

# Lack of association between polymorphisms of the DNA base excision repair genes *MUTYH* and *hOGG1* and keratoconus in a Polish subpopulation

Ewelina Synowiec<sup>1</sup>, Katarzyna A. Wójcik<sup>1</sup>, Anna Czubatka<sup>1</sup>, Piotr Polakowski<sup>2</sup>, Justyna Izdebska<sup>2</sup>, Jerzy Szaflik<sup>2</sup>, Janusz Błasiak<sup>1</sup>, Jacek P. Szaflik<sup>2</sup>

<sup>1</sup>Department of Molecular Genetics, University of Lodz, Lodz, Poland

<sup>2</sup>Department of Ophthalmology, Medical University of Warsaw, SPKSO Ophthalmic University Hospital, Warsaw, Poland

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## Corresponding author:

Jacek P. Szaflik  
Department  
of Ophthalmology  
Medical University of Warsaw  
SPKSO Ophthalmic  
University Hospital  
13 Sierakowskiego St  
03-710 Warsaw, Poland  
Phone: +48 22 511 63 00  
Fax: +48 22 511 63 01  
E-mail: szaflik@  
ophthalmology.pl

## Abstract

**Introduction:** Keratoconus (KC) is a non-inflammatory thinning of the cornea and a leading indication for corneal transplantation. Oxidative stress plays a role in the pathogenesis of this disease. The products of the *hOGG1* and *MUTYH* genes play an important role in the repair of oxidatively modified DNA in the base excision repair pathway. We hypothesized that variability in these genes may change susceptibility to oxidative stress and predispose individuals to the development of KC. We investigated the possible association between the c.977C>G polymorphism of the *hOGG1* gene (rs1052133) and the c.972G>C polymorphism of the *MUTYH* gene (rs3219489) and KC occurrence as well as the modulation of this association by some KC risk factors.

**Material and methods:** A total of 205 patients with KC and 220 controls were included in this study. The polymorphisms were genotyped with polymerase chain reaction (PCR) restriction fragment length polymorphism and PCR-confronting two-pair primer techniques. Differences in genotype and allele frequency distributions were evaluated using the  $\chi^2$  test, and KC risk was estimated with an unconditional multiple logistic regression with and without adjustment for co-occurrence of visual impairment, allergies, sex and family history for KC.

**Results:** We did not find any association between the genotypes and combined genotypes of the c.977C>G polymorphism of the *hOGG1* gene and the c.972G>C polymorphism of the *MUTYH* gene and the occurrence of KC.

**Conclusions:** Our findings suggest that the c.977C>G-*hOGG1* polymorphism and the c.972G>C-*MUTYH* polymorphism may not be linked with KC occurrence in this Polish subpopulation.

**Key words:** oxidative stress, genetic variability, DNA glycosylases, oxidative DNA damage, DNA repair.

## Introduction

Keratoconus (KC; MIM #148300) is a progressive, non-inflammatory disease of the cornea that usually develops in adolescence and can lead to severe visual impairment or blindness. In about 20% of cases, it progresses to the point of legal blindness and then can be treated only by corneal transplantation [1–3]. To date, KC is a major cause of cornea transplantation in Western countries [4, 5]. Keratoconus usu-

ally presents in the second decade of life and progresses into the third and fourth decades, with decreasing visual function [3–6]. The estimated prevalence of this disease is ~50–230 per 100 000, depending on the clinical detection methods and diagnostic criteria [3]. Keratoconus is observed in all ethnic groups, with no female or male predominance [7]. It was found that the incidence of KC in Asians was 25 per 100 000 (1 per 4000) per year, compared with 3.3 per 100 000 (1 in 30 000) per year in Caucasians [8].

Although the aetiology of KC remains unclear, a growing body of evidence suggests that both genetic and environmental factors and interaction between them are responsible for the disease induction and progression. It was shown that environmental factors include contact lens wear, chronic eye rubbing and atopy of the eye [9, 10]. Genetic factors include familial inheritance, discordance between dizygotic twins and KC association with other genetic disorders. The prevalence of KC in first degree relatives is 3.34%, which is 15–67 times higher than that of general population [11]. Keratoconus is found in 0.5–15% of patients with Down syndrome, and was also reported in patients with Leber's congenital amaurosis, Ehlers-Danlos syndrome and osteogenesis imperfecta [12–14]. In addition, KC occurs with a higher concordance rate of the trait in monozygotic twins than in dizygotic ones [15]. A number of gene loci/chromosomal regions for familial KC have been described [16–19]. Taken together, these data suggest that KC is a multifactorial disease.

