

Research Article

Polymorphisms of the Homologous Recombination Gene *RAD51* in Keratoconus and Fuchs Endothelial Corneal Dystrophy

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Purpose. We investigated the association between genotypes and haplotypes of the c.-61G>T (rs 1801320) and c.-98G>C (rs 1801321) polymorphisms of the *RAD51* gene and the occurrence of keratoconus (KC) and Fuchs endothelial corneal dystrophy (FECD) in dependence on some environmental factors. **Methods.** The polymorphisms were genotyped in peripheral blood lymphocytes of 100 KC and 100 FECD patients as well as 150 controls with PCR-RFLP. **Results.** The G/T genotype of the c.-61G>T polymorphism was associated with significantly increased frequency occurrence of KC (crude OR 2.99, 95% CI 1.75–5.13). On the other hand, the G/G genotype of this polymorphism was positively correlated with a decreased occurrence of this disease (crude OR 0.52, 95% CI 0.31–0.88). We did not find any correlation between genotypes/alleles of the c.-98G>C polymorphism and the occurrence of KC. We also found that the G/G genotype and G allele of the c.-98G>C polymorphism had a protective effect against FECD (crude OR 0.51, 95% CI 0.28–0.92; crude OR 0.53, 95% CI 0.30–0.92, resp.), while the G/C genotype and the C allele increased FECD occurrence (crude OR 1.85, 95% CI 1.01–3.36; crude OR 1.90, 95% CI 1.09–3.29, resp.). **Conclusions.** The c.-61T/T and c.-98G>C polymorphisms of the *RAD51* gene may have a role in the KC and FECD pathogenesis and can be considered as markers in these diseases.

1. Introduction

Keratoconus (KC, MIM no. 148300) is a chronic, noninflammatory, degenerative disease of the cornea and can lead to severe visual impairment or blindness. KC is a leading cause of corneal transplantation in Western countries [1]. It is estimated that in ~20% of cases, it progresses to the point of legal blindness and then can be treated only by corneal transplantation. KC typically progresses until the third to fourth decade of life [2]. This disease is observed in all ethnic groups with no female or male predominance [3]. It was found that the incidence of KC in Asians is 25 per 100 000 (1 in 4000) per year, compared with 3.3 per 100 000 (1 in 30 000) per year in Caucasians ($P < 0.001$) [4]. Although the exact pathogenesis of KC is unclear, genetic, environmental,

and behavioral factors as well as interaction between them may be involved. Genetic factors include familial inheritance, discordance between dizygotic twins, and their association with other genetic disorders. The familial nature of KC has been reported in several studies; the prevalence of KC in first degree relatives is 3.34%, which is 15–67 times higher than that of the general population [5]. In such cases, both autosomal dominant and recessive patterns of inheritance have been described [5]. KC is also associated with genetic disorders, such as Leber's congenital amaurosis [6], Down syndrome [7], Ehlers-Danlos syndrome [8], osteogenesis imperfecta [9], and Turner syndrome [10]. It occurs with a higher concordance rate of the trait in monozygotic than in dizygotic twins [11]. Genome-wide linkage analyses have identified several chromosomal regions/gene loci that may be

associated with KC. To date, chromosomal regions 20p11-q1, 16q22.3-3-q23.1, 3p14-q13, 2p24, and 15q22.23-24 as well as the *V SXI* (visual system homeobox 1) gene on 20p11.2 and the *SODI* (superoxide dismutase 1) gene on 21q22.11 have been implicated in KC pathogenesis [12–15]. These findings show a heterogeneous and complex genetic nature of KC. The disease may be also associated with various environmental factors, including hard contact lenses, chronic eye rubbing, and atopy of the eye [16, 17].

