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Polymorphism of the *LIG3* gene in keratoconus and Fuchs endothelial corneal dystrophy

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Abstract

The product of the *LIG3* gene encodes DNA ligase III, which is involved in the repair of oxidatively damaged DNA in the base excision repair pathway. We hypothesized that polymorphism in this gene may change susceptibility to oxidative stress and predispose individuals to the development of keratoconus (KC) and Fuchs endothelial corneal dystrophy (FECD). Therefore, we investigated the association between genotypes and haplotypes of the g.29661G>A polymorphism (rs1003918) and the g.29059C>T polymorphism (rs1052536) of the *LIG3* gene and the occurrence of KC and FECD in patients with FECD (258 individuals) or KC (283) and ethnically matched controls (300). The A/A genotype and the A allele of the g.29661G>A polymorphism were associated with increased occurrence of KC, while the G allele of this polymorphism was positively correlated with a decreased occurrence of this disease. The T/C genotype of the g.29059C>T polymorphism was associated with decreased FECD occurrence. In addition, the AT haplotype was associated with increased occurrence of KC and FECD, while the GT haplotype was associated with decreased occurrence of these diseases. The g.29661G>A and g.29059C>T polymorphisms may play a role in the KC and FECD pathogenesis and can be considered as markers in these diseases.

Key words: KC, FECD, LIG3, gene polymorphism, DNA repair, keratoconus, Fuchs endothelial corneal dystrophy, genetic susceptibility, DNA ligase III.

Introduction

Keratoconus (KC) is a common and progressive corneal ectasia, affecting both genders and all ethnic groups throughout the world. Clinically, it is characterized as noninflammatory thinning and asymmetrical conical protrusion of the cornea, which causes variable and severe visual impairment. It is estimated that the incidence of KC in the European Caucasian population is between 5 and 23 with a mean prevalence of 54 per 100 000 (1, 2). KC usually appears during puberty or the second decade of the life and normally progresses for the following two decades until it stabilizes (3). Owing to the limited availability of medical treatments, KC is one of the leading causes of corneal transplantation in the Western countries (4). It has been shown KC is a complex heterogeneous disease with multifactorial etiology, associated with genetic and environmental factors (5, 6). There are several potential KC susceptibility chromosomal *loci* and genes, including 5q21.2 (7, 8), 14q11.2, 16q23, 17p13 (9), *VSX1* gene (visual system homeobox 1, location 20p11-q11) (10), *DOCK9* gene (dedicator of cytocines 9, location 13q32.3) (11) and *SOD1* gene (superoxide dismutase 1, location 20p11.2) (12). Moreover, environmental factors, such as contact lens wear, chronic eye rubbing, magnesium deficiency and atopy of the eye, have also been reported to contribute to this disease (2, 5, 13).

Fuchs endothelial corneal dystrophy (FECD) is a degenerative, slowly progressive corneal disease affecting elderly patients, with a preference for women (14, 15). The most common first sign of the disease is the formation of central focal excrescences of Descemet's membrane termed guttata. Gradual impairment of corneal endothelial cells (CECs) with disease progression leads to a stromal oedema and impaired vision. CECs are derived from neural crest and are arrested in the post-mitotic state (16). FECD becomes clinically evident in 4th to 5th decade of life, but visual symptoms appear approximately a decade later. At present, the only effective treatment of restoring vision in FECD patients with late-onset is corneal transplantation (4). The knowledge of FECD etiology and disease progression is still limited. Some data suggest that the single major risk factor associated with FECD is genetic predisposition, with no consistent systemic or environmental correlates (17, 18). To date, several genome-wide linkage analyses identified many chromosomal loci associated with late-onset FECD, including 13pTel-13q12.13 (19), 18q21.2-q21.3 (20), 5q33.1-q35.2 (21) and 9p24.1-22.1 (22). Moreover, Afshari *et al*. revealed additional chromosomes 1, 7, 15, 17, and X as potentially being involved in FECD (23).