Results of several studies indicate that oxidative stress may play a role in the development of KC [20–26]. The cornea is exposed to a wide spectrum of light, including ultraviolet (UV) radiation. UV exposure is a well-characterized environmental stress factor that generates reactive oxygen species (ROS), including free radicals [27]. As the cornea absorbs most of the UV reaching the eye, it would be especially susceptible to ROS-induced damage. In the healthy cornea, several defence mechanisms are present to minimize such damage [23]. In general, the cornea has antioxidant enzymes, including superoxide dismutase, glutathione reductase and glutathione peroxidase, that neutralize ROS before they damage cells [28]. It was demonstrated that KC corneas displayed altered activity of antioxidant enzymes [20, 21], accumulation of cytotoxic ROS [23] and mitochondrial DNA damage [29]. Oxidation may involve various biomolecules – membrane phospholipids, proteins and DNA [30] – and has been implicated in a wide range of pathological conditions, including premature aging and systemic diseases, such as cancer, neurodegenerative and ocular diseases [31–36]. Reactive oxygen species can cause a variety of DNA damage, including sin-

gle and double strand breaks (SSBs and DSBs, respectively) and DNA base modifications; therefore efficient repair of ROS-induced DNA damage is important for preventing mutations and maintaining the stability of the genome [37]. One of the most commonly produced DNA base lesions, frequently applied as a hallmark of oxidative DNA damage, is 8-oxoguanine (8-OH-G) [38]. About one thousand 8-OH-Gs are estimated to be generated in normal human cells per day [39]. 8-OH-G can pair with adenine, instead of cytosine, during DNA replication. If this mispairing is not repaired, it leads to a G:C to T:A transversion mutation [40]. In mammalian cells two base excision repair (BER) DNA glycosylases, hOGG1 and MUTYH, initiate the repair of such lesions – hOGG1 removes 8-OH-G and MUTYH removes adenine paired with 8-OH-G, leaving an apurinic site in the DNA.

In recent years, the role of genetic factors in KC has been extensively investigated. Several case-control studies have confirmed the association between single nucleotide polymorphisms (SNPs)/mutations and this disease [41–46]. In the present study we assessed the association between polymorphism of two BER genes, *MUTYH* (c.972G>C; rs3219489) and *hOGG1* (c.977C>G; rs1052133), and KC occurrence as well as modulation of this association by some demographic and risk factors for KC. Both polymorphisms are located in the coding regions of their genes, resulting in amino acid substitutions. The c.977G>C polymorphism causes change of serine to cysteine at position 326 (Ser326Cys) of the hOGG1 protein, whereas the c.972C>G polymorphism is associated with substitution of glutamine to histidine at position 324 (Gln324His) of the MUTYH protein. These SNPs may exert a functional effect according to results of association and/or *in vitro* studies [47–55].

## Material and methods

### Ethics

The study design was approved by the Bioethics Committee of the Medical University of Warsaw and each patient and control individual gave a written informed consent and approval form for genetic analysis.

### Patients and controls

A total of 205 patients with KC and 220 individuals with normal corneas (controls) were enrolled in this study. All patients and controls were examined in the Department of Ophthalmology, Medical University of Warsaw (Warsaw, Poland). They underwent ophthalmic examination, including best-corrected visual acuity, intraocular pressure, slit lamp examination, fundus examination, corneal topography (TMS4, Tomey, Nagoya, Japan),

and Orbscan corneal topographical and pachymetric maps (Orbscan IIz, Bausch & Lomb, USA). The diagnosis of KC was based on clinical signs and topographical and pachymetric parameters on Topographic Modeling System (TMS) topography and Orbscan examinations [3, 56, 57]. The map patterns were carefully interpreted manually in all cases. The patients with an unmistakable diagnosis of KC were included in the study group, and the patients with normal corneal topography and pachymetry were included in the control group. Medical history was obtained from all subjects, and no one reported any genetic disease.

After informed consent, 5 ml of venous blood from participants was collected into EDTA tubes, coded and stored at  $-20^{\circ}\text{C}$  until further use. In addition, all subjects were interviewed using a structural questionnaire to determine demographic and potential risk factors for KC. Study subjects 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 family history among 1st degree relatives for KC. Smoking was categorized as current, former or never smokers. The validity and reliability of the ques-

tionnaires were checked whenever possible. Characteristics of patients and controls are presented in Table I. All individuals employed in our research were unrelated and were of Polish nationality, belonging to the Polish ethnic group.