Fuchs endothelial corneal dystrophy (FECD, MIM no. 136800) is a degenerative, slowly progressive corneal disease leading to blindness in its advanced stage [18]. It is characterized by an accelerated loss of corneal endothelial cells, morphological changes in the cornea, and increased deposition of extracellular matrix at the Descemet membrane (a collagen-rich basal lamina) secreted by corneal endothelium, called corneal *guttae* [19, 20]. These changes in the structure of the cornea block ion transport and solute barrier functions in the endothelial layer, resulting in the progressive dysfunction of the corneal endothelium [21, 22]. The endothelial dysfunction and loss of corneal endothelial cells lead to corneal decompensation and impaired vision [23]. Based on its clinical and pathologic features, FECD is classified into two forms: early-onset FECD and most typical late-onset form of this disease, which can lead to visual loss [24, 25]. FECD is considered as a disease of aging that typically appears over the age of 50 years. Generally, FECD begins in the 5th decade of life and can progress slowly over the next 2 to 3 decades [26]. This disease occurs predominantly in women at a ratio 2.5–3:1 [18], who comprise approximately 75% of cases [27]. Several reports have shown that FECD is often inherited as an autosomal dominant trait [28–30], and 50% of affected patients are estimated to show a familial clustering [31]. At present, the only treatment modality to restore lost vision is corneal transplantation in the form of penetrating keratoplasty or Descemet's stripping endothelial keratoplasty. The disease is one of the most common indications for corneal transplantation performed in the USA [22]. Despite these findings, the pathogenesis of FECD remains poorly understood. Some evidence suggests that FECD may develop independently of systemic or environmental factors [29, 31]. Therefore, understanding genetic risk factors that cause the disease is crucial for establishing strategies to prevent and treat it. The first gene to be causally linked with FECD was *COL8A2*. This gene is located on chromosome 1 (1p34.3-p32) and encodes the alpha 2 chain of type VIII collagen, a major component of Descemet membrane. Two missense mutations, p.L450W and p.Q455V/Q455K, have been identified in early-onset form multigenerational families with FECD [32–34] and in atypical sporadic FECD cases [34]. Several genome-wide linkage scans in large multigenerational families have mapped late-onset FECD susceptibility loci on chromosomes 13 (*FCD1*), 18 (*FCD2*), 5 (*FCD3*), and 9 (*FCD4*) [35–37].

A growing body of evidence suggests that oxidative stress may play an important role in the pathogenesis of KC and FECD [38–45]. The cornea is a transparent, avascular structure of the eye that is exposed to a wide spectrum of light, including the ultraviolet (UV) radiation. UV exposure is

a well-characterized environmental stress factor that generates free radicals and reactive oxygen species (ROS), harmful to most cells and tissues [46]. Since the cornea is the first target of UV-light entering the eye, it is especially susceptible to damage from ROS. An excess of ROS may induce mutations, cancer, or cell death [47]. In normal conditions, the cornea has natural antioxidant enzymes, such as superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase that eliminate ROS [48]. On the other hand, in disease state, the cornea displays an altered activity of antioxidant enzymes, accumulation of ROS, and mitochondrial DNA damage [39, 41, 42, 49].

ROS can cause a variety of DNA damage, including DNA single and double strand breaks (DSBs) and DNA base modifications. Therefore efficient repair of ROS-induced DNA damage is important for preventing mutations and maintaining the stability of the genome. It has been demonstrated that DNA damage has been implicated as a causative factor in a wide variety of degenerative diseases, cancer, and premature aging [50–54]. The most dangerous and lethal types of DNA damage are DSBs, which if left unrepaired can result in permanent cell cycle arrest, apoptosis, or mitotic cell death caused by the loss of genomic material [55]; if misrepaired, they can lead to cancer and other disorders through translocations, inversions, or deletions [56, 57]. In mammalian cells, DSBs can be repaired by nonhomologous end joining (NHEJ) or homologous recombination (HRR) pathways. It was shown that single-nucleotide polymorphisms (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 [42, 58–63].

Several case-control studies have confirmed the association between genetic polymorphisms or mutations and corneal diseases [22, 27, 64–69]. We hypothesize that genetic variations in the *RAD51* gene may also contribute to the development of KC and FECD. The *RAD51* protein encoded by this gene is the central protein involved in homologous recombination and repair of DSBs in humans. In the present work we checked whether two polymorphisms, c.-61G>T and c.-98G>C, of the *RAD51* gene were associated with KC/FECD occurrence and whether this association was modulated by some demographic and potential risk factors for KC/FECD. Both polymorphisms are SNPs located in the 5' untranslated region (5' UTR) of the gene. These SNPs have been chosen as they were reported to be associated with altered gene transcription [70].

