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# Variation in DNA Base Excision Repair Genes in Fuchs Endothelial Corneal Dystrophy

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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**Background:** Fuchs endothelial corneal dystrophy (FECD) is a corneal disease characterized by abnormalities in the Descemet membrane and the corneal endothelium. The etiology of this disease is poorly understood. An increased level of oxidative DNA damage reported in FECD corneas suggests a role of DNA base excision repair (BER) genes in its pathogenesis. In this work, we searched for the association between variation of the *PARP-1*, *NEIL1*, *POLG*, and *XRCC1* genes and FECD occurrence.

**Material/Methods:** This study was conducted on 250 FECD patients and 353 controls using polymerase chain reaction-restriction fragment length polymorphism, high-resolution melting analysis, and the TaqMan® SNP Genotyping Assay.

**Results:** We observed that the A/A genotype and the A allele of the c.1196A>G polymorphism of the *XRCC1* gene were positively correlated with an increased FECD occurrence, whereas the G allele had the opposite effect. A weak association between the C/G genotype of the g.46438521G>C polymorphism of the *NEIL1* gene and an increased incidence of FECD was also detected. Haplotypes of both polymorphisms of the *XRCC1* were associated with FECD occurrence. No association of the c.2285T>C, c.–1370T>A and c.580C>T polymorphisms of the *PARP-1*, *POLG* and *XRCC1* genes, respectively, with FECD occurrence was observed.

**Conclusions:** Our results suggest that the c.1196A>G polymorphism in the *XRCC1* gene may be an independent genetic risk factor for FECD.

**MeSH Keywords:** DNA Repair • Fuchs' Endothelial Dystrophy • Oxidative Stress

**Full-text PDF:** http://www.medscimonit.com/abstract/index/idArt/894273

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## Background

Fuchs endothelial corneal dystrophy (FECD) is a posterior corneal dystrophy characterized by loss of endothelial cell density, changes in Descemet membrane, and corneal edema [1]. The signs of the disease include the formation of excrescences on a thickened Descemet membrane called 'cornea guttae', edema of the stroma, and loss of corneal clarity and visual acuity [2,3]. FECD is usually a late-onset disorder; it affects approximately 4% of individuals above 40 years of age, although a higher prevalence was reported in some populations, including European and American ones [4]. The majority of patients with FECD are classified as sporadic, but an autosomal dominant pattern of inheritance was also reported in several familial studies [5].

Despite intensive research, the etiology of FECD is not completely known. It seems that interaction of genetic and environmental factors contribute to development and progression of FECD [6,7]. Several causal genes were proposed as involved in the pathogenesis of FECD [1, 4]. A rare early-onset form of FECD is linked with mutations in *COL8A2*, encoding collagen type VIII [8,9], whereas genetic linkage analysis revealed 13pTel–13q12.13, 18q21.2–18q21.32, 5q33.1–5q35.2 and 9p22.1–9p24.1 *loci* as associated with more common late-onset FECD [10–13]. In addition, mutations in the *ZEB1* and *TCF4* genes that encode transcription factors, as well as the *SLC4A11* and *LOXHD1* genes encoding membrane transport proteins, were also reported to be associated with sporadic and familial cases of late-onset FECD [14–20].

A growing body of evidence shows that oxidative stress plays a role in the pathogenesis of FECD [6,21]. Proteomic analysis of corneal endothelium showed a decreased level of certain antioxidants, including peroxiredoxins, thioredoxin reductase, metallothionein 3, superoxide dismutase 2, nuclear ferritin, and glutathione S-transferase π in FECD corneas [6,16]. FECD corneas may be more susceptible to oxidative damage resulting from oxidant-antioxidant imbalance. Reactive oxygen species (ROS) induce various types of DNA damage, including oxidized bases, abasic sites, and single- and double-strand breaks [22]. Results of studies on FECD corneal endothelium showed that a level of 8-hydroxy-2'-deoxyguanosine (8-oxoG), which is an oxidative damage marker in DNA, is increased compared to normal age-matched controls [6]. Moreover, the majority of 8-oxoGs is located in the mitochondrial DNA (mtDNA), suggesting that it is a key target of alterations observed in FECD. A decreased number of mitochondria in endothelium and a decreased activity of cytochrome *c* oxidase – the major respiratory chain enzyme – were also found in FECD corneas [23,24]. Oxidative mtDNA damage may cause dysfunctional mitochondrial protein synthesis, loss of integrity of inner mitochondrial membrane potential, and apoptotic cell death. Increased apoptotic cell death detected in FECD endothelium suggests that oxidative stress-induced apoptosis may be involved in the causal mechanism of FECD [25].

Base excision repair is involved in the repair of many oxidative modifications from both nDNA and mtDNA. Defects in base excision repair (BER) affect genome stability and may induce various disorders [26]. The aim of this study was to assess whether change in the sequence of gene(s) involved in BER are associated with FECD occurrence. In this study, we investigated the association between 5 polymorphisms of the BER genes: the g.46438521G>C (rs4462560) in *NEIL1*, c.2285T>C (rs1136410) in *PARP-1*, c.–1370T>A (rs1054875) in *POLG*, c.580C>T (rs1799782), and c.1196A>G (rs25487) in *XRCC1* and FECD in the Polish population, as well as the modulation of this association by demographic and environmental risk factors.

## Material and Methods

### Study population

This study included 250 patients with FECD and 353 controls who were enrolled in the Department of Ophthalmology, Medical University of Warsaw (Warsaw, Poland).

The diagnosis of FECD was determined on the basis of clinical signs on the slit lamp examination, including occurrence of endothelial guttae and corneal edema [27,28]. In addition, presence of specific lesions, polymegathism, and pleomorphism of the endothelial cells were detected using *in vivo* confocal microscopy (IVCM) examination. All subjects had ophthalmic examination, including intraocular pressure, best-corrected visual acuity, slit lamp examination, fundus examination, anterior segment optical coherence tomography including pachymetry maps (AS-OCT), and IVCM as described previously [29]. The AS-OCT was carried out by Swept Source Anterior Segment Casia OCT (Tomey, Nagoya, Japan). The IVCM was carried out by the white light scanning slit confocal microscopy system (ConfoScan 3 or ConfoScan 4, Nidek Techologies, Padova, Italy). FECD patients were also divided according to a new classification proposed by Professor Jacek P. Szaflik, depending on the size and location of corneal lesions: central, scattered, or undefined.

No clinical evidence of FECD, as well as healthy corneal endothelium on IVCM and normal corneal pachymetry and topography, were observed in all control subjects.

The Bioethics Committee (Medical University of Warsaw, Poland) approved this study and informed consent was obtained from each participant. All individuals gave information on demographic data and potential risk factors for FECD. All subjects

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**Table 1.** Characteristics of Fuchs endothelial corneal dystrophy (FECD) patients and controls enrolled in this study.

| Feature | Controls (n=353) | FECD (n=250) | *p* |
|---|---|---|---|
| | Number (frequency) | | |
| Sex | | | **<0.001** |
| Females | 223 (0.63) | 192 (0.77) | |
| Males | 130 (0.37) | 58 (0.23) | |
| Age | | | **<0.001*** |
| Mean ±SD | 63±18.9 | 70±9.9 | |
| Range | 19–100 | 37–91 | |
| Smoking | | | 0.838 |
| Yes (current/former) | 116 (0.33) | 85 (0.34) | |
| Never | 237 (0.67) | 165 (0.66) | |
| FECD in family | | | **<0.001** |
| Yes | 3 (0.01) | 38 (0.15) | |
| No | 350 (0.99) | 212 (0.85) | |
| BMI | | | 0.952 |
| ≤25 | 149 (0.42) | 104 (0.42) | |
| 25–30 | 119 (0.34) | 83 (0.33) | |
| ≥30 | 85 (0.24) | 63 (0.25) | |
| Visual impairment | | | **<0.001** |
| Yes | 114 (0.32) | 148 (0.59) | |
| No | 239 (0.68) | 102 (0.41) | |
| Allergies | | | 0.130 |
| Yes | 44 (0.12) | 43 (0.17) | |
| No | 309 (0.88) | 207 (0.83) | |
| Heart and vascular diseases | | | 0.231 |
| Yes | 189 (0.54) | 147 (0.59) | |
| No | 164 (0.46) | 103 (0.41) | |

*p* Values for two-side $\chi^2$ test, except * *p* values for t-test, $p<0.05$ are in bold.

had detailed medical history taken and none had any genetic disease. Collected data included sex, age, allergy, co-occurrence of heart or vascular diseases, visual impairment (including hyperopia, astigmatism, or myopia), smoking, body mass index (BMI), and family history among first-degree relatives for FECD. Smoking was categorized as current, former, or never smokers. Characteristics of all subjects are presented in Table 1.

Each patient gave 5 milliliters of venous blood to EDTA-containing tubes, which were stored at –20°C.

### Selection of SNPs and primer design

The National Center for Biotechnology Information at (*http://www.ncbi.nlm.nih.gov/snp*) was used to select SNPs in BER genes. We chose 5 polymorphisms: the g.46438521G>C in *NEIL1,* c.2285T>C in *PARP-1*, c.–1370T>A in *POLG*, c.580C>T, and c.1196A>G in *XRCC1*, which have expected minor allele frequency (MAF) higher than 0.05 in Caucasians (*http://www.ncbi.nlm.nih.gov/snp*). All polymorphisms are located in the coding or regulatory regions of genes and might have functional significance for transcription and protein function. We used the nucleotide sequences published in ENSEMBL (*http://*

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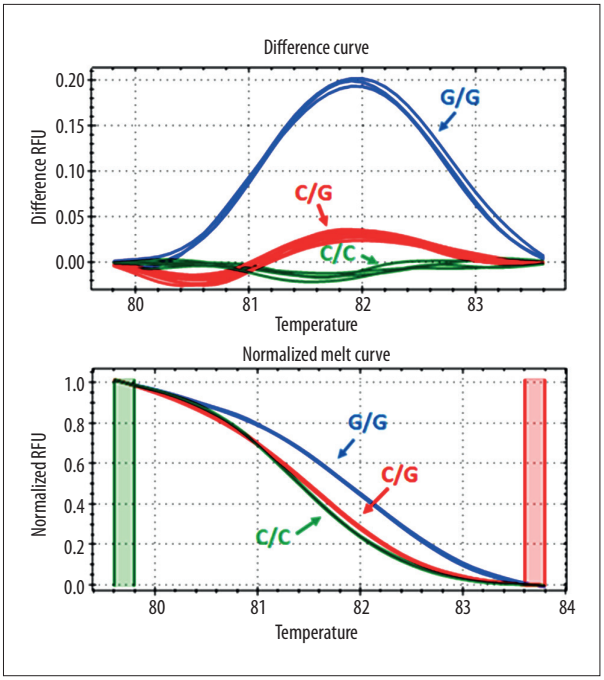



**Supplementary Figure 1.** Results of high resolution melting (HRM) analysis of the g.46438521G>C polymorphism (rs4462560) of the *NEIL1* gene. Homozygous G/G and C/G, and heterozygous C/C samples are shown on a standard normalized melt curve and a difference curves. Arrows indicate the different genotypes.