Although the knowledge of KC and FECD etiology and progression is still limited, oxidative stress may play an important role in the pathogenesis of both diseases (24-30). The cornea is a transparent, avascular structure of the eye that is exposed to a wide spectrum of light, including the ultraviolet radiation (UVR). UVR is one of the most powerful agents, that can induce a variety of mutagenic and cytotoxic DNA damage, such as cyclobutane-pirimidine dimers, 6-4 photoproducts, DNA single (SSBs) and double strand breaks (DSBs) (31). UV exposure is a well-characterized environmental stress factor that generates free radicals and reactive oxygen species (ROS), harmful to most cells and tissues (32). ROS can cause a variety of DNA damage, including SSBs, DSBs and DNA base modifications. There-

fore efficient repair of UV/ROS-induced DNA damage is important for preventing mutations and maintaining the stability of the genome. Base-excision repair (BER) is the primary DNA repair pathway responsible for repair of DNA modifications caused by UVR/ROS. Ligase III DNA is an essential component in the BER pathway and is encoded by the *LIG3* gene (official full name: ligase III, DNA, ATP-dependent, gene ID: 3980; also known as: *LIG2*), which is located at 17q11.2-q12, comprises 22 exons spanning 24,551 bb, and encodes a polypeptide of 862 amino acids (33, 34). *LIG3* is highly polymorphic, at least 1728 single nucleotide polymorphisms (SNPs) of this gene have been registered in the public domain of the Single Nucleotide Polymorphism database (dbSNP) at the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/snp).

It was shown that SNPs in DNA repair genes could modulate individual DNA repair capacity and therefore affected individual genetic susceptibility to cancer and other disorders, including eye diseases (35-40). Our study was designed to examine the association between the g. 29661G>A (rs1003918) and the g.29059C>T (rs1052536) polymorphisms of the *LIG3* gene and KC and FECD occurrence. Despite the identification of multiple SNPs in the *LIG3* gene, there is little information on their phenotypic consequences. We selected these two polymorphisms because of the known distribution in European population, where minor allele frequency > 4%. The g. 29661G>A SNP is located in the 3' untranslated region (3' UTR) of the *LIG3* gene, while the other SNP is in 3' near gene region. The polymorphisms in these regions can affect mRNA stability, its half-life and degradation and thus can results in alteration of gene expression level (41).

Materials and methods

Ethics

The study design was approved by the Bioethics Committee of the Medical University of Warsaw, Poland and each patient or control individual enrolled in this study gave a written informed consent and approval form for genetic analysis in compliance with the Helsinki declaration.

Study population

The study population comprised 283 patients with KC, 258 patients with FECD and 300 individuals with healthy corneas (controls).All patients and controls were examined in the Department of Ophthalmology, Medical University of Warsaw (Warsaw, Poland), as described previously (42).

Data collection

All participants were interviewed using a structural questionnaire to determine demographic and potential risk factors for KC and FECD. Study cases and controls provided information on their age, lifestyle habits, including smoking, body mass index (BMI), allergy, co-occurrence of visual impairment (hyperopia, astigmatism, myopia) and cardiovascular diseases, family history among 1st degree relatives for KC or FECD. Smoking was categorized due to current, former or never smokers. Characteristics of patients and controls are presented in Table 1. All individuals employed in our research were unrelated.

SNP selection

We searched the public domain of the Single Nucleotide Polymorphism database (dbSNP) at the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/snp) and the related literature to identify the common, potentially functional polymor-