2. Materials and Methods

2.1. Ethics. The study design was approved by the Bioethics Committee of the Medical University of Warsaw, 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.

2.2. Study Population and Sample Collection. The study population comprised 100 patients with KC, 100 patients with FECD, and 150 individuals with healthy corneas (controls).

TABLE 1: Characteristics of KC and FECD patients and controls enrolled in this study.

Feature	Controls (<i>n</i> = 150)		KC (<i>n</i> = 100)		<i>P</i>	FECD (<i>n</i> = 100)		<i>P</i>
	Number	Frequency	Number	Frequency		Number	Frequency	
Sex								
females	96	0.96	30	0.30	<0.001	73	0.73	0.176
males	54	0.54	70	0.70		27	0.27	
Age								
Mean ± SD	67.02 ± 16.83		36.54 ± 11.12		<0.001*	71.76 ± 9.25		<0.001*
Range	20–100		20–63			47–91		
Smoking								
yes (current/former)	20	0.13	24	0.24	0.045	32	0.32	0.0007
never	130	0.87	76	0.76		68	0.68	
KC/FECD in family								
yes	3	0.02	9	0.09	0.0254	10	0.10	0.0124
no	147	0.98	91	0.91		90	0.90	
BMI								
≤25	64	0.44	43	0.43	0.993	40	0.40	0.838
25–30	46	0.31	32	0.32		31	0.31	
≥30	37	0.25	25	0.25		28	0.28	
Visual impairment								
yes	44	0.29	67	0.67	<0.001	52	0.52	<0.001
no	106	0.71	33	0.33		48	0.48	
Allergies								
yes	23	0.15	23	0.23	0.172	14	0.14	0.913
no	127	0.85	77	0.77		86	0.86	

P values for a two-sided χ^2 -test; * *P* values for *t*-test. *P* < 0.05 are in bold.

All patients and controls were examined in the Department of Ophthalmology, Medical University of Warsaw (Warsaw, Poland). Medical history was obtained from all subjects, and no one reported any genetic disease.

The diagnosis of KC was based on clinical signs and topographical and pachymetric parameters on TMS corneal topography and Orbscan examinations [2, 71, 72]. The map patterns were carefully interpreted manually in all cases. Patients underwent ophthalmic examination, including best-corrected visual acuity, intraocular pressure, slit lamp examination, fundus examination, corneal topography (TMS4, Tomey, Nagoya, Japan), Orbscan corneal topographical, and pachymetric maps (Orbscan IIz, Bausch & Lomb, USA).

The diagnosis of FECD was based on clinical signs on the slit lamp examination (occurrence of endothelial guttae, corneal edema) and in all the cases was confirmed by the presence of specific lesions, polymegathism, and pleomorphism of the endothelial cells in *in vivo* confocal microscopy (IVCM) examination [73, 74]. Patients underwent ophthalmic examination, including best-corrected visual acuity, intraocular pressure, slit lamp examination, fundus examination, IVCM, and anterior segment optical coherence tomography including pachymetry maps (AS-OCT). The IVCM was performed by white light scanning slit confocal microscopy system (ConfoScan 3 or ConfoScan 4, Nidek Technologies, Padova, Italy). The AS-OCT was performed by Swept Source Anterior Segment Casia OCT (Tomey, Nagoya, Japan).

The control subjects had no clinical evidence of FECD/KC and presented with healthy corneal endothelium on IVCM and normal corneal topography and pachymetry.

Five mL of venous blood was collected from each individual enrolled in this study into EDTA-containing tubes, coded and stored at -20°C until further use.

2.3. Interviews. 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, and myopia), and 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.