*www.ensembl.org/index.html*) and Primer3 software (*http://frodo.wi.mit.edu/*) to design the primer. The Primer-BLAST software (*http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi*) was used to analyze specificities of primers for the high-resolution melting analysis (HRM). We employed TaqMan probe for c.2285T>C SNP (Life Technologies, Foster City, CA, USA).

### DNA isolation

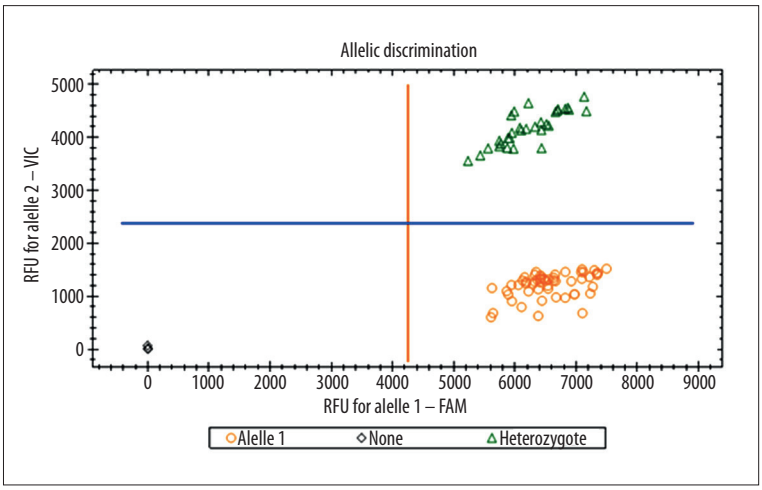
DNA was isolated from PBL using AxyPrep™ Blood Genomic DNA Miniprep Kit (Axygen Biosciences, Union City, CA, USA).

### Genotyping of g.46438521G>C of NEIL1

The g.46438521G>C polymorphism was genotyped by HRM on a C1000™ Thermal Cycler (Bio-Rad, Hercules, CA, USA). PCR reaction was carried out in a volume of 10 µl containing 25 ng of genomic DNA, 1× KAPA HRM FAST Master Mix (with EvaGreen® dye) and 0.25 µM of each primer (Sigma-Aldrich, St. Louis, MO, USA), and 2.5 mM $MgCl_2$ (Kapa Biosystems, Woburn, MA, USA). The sequences of the primers used in PCR were as follows: forward 5'-GGG CTT CTC AAC TCA TGG TC-3' and reverse 5'-ACA GGA GAG ACT GGG GAC CT-3. Amplification conditions were as follows: 2 min denaturation at 95°C, followed by 49 cycles of denaturation 5 s at 95°C; and annealing 30 s at 60.3°C. The products were heated to 95°C for 30 s and then sample temperature was reduced to 60°C for 1 min. HRM data were acquired at 71–85°C, increment 0.2°C, for 0:10 min (Supplementary Figure 1). Analysis was performed using Bio-Rad Precision Melt Analysis™ software.

### Genotyping of c.2285T>C of *PARP-1*

The c.2285T>C polymorphism in the *PARP-1* gene was genotyped using the TaqMan® SNP Genotyping Assay. Subjects were genotyped using ID: C_11468118_10 assay (Life Technologies, Foster City, CA, USA), which consists of forward and reverse primers and 2 TaqMan probes: 1 probe labeled with VIC®-dye specific to the C allele, and 1 probe labeled with FAM™ dye specific to the T allele. Real-time PCR was carried out on the same thermal cycler as in HRM analysis, using the recommended conditions, including 10 min incubation at 95°C, followed by 40 cycles, 15 s denaturation at 92°C, and 1 min




**Supplementary Figure 2.** Results of the TaqMan® SNP Genotyping Assay of the c.2285T>C polymorphism (rs1136410) of the *PARP-1* gene. The X-axis represents the relative fluorescent emission for the T allele-specific probe labeled with 6-carboxyfluorescein (FAM), and the Y-axis represents the emission for the C allele-specific probe labeled with 2'-chloro-7'-phenyl-1,4-dichloro–6-carboxyfluorescein (VIC). Circles – homozygous GG; triangles– heterozygous AG. Diamonds represent no template controls.

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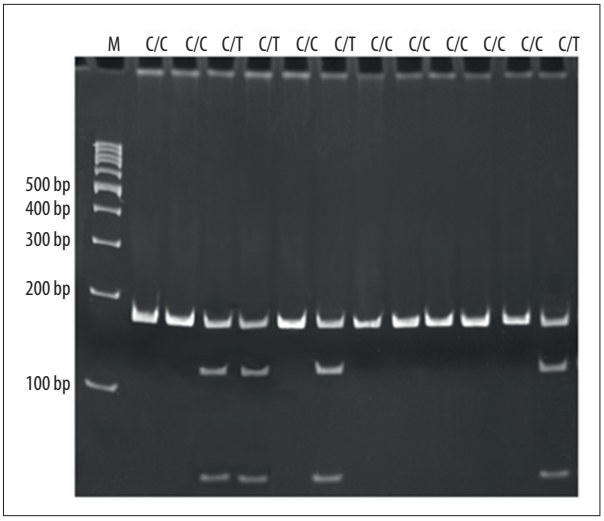


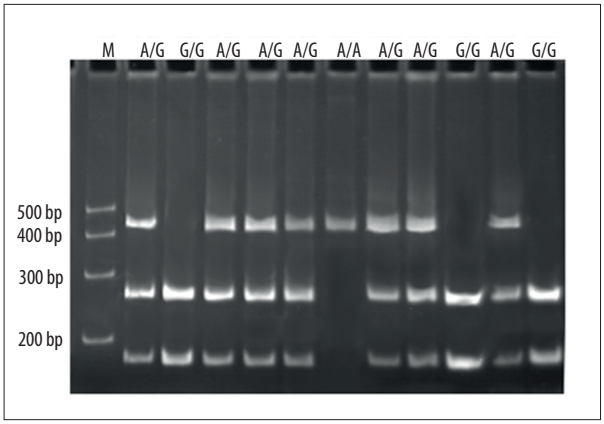

**Supplementary Figure 3.** Results of the restriction fragments length polymorphism analysis of the c.580C>T polymorphism (rs1799782) of the *XRCC1* gene on an 8% polyacrylamide gel. Lane M shows a GeneRuler™ 100 bp marker ladder, with length of the fragment indicated left to the picture. Genotypes are shown above the picture.

annealing/extension at 60°C. Levels of FAM and VIC fluorescence of the PCR products were measured at each PCR cycle at 60°C. CFX Manager Software was used to analyze all samples based on the dye component fluorescent emission data (Supplementary Figure 2).

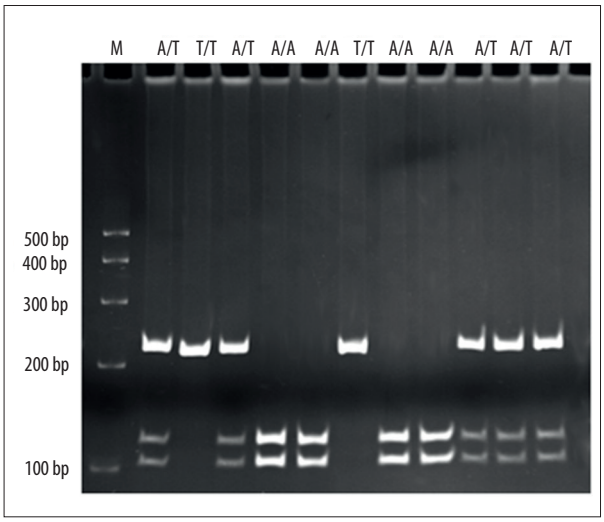
### Genotyping of c.580C>T and c.1196A>G of XRCC1and c.–1370T>A of POLG

The genotypes of the c.580C>T, c.1196A>G, and c.–1370T>A polymorphisms were performed by polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP). PCR amplification was carried out in 10-µl PCR reactions, including 0.25 U HotStarTaq Plus DNA Polymerase (Qiagen, Venlo, The Netherlands), 0.25µM of each primer, 200 µM of dNTPs, 25 ng DNA, and 1 µl of 10× PCR buffer.

Following primers were used for DNA amplification of the c.580C>T polymorphism: forward 5'-TGA AGG AGG AGG ATG AGA GC-3'and reverse 5'-TCA GAC CCA GGA ATC TGA GC-3'. PCR conditions were as follows: an initial denaturation step at 95°C for 5 min, 40 cycles at 95°C for 30 s, 64°C for 30 s, 72°C for 1 min, and a final elongation step at 72°C for 5 min. Amplified fragments were digested with 2 U of *Pvu*II restriction endonuclease (New England Biolabs, Ipswich, UK) in a final volume of 15 µl for 16 h at 37°C. The samples were genotyped according to the size of the PCR products: products amplified with the C/C genotype were 176 bp in length, those with the T/T genotype were 120 and 56 bp, and those with the C/T genotype were 176, 120, and 56 bp (Supplementary Figure 3).




**Supplementary Figure 4.** Results of the restriction fragments length polymorphism analysis of the c.1196A>G polymorphism (rs25487) of the *XRCC1* gene on an 8% polyacrylamide gel. Lane M shows a M100-500 DNA marker ladder, with length of the fragment indicated left to the picture. Genotypes are shown above the picture.




**Supplementary Figure 5.** Results of the restriction fragments length polymorphism analysis of the c.–1370T>A polymorphism (rs1054875) of the *POLG* gene on an 8% polyacrylamide gel. Lane M shows a M100–500 DNA marker ladder, with length of the fragment indicated left to the picture. Genotypes are shown above the picture.