#### Selection of SNPs and primer design

We searched the public domain of the National Center for Biotechnology Information 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.972G>C and the c.977C>G polymorphisms with minor allele frequency (MAF) of 0.284 and 0.224 in the 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 the ENSEMBL database (gene ID for *MUTYH* ENSG00000132781 and ENSG00000114026 for *hOGG1*) and using Primer3 software for *hOGG1* SNP (<http://frodo.wi.mit.edu/>) and Web-based allele-specific primer software for *MUTYH* SNP (<http://bioinfo.biotec.or.th/WASP>).

**Table I.** Characteristics of KC patients and controls

Feature	Controls (n = 220)		KC (n = 205)		Value of p
	Number	Frequency	Number	Frequency	
Gender:					
Females	129	0.59	64	0.31	<b>&lt; 0.001</b>
Males	91	0.41	141	0.69	
Age:					
Mean $\pm$ SD	60.71 $\pm$ 20.25		36.39 $\pm$ 11.87		<b>&lt; 0.001*</b>
Range	19–91		14–63		
Smoking:					
Current/former	68	0.31	67	0.33	0.7732
Never	152	0.69	138	0.67	
KC in family:					
Yes	9	0.04	22	0.11	<b>0.0145</b>
No	21	0.96	183	0.89	
BMI [kg/m <sup>2</sup> ]:					
$\leq$ 25	94	0.44	92	0.45	0.9227
25–30	69	0.32	66	0.32	
$\geq$ 30	53	0.25	47	0.23	
Visual impairment:					
Yes	106	0.48	150	0.26	<b>&lt; 0.001</b>
No	114	0.52	55	0.74	
Allergies:					
Yes	32	0.15	53	0.26	<b>0.0053</b>
No	188	0.85	152	0.74	

Values of p for a two-sided  $\chi^2$  test; \*p values for t-test; p values < 0.05 are in bold.

## DNA isolation

Genomic DNA was extracted from venous blood 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  $-20^{\circ}\text{C}$  until further analysis.

## SNPs genotyping

Genotyping of the c.972G>C-*MUTYH* polymorphism was carried out by polymerase chain reaction (PCR) with confronting two-pair primers (PCR-CTPP). The amplification of allele-specific DNA products of different lengths by adding four (two pairs) primers into one tube containing a PCR mixture enables direct genotyping by gel electrophoresis [58]. Polymerase chain reaction was performed in a 25  $\mu\text{l}$  reaction volume. The reaction mixture contained 50 ng of genomic DNA, 1 U of Biotools DNA polymerase (Biotools, Madrid, Spain), 1  $\times$  reaction buffer (750 mM Tris-HCl, pH 9.0, 500 mM KCl, 200 mM  $(\text{NH}_4)_2\text{SO}_4$ ), 0.2 mM of each dNTP, 1.5 mM  $\text{MgCl}_2$ , and 0.25  $\mu\text{M}$  of each primer (Metabion, Martinsried, Germany). The primers designed to detect the c.972G>C SNP were: F1: 5'-CCTGTCTGGGAGTCCTGACG-3' and R2: 5'-GAGGCAGGC ACAGGTGGCAC-3' for the amplification of the G allele (241-bp band), and F2 5'-CCCAGC TCCCAACTGGACAC-3' and R1 5'-CGCTGAAGCTGCTCTGAGGGC-3' for the amplification of the C allele (362-bp band). F1 and R1 give a common 562-bp PCR product. F2 and R2 confront each other at the 3' end with the base specific to the allele. The PCR-CTPP conditions were as follows: 5 min of initial denaturation at  $95^{\circ}\text{C}$ , followed by 30 cycles of 30 s denaturation at  $95^{\circ}\text{C}$ , 30 s annealing at  $65^{\circ}\text{C}$  and 1 min extension

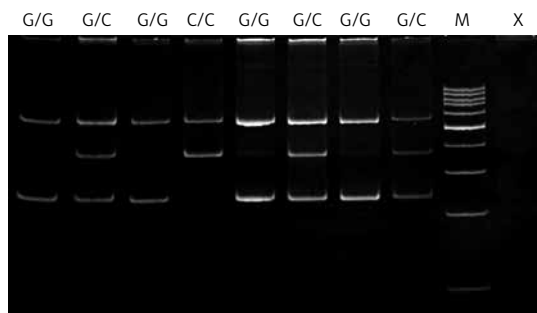
at  $72^{\circ}\text{C}$ . A final extension step at  $72^{\circ}\text{C}$  for 10 min was included. The PCR products were fractionated by electrophoresis on 3% agarose gel, stained with ethidium bromide and viewed under UV light. The samples were genotyped according to the size of the PCR products: products amplified with the G/G genotype were 562 and 241 bp in length, those with the C/C genotype were 562 and 362 bp, and those with the G/C genotype were 562, 362 and 241 bp. Figure 1 presents a representative gel for analysis of this polymorphism.