2.4. SNP Selection and Primers Design. We searched the public domain of the National Center for Biotechnology Information the Single Nucleotide Polymorphisms database (NCBI dbSNP) at <http://www.ncbi.nlm.nih.gov/snp> to identify potentially functional polymorphisms in the DNA repair genes. We chose to genotype the c.-61G>T (rs1801321) and the c.-98G>C (rs1801320) polymorphisms of the *RAD51* gene

with a minor allele frequency (MAF) 0.467 and 0.067 in European population, respectively (submitter population ID: HapMap-CEU for both; <http://www.ncbi.nlm.nih.gov/snp>). Primers were designed according to the published nucleotide sequence in ENSEMBL database (gene ID ENSG00-000051180) and using Primer3 software (<http://frodo.wi.mit.edu/>).

We chose polymorphisms of the known distribution in the European population. SNPs selection favored those with a minor allele frequency not less than 5%. Our choice was mainly determined by a potential biological significance of the polymorphisms following from their location. Both SNPs are located in the 5' UTR of the gene and may be associated with altered gene transcription.

2.5. DNA Extraction. Genomic DNA was extracted from venous blood by using the commercially available AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Biosciences, Union City, CA, USA), according to the manufacturer's instructions. DNA was directly isolated from the white blood cells. DNA purity and concentration were determined by comparing the absorbance at 260 and 280 nm. The purified genomic DNA was stored in TE buffer (5 mM Tris-HCl, 0.1 mM EDTA, pH 8.5), at -20°C until further analysis.

2.6. SNP Genotyping. The c.-61G>T and c.-98G>C polymorphisms were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The total reaction volume of $20\ \mu\text{L}$ contained 50 ng of genomic DNA, $1 \times$ Kapa Taq Ready Mix containing KapaTag DNA polymerase ($0.025\ \text{U}/\mu\text{L}$), reaction buffer with MgCl_2 and 0.2 mM each dNTP (Kapa Biosystems, Woburn, MA, USA), and 0.25 μM of each primer (Metabion, Martinsried, Germany). 131-bp length fragments containing two SNPs were amplified using the following primers: forward 5'-TGG GAA CTG CAA CTC ATC TGG-3' and reverse 5'-GCT CCG ACT CCC CGC CGG-3'. The PCR profile consisted of an initial denaturation step for 5 min at 95°C , 30 cycles at 95°C for 30 s, 30 s at 65°C annealing temperature and 60 s at 72°C , and the final extension step for 5 min at 72°C . The amplified 131 bp fragments were analyzed on a 3% agarose gel and separated into two groups. One group was digested with 2 U of *Ngo*MIV restriction endonuclease (New England BioLabs, Beverly, MA, USA) in a final volume of $15\ \mu\text{L}$ for 16 h at 37°C to analyse c.-61G>T polymorphism, while the second group was digested with 2 U of *Mva*I (BstNI) restriction endonuclease (Fermentas, Hanover, MD, USA) to analyse c.-98G>C polymorphisms under the same conditions. The G/G genotype of the c.-61G>T polymorphism produced two fragments (110 and 21 bp), whereas the G/T genotype yielded three fragments (131, 110, and 21 bp), and the homozygote T/T resulted in one 131 bp fragment. In the case of the c.-98G>C polymorphism, the G/G genotype produced two fragments (71 and 60 bp) and the C/C genotype only one fragments, while the heterozygote G/C displayed all three fragments (131, 71 and 60 bp). After digestion, the reaction mixture was subjected to electrophoresis on an 8% polyacrylamide gel and visualized by ethidium bromide staining using a GeneRuler

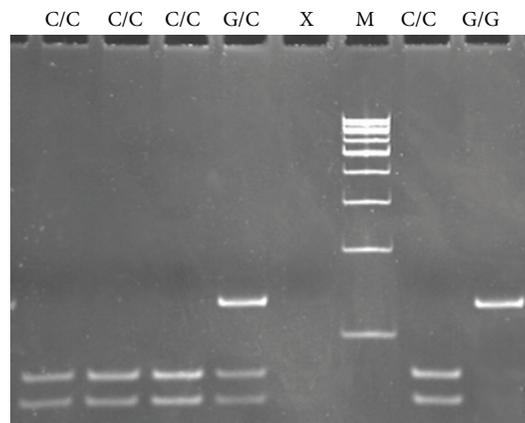


FIGURE 1: Genotyping of the *RAD51* c.-98G>C (rs1801320) polymorphism. Genotypes are indicated in the upper part of the picture. Lane M shows GeneRuler 100 bp molecular length marker; lane X shows a negative control—reaction mixture without target DNA.