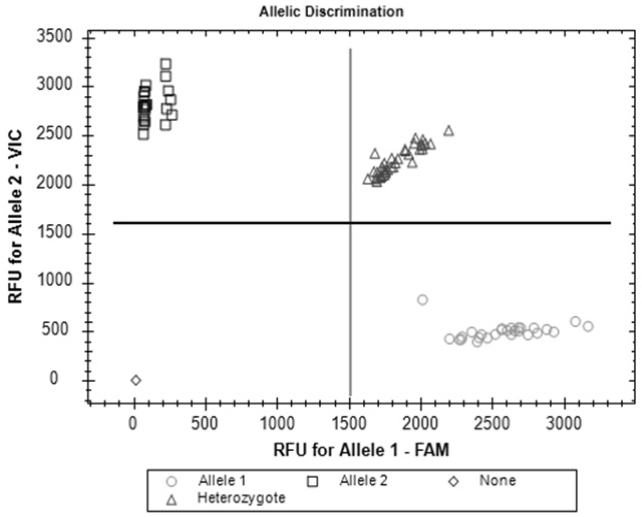

Figure 1. Allelic discrimination X-Y scatter-plot of the g. 29661G>A SNP (rs1003918) of the *LIG3* gene. The TaqMan® SNP Genotyping Assay (ID: C___2557690_10_) was used for genotyping of this SNP. The X-axis represents the relative fluorescent emission for the G allele-specific probe labeled with 6-carboxyfluorescein (FAM), and the Y-axis represents the emission for the A allele-specific probe labeled with 2'-chloro-7'-phenyl-1,4-dichloro-6-carboxyfluorescein (VIC). Circles: homozygous GG; squares: homozygous AA; triangles: heterozygous AG. Diamonds represent no template controls.

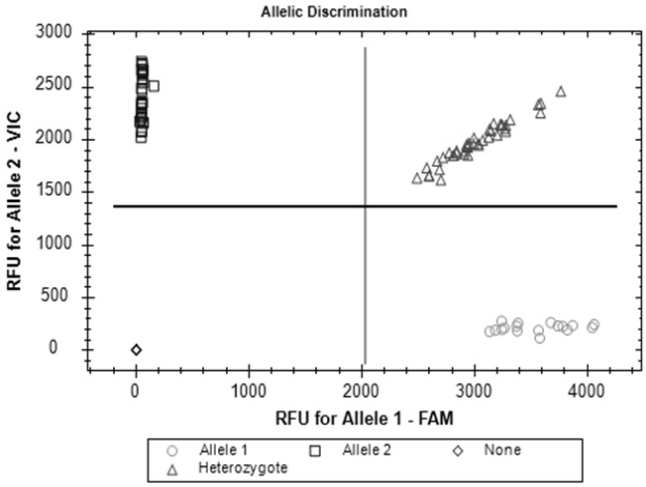

Figure 2. Allelic discrimination X-Y scatter-plot of the g.29059C>T SNP (rs1052536) of the *LIG3* gene. The TaqMan® SNP Genotyping Assay (ID: C___2557692_1_) was used for genotyping of this SNP. The X-axis represents the relative fluorescent emission for the T allele-specific probe labeled with 6-carboxyfluorescein (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 CC; squares: homozygous TT; triangles: heterozygous CT. Diamonds show no template controls.

phisms in the *LIG3* gene. Our searches were conducted based on the following criteria:

a) a minor allele frequency ≥3% in European population reported in the dsSNP database
b) location at the regulatory region of genes, i.e. the 5' near gene, the 5' UTR, the 3' UTR and 3' near gene, or coding region

Based on the above criteria we chose to examine the g.29661G>A SNP (rs1003918) and the g.29059 C>T SNP (rs1052536) polymorphisms of the *LIG3* gene with a minor allele frequency 0.458 and 0.491 in European population, respectively. The g. 29661G>A SNP is located in the 3' untranslated region (3' UTR) of the *LIG3* gene, while the second SNP is in 3' near gene region.

Genotyping

Genomic DNA was extracted from venous blood by using the AxyPrep™ Blood Genomic DNA Miniprep Kit (Axygen Biosciences, Union City, CA, USA), according to the manufacturer's instructions. The TaqMan® SNP Genotyping Assay (ID: C___2557690_10_ and C___2557692_1_) was used for analysis of the g.29661G>A and g.29059C>T polymorphisms, according to manufacturer protocol (Life Technologies, Foster City, CA, USA). The thermal cycling conditions were as follows: initial denaturing at 95°C for 10 min, 40 cycles of 92°C for 15 sec and 60°C for 60 sec. The reactions were carried out in a thermal cycler CFX96 ™ Real-Time PCR Detection System (BIO-RAD Laboratories, Hercules, CA, USA). The genotypes were determined automatically based on dye-component fluorescent emission data depicted in the X-Y scatter-plot of the CFX Manager software. The genotyping results of the two SNPs in the *LIG3* gene are presented as allelic discrimination plots in Figures 1 and 2. Negative controls containing no DNA template were included for each gene within each PCR run. For quality control, 10% of samples were randomly genotyped again and the results were 100% concordant.