The primers to detect the c.1196A>G SNP were as follows: forward: 5'-GGT CCT CCT TCC CTC ATC TG-3'; reverse: 5'-TGC ATC TCT CCC TTG GTC TC-3'. The PCR conditions were: 5 min of initial denaturation at 95 °C, followed by 40 cycles of 30 s denaturation at 95°C, 30 s annealing at 64.5°C, and 1 min extension at 72°C, and the final extension step at 72°C for 10 min. Amplification products were digested with 2 U of *Hpy*II restriction enzyme for 16 h at 37°C. Digestion of the PCR products yielded bands of 277 and 182 bp in G/G homozygotes,

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**Table 2.** Occurrence of FECD associated with age, sex, tobacco smoking, co-occurrence of visual disturbances, BMI, heart and vascular diseases, allergies and family history of FECD.

| Characteristics | Controls (n=353) | FECD (n=250) | OR (95% CI) | *p* |
|---|---|---|---|---|
| | Number (frequency) | | | |
| Sex | | | | |
| females | 223 (0.63) | 192 (0.77) | **1.91 (1.33–2.76)** | **<0.001** |
| males | 130 (0.37) | 58 (0.23) | **0.52 (0.36–0.75)** | **<0.001** |
| Age | 63±18.9 | 70±9.9 | **1.03 (1.02–1.04)** | **<0.001** |
| Smoking | | | | |
| yes (current/former) | 116 (0.33) | 85 (0.34) | 1.07 (0.76–1.51) | 0.702 |
| never | 237 (0.67) | 165 (0.66) | 0.93 (0.66–1.32) | 0.702 |
| FECD in family | | | | |
| yes | 3 (0.01) | 38 (0.15) | **21.21 (6.47–69.59)** | **<0.001** |
| no | 350 (0.99) | 212 (0.85) | **0.04 (0.01–0.15)** | **<0.001** |
| BMI | | | | |
| ≤25 | 149 (0.42) | 104 (0.42) | 0.98 (0.70–1.36) | 0.904 |
| 25–30 | 119 (0.34) | 83 (0.33) | 0.97 (0.69–1.37) | 0.875 |
| ≥30 | 85 (0.24) | 63 (0.25) | 1.06 (0.73–1.55) | 0.756 |
| Visual impairment | | | | |
| yes | 114 (0.32) | 148 (0.59) | **3.31 (2.35–4.65)** | **<0.001** |
| no | 239 (0.68) | 102 (0.41) | **0.30 (0.22–0.43)** | **<0.001** |
| Allergies | | | | |
| yes | 44 (0.12) | 43 (0.17) | 1.45 (0.92–2.29) | 0.106 |
| no | 309 (0.88) | 207 (0.83) | 0.69 (0.47–1.08) | 0.106 |
| Heart and vascular diseases | | | | |
| yes | 189 (0.54) | 147 (0.59) | 1.26 (0.91–1.75) | 0.164 |
| no | 164 (0.46) | 103 (0.41) | 0.79 (0.57–1.10) | 0.164 |

OR – odds ratio; 95% CI – 95% confidence interval; *p* values <0.05 along with corresponding ORs are in bold.

459 bp in A/A homozygotes, and all 3 bands in heterozygotes (Supplementary Figure 4).

Genotypic analysis of the c.–1370T>A polymorphism was performed using primers: forward 5'-TGA AGG AGG AGG ATG AGA GC-3'and reverse 5'-TCA GAC CCA GGA ATC TGA GC-3'. The cycling program consisted of preliminary denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 30 s, and annealing at 66°C for 30 s, extension at 72°C for 1 minutes, and a final extension at 72°C for 5 min. The PCR product of 224 bp was digested with 2 U of *Hpy*188I restriction enzyme at 37°C for 16 h, resulting in fragments of 121 and 103 bp for the A allele and 224 bp for the T allele (Supplementary Figure 5).

Restriction DNA fragments were separated by electrophoresis on an 8% polyacrylamide gel and stained with ethidium bromide (0.5 mg/ml) using M100-500 DNA Ladder (BLIRT S.A., Gdansk, Poland) or a GeneRuler™ 100 bp (Fermentas, Hanover, MD, USA) as a size marker. Electrophoresis was carried out at 5 V/cm in TBE buffer. The gel was then visualized under UV illumination. All PCR amplifications were conducted in the C1000 Thermal Cycler.

### Statistical analysis

We verified the Hardy-Weinberg equilibrium of genotype distributions separately among patients and controls by the chi-square ($\chi^2$) test [29,30]. In addition, the $\chi^2$ test was used to

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**Table 3.** Distribution of genotypes and alleles of the g.46438521G>C – *NEIL1*, c.2285T>C – *PARP-1*, c.–1370T>A – *POLG*, c.580C>T – *XRCC1* and c.1196A>G – *XRCC1* polymorphisms and odds ratio (OR) with 95% confidence interval (95% CI) in patients with Fuchs endothelial corneal dystrophy (FECD) and controls.

| Polymorphism genotype/allele | Controls (n=353) Number (frequency) | | FECD (n=250) Number (frequency) | | Crude OR (95% CI) | p | Adjusted *OR (95% CI) | p |
|---|---|---|---|---|---|---|---|---|
| g.46438521G>C *NEIL1* | | | | | | | | |
| C/C | 98 | (0.28) | 53 | (0.21) | 0.70 (0.48–1.03) | 0.068 | 0.66 (0.42–1.03) | 0.067 |
| C/G | 240 | (0.68) | 188 | (0.75) | 1.43 (0.99–2.05) | 0.055 | **1.53 (1.01–2.34)** | **0.047** |
| G/G | 15 | (0.04) | 9 | (0.04) | 0.84 (0.36–1.95) | 0.688 | 0.77 (0.30–1.95) | 0.581 |
| $\chi^2$=3.744; p=0.1538 | | | | | | | | |
| C | 436 | (0.62) | 294 | (0.59) | 0.78 (0.56–1.09) | 0.150 | 0.77 (0.52–1.12) | 0.176 |
| G | 270 | (0.38) | 206 | (0.41) | 1.27 (0.92–1.77) | 0.150 | 1.30 (0.89–1.90) | 0.176 |
| c.2285T>C *PARP-1* | | | | | | | | |
| A/A | 239 | (0.68) | 166 | (0.66) | 0.94 (0.67–1.33) | 0.737 | 1.09 (0.73–1.62) | 0.685 |
| A/G | 114 | (0.32) | 84 | (0.34) | 1.06 (0.75–1.49) | 0.737 | 0.92 (0.62–1.37) | 0.685 |
| G/G | 0 | (0.00) | 0 | (0.00) | – | – | – | – |
| $\chi^2$=0.062; p=0.8039 | | | | | | | | |
| A | 592 | (0.84) | 416 | (0.83) | 0.94 (0.67–1.33) | 0.737 | 1.09 (0.73–1.62) | 0.685 |
| G | 114 | (0.16) | 84 | (0.17) | 1.06 (0.75–1.49) | 0.737 | 0.92 (0.62–1.37) | 0.685 |
| c.–1370T>A *POLG* | | | | | | | | |
| A/A | 46 | (0.13) | 35 | (0.14) | 1.07 (0.68–1.74) | 0.731 | 1.08 (0.63–1.86) | 0.775 |
| A/T | 203 | (0.57) | 144 | (0.58) | 1.00 (0.72–1.39) | 0.982 | 1.27 (0.87–1.86) | 0.210 |
| T/T | 104 | (0.30) | 71 | (0.28) | 0.95 (0.66–1.36) | 0.777 | 0.71 (0.47–1.08) | 0.113 |
| $\chi^2$=0.159; p=0.9234 | | | | | | | | |
| A | 295 | (0.42) | 214 | (0.43) | 1.05 (0.81–1.36) | 0.698 | 1.21 (0.90–1.63) | 0.198 |
| T | 411 | (0.58) | 286 | (0.57) | 0.95 (0.74–1.23) | 0.698 | 0.82 (0.61–1.11) | 0.198 |
| c.580C>T *XRCC1* | | | | | | | | |
| C/C | 305 | (0.86) | 223 | (0.89) | 1.30 (0.79–2.15) | 0.306 | 0.10 (0.61–1.99) | 0.742 |
| C/T | 48 | (0.14) | 27 | (0.11) | 0.77 (0.47–1.27) | 0.306 | 1.36 (0.89–2.07) | 0.742 |
| T/T | 0 | (0.00) | 0 | (0.00) | – | – | – | – |
| $\chi^2$=0.811; p=0.3678 | | | | | | | | |
| C | 653 | (0.92) | 468 | (0.94) | 1.20 (0.75–1.93) | 0.442 | 0.96 (0.55–1.68) | 0.885 |
| T | 53 | (0.08) | 32 | (0.06) | 0.83 (0.52–1.33) | 0.442 | 1.04 (0.60–1.82) | 0.885 |
| c.1196A>G *XRCC1* | | | | | | | | |
| A/A | 72 | (0.20) | 74 | (0.30) | **1.64 (1.13–2.39)** | **0.010** | **1.59 (1.04–2.44)** | **0.033** |
| A/G | 218 | (0.62) | 143 | (0.57) | 0.83 (0.59–1.15) | 0.261 | 0.82 (0.56–1.20) | 0.308 |
| G/G | 63 | (0.18) | 33 | (0.13) | 0.70 (0.44–1.10) | 0.126 | 0.71 (0.42–1.23) | 0.230 |
| $\chi^2$=7.613; p=0.0222 | | | | | | | | |
| A | 362 | (0.51) | 291 | (0.58) | **1.43 (1.10–1.85)** | **0.008** | **1.40 (1.03–1.90)** | **0.030** |
| G | 344 | (0.49) | 209 | (0.42) | **0.70 (0.54–0.91)** | **0.008** | **0.71 (0.53–0.97)** | **0.030** |

*p*<0.05 along with corresponding ORs are in bold; * OR adjusted for co-occurrence of visual impairment, sex, age and family history for FECD.

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**Supplementary Table 1.** Distribution of combined genotypes of the g.46438521G>C – *NEIL1* and c.2285T>C – *PARP-1* polymorphisms and odds ratio (OR) with 95% confidence interval (95% CI) in patients with Fuchs endothelial corneal dystrophy (FECD) and controls.

| Combined genotypes | Controls (n=353) Number (frequency) | FECD (n=250) Number (frequency) | Crude OR (95% CI) | *p* | Adjusted OR* (95% CI) | *p* |
|---|---|---|---|---|---|---|
| C/C – A/A | 64 (0.18) | 35 (0.14) | 0.73 (0.47–1.15) | 0.178 | 0.90 (0.53–1.53) | 0.702 |
| C/C – A/G | 34 (0.10) | 18 (0.07) | 0.73 (0.40–1.32) | 0.296 | **0.42 (0.20–0.88)** | **0.021** |
| C/C – G/G | 0 (0.00) | 0 (0.00) | – | – | – | – |
| C/G – A/A | 167 (0.47) | 126 (0.50) | 1.13 (0.82–1.56) | 0.454 | 1.11 (0.76–1.62) | 0.595 |
| C/G – A/G | 73 (0.21) | 62 (0.25) | 1.26 (0.86–1.86) | 0.232 | 1.42 (0.91–2.25) | 0.122 |
| C/G – G/G | 0 (0.00) | 0 (0.00) | – | – | – | – |
| G/G – A/A | 8 (0.02) | 5 (0.02) | 0.88 (0.28–2.73) | 0.825 | 1.38 (0.90–2.11) | 0.924 |
| G/G – A/G | 7 (0.02) | 4 (0.02) | 0.80 (0.23–2.77) | 0.730 | 0.53 (0.13–2.15) | 0.376 |
| G/G – G/G | 0 (0.00) | 0 (0.00) | – | – | – | – |

*p* values <0.05 along with corresponding ORs are in bold; * OR adjusted for co-occurrence of visual impairment, sex, age and family history for FECD.