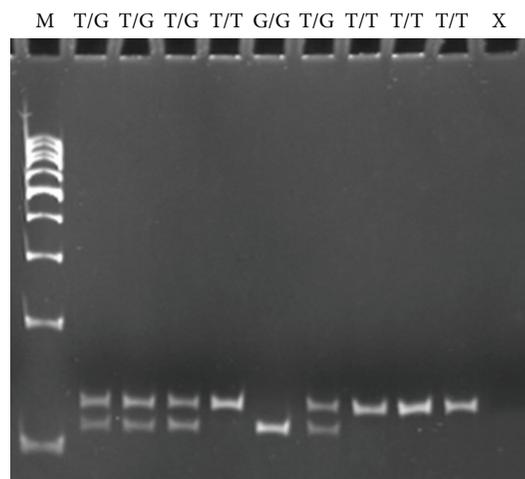


FIGURE 2: Genotyping of the *RAD51* c.-61G>T (rs1801321) polymorphism. Genotypes are indicated in the upper part of the picture. Lane M shows GeneRuler 100 bp molecular length marker; lane X shows a negative control—reaction mixture without target DNA.

100 bp (Fermentas, Hanover, MD, USA) as a molecular mass marker. Representative gels for these polymorphisms are presented in Figures 1 and 2.

All PCR amplifications were conducted in a C1000 Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA). Positive and negative (no template) controls were included in all sets. For quality control, 10% of samples were randomly genotyped again, and the results were 100% concordant.

2.7. Statistical Analysis. The statistical analyses were carried out with the SigmaPlot version 11.0 (Systat Software, Inc., San Jose, CA, USA), statistical software package. 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

TABLE 2: Distribution of genotypes and alleles of the c.-61G>T and c.-98G>C polymorphisms of the *RAD51* gene and odds ratio (OR) with 95% confidence interval (95% CI) in patients with KC and controls.

Genotype/allele polymorphisms	Controls (<i>n</i> = 150)		KC (<i>n</i> = 100)		Crude OR (95% CI)	<i>P</i>	Adjusted OR ^a (95% CI)	<i>P</i>
	Number	Frequency	Number	Frequency				
c.-61G>T								
G/G	76	0.51	35	0.35	0.52 (0.31–0.88)	0.015	0.54 (0.31–0.98)	0.043
G/T	39	0.26	51	0.51	2.99 (1.75–5.13)	<0.001	2.54 (1.41–4.58)	0.002
T/T	35	0.23	14	0.14	0.53 (0.27–1.06)	0.071	0.65 (0.31–1.36)	0.251
$\chi^2 = 16.400; P = 0.0003$								
G	191	0.64	121	0.60	0.75 (0.47–1.22)	0.563	0.89 (0.61–1.30)	0.530
T	109	0.36	79	0.40	1.10 (0.79–1.54)	0.563	1.14 (0.79–1.65)	0.477
c.-98G>C								
G/G	122	0.81	80	0.80	0.92 (0.48–1.74)	0.793	0.82 (0.40–8.33)	0.596
G/C	27	0.18	19	0.19	1.06 (0.55–2.03)	0.861	1.18 (0.56–2.46)	0.665
C/C	1	0.01	1	0.01	1.51 (0.09–24.33)	0.773	1.79 (0.09–35.84)	0.703
$\chi^2 = 0.129; P = 0.9375$								
G	271	0.90	179	0.90	0.91 (0.50–1.66)	0.758	0.82 (0.42–1.61)	0.562
C	29	0.10	21	0.10	1.09 (0.60–2.00)	0.758	1.22 (0.62–2.39)	0.562

P < 0.05 along with corresponding ORs are in bold; ^aOR adjusted for cooccurrence of visual impairment, smoking, and family history for KC.

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. The association between case-control status and each polymorphism, measured by the odds ratio (OR) and its corresponding 95% confidence interval (CI), was estimated using an unconditional multiple logistic regression model, both with and without adjustment for cooccurrence of visual, smoking, and family status of KC/FECD.

