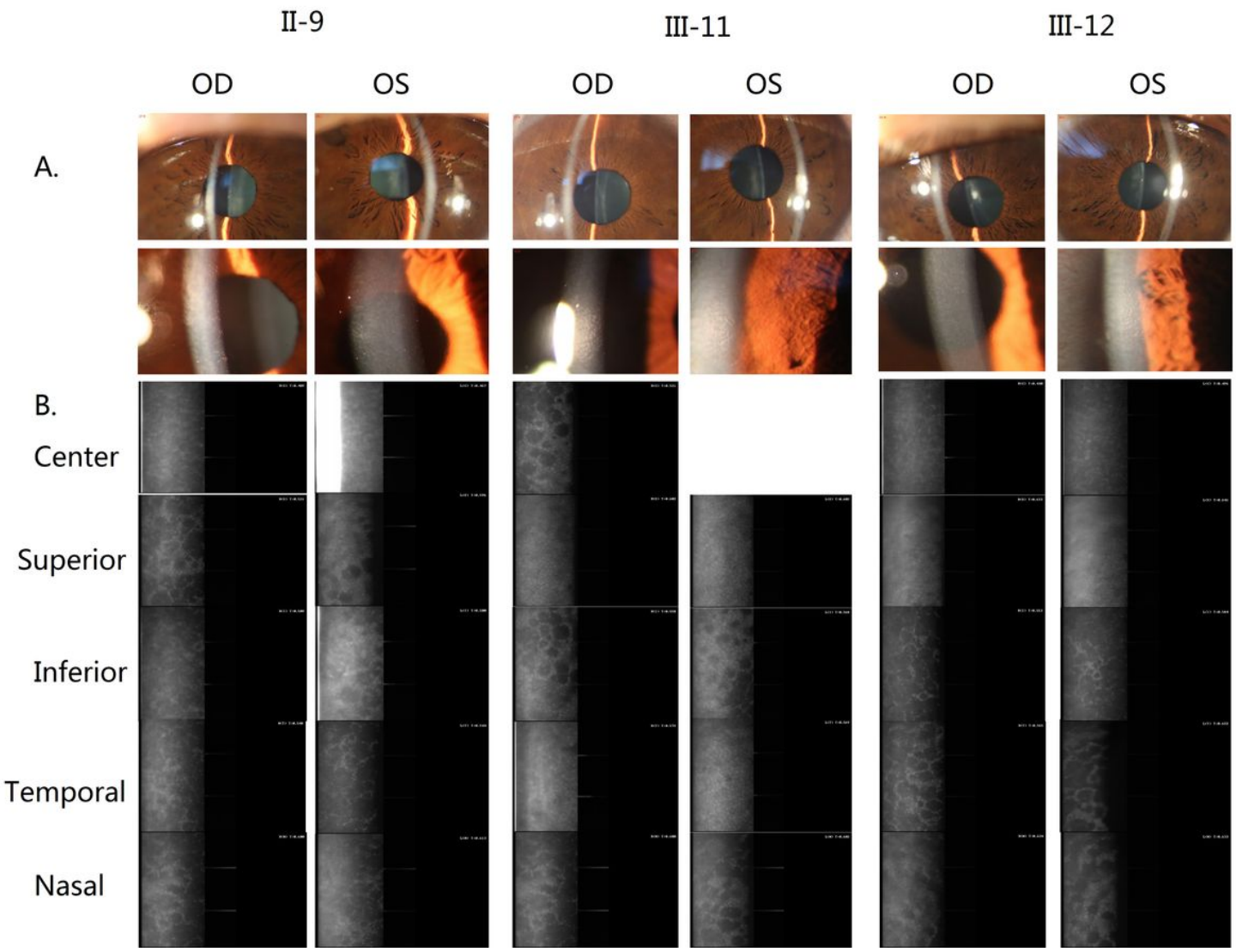

Figure 4

Slit-lamp photography (A) and specular microscope (B) of patients who had only corneal abnormalities were shown. OD: Oculus Dexter OS: Oculus Sinister. Specular microscope images were taken at five points: Central, upper, lower, nasal, temporal.