**Supplementary Table 2.** Distribution of combined genotypes of the g.46438521G>C – *NEIL1* and c.–1370T>A – *POLG* polymorphisms and odds ratio (OR) with 95% confidence interval (95% CI) in patients with Fuchs endothelial corneal dystrophy (FECD) and controls.

| Combined genotypes | Controls (n=353) Number (frequency) | FECD (n=250) Number (frequency) | Crude OR (95% CI) | *p* | Adjusted OR* (95% CI) | *p* |
|---|---|---|---|---|---|---|
| C/C – A/A | 14 (0.04) | 9 (0.04) | 0.90 (0.38–2.12) | 0.817 | 0.81 (0.29–2.29) | 0.691 |
| C/C – A/T | 30 (0.08) | 25 (0.10) | 1.20 (0.68–2.09) | 0.529 | 1.25 (0.67–2.32) | 0.486 |
| C/C – T/T | 2 (0.01) | 1 (0.01) | 0.70 (0.06–7.82) | 0.776 | 0.71(0.04–11.14) | 0.807 |
| C/G – A/A | 57 (0.16) | 30 (0.12) | 0.71 (0.44–1.14) | 0.155 | 0.85 (0.49–1.47) | 0.562 |
| C/G – A/T | 140 (0.40) | 109 (0.44) | 1.17 (0.85–1.63) | 0.333 | 1.34 (0.94–1.96) | 0.133 |
| C/G – T/T | 6 (0.02) | 5 (0.02) | 1.18 (0.36–3.91) | 0.786 | 1.56 (0.42–5.75) | 0.502 |
| G/G – A/A | 27 (0.08) | 14 (0.06) | 0.72 (0.37–1.39) | 0.327 | **0.40 (0.17–0.95)** | **0.039** |
| G/G – A/T | 70 (0.20) | 54 (0.22) | 1.11 (0.74–1.66) | 0.596 | 0.97 (0.60–1.56) | 0.906 |
| G/G – T/T | 7 (0.02) | 3 (0.01) | 0.60 (0.15–2.34) | 0.463 | 0.33 (0.07–1.60) | 0.170 |

*p* values <0.05 along with corresponding ORs are in bold; * OR adjusted for co-occurrence of visual impairment, sex, age and family history for FECD.

estimate the differences in frequency distributions of demographic and potential risk factors for FECD and genotypes and alleles between the these 2 groups. Unconditional logistic regression, both with and without adjustment for sex, age, family history of FECD, and co-occurrence of visual disturbances, was used to estimate the odds ratios (ORs) and their 95% confidence intervals (CIs) for the association between the genotypes/ combined genotypes and risk of FECD. Moreover, the data were analyzed stratified by sex and form of FECD. PHASE software (*http://stephenslab.uchicago.edu/software.html*) was used to assess the association of haplotypes with FECD. Data were analyzed with the SigmaPlot v 11.0 (Systat Software, Inc., San Jose, CA, USA).

## Results

### Characteristics of study population

Table 1 show demographic variables and potential risk factors for FECD of patients and controls. The occurrence of the disease among first-degree relatives in the patients was significantly

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**Supplementary Table 3.** Distribution of combined genotypes of the g.46438521G>C – *NEIL1* and c.580C>T – *XRCC1* polymorphisms and odds ratio (OR) with 95% confidence interval (95% CI) in patients with Fuchs endothelial corneal dystrophy (FECD) and controls.

| Combined genotypes | Controls (n=353) Number (frequency) | FECD (n=250) Number (frequency) | Crude OR (95% CI) | *p* | Adjusted OR* (95% CI) | *p* |
|---|---|---|---|---|---|---|
| C/C – C/C | 82 (0.23) | 49 (0.20) | 0.81 (0.54–1.20) | 0.287 | 0.73 (0.46–1.17) | 0.193 |
| C/C – C/T | 16 (0.05) | 4 (0.02) | 0.34 (0.11–1.04) | 0.058 | 0.33 (0.08–1.32) | 0.118 |
| C/C – T/T | 0 (0.00) | 0 (0.00) | – | – | – | – |
| C/G – C/C | 210 (0.59) | 167 (0.67) | 1.37 (0.98–1.92) | 0.068 | 1.42 (0.95–2.10) | 0.084 |
| C/G – C/T | 30 (0.08) | 21 (0.08) | 0.99 (0.55–1.77) | 0.966 | 1.09 (0.55–2.16) | 0.809 |
| C/G – T/T | 0 (0.00) | 0 (0.00) | – | – | – | – |
| G/G – C/C | 13 (0.04) | 7 (0.03) | 0.75 (0.30–1.92) | 0.552 | 0.67 (0.24–1.89) | 0.452 |
| G/G – C/T | 2 (0.01) | 2 (0.01) | 1.41(0.20–10.11) | 0.729 | 1.45(0.16–12.87) | 0.735 |
| G/G – T/T | 0 (0.00) | 0 (0.00) | – | – | – | – |

*p* values <0.05 along with corresponding ORs are in bold; * OR adjusted for co-occurrence of visual impairment, sex, age and family history for FECD.

**Supplementary Table 4.** Distribution of combined genotypes of the g.46438521G>C – *NEIL1* and c.1196A>G – *XRCC1* polymorphisms and odds ratio (OR) with 95% confidence interval (95% CI) in patients with Fuchs endothelial corneal dystrophy (FECD) and controls.

| Combined genotypes | Controls (n=353) Number (frequency) | FECD (n=250) Number (frequency) | Crude OR (95% CI) | *p* | Adjusted OR* (95% CI) | *p* |
|---|---|---|---|---|---|---|
| C/C – A/A | 24 (0.07) | 16 (0.06) | 0.93 (0.48–1.80) | 0.846 | 1.05 (0.50–2.20) | 0.903 |
| C/C – A/G | 59 (0.17) | 29 (0.12) | 0.65 (0.41–1.05) | 0.081 | 0.56 (0.31–1.01) | 0.054 |
| C/C – G/G | 15 (0.04) | 8 (0.03) | 0.74 (0.31–1.78) | 0.509 | 0.65 (0.23–1.85) | 0.417 |
| C/G – A/A | 48 (0.14) | 55 (0.22) | **1.79 (1.17–2.74)** | **0.007** | 1.61 (0.99–2.62) | 0.053 |
| C/G – A/G | 146 (0.41) | 108 (0.43) | 1.08 (0.77–1.50) | 0.652 | 1.14 (0.78–1.68) | 0.477 |
| C/G – G/G | 46 (0.13) | 25 (0.10) | 0.74 (0.44–1.24) | 0.256 | 0.81 (0.43–1.53) | 0.525 |
| G/G – A/A | 0 (0.00) | 3 (0.01) | – | – | – | – |
| G/G – A/G | 13 (0.04) | 6 (0.02) | 0.64 (0.24–1.72) | 0.378 | 0.49 (0.16–1.47) | 0.205 |
| G/G – G/G | 2 (0.01) | 0 (0.00) | – | – | – | – |

*p* values <0.05 along with corresponding ORs are in bold; * OR adjusted for co-occurrence of visual impairment, sex, age and family history for FECD.

higher compared to controls (15% *vs.* 1%, $p<0.001$). We detected a significant difference between distributions of sex, age, family history for FECD (positive *vs* negative), and co-occurrence of visual impairment (yes *vs.* no). These variables were further adjusted in the multivariate logistic regression analysis.

### Relationship between risk factor of FECD and the occurrence of FECD independently of genotype

Age, sex, tobacco smoking, co-occurrence of FECD in family, visual disturbances, heart and vascular diseases, allergies, and BMI were analyzed for association with FECD independently of genotype. We compared FECD patients with controls according to these parameters (Table 2). We observed positive correlations between females, higher age, FECD in family, and co-occurrence of visual disturbances and increased the occurrence of FECD. No significant difference was found in the distribution of BMI, smoking status, co-occurrence of allergies, or heart and vascular diseases in FECD patients and controls.

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**Supplementary Table 5.** Distribution of combined genotypes of the c.2285T>C – *PARP-1* and c.–1370T>A – *POLG* polymorphisms and odds ratio (OR) with 95% confidence interval (95% CI) in patients with Fuchs endothelial corneal dystrophy (FECD) and controls.

| Combined genotypes | Controls (n=353) | FECD (n=250) | Crude OR (95% CI) | *p* | Adjusted OR* (95% CI) | *p* |
|---|---|---|---|---|---|---|
| | Number (frequency) | | | | | |
| A/A – A/A | 29 (0.08) | 26 (0.10) | 1.30 (0.74–2.61) | 0.360 | 1.16 (0.62–2.18) | 0.645 |
| A/A – A/T | 17 (0.05) | 9 (0.04) | 0.74 (0.32–1.68) | 0.470 | 0.96 (0.38–2.43) | 0.929 |
| A/A – T/T | 0 (0.00) | 0 (0.00) | – | – | – | – |
| A/G – A/A | 143 (0.41) | 104 (0.42) | 1.05 (0.75–1.45) | 0.789 | 1.33 (0.90–1.95) | 0.150 |
| A/G – A/T | 60 (0.17) | 40 (0.16) | 0.93 (0.60–1.44) | 0.746 | 0.95 (0.57–1.58) | 0.849 |
| A/G – T/T | 0 (0.00) | 0 (0.00) | – | – | – | – |
| G/G – A/A | 67 (0.19) | 36 (0.14) | 0.72 (0.46–1.12) | 0.142 | 0.61 (0.37–1.03) | 0.065 |
| G/G – A/T | 37 (0.10) | 35 (0.14) | 1.39 (0.85–2.28) | 0.191 | 0.94 (0.52–1.74) | 0.867 |
| G/G – T/T | 0 (0.00) | 0 (0.00) | – | – | – | – |

*p* values <0.05 along with corresponding ORs are in bold; * OR adjusted for co-occurrence of visual impairment, sex, age and family history for FECD.