3. Results

The PCR-RFLP analyses were successful for all 250 DNA samples (100 KC/FECD patients and 150 KC/FECD free controls).

3.1. Characteristics of the Study Subjects. Table 1 presents demographic and potential risk factors for KC and FECD of the study patients and controls. The mean \pm SD age for KC patients were 36.54 ± 11.12 (range 20–63), 71.76 ± 9.25 (range 47–91) for FECD patients, and 67.02 ± 16.83 (range 20–100) for controls. Moreover, there were significantly more subjects with positive family history for KC and FECD (1st degree relatives) among the patients in comparison to controls (9% and 10% versus 2%, *P* < 0.05). We demonstrated significant differences between distribution of family history for KC/FECD (positive versus negative family history), smoking (yes versus never), and cooccurrence of visual impairment (yes versus no) among patients and controls. These parameters were further adjusted in multivariate logistic regression model for possible confounding factors of the main effect of the SNPs.

3.2. The c.-61G>T and c.-98G>C Polymorphisms of the *RAD51* Gene and KC Occurrence. The genotype and allele

distributions of the c.-61G>T polymorphism of the *RAD51* gene 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 each group. The difference in the frequency distributions of genotypes of the c.-61G>T polymorphism between the cases and controls was statistically significant (*P* < 0.05). The presence of the G/T genotype significantly increased the occurrence of KC (crude OR 2.99, 95% CI 1.75–5.13). On the other hand, the G/G genotype decreased it (crude OR 0.52, 95% CI 0.31–0.88). We did not find any correlation between genotypes/alleles of the other polymorphism and KC occurrence.

3.3. The c.-61G>T and c.-98G>C Polymorphisms of the *RAD51* Gene and FECD Occurrence. Details of genotype and allele frequencies of the c.-98G>C polymorphisms of the *RAD51* gene and summary of statistical analysis are shown in Table 3. There was not any difference in the frequency distributions of genotypes of the c.-98G>C polymorphism 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 each group. The presence of the T/T genotype and the T allele of the c.-61G>T polymorphism was associated with a decreased occurrence of KC (crude OR 0.41, 95% CI 0.19–0.84; OR 0.50, 95% CI 0.36–0.69, resp.), but on the other hand the G/T genotype increased it (crude OR 2.50, 95% CI 1.46–4.28). In the case of the c.-98G>C polymorphism, we found that the G/G genotype and G allele were associated with a protective effect (crude OR 0.51, 95% CI 0.28–0.92; crude OR 0.53, 95% CI 0.30–0.92, resp.), while the G/C genotype and the C allele increased FECD occurrence (crude OR 1.85, 95% CI 1.01–3.36; crude OR 1.90, 95% CI 1.09–3.29).

TABLE 3: Distribution of genotypes and alleles of the c.-61G>T and c.-98G>C polymorphisms of the *RAD51* gene and odds ratio (OR) with 95% confidence interval (95% CI) in patients with FECD and controls.

Genotype/allele polymorphisms	Controls (<i>n</i> = 150)		FECD (<i>n</i> = 100)		Crude OR (95% CI)	<i>P</i>	Adjusted OR ^a (95% CI)	<i>P</i>
	Number	Frequency	Number	Frequency				
c.-61G>T								
G/G	76	0.51	42	0.42	0.71 (0.42–1.17)	0.179	0.76 (0.44–1.32)	0.334
G/T	39	0.26	47	0.47	2.50 (1.46–4.28)	<0.001	2.30 (1.27–4.16)	0.006
T/T	35	0.23	11	0.11	0.41 (0.19–0.84)	0.016	0.44 (0.19–0.98)	0.045
$\chi^2 = 13.607$; <i>P</i> = 0.0011								
G	191	0.64	131	0.66	1.09 (0.78–1.52)	0.610	1.08 (0.74–1.58)	0.684
T	109	0.36	69	0.34	0.50 (0.36–0.69)	<0.001	0.93 (0.63–1.35)	0.684
c.-98G>C								
G/G	122	0.81	69	0.69	0.51 (0.28–0.92)	0.026	0.54 (0.28–1.05)	0.069
G/C	27	0.18	29	0.29	1.85 (1.01–3.36)	0.045	1.73 (0.88–3.38)	0.112
C/C	1	0.01	2	0.02	3.04 (0.27–33.99)	0.367	3.12 (0.24–40.21)	0.383
$\chi^2 = 5.325$; <i>P</i> = 0.0698								
G	271	0.90	167	0.84	0.53 (0.30–0.92)	0.023	0.55 (0.30–1.02)	0.059
C	29	0.10	33	0.16	1.90 (1.09–3.29)	0.023	1.79 (0.99–3.24)	0.056