Statistical analysis

To compare the distributions of demographic variables and potential risk factors between patients and controls Chi-square (χ^2) test was used. Hardy-Weinberg equilibrium was checked using χ^2 test to compare the observed genotype frequencies with the expected frequencies among the case and control subjects. The χ^2 analysis was also used to test the significance of the differences between distributions of genotypes and alleles in KC/FECD patients and controls. Unconditional multiple logistic regression analyses were used to obtain the crude and adjusted odds ratio (OR) and its corresponding 95% confidence interval (CI), for the risk of KC/FECD. Multivariable adjustment was conditional on effects of age, sex, co-occurrence of visual, smoking and family status of KC/FECD. Haplotypes were assessed for each subject on the basic of known genotypes and the PHASE software (http://stephenslab.uchicago.edu/software.html) was used. Genetic effects of inferred haplotypes were analyzed in the same way as SNPs. All statistical analyses were performed using SigmaPlot version 11.0 for Windows (Systat Software, Inc., San Jose, CA, USA).

Results

Characteristics of the study population

The frequency distributions of selected characteristics of the case patients and control subjects are presented in Table 1. The mean ± SD age were 36.14 ± 12.13 for KC patients, 70.08 ± 9.73 for FECD patients and 63 ± 19 for controls. We demonstrated a significant differences between distribution of family history for KC (positive vs negative family history), allergies (yes vs no), co-occurrence of visual impairment and cardiovascular diseases (yes vs no) among KC patients

Table 1. Demographic variables and risk factors of the study population.

Feature	Controls (n = 300)		KC (n = 283)		*p*	FECD (n = 258)		*p*
	Number	Frequency	Number	Frequency		Number	Frequency	
Sex								
females	194	0.65	84	0.30	**< 0.001**	197	0.76	**0.004**
males	106	0.35	199	0.70		61	0.24	
Age								
Mean ± SD	63 ± 19		36.14 ± 12.13		**< 0.001***	70.08 ± 9.73		**< 0.001***
Range	20-100		20-63			47-91		
Smoking								
yes (current/former)	96	0.32	89	0.31	0.957	89	0.34	0.593
never	204	0.68	194	0.69		169	0.66	
KC/FECD in family								
yes	10	0.03	33	0.12	**< 0.001**	40	0.16	**< 0.001**
no	290	0.97	250	0.88		218	0.84	
BMI								
≤25	128	0.43	130	0.46	0.708	102	0.40	0.451
25-30	100	0.33	91	0.32		82	0.32	
≥30	72	0.24	62	0.22		74	0.28	
Visual impairment								
yes	103	0.34	196	0.69	**< 0.001**	154	0.60	**< 0.001**
no	197	0.66	87	0.31		104	0.40	
Allergies								
yes	40	0.13	80	0.28	**< 0.001**	48	0.19	0.113
no	260	0.87	203	0.72		210	0.81	
Cardiovascular diseases								
yes	166	0.55	58	0.20	**< 0.001**	149	0.58	0.570
no	135	0.45	225	0.80		109	0.42	

p - values for a two-sided χ^2 –test; except for * for t-test; $p < 0.05$ are in bold.

vs controls. Among FECD patients, we observed significant differences between distribution of family history for FECD (positive vs negative family history) and co-occurrence of visual impairment (yes vs no) among FECD patients compared to controls. Therefore, these variables were further adjusted for in the multivariate logistic regression analysis.