**Supplementary Table 6.** Distribution of combined genotypes of the c.2285T>C – *PARP-1* and c.580C>T – *XRCC1* polymorphisms and odds ratio (OR) with 95% confidence interval (95% CI) in patients with Fuchs endothelial corneal dystrophy (FECD) and controls.

| Combined genotypes | Controls (n=353) | FECD (n=250) | Crude OR (95% CI) | *p* | Adjusted OR* (95% CI) | *p* |
|---|---|---|---|---|---|---|
| | Number (frequency) | | | | | |
| A/A – C/C | 204 (0.58) | 150 (0.60) | 1.18 (0.84–1.65) | 0.344 | 1.22 (0.82–1.80) | 0.329 |
| A/A – C/T | 35 (0.10) | 16 (0.06) | 0.58 (0.31–1.08) | 0.086 | 1.43 (0.92–2.21) | 0.084 |
| A/A – T/T | 0 (0.00) | 0 (0.00) | – | | | |
| A/G – C/C | 101 (0.29) | 73 (0.29) | 0.99 (0.69–1.43) | 0.963 | 0.99 (0.65–1.53) | 0.994 |
| A/G – C/T | 13 (0.04) | 11 (0.04) | 1.10 (0.48–2.49) | 0.824 | 0.99 (0.39–2.49) | 0.982 |
| A/G – T/T | 0 (0.00) | 0 (0.00) | – | – | – | – |
| G/G – C/C | 0 (0.00) | 0 (0.00) | – | – | – | – |
| G/G – C/T | 0 (0.00) | 0 (0.00) | – | – | – | – |
| G/G – T/T | 0 (0.00) | 0 (0.00) | – | – | – | – |

*p* values <0.05 along with corresponding ORs are in bold; * OR adjusted for co-occurrence of visual impairment, sex, age and family history for FECD.

### Polymorphisms of the *NEIL1*, *PARP-1*, *POLG* and *XRCC1* genes and FECD occurrence

The genotype and allele distributions of 5 polymorphisms in *NEIL1, PARP-1*, *POLG,* and *XRCC1* genes in FECD patients and controls are presented in Table 3. The observed genotypes frequencies for the c.580C>T were in agreement with Hardy-Weinberg equilibrium ($p>0.05$, data not shown) for the controls and patients. A positive association between FECD occurrence and the C/G genotype of the g.46438521G>C polymorphism was found. Moreover, the A/A genotype and the A allele of the c.1196A>G polymorphism were positively correlated with the occurrence of FECD, whereas the G allele had a protective effect against this disease. No association between genotypes/alleles of the c.2285T>C, c.–1370T>A and c.580C>T and FECD occurrence were found.

### Gene-gene interaction and FECD occurrence

The association between the occurrence of FECD and combined genotypes of the c.2285T>C, g.46438521G>C, c.–1370T>A, c.1196A>G, and c.580C>T polymorphisms were

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**Supplementary Table 7.** Distribution of combined genotypes of the c.2285T>C – *PARP-1* and c.1196A>G – *XRCC1* polymorphisms and odds ratio (OR) with 95% confidence interval (95% CI) in patients with Fuchs endothelial corneal dystrophy (FECD) and controls.

| Combined genotypes | Controls (n=353) | FECD (n=250) | Crude OR (95% CI) | *p* | Adjusted OR* (95% CI) | *p* |
|---|---|---|---|---|---|---|
| | Number (frequency) | | | | | |
| A/A – A/A | 52 (0.15) | 48 (0.19) | 1.47 (0.94–2.26) | 0.087 | 1.57 (0.92–2.67) | 0.096 |
| A/A – A/G | 144 (0.41) | 97 (0.39) | 1.03 (0.73–1.44) | 0.859 | 0.96 (0.65–1.43) | 0.843 |
| A/A – G/G | 43 (0.12) | 21 (0.08) | 0.58 (0.33–1.03) | 0.062 | **0.46 (0.23–0.92)** | **0.029** |
| A/G – A/A | 20 (0.06) | 26 (0.10) | 1.29 (0.68–2.46) | 0.436 | 1.58 (0.75–3.32) | 0.230 |
| A/G – A/G | 74 (0.21) | 46 (0.18) | 0.80 (0.52–1.22) | 0.293 | 0.86 (0.53–1.40) | 0.549 |
| A/G – G/G | 20 (0.06) | 12 (0.05) | 1.13 (0.55–2.30) | 0.744 | 1.18 (0.51–2.74) | 0.705 |
| G/G – A/A | 0 (0.00) | 0 (0.00) | – | – | – | – |
| G/G – A/G | 0 (0.00) | 0 (0.00) | – | – | – | – |
| G/G – G/G | 0 (0.00) | 0 (0.00) | – | – | – | – |

*p* values <0.05 along with corresponding ORs are in bold; * OR adjusted for co-occurrence of visual impairment, sex, age and family history for FECD.

**Supplementary Table 8.** Distribution of combined genotypes of the c.–1370T>A – *POLG* and c.580C>T – *XRCC1* polymorphisms and odds ratio (OR) with 95% confidence interval (95% CI) in patients with Fuchs endothelial corneal dystrophy (FECD) and controls.

| Combined genotypes | Controls (n=353) | FECD (n=250) | Crude OR (95% CI) | *p* | Adjusted OR* (95% CI) | *p* |
|---|---|---|---|---|---|---|
| | Number (frequency) | | | | | |
| A/A – C/C | 41 (0.12) | 32 (0.13) | 1.12 (0.68–1.83) | 0.660 | 1.07 (0.61–1.88) | 0.804 |
| A/A – C/T | 5 (0.01) | 3 (0.01) | 0.84 (0.20–3.57) | 0.819 | 1.38 (0.90–2.13) | 0.745 |
| A/A – T/T | 0 (0.00) | 0 (0.00) | – | – | – | – |
| A/T – C/C | 175 (0.50) | 130 (0.52) | 1.10 (0.80–1.52) | 0.557 | 1.39 (0.92–1.96) | 0.129 |
| A/T – C/T | 28 (0.08) | 14 (0.06) | 0.70 (0.35–1.33) | 0.270 | 0.81 (0.36–1.80) | 0.601 |
| A/T – T/T | 0 (0.00) | 0 (0.00) | – | – | – | – |
| T/T – C/C | 89 (0.25) | 61 (0.24) | 0.96 (0.66–1.39) | 0.820 | 0.69 (0.44–1.09) | 0.110 |
| T/T – C/T | 15 (0.04) | 10 (0.04) | 0.94 (0.41–2.13) | 0.880 | 0.85 (0.32–2.20) | 0.732 |
| T/T – T/T | 0 (0.00) | 0 (0.00) | – | – | – | – |

*p* values <0.05 along with corresponding ORs are in bold; * OR adjusted for co-occurrence of visual impairment, sex, age and family history for FECD.

also investigated. The distribution of these genotypes is presented in Supplementary Tables 1–9. We found an association between the presence of the C/C-A/G genotype of g.46438521G>C and c.2285T>C polymorphisms and decreased FECD occurrence. The association the G/G-A/A genotype of g.46438521G>C and c.–1370T>A polymorphisms and decreased FECD occurrence was detected. The A/A-G/G genotype of c.2285T>C and c.580C>T polymorphisms was correlated with decreased FECD occurrence. In addition, the T/T-A/G genotype of the c.–1370T>A and c.1196A>G polymorphisms was negatively correlated with FECD, while the A/A-A/A genotypes increased the risk of this disease.

### Haplotypes and FECD occurrence

In this study we also checked the association between the occurrence of FECD and haplotypes of the c.580C>T and c.1196A>G polymorphisms of the *XRCC1* gene (Table 4). The CA haplotype was associated with an increased occurrence of FECD, while the CG haplotype was associated with decreased occurrence.

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**Supplementary Table 9.** Distribution of combined genotypes of the c.–1370T>A – *POLG* and c.1196A>G – *XRCC1* polymorphisms and odds ratio (OR) with 95% confidence interval (95% CI) in patients with Fuchs endothelial corneal dystrophy (FECD) and controls.

| Combined genotypes | Controls (n=353) | FECD (n=250) | Crude OR (95% CI) | *p* | Adjusted OR* (95% CI) | *p* |
|---|---|---|---|---|---|---|
| | Number (frequency) | | | | | |
| A/A – A/A | 6 (0.02) | 14 (0.06) | **3.43 (1.30–9.05)** | **0.013** | **3.29 (1.16–9.32)** | **0.025** |
| A/A – A/G | 31 (0.09) | 18 (0.07) | 0.81 (0.44–1.48) | 0.484 | 0.71 (0.35–1.44) | 0.344 |
| A/A – G/G | 9 (0.03) | 3 (0.01) | 0.46 (0.12–1.73) | 0.253 | **0.71 (0.17–2.87)** | **0.627** |
| A/T – A/A | 41 (0.12) | 43 (0.17) | 1.58 (0.99–2.51) | 0.052 | 1.65 (0.97–2.80) | 0.066 |
| A/T – A/G | 118 (0.33) | 88 (0.35) | 1.08 (0.77–1.52) | 0.651 | 1.27 (0.84–1.85) | 0.274 |
| A/T – G/G | 44 (0.12) | 13 (0.05) | **0.38 (0.20–0.73)** | **0.004** | 0.49 (0.24–1.03) | 0.060 |
| T/T – A/A | 25 (0.07) | 17 (0.07) | 0.96 (0.50–1.81) | 0.893 | 0.84 (0.42–1.67) | 0.615 |
| T/T – A/G | 69 (0.20) | 37 (0.15) | 0.71 (0.46–1.11) | 0.132 | **0.56 (0.33–0.95)** | **0.033** |
| T/T – G/G | 10 (0.03) | 17 (0.07) | 2.50 (1.13–5.56) | 0.024 | 1.70 (0.64–4.50) | 0.283 |

*p* values <0.05 along with corresponding ORs are in bold; * OR adjusted for co-occurrence of visual impairment, sex, age and family history for FECD.

**Table 4.** Distribution of haplotypes of the c.580C>T and c.1196A>G polymorphisms of the *XRCC1* gene and odds ratio (OR) with 95% confidence interval (95% CI) in patients with FECD and controls.

| Haplotype | Controls (n=353) | FECD (n=250) | OR (95% CI) | *p* |
|---|---|---|---|---|
| | Number (frequency) | | | |
| CA | 675 (0.48) | 556 (0.56) | **1.37 (1.16–1.61)** | **<0.001** |
| CG | 641 (0.45) | 390 (0.39) | **0.77 (0.65–0.91)** | **0.002** |
| TA | 49 (0.03) | 26 (0.03) | 0.74 (0.46–1.20) | 0.227 |
| TG | 41 (0.03) | 28 (0.03) | 0.96 (0.59–1.57) | 0.880 |

*p*<0.05 along with corresponding ORs are in bold.