P < 0.05 along with corresponding ORs are in bold; ^aOR adjusted for cooccurrence of visual impairment, smoking, and family history for FECD.

TABLE 4: Distribution of haplotypes of the c.-61G>T and c.-98G>C polymorphisms of the *RAD51* gene and odds ratio (OR) with 95% confidence interval (95% CI) in patients with KC and FECD and controls.

Haplotype	Controls (<i>n</i> = 150)		KC (<i>n</i> = 100)		OR (95% CI)	<i>P</i>	FECD (<i>n</i> = 100)		OR (95% CI)	<i>P</i>
	Number	Frequency	Number	Frequency			Number	Frequency		
GG	324	0.54	209	0.52	0.93 (0.72–1.20)	0.566	207	0.52	0.91 (0.71–1.17)	0.566
GC	218	0.36	149	0.37	1.04 (0.80–1.35)	0.588	127	0.32	0.82 (0.62–1.07)	0.588
TG	58	0.10	33	0.08	0.89 (0.57–1.40)	0.544	55	0.14	1.23 (0.51–3.00)	0.544
TC	0	0	9	0.02	—	—	11	0	—	—

3.4. Haplotypes and KC/FECD Occurrence. We also assessed the association between the occurrence of KC/FECD and haplotypes of the c.-61G>T and c.-98G>C polymorphisms of the *RAD51* gene. The distribution of such haplotypes is shown in Table 4. We did not find any correlation between haplotypes of both polymorphisms and the occurrence of KC and FECD.

4. Discussion

Several studies have confirmed that genetic factors play a pivotal role in corneal diseases development, as indicated by the association of such diseases with genetic syndromes or genetic disorders, segregation analyses, genetic epidemiological data, and gene mapping studies [5, 75–77]. Because DNA damage may be associated with development of the degenerative diseases, it was hypothesized that genes involved in DNA repair may influence an individual's susceptibility to these diseases [78, 79]. Increasing evidence has shown that genetic polymorphisms in DNA repair genes could modulate DNA repair capacity, resulting in DNA damage accumulation, and have an effect on the sensitivity of the organism to environmental mutagens [80–84], and then they contribute to some complex disorders, including corneal

diseases [59–62, 85]. Given the potential roles of the c.-61G>T and c.-98G>C polymorphisms of the *RAD51* gene in the DNA repair, we examined the association between these two SNPs and KC/FECD occurrence.

The *RAD51* gene (full name: RAD51 homolog (*S. cerevisiae*); gene ID: 5888, also known as ECA, BRCC5, MRMV2, HRAD51, RAD51A, HsRad51, and HsT16930) is a eukaryotic homologue of *Rad51 Saccharomyces cerevisiae*. This gene consists of 16 exons spanning a region >30 kb and encodes a 339 amino acid protein. The first exon and part of the second exon of *RAD51* are located at its 5' untranslated region (5' UTR), which may regulate gene expression [86]. The protein encoded by this gene is a member of the RAD51 protein family, which is known to be involved in homologous recombination and DNA repair [87]. Homologous recombination repair is a crucial DNA repair pathway to the maintenance of genomic stability. HRR repairs damaged DNA by identifying a stretch of homologous sequence on an undamaged sister chromatid, using that chromatid as a template for reconstruction of the broken DNA strands. One of the first steps during HRR is recognition of DSBs by the MRN complex comprising MRE11, RAD50, and NBS1 proteins. The central HRR protein-RAD51 recognizes the homology, forms a nucleoprotein filament with the 3' ssDNA