The g.29661G>A and the g.29059C>T polymorphisms of the LIG3 gene and KC occurrence

The genotype and allele distributions of the g.29661G>A and the g.29059C>T polymorphisms in KC patients and controls are presented in Table 2. The observed genotypes frequencies did not differ significantly from Hardy-Weinberg equilibrium ($p > 0.05$, data not shown) for KC subjects. The difference in the frequency distributions of genotypes of these polymorphisms between the cases and controls was statistically significant ($p < 0.05$). The presence of the A/A genotype and A allele of the g.29661G>A polymorphism was associated with increased occurrence of KC. On the other hand, the G allele genotype of this polymorphism was associated with decreased occurrence of the disease. The T/T genotype of the g.29059C>T polymorphism showed borderline association with increased occurrence of KC.

The g.29661G>A and the g.29059C>T polymorphisms of the LIG3 gene and FECD occurrence

Details of genotype and allele frequencies of the g.29661G>A and the g.29059C>T polymorphisms of the *LIG3* gene and results of statistical analysis are shown in Table 3. There was difference in the frequency distributions of genotypes of these polymorphisms between patients and controls ($p < 0.05$). The observed genotypes frequencies did not differ significantly from Hardy-Weinberg equilibrium ($p > 0.05$, data not shown) for patient groups. The presence of the T/C genotype of the g.29059C>T polymorphism was associated with decreased occurrence of FECD. Moreover, we did not find any correlation between genotypes/alleles of the other polymorphism and FECD occurrence.

Haplotypes and KC/FECD occurrence

We also investigated the association between the occurrence of KC/FECD and haplotypes of the g.29661G>A and the g.29059C>T polymorphisms of the *LIG3* gene. The distribution of such haplotypes is shown in Table 4. The AT haplotype was associated with increased occurrence of KC as well as FECD, while GT

Table 2. Distribution of genotypes and alleles of the g.29661G>A polymorphism and the g.29059C>T polymorphisms of the *LIG3* gene and odds ratio (OR) with 95% confidence interval (95% CI) in patients with keratoconus (KC) and controls.

Genotype/allele Polymorphisms	Controls (n = 300)		KC (n = 283)		Crude OR (95% CI)	*p*	Adjusted OR [a](95% CI)	*p*
	Number	Frequency	Number	Frequency				
g.29661G>A								
G/G	87	0.29	73	0.23	0.85 (0.59-1.22)	0.386	0.74 (0.44-1.26)	0.266
G/A	170	0.57	134	0.53	0.69 (0.50-0.95)	0.025	0.86 (0.42-1.08)	0.102
A/A	43	0.14	76	0.24	2.19 (1.45-3.33)	<0.001	**2.60 (1.43-4.69)**	**0.002**
	$\chi^2 = 14.16$; $p < 0.001$							
G	344	0.57	280	0.49	0.72 (0.56-0.91)	0.006	**0.64 (0.45-0.89)**	**0.010**
A	256	0.43	286	0.51	1.40 (1.10-1.78)	0.006	**1.57 (1.11-2.21)**	**0.010**
g.29059C>T								
T/T	47	0.16	69	0.24	1.74 (1.15-2.63)	0.009	**1.82 (1.01-3.20)**	**0.048**
T/C	171	0.57	135	0.48	0.69 (0.5-0.95)	0.025	0.81 (0.51-1.28)	0.861
C/C	82	0.27	79	0.28	1.03 (0.72-1.48)	0.875	0.82 (0.48-1.37)	0.442
	$\chi^2 = 7.96$; $p = 0.019$							
T	265	0.44	164	0.31	1.19 (0.93-1.51)	0.152	1.34 (0.95-1.88)	0.098
C	335	0.56	364	0.69	0.84 (0.66-1.07)	0.152	0.75 (0.53-1.06)	0.098

$p < 0.05$ along with corresponding ORs are in bold; [a] OR adjusted for age, sex, allergies, co-occurrence of visual impairment and cardiovascular diseases, and family history for KC.

Table 3. Distribution of genotypes and alleles of the g.29661G>A polymorphism and the g.29059C>T polymorphisms of the *LIG3* gene and odds ratio (OR) with 95% confidence interval (95% CI) in patients with FECD and controls.