### Stratification analysis of the *PARP-1*, *NEIL1*, *POLG* and *XRCC1* and FECD occurrence

The distribution of genotypes and allele frequencies of polymorphisms in the *NEIL1*, *PARP-1*, *POLG, and XRCC1* genes and the values obtained by the analysis of OR in groups of females and males are reported in Supplementary Table 10. In analysis for the c.1196A>G polymorphisms, the A/A genotype and the A allele were correlated with increased FECD occurrence, whereas the G allele was negatively correlated with FECD in males. We found no association between studied polymorphisms and FECD occurrence in females. We also analyzed the frequencies of genotypes/alleles and the risk of FECD stratified by form of FECD (Supplementary Tables 11–13). We detected that the T/T and the T allele were associated with decreased occurrence of the scattered form of FECD, whereas the A allele increased it. We did not detect any correlations between studied polymorphisms and central form of FECD. On the other hand, the A/A genotype and the A allele of the c.1196A>G polymorphism were positively correlated with increased occurrence of the undefined form of FECD, whereas the G allele had a protective effect against it.

## Discussion

The analysis of potential risk factors independently from genotypes in our study showed a significant influence of sex, age, visual impairment, and positive FECD family history on FECD occurrence. Our results are in general accordance with results obtained in other laboratories. Correlation between positive FECD family history in first-degree relatives and FECD occurrence was detected in several other studies [9]. A significant association between visual impairment and FECD occurrence was also reported [31]. In addition, a number of studies indicated that female sex may be a risk factor of FECD [8,9].

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**Supplementary Table 10.** Distribution of genotypes of the 46438521G>C – *NEIL1*, c.2285T>C – *PARP-1*, c.–1370T>A – *POLG*, c.580C>T – *XRCC1* and c.1196A>G – *XRCC1* polymorphisms stratified by sex in patients with Fuchs endothelial corneal dystrophy (FECD) and controls.

| Polymorphism genotype/allele | Controls | | FECD | | Crude OR (95% CI) | | *p* | Adjusted OR* (95% CI) | | *p* |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number (frequency) | | | | | | | | | |
| g.46438521G>C *NEIL1* | | | | | | | | | | |
| Women | | | | | | | | | | |
| C/C | 60 | (0.27) | 43 | (0.22) | 0.79 | (0.50–1.24) | 0.302 | 0.67 | (0.40–1.13) | 0.136 |
| C/G | 153 | (0.68) | 142 | (0.74) | 1.32 | (0.86–2.02) | 0.206 | 1.48 | (0.91–2.42) | 0.114 |
| G/G | 11 | (0.05) | 7 | (0.04) | 0.73 | (0.28–1.93) | 0.529 | 0.82 | (0.28–2.40) | 0.713 |
| C | 273 | (0.61) | 228 | (0.59) | 0.88 | (0.60–1.30) | 0.525 | 0.77 | (0.50–1.20) | 0.256 |
| G | 175 | (0.39) | 156 | (0.41) | 1.13 | (0.77–1.67) | 0.525 | 1.29 | (0.83–2.01) | 0.256 |
| Men | | | | | | | | | | |
| C/C | 38 | (0.29) | 10 | (0.17) | 0.50 | (0.33–1.09) | 0.080 | 0.66 | (0.27–1.62) | 0.364 |
| C/G | 87 | (0.67) | 46 | (0.79) | 1.85 | (0.89–3.86) | 0.100 | 1.60 | (0.69–3.76) | 0.282 |
| G/G | 4 | (0.03) | 2 | (0.03) | 1.12 | (0.20–6.27) | 0.901 | 0.54 | (0.09–3.41) | 0.516 |
| C | 163 | (0.63) | 66 | (0.57) | 0.58 | (0.30–1.12) | 0.107 | 0.80 | (0.38–1.69) | 0.557 |
| G | 95 | (0.37) | 50 | (0.43) | 1.73 | (0.89–3.37) | 0.107 | 1.25 | (0.59–2.64) | 0.557 |
| c.2285T>C *PARP-1* | | | | | | | | | | |
| Women | | | | | | | | | | |
| A/A | 157 | (0.70) | 125 | (0.65) | 0.80 | (0.53–1.20) | 0.278 | 0.95 | (0.59–1.52) | 0.836 |
| A/G | 67 | (0.30) | 67 | (0.35) | 1.25 | (0.83–1.90) | 0.278 | 1.05 | (0.66–1.67) | 0.836 |
| G/G | 0 | (0.00) | 0 | (0.00) | | – | – | | – | – |
| A | 381 | (0.85) | 317 | (0.83) | 0.80 | (0.53–1.20) | 0.278 | 0.95 | (0.59–1.52) | 0.836 |
| G | 67 | (0.15) | 67 | (0.17) | 1.25 | (0.83–1.90) | 0.278 | 1.05 | (0.66–1.67) | 0.836 |
| Men | | | | | | | | | | |
| A/A | 82 | (0.64) | 41 | (0.71) | 1.27 | (0.65–2.49) | 0.479 | 1.60 | (0.72–3.53) | 0.245 |
| A/G | 47 | (0.36) | 17 | (0.29) | 0.78 | (0.40–1.53) | 0.479 | 0.62 | (0.28–1.38) | 0.245 |
| G/G | 0 | (0.00) | 0 | (0.00) | | – | – | | – | – |
| A | 211 | (0.82) | 99 | (0.85) | 1.27 | (0.65–2.49) | 0.479 | 1.60 | (0.72–3.53) | 0.245 |
| G | 47 | (0.18) | 17 | (0.15) | 0.78 | (0.40–1.53) | 0.479 | 0.62 | (0.28–1.38) | 0.245 |
| c.–1370T>A *POLG* | | | | | | | | | | |
| Women | | | | | | | | | | |
| A/A | 30 | (0.13) | 30 | (0.16) | 1.19 | (0.69–2.07) | 0.519 | 1.16 | (0.62–2.14) | 0.645 |
| A/T | 125 | (0.56) | 108 | (0.56) | 1.02 | (0.69–1.50) | 0.927 | 1.25 | (0.81–1.95) | 0.316 |
| T/T | 69 | (0.31) | 54 | (0.28) | 0.88 | (0.57–1.34) | 0.551 | 0.70 | (0.43–1.13) | 0.146 |
| A | 185 | (0.41) | 168 | (0.44) | 1.12 | (0.83–1.52) | 0.440 | 1.25 | (0.89–1.76) | 0.200 |
| T | 263 | (0.59) | 216 | (0.56) | 0.89 | (0.66–1.20) | 0.440 | 0.80 | (0.57–1.13) | 0.200 |
| Men | | | | | | | | | | |
| A/A | 16 | (0.12) | 5 | (0.09) | 0.67 | (0.23–1.91) | 0.451 | 0.84 | (0.26–2.67) | 0.770 |
| A/T | 78 | (0.60) | 36 | (0.62) | 1.07 | (0.57–2.02) | 0.835 | 1.38 | (0.65–2.92) | 0.406 |
| T/T | 35 | (0.27) | 17 | (0.29) | 1.11 | (0.56–2.21) | 0.758 | 9.75 | (0.33–1.70) | 0.492 |
| A | 110 | (0.43) | 46 | (0.40) | 0.85 | (0.51–1.42) | 0.532 | 1.11 | (0.62–2.00) | 0.724 |
| T | 148 | (0.57) | 70 | (0.60) | 1.18 | (0.79–1.98) | 0.532 | 0.90 | (0.50–1.62) | 0.724 |

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**Supplementary Table 10 continued.** Distribution of genotypes of the 46438521G>C – *NEIL1*, c.2285T>C – *PARP-1*, c.–1370T>A – *POLG*, c.580C>T – *XRCC1* and c.1196A>G – *XRCC1* polymorphisms stratified by sex in patients with Fuchs endothelial corneal dystrophy (FECD) and controls.

| Polymorphism genotype/allele | Controls | | FECD | | Crude OR (95% CI) | *p* | Adjusted OR* (95% CI) | *p* |
|---|---|---|---|---|---|---|---|---|
| | Number (frequency) | | | | | | | |
| c.580C>T *XRCC1* | | | | | | | | |
| Women | | | | | | | | |
| C/C | 198 | (0.88) | 169 | (0.88) | 0.96 (0.53–1.75) | 0.907 | 1.03 (0.53–1.98) | 0.937 |
| C/T | 26 | (0.12) | 23 | (0.12) | 1.04 (0.57–1.88) | 0.907 | 0.97 (0.50–1.87) | 0.937 |
| T/T | 0 | (0.00) | 0 | (0.00) | – | – | – | – |
| C | 418 | (0.93) | 357 | (0.93) | 0.94 (0.54–1.65) | 0.843 | 0.89 (0.48–1.65) | 0.704 |
| T | 30 | (0.07) | 27 | (0.07) | 1.06 (0.60–1.85) | 0.843 | 1.13 (0.61–2.09) | 0.704 |
| Men | | | | | | | | |
| C/C | 107 | (0.83) | 54 | (0.93) | 2.78 (0.91–8.46) | 0.073 | 1.56 (0.35–6.88) | 0.557 |
| C/T | 22 | (0.17) | 4 | (0.07) | 0.36 (0.12–1.10) | 0.073 | 0.64 (0.145–2.83) | 0.557 |
| T/T | 0 | (0.00) | 0 | (0.00) | – | – | – | – |
| C | 235 | (0.91) | 111 | (0.96) | 2.30 (0.83–6.39) | 0.110 | 1.53 (0.36–6.53) | 0.564 |
| T | 23 | (0.09) | 5 | (0.04) | 0.43 (0.16–1.21) | 0.110 | 0.65 (0.15–2.78) | 0.564 |
| c.1196A>G *XRCC1* | | | | | | | | |
| Women | | | | | | | | |
| A/A | 50 | (0.22) | 54 | (0.28) | 1.36 (0.87–2.12) | 0.174 | 1.39 (0.84–2.28) | 0.200 |
| A/G | 137 | (0.61) | 112 | (0.58) | 0.89 (0.60–1.32) | 0.558 | 0.82 (0.53–1.28) | 0.391 |
| G/G | 37 | (0.17) | 26 | (0.14) | 0.79 (0.46–1.36) | 0.399 | 0.89 (0.48–1.64) | 0.702 |
| A | 237 | (0.53) | 220 | (0.57) | 1.25 (0.91–1.71) | 0.155 | 1.22 (0.86–1.73) | 0.267 |
| G | 211 | (0.47) | 164 | (0.43) | 0.80 (0.59–1.09) | 0.155 | 0.82 (0.58–1.16) | 0.267 |
| Men | | | | | | | | |
| A/A | 22 | (0.17) | 20 | (0.34) | **2.56(1.26–5.20)** | **0.009** | **1.05(1.02–1.08)** | **<0.001** |
| A/G | 81 | (0.63) | 31 | (0.53) | 0.68 (0.36–1.27) | 0.229 | 0.79 (0.37–1.66) | 0.532 |
| G/G | 26 | (0.20) | 7 | (0.12) | 0.54 (0.22–1.34) | 0.184 | 0.38 (0.11–1.34) | 0.133 |
| A | 125 | (0.48) | 71 | (0.61) | **1.94(0.16–3.30)** | **0.012** | **2.07(1.09–3.92)** | **0.026** |
| G | 133 | (0.52) | 45 | (0.39) | **0.52(0.31–0.86)** | **0.012** | **0.48(0.25–0.92)** | **0.026** |

*p* values <0.05 along with corresponding ORs are in bold; * OR adjusted for co-occurrence of visual impairment, sex, age and family history for FECD.