overhangs of the resected DSB and catalyzes homologous pairing and strand exchange [88]. RAD51 can interact with BRCA1 and BRCA2, which may be important for the cellular response to DNA damage. It was shown that BRCA2 regulates both the intracellular localization and DNA-binding ability of the RAD51 [89–92]. RAD51 knockout mouse ($RAD51^{-/-}$) show early embryonic lethality, suggesting that the RAD51 is fundamental for HRR and genomic integrity [93]. It has been demonstrated that loss of RAD51 expression predisposes cells to chromosome breaks or aberrations, mutagenesis, and cell death [94]. In general, even minor changes or deregulation of RAD51 may lead to genetic instability and cancer [95, 96].

The human *RAD51* gene is highly polymorphic, at least its 733 SNPs have been registered in the public domain of the National Center for Biotechnology Information the Single Nucleotide Polymorphisms database (NCBI dbSNP; <http://www.ncbi.nlm.nih.gov/snp>). Among them, c.-61G>T (rs1801321; 172G>T) and c.-98G>C (rs1801320; 135G>C) belong to the most common polymorphisms and are located at 5' UTR. Interestingly, only a few SNPs are located in the coding region of this gene, none of which has a minor allele frequency (MAF) >5%, indicating that its protein structure is well conserved [86, 97]. Only a few studies have investigated the association between the c.-61G>T polymorphisms and risk of cancer. In a large European case-control study of 2205 patients with breast cancer and 1805 controls, the c.-61T variant genotypes were found to be associated with nonsignificantly reduced risk of breast cancer [98]. Another case-control study conducted in Korea also brought similar results [99]. As shown by bioinformatics analysis, c.-61T allele is located in a binding site for the transcription factor P300/CBP, a cofactor of nuclear receptor signaling that processes strong histone acetyltransferase activities and functions as an adaptor protein that enhances transcription of the genes to which the ligand is bound [100]. In contrast, the c.61G allele does not form a binding site of *cis*-transcriptional elements for P300/CBP. Thus, the presence of the T allele shows a greater effect on *RAD51* gene expression. In another study, using luciferase reporter gene assay, it was shown that the c.61T allele was associated with a significantly higher activity of the *RAD51* promoter as compared with the c.-61G allele [70]. As mentioned, the c.-98G>C polymorphism is also located at 5'UTR. Although functional consequence of this variation is unknown, it is speculated that because it alters a CpG island pattern in the promoter, it may regulate expression and affect mRNA levels [70, 101]. Case-control studies have shown that the c.-98C allele was associated with a 2-fold lower risk of breast and ovarian cancer than the c.-98G allele, in *BRCA1* 5382insC mutation carriers [102] and other mutation carriers [103], whereas no association was found in studies of sporadic breast cancer [104, 105].

The effect of genetic variations in the *RAD51* gene on the corneal endothelial cells has not yet been examined, so we chose the c.-61G>T and c.-98G>C SNPs to study in our research. We observed that the occurrence of KC was positively correlated with the G/G and G/T genotype and the T allele of the c.-61G>T SNP. In the case of the other polymorphism, c.-98G>C, we did not find any correlation

between genotypes/alleles and the occurrence of KC. We also found that the G/G genotype and the G allele of the c.-98G>C polymorphism decreased FECD occurrence, while the G/C genotype and the C allele increased it. Furthermore, the presence of the T/T genotype and the T allele of the c.-61G>T polymorphism was associated with a decreased occurrence of KC, but on the other hand the G/T genotype increased it.

In summary, our results showed that the c.-61G>T and c.-98G>C SNPs of the *RAD51* gene may be associated with individual susceptibility to keratoconus and Fuchs endothelial corneal dystrophy and suggested that DNA damage may be involved in the pathogenesis of these diseases.

5. Conclusion

This study suggests that the c.-61T/T and c.-98G>C polymorphisms of the *RAD51* gene may play a role in KC and FECD pathogenesis and are considered as markers in these diseases, but further studies in other independent cohorts are needed to confirm or exclude these associations.

Conflict of Interests

The authors report that they have no conflict of interests.

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