Genotype/allele Polymorphisms	Controls (n = 300)		FECD (n = 258)		Crude OR (95% CI)	*p*	Adjusted OR[a] (95% CI)	*p*
	Number	Frequency	Number	Frequency				
g.29661G>A								
G/G	87	0.29	69	0.27	0.89 (0.62-1.30)	0.554	1.01 (0.66-1.55)	0.957
G/A	170	0.57	124	0.48	0.71 (0.51-0.99)	0.043	0.84 (0.57-1.24)	0.380
A/A	43	0.14	65	0.25	2.01 (1.31-3.09)	0.001	1.31 (0.80-2.14)	0.289
	$\chi^2 = 10.66$; $p = 0.005$							
G	344	0.57	262	0.51	0.75 (0.59-0.96)	0.024	0.92 (0.69-1.22)	0.567
A	256	0.43	254	0.49	1.33 (1.04-1.70)	0.024	1.09 (0.82-1.44)	0.567
g.29059C>T								
T/T	47	0.16	70	0.27	2.00 (1.32-3.04)	0.001	1.60 (0.97-2.54)	0.287
T/C	171	0.57	114	0.44	0.60 (0.43-0.84)	0.003	**0.61 (0.42-0.90)**	**0.013**
C/C	82	0.27	74	0.29	1.07 (0.74-1.55)	0.723	1.28 (0.84-1.96)	0.252
	$\chi^2 = 13.245$; $p = 0.001$							
T	265	0.44	254	0.49	1.23 (0.97-1.57)	0.087	1.05 (0.79-1.38)	0.751
C	335	0.56	262	0.51	0.81 (0.64-1.03)	0.087	0.96 (0.72-1.26)	0.751

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

Table 4. Distribution of combined genotypes of the g.29661G>A polymorphism and the g.29059C>T polymorphisms of the *LIG3* gene and odds ratio (OR) with 95% confidence interval (95% CI) in patients with KC and controls.

Haplotype	Controls (n = 300)		KC (n = 283)		OR (95% CI)	*p*	FECD (n =258)		OR (95% CI)	*p*
	number	frequency	number	frequency			number	frequency		
GT	201	0.17	136	0.12	**0.68 (0.54-0.86)**	**0.001**	131	0.13	**0.72 (0.57-0.92)**	**0.007**
GC	487	0.41	424	0.37	0.88 (0.74-1.04)	0.129	393	0.38	0.90 (0.76-1.07)	0.228
AT	329	0.27	410	0.36	**1.50 (1.26-1.79)**	**<0.001**	377	0.37	**1.56 (1.31-1.87)**	**<0.001**
AC	183	0.15	162	0.14	0.93 (0.74-1.17)	0.523	131	0.13	0.81 (0.63-1.03)	0.084

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

haplotype was associated with decreased occurrence of these diseases.

Discussion

In the present study we assessed the association between two polymorphisms in the *LIG3* gene: g.29661G>A (rs1003918) and g.29059C>T (rs1052536), and the occurrence of KC and FECD in a case-control study involving 841 individuals, including 283 patients with KC, 258 patients with FECD and 300 controls (KC/FECD free). In our study, subjects in the control group were not matched in terms of age of the KC patients (36.14 ± 12.13 versus 63 ± 19), but in our opinion this is not a serious drawback of these studies, because we believe that age-matched control individuals would not guarantee the lack of this disease at a later time.