As mentioned above, oxidative stress is also implicated in FECD pathogenesis. The presence of DNA oxidative damage in FECD cornea may also suggest the role of DNA repair genes. Therefore, we checked a role of 5 polymorphisms in 4 BER-associated genes in FECD occurrence in the Polish population.

Nei endonuclease VIII-like 1 (NEIL1) is 1 of 11 known human DNA glycosylases involved in the BER pathway [32]. It is responsible for recognizing and removal of a wide range of substrates, including 8-hydroxyguanine, thymine glycol (Tg), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG), and 4,6-diamino-5-formamidopyrimidine (FapyA) [10, 33, 34]. Moreover, NEIL1 also possesses apurinic/apyrimidinic (AP) lyase activity, cleaving a DNA strand at the AP site via beta- and delta-elimination [35]. Currently, the role of genetic variation in the *NEIL1* gene is poorly studied. Mutations in *NEIL1* and reduced expression of this protein are associated with gastric cancers, indicating the potential biological importance of the enzyme [36]. In this study, we investigated the role the g.46438521G>C polymorphism located in the 3' near gene.

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**Supplementary Table 11.** Distribution of genotypes of the 46438521G>C – *NEIL1*, c.2285T>C – *PARP-1*, c.–1370T>A – *POLG*, c.580C>T – *XRCC1* and c.1196A>G – *XRCC1* polymorphisms in patients with scattered form of Fuchs dystrophy and controls.

| Polymorphism genotype/allele | Controls | FECD | Crude OR (95% CI) | *p* | Adjusted OR* (95% CI) | *p* |
|---|---|---|---|---|---|---|
| | Number (frequency) | | | | | |
| g.46438521G>C *NEIL1* | | | | | | |
| C/C | 98 (0.28) | 14 (0.18) | 0.58 (0.31–1.08) | 0.085 | 0.54 (0.26–1.11) | 0.095 |
| C/G | 240 (0.68) | 60 (0.78) | 1.66 (0.93–2.98) | 0.088 | 1.85 (0.93–3.67) | 0.077 |
| G/G | 15 (0.04) | 3 (0.04) | 0.91 (0.26–3.24) | 0.889 | 0.74 (0.17–3.28) | 0.691 |
| C | 436 (0.62) | 88 (0.57) | 0.69 (0.42–1.14) | 0.147 | 0.68 (0.38–1.22) | 0.199 |
| G | 270 (0.38) | 66 (0.43) | 1.45(90.88–2.40) | 0.147 | 1.46 (0.82–2.61) | 0.199 |
| c.2285T>C *PARP-1* | | | | | | |
| A/A | 239 (0.68) | 50 (0.65) | 0.88 (0.52–1.48) | 0.639 | 1.09 (0.59–2.01) | 0.790 |
| A/G | 114 (0.32) | 27 (0.35) | 1.13 (0.67–1.90) | 0.639 | 0.92 (0.50–1.70) | 0.790 |
| G/G | 0 (0.00) | 0 (0.00) | – | – | – | – |
| A | 592 (0.84) | 127 (0.82) | 0.88 (0.53–1.48) | 0.639 | 1.09 (0.59–2.01) | 0.790 |
| G | 114 (0.16) | 27 (0.18) | 1.13 (0.67–1.90) | 0.639 | 0.92 (0.50–1.70) | 0.790 |
| c.–1370T>A *POLG* | | | | | | |
| A/A | 46 (0.13) | 13 (0.17) | 1.36 (0.69–2.65) | 0.375 | 1.52 (0.72–3.23) | 0.274 |
| A/T | 203 (0.57) | 45 (0.58) | 1.04 (0.63–1.71) | 0.880 | 1.46 (0.81–2.63) | 0.211 |
| T/T | 104 (0.30) | 19 (0.25) | 0.78 (0.44–1.38) | 0.401 | **0.44(0.21–0.92)** | **0.028** |
| A | 295 (0.42) | 71 (0.46) | 1.24 (0.84–1.83) | 0.279 | **1.64(1.04–2.57)** | **0.031** |
| T | 411 (0.58) | 83 (0.54) | 0.81 (0.55–1.19) | 0.279 | **0.61(0.39–0.95)** | **0.031** |
| c.580C>T *XRCC1* | | | | | | |
| C/C | 305 (0.86) | 66 (0.86) | 0.94 (0.46–1.91) | 0.874 | 0.87 (0.37–2.05) | 0.754 |
| C/T | 48 (0.14) | 11 (0.14) | 1.06 (0.52–2.15) | 0.874 | 1.15 (0.49–2.69) | 0.754 |
| T/T | 0 (0.00) | 0 (0.00) | – | – | – | – |
| C | 653 (0.92) | 140 (0.91) | 0.79 (0.42–1.52) | 0.488 | 0.63 (0.29–1.38) | 0.251 |
| T | 53 (0.08) | 14 (0.09) | 1.26 (0.66–2.41) | 0.488 | 1.58 (0.72–3.42) | 0.251 |
| c.1196A>G *XRCC1* | | | | | | |
| A/A | 72 (0.20) | 23 (0.30) | 1.66 (0.95–2.89) | 0.071 | 1.52 (0.80–2.89) | 0.204 |
| A/G | 218 (0.62) | 44 (0.57) | 0.82 (0.50–1.36) | 0.453 | 0.73 (0.41–1.31) | 0.291 |
| G/G | 63 (0.18) | 10 (0.13) | 0.69 (0.33–1.41) | 0.306 | 0.97 (0.43–2.19) | 0.941 |
| A | 362 (0.51) | 90 (0.58) | 1.45 (0.97–2.17) | 0.068 | 1.40 (0.89–2.20) | 0.145 |
| G | 344 (0.49) | 64 (0.42) | 0.69 (0.46–1.03) | 0.068 | 0.80 (0.50–1.29) | 0.355 |

*p* values <0.05 along with corresponding ORs are in bold; * OR adjusted for co-occurrence of visual impairment, sex, age and family history for FECD.

It was found that this polymorphism has a protective effect on radiation-induced toxicity [37]. We showed a weak association between the C/G genotype of the g.46438521G>C polymorphism of the *NEIL1* gene and increased FECD occurrence, but we do not think it is biologically important. The lower limit of the 95% CI was close to 1 and we did not find any other association in this polymorphism. Therefore, future studies are needed to determine if this SNP is associated with FECD.

Poly (ADP-ribose) polymerase family member 1 (PARP-1) has a key role in the repair of DNA damage [38]. It recognizes DNA single-strand breaks and then catalyzes the poly(ADP-ribosyl)ation, giving a signal for other repair proteins. PARP-1 is also

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**Supplementary Table 12.** Distribution of genotypes of the 46438521G>C – *NEIL1*, c.2285T>C – *PARP-1*, c.–1370T>A – *POLG*, c.580C>T – *XRCC1* and c.1196A>G – *XRCC1* polymorphisms in patients with central form of Fuchs dystrophy and controls.

| Polymorphism genotype/allele | Controls | | FECD | | Crude OR (95% CI) | *p* | Adjusted OR* (95% CI) | *p* |
|---|---|---|---|---|---|---|---|---|
| | Number (frequency) | | | | | | | |
| g.46438521G>C *NEIL1* | | | | | | | | |
| C/C | 98 | (0.28) | 33 | (0.22) | 0.75 (0.47–1.17) | 0.205 | 0.74 (0.44–1.25) | 0.261 |
| C/G | 240 | (0.68) | 109 | (0.74) | 1.32 (0.86–2.02) | 0.209 | 1.29 (0.79–2.11) | 0.306 |
| G/G | 15 | (0.04) | 6 | (0.04) | 0.95 (0.36–2.50) | 0.921 | 1.01 (0.36–2.80) | 0.993 |
| C | 436 | (0.62) | 175 | (0.59) | 0.81 (0.55–1.19) | 0.287 | 0.81 (0.52–1.25) | 0.336 |
| G | 270 | (0.38) | 121 | (0.41) | 1.23 (0.84–1.81) | 0.287 | 1.24 (0.80–1.92) | 0.336 |
| c.2285T>C *PARP-1* | | | | | | | | |
| A/A | 239 | (0.68) | 95 | (0.64) | 0.85 (0.57–1.28) | 0.446 | 0.89 (0.56–1.41) | 0.621 |
| A/G | 114 | (0.32) | 53 | (0.36) | 1.17 (0.78–1.75) | 0.446 | 0.12 (0.71–1.79) | 0.621 |
| G/G | 0 | (0.00) | 0 | (0.00) | – | – | – | – |
| A | 592 | (0.84) | 0.82 | (1.64) | 0.85 (0.57–1.28) | 0.446 | 0.89 (0.56–1.41) | 0.621 |
| G | 114 | (0.16) | 0.18 | (0.36) | 1.17 (0.78–1.75) | 0.446 | 0.12 (0.71–1.79) | 0.621 |
| c.–1370T>A *POLG* | | | | | | | | |
| *A/A* | 46 | (0.13) | 14 | (0.09) | 0.70 (0.37–1.31) | 0.263 | 0.71 (0.35–1.44) | 0.338 |
| A/T | 203 | (0.57) | 91 | (0.61) | 1.18 (0.80–1.74) | 0.409 | 1.36 (0.86–2.15) | 0.183 |
| T/T | 104 | (0.30) | 43 | (0.29) | 0.98 (0.64–1.49) | 0.927 | 0.83 (0.51–1.35) | 0.459 |
| A | 295 | (0.42) | 119 | (0.40) | 0.92 (0.67–1.26) | 0.602 | 1.01 (0.71–1.43) | 0.970 |
| T | 411 | (0.58) | 177 | (0.60) | 1.09(0.079–1.48) | 0.602 | 0.99 (0.70–1.41) | 0.970 |
| c.580C>T *XRCC1* | | | | | | | | |
| C/C | 305 | (0.86) | 131 | (0.89) | 1.21 (0.67–2.19) | 0.522 | 1.10 (0.55–2.19) | 0.784 |
| C/T | 48 | (0.14) | 17 | (0.11) | 0.82 (0.46–1.49) | 0.522 | 0.91 (0.46–1.81) | 0.784 |
| T/T | 0 | (0.00) | 0 | (0.00) | – | – | – | – |
| C | 653 | (0.92) | 277 | (0.94) | 1.20 (0.68–2.11) | 0.527 | 1.05 (0.54–2.03) | 0.888 |
| T | 53 | (0.08) | 19 | (0.06) | 0.83 (0.47–1.46) | 0.527 | 0.95 (0.49–1.85) | 0.888 |
| c.1196A>G *XRCC1* | | | | | | | | |
| A/A | 72 | (0.20) | 42 | (0.28) | 1.55 (0.99–2.40) | 0.053 | 1.44 (0.87–2.39) | 0.154 |
| *A/G* | 218 | (0.62) | 83 | (0.56) | 0.79 (0.54–1.17) | 0.237 | 0.82 (0.52–1.29) | 0.396 |
| G/G | 63 | (0.18) | 23 | (0.16) | 0.85 (0.50–1.43) | 0.533 | 0.84 (0.45–1.58) | 0.590 |
| A | 362 | (0.51) | 167 | (0.56) | 1.30 (0.95–1.77) | 0.096 | 1.31 (0.93–1.84) | 0.127 |
| G | 344 | (0.49) | 129 | (0.44) | 0.77 (0.57–1.05) | 0.096 | 0.79 (0.55–1.13) | 0.196 |