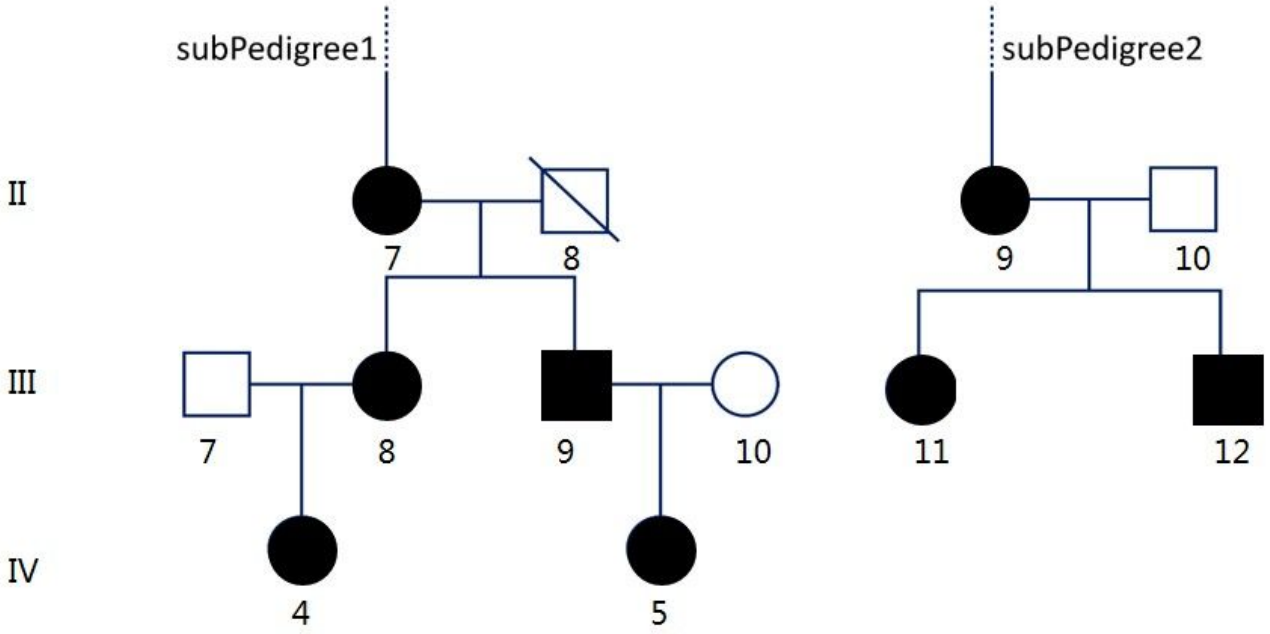

Figure 5

The map of the two sub-pedigrees in this family who performed WES.

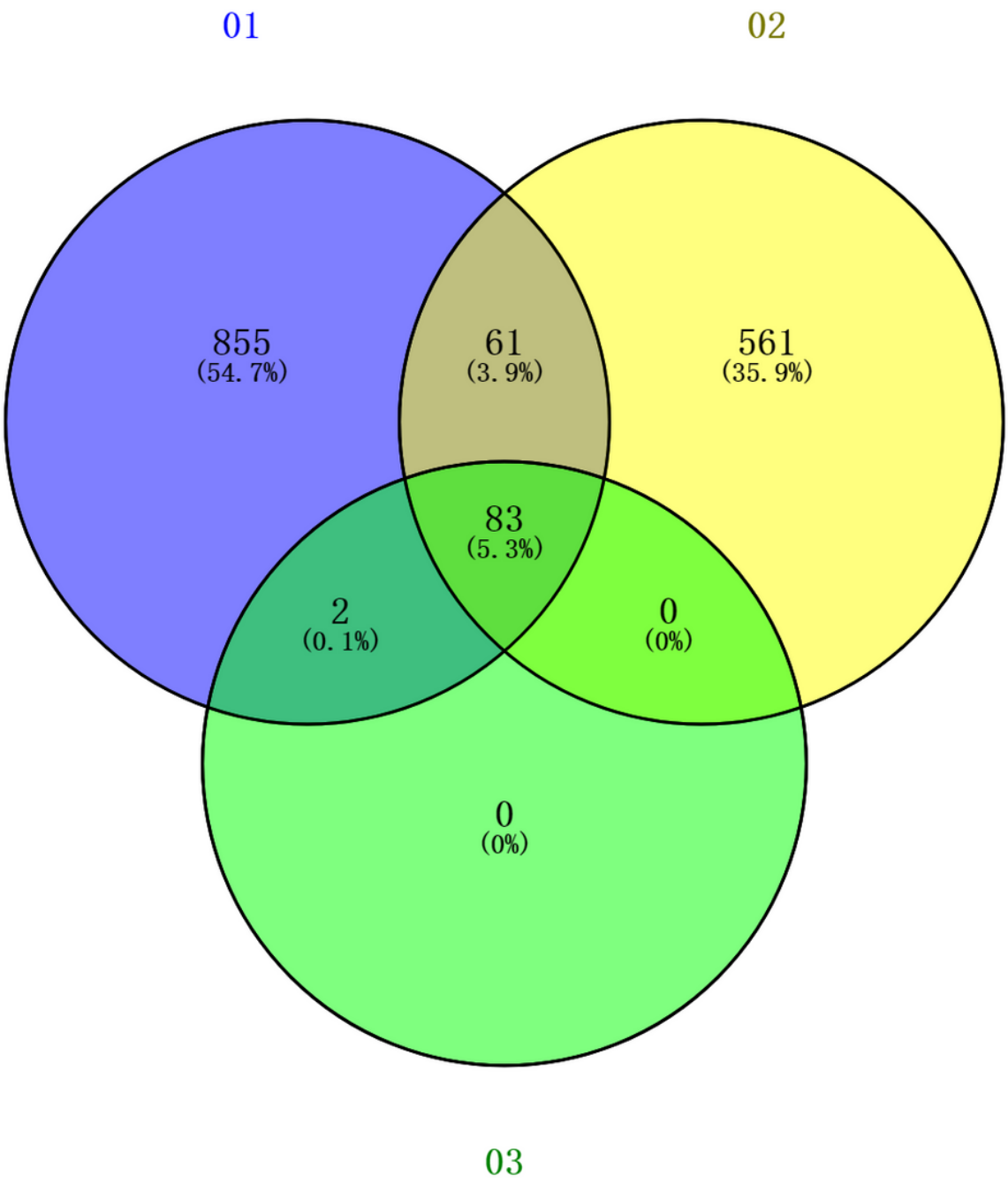

Figure 6

The Venn Diagram for the genes of all the variations with functional influence screened under three dominant modes. There were 83 genes shared by 01, 02 and 03.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- All.INDEL.Annotationresult.xls
- All.SNV.Annotationresult.xls
- SupplementaryTable.docx