As mentioned earlier, increasing evidence has shown that oxidative stress play a pivotal role in the development of KC and FECD (26, 29, 43-46). Oxidative stress can be considered as a disturbance in the balance between the production of ROS and antioxidant defenses, resulting in an ROS excess. It has been demonstrated that, in response to oxidative stress, the mammalian cells counteracted oxidant effects and restored the redox balance by activating or silencing genes encoding defense enzymes, transcription factors, stress induced enzymes and apoptosis (47-49). The susceptibility of KC cornea to cellular damage due to chronic oxidative stress was supported by detection of oxidant-antioxidant imbalance and oxidant-induced DNA damage, mainly mitochondrial DNA (mtDNA), in KC corneas. Furthermore, abnormal oxidative stress-related properties were found in KC corneal cells cultured *in vitro* (43, 50). Abnormal expression of antioxidant enzymes, including catalase has been observed in KC corneas compared to normal human corneas (27). Moreover, altered levels of glutathione reductase and glutathione S-transferase activities and an aberrant banding pattern of aldehyde dehydrogenase were detected in KC specimens (25). Other reports showed that KC corneas had decreased levels of extracellular superoxide dismutase (EC-SOD) (51, 52). Nuclear DNA damage is also observed in corneal endothelium, which may account for the decline in proliferative capacity of these cells (44).

Several studies also demonstrated an oxidant-antioxidant imbalance and accumulation of oxidized DNA damage in FECD endothelium compared with normal corneal endothelium (28, 29). As known, corneal endothelium is arrested in post-mitotic state and does not divide, thus it is especially susceptible to ROS. In addition, immunocytochemistry studies showed co-localization of 8-hydroxyguanine, a marker of oxidative DNA damage, and mitochondria, suggesting that mtDNA damage may be a key component of alteration seen in FECD corneal endothelial cells (29). PCR array analyses discovered transcriptional downregulation of antioxidants and oxidative-stress related genes, including peroxiredoxins, thioredoxin reductase, superoxide dismutase isoforms, nuclear ferritin and glutathione S-transferase π and metallothionein (53).

Because the accumulation of ROS-induced DNA damage and DNA mutations over time are the main contributors to deleterious changes leading to cellular dysfunction, the efficient repair of these DNA damage by BER pathway is crucial. BER takes place both in the nucleus and mitochondria, but all components of these pathways are encoded by nuclear genes. Two different BER subpathways exist depending on whether polymerase inserts one or more nucleotide: short-patch BER (SP-BER) or long-patch BER (LP-BER) (54). The final step in this pathway – sealing the nick, is catalyzed by ATP-dependent DNA ligase (Ligase I or III), which completes the repair process and restores the integrity of the DNA double helix. It was shown that in nucleus DNA ligase I has been implicated in LP-BER, while DNA ligase III in SP-BER. DNA ligase III is a splice variant from the *LIG3* gene, which encodes multiple DNA ligase polypeptides with translation initiation from the upstream start codon allowing for transport to the mitochondria and translation initiation from a downstream start codon allowing for transport to the nucleus. The localization of this ligase in mitochondria suggests that this enzyme may also perform the ligation step in mitochondrial BER (55). Earlier studies showed that depletion of DNA ligase III levels by siRNA resulted in a reduced number of mtDNA copies and increased accumulation of DNA single strand breaks in mtDNA (56).

It was shown that polymorphisms in DNA repair genes, which affect gene expression, may lead to genomic instability and thus increased risk of many diseases. Over the last decade, the role of genetic variations in DNA repair pathway genes in various human disorders, including cancer, have been studied extensively (57-61). The strongest association between the TT homozygote carriers of *LIG3* rs1052536 and the risk of young-onset lung cancer was shown (62). The effect of genetic variations in the *LIG3* gene on the corneal endothelial cells has not yet been examined, so we chose the g.29661G>A polymorphism (rs1003918) and the g.29059C>T polymorphism (rs1052536) to study in our research. Given the role of the DNA damage in corneal diseases (63-65), it is likely that the rs1052536 and rs1003918 polymorphisms of the *LIG3* gene may modulate the occurrence of KC/FECD. In the present study, we observed that these polymorphisms may be associated with KC and FECD. We postulated that these polymorphisms can cause changes in the level or the activity of the enzymatic product, which can lead to a de-

creased protection against ROS-induced DNA damage.

In conclusion, the g. 29661G>A (rs1003918) and g.29059C>T (rs1052536) SNPs of the *LIG3* gene may be associated with individual susceptibility to keratoconus and Fuchs endothelial corneal dystrophy and can be considered as molecular markers in these diseases.

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