*p* values <0.05 along with corresponding ORs are in bold; * OR adjusted for co-occurrence of visual impairment, sex, age and family history for FECD.

involved in maintenance of chromatin structure and DNA metabolism [39,40]. The c.2285T>C polymorphism is one of the best characterized variations in *PARP-1*. This polymorphism causes substitution of valine to alanine in the catalytic domain of PARP-1, resulting in reduced enzymatic activity [41]. Several studies reported an association between the c.2285T>C polymorphism and development of bladder, prostate, cervical, and thyroid cancer [42–45]. Variants at the promoter of the *PARP-1* gene were also significantly associated with the development of Parkinson's disease and Alzheimer's disease, which, like FECD, are oxidative stress-related diseases [46,47].

X-ray repair cross complementing group 1 (XRCC1) is another important component of BER [48]. XRCC1 is a crucial scaffold protein that interacts with PARP-1, DNA ligase III (LIG3), DNA polymerase β (POLB), and APEX nuclease 1 (APEX1) [48–51]. XRCC1 also takes part in the stimulation of the 3'-DNA phosphatase and 5'-DNA kinase activities of PNK, and accelerates the

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**Supplementary Table 13.** Distribution of genotypes of the 46438521G>C – *NEIL1*, c.2285T>C – *PARP-1*, c.–1370T>A – *POLG*, c.580C>T – *XRCC1* and c.1196A>G – *XRCC1* polymorphisms in patients with undefined form of Fuchs dystrophy and controls.

| Polymorphism genotype/allele | Controls | | FECD | | Crude OR (95% CI) | *p* | Adjusted OR* (95% CI) | *p* |
|---|---|---|---|---|---|---|---|---|
| | Number (frequency) | | | | | | | |
| g.46438521G>C *NEIL1* | | | | | | | | |
| C/C | 98 | (0.28) | 6 | (0.22) | 0.61 (0.20–1.83) | 0.379 | 0.61 (0.20–1.83) | 0.379 |
| C/G | 240 | (0.68) | 20 | (0.74) | 1.34 (0.55–3.27) | 0.513 | 1.66 (0.60–4.62) | 0.331 |
| G/G | 15 | (0.04) | 1 | (0.04) | 0.86 (0.11–6.82) | 0.892 | 0.73 (0.08–6.34) | 0.773 |
| C | 436 | (0.62) | 32 | (0.59) | 0.82 (0.38–1.79) | 0.625 | 0.76 (0.32–1.79) | 0.532 |
| G | 270 | (0.38) | 22 | (0.41) | 1.21 (0.56–2.63) | 0.625 | 0.31 (0.56–3.07) | 0.532 |
| c.2285T>C *PARP-1* | | | | | | | | |
| A/A | 239 | (0.68) | 22 | (0.81) | 2.10 (0.77–5.68) | 0.145 | 2.65 (0.85–8.30) | 0.093 |
| A/G | 114 | (0.32) | 5 | (0.19) | 0.48 (0.18–1.29) | 0.145 | 0.38 (0.12–1.18) | 0.093 |
| G/G | 0 | (0.00) | 0 | (0.00) | – | – | – | – |
| A | 592 | (0.84) | 49 | (0.91) | 2.10 (0.77–5.68) | 0.145 | 2.65 (0.85–8.30) | 0.093 |
| G | 114 | (0.16) | 5 | (0.09) | 0.48 (0.18–1.29) | 0.145 | 0.38 (0.12–1.18) | 0.093 |
| c.–1370T>A *POLG* | | | | | | | | |
| A/A | 46 | (0.13) | 6 | (0.22) | 1.91 (0.73–4.97) | 0.187 | 1.63(0.544–4.88) | 0.382 |
| A/T | 203 | (0.57) | 14 | (0.52) | 0.80 (0.36–1.74) | 0.568 | 1.34 (0.55–3.29) | 0.519 |
| T/T | 104 | (0.30) | 7 | (0.26) | 0.84 (0.34–2.04) | 0.697 | 0.47 (0.16–1.40) | 0.178 |
| A | 295 | (0.42) | 26 | (0.48) | 1.37 (0.74–2.52) | 0.318 | 1.61 (0.83–3.13) | 0.156 |
| T | 411 | (0.58) | 28 | (0.52) | 0.62 (0.32–1.20) | 0.156 | 0.62 (0.32–1.20) | 0.156 |
| c.580C>T *XRCC1* | | | | | | | | |
| C/C | 305 | (0.86) | 25 | (0.93) | 1.97 (0.45–8.57) | 0.368 | 1.38 (0.29–6.43) | 0.678 |
| C/T | 48 | (0.14) | 2 | (0.07) | 0.51 (0.12–2.21) | 0.368 | 0.72 (0.15–3.36) | 0.678 |
| T/T | 0 | (0.00) | 0 | (0.00) | – | – | – | – |
| C | 653 | (0.92) | 52 | (0.96) | 2.21 (0.51–9.60) | 0.291 | 1.40 (0.30–6.49) | 0.668 |
| T | 53 | (0.08) | 2 | (0.04) | 0.45 (0.10–1.97) | 0.291 | 0.71 (0.15–3.31) | 0.668 |
| c.1196A>G *XRCC1* | | | | | | | | |
| A/A | 72 | (0.20) | 10 | (0.37) | **2.30(1.01–5.23)** | **0.048** | **2.16(1.16–7.26)** | **0.022** |
| A/G | 218 | (0.62) | 15 | (0.56) | 0.77(0.35 0 1.70) | 0.525 | 0.58 (0.24–1.41) | 0.228 |
| G/G | 63 | (0.18) | 2 | (0.07) | 0.39 (0.08–1.59) | 0.182 | 0.29 (0.03–2.25) | 0.235 |
| A | 362 | (0.51) | 35 | (0.65) | **2.06(1.07–3.96)** | **0.030** | **2.58(1.18–5.67)** | **0.018** |
| G | 344 | (0.49) | 19 | (0.35) | **0.49(0.25–0.93)** | **0.030** | **0.39(0.18–0.85)** | **0.018** |

*p* values <0.05 along with corresponding ORs are in bold; * OR adjusted for co-occurrence of visual impairment, sex, age and family history for FECD.

single-strand break repair reaction *in vitro* [52]. The c.580C>T and the c.1196A>G polymorphisms were extensively studied in *XRCC1* [53,54]. The c.580C>T polymorphism causes a change from arginine to tryptophan in an evolutionarily conserved linker region that coordinates protein interactions [53]. In turn, the c.1196A>G polymorphism is a missense mutation in the poly (ADP-ribose) polymerase-binding domain. An association between these polymorphisms and several diseases, including cervical cancer, glioma, and lung cancer, was reported [47,55,56]. It was also reported that there is a correlation between the c.1196A>G polymorphism and increased risk of eye diseases, including age-related cataract and primary open-angle glaucoma [57,58]. Our previous studies showed a significant association between the c.580C>T and c.1196A>G polymorphisms and the occurrence of another corneal disease – keratoconus [30]. In this work, we did not find any association between the c.580C>T polymorphism and FECD occurrence. However, analysis of the impact of the c.1196A>G polymorphism on FECD

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risk showed a correlation of the A/A genotype and the A allele with increased FECD occurrence, whereas the G allele was associated with decreased occurrence. Stratification analysis showed similar association only among patients with Szaflik's undefined form of FECD. Therefore, the c.1196A>G polymorphism should rather be attributed to undefined the form of FECD than to FECD in general. The presence of the A allele may induce alternation in the active site of the XRCC1 protein, resulting in deficient DNA repair. In oxidative stress conditions, this modification may increase the susceptibility of the cornea to oxidative DNA damage, leading to a pathological state in this tissue and FECD development.

*POLG* encodes alpha subunit of DNA polymerase γ on chromosome 15q25. Polymerase γ possesses both 3'-5' exonuclease and 5'dRP lyase activities [59]. This enzyme occurs in mitochondria, where it is responsible for mitochondrial DNA replication and plays critical roles in mtDNA repair [60]. Alternation in the *POLG* gene may lead to mutagenesis of mtDNA, which in turn causes disturbance in oxidative phosphorylation. Mutations in the *POLG* gene were commonly associated with human mitochondrial diseases, including myoclonus epilepsy myopathy sensory ataxia (MEMSA), Alpers-Huttenlocher syndrome, and myocerebrohepatopathy spectrum disorder (MCHS) [61,62]. Changes in *POLG* are also responsible for an eye disease – progressive external ophthalmoplegia [63]. In a previously study, we reported an association of the c.–1370T>A polymorphism with keratoconus [30]. We found that Szaflik's scattered form of FECD was positively correlated with the A allele and negatively correlated with the T allele of c.–1370T>A. Although functional studies of this polymorphism have not been performed, its localization in the regulatory region of *POLG* may indicate that the A allele influences its transcription level and may lead to reduction in its activity, accumulation of mtDNA damage, apoptosis, and, finally, to corneal lesions typical for FECD.

## Conclusions

Polymorphisms in BER genes may play a role in FECD pathogenesis in the Polish population.

### Conflict of interest

The authors report no conflict of interest

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