The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to determine the genotypes of the c.977C>G-*hOGG1* polymorphism. PCR assay was performed in a total reaction volume of 25  $\mu\text{l}$  containing the same reagents as in the previous analysis except for primers. 200-bp length fragments containing the polymorphic site were amplified using the following primers: sense 5'-ACTGTCACTAGTCTCACCAG-3' and antisense 5'-GGAAGGTGCTTGGGAAT-3'. The PCR profile contained an initial denaturation step for 5 min at  $95^{\circ}\text{C}$ , 30 cycles at  $95^{\circ}\text{C}$  for 30 s, 30 s at  $58^{\circ}\text{C}$  annealing temperature and 60 s at  $72^{\circ}\text{C}$  and the final extension step for 5 min at  $72^{\circ}\text{C}$ . After amplification, 200-bp PCR products were analyzed on 3% agarose gel and digested with 2U of *SatI* (*Fnu4HI*) restriction endonuclease (Fermentas, Hanover, MD, USA) in a final volume of 15  $\mu\text{l}$  for 16 h at  $37^{\circ}\text{C}$ . PCR products with a G at the polymorphic site were digested into two 100-bp fragments, while those with a C were not, because of the lack of a *SatI* restriction site. The G/G genotype produced one fragment (100 bp), whereas the G/C genotype yielded two fragments (200, 100 bp) and the homozygote C/C resulted in one 200 bp fragment. Digested PCR products were separated by electrophoresis on 8% polyacrylamide gel and visualized by ethidium bromide staining using a GeneRuler 100 bp ladder (Fermentas, Hanover, MD, USA) as a length marker. A representative gel for this polymorphism is presented in Figure 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.

## Statistical analysis

The statistical analyses were performed using Statistica 9.0 software (StatSoft, Tulsa, OK, USA) and SigmaPlot v11.0 software (Systat Software, Inc., San Jose, CA, USA). To compare the distributions of demographic variables and potential risk factors between patients and controls the  $\chi^2$  test was used. Hardy-Weinberg equilibrium was



**Figure 1.** Detection of the *MUTYH* – c.972G>C (rs3219489) 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 comprising reaction mixture without target DNA

checked using the  $\chi^2$  test to compare the observed genotype frequencies with the expected frequencies among the case and control subjects. The  $\chi^2$  analysis was also used to test the significance of the differences between distributions of genotypes and alleles in KC 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 co-occurrence of visual impairment, allergies, sex and family status of KC.

## Results

The PCR-RFLP and PCR-CTPP analyses were successful for all 425 DNA samples (205 KC patients and 220 KC-free controls).

### Characteristics of study subjects

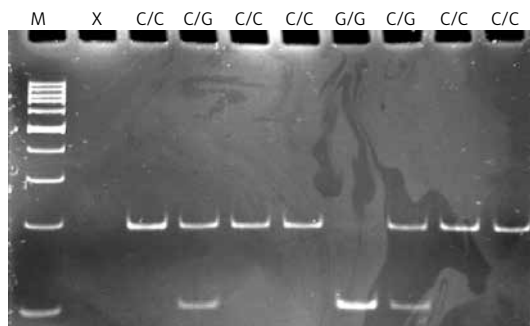
Table I presents demographic and risk factors for KC of the study patients and controls. The mean  $\pm$  SD age for KC patients were  $36.39 \pm 11.87$  (range: 14–63) and  $60.71 \pm 20.25$  (range: 19–91) for controls. Moreover, there were significantly more subjects with a positive family history for KC (1<sup>st</sup> degree relatives) among the patients in comparison to controls (11% vs. 4%,  $p = 0.0145$ ). We observed a significant difference between distribution of family history for KC (positive vs. negative family history), gender (females vs. males), allergies (yes vs. no) and co-occurrence of visual impairment (yes vs. no) among patients and controls. These parameters were further adjusted in the multivariate logistic regression model for possible confounding factors of the main effect of the SNPs.

### The c.972G>C polymorphism of the *MUTYH* gene and KC occurrence

The genotype and allele distributions of the c.972G>C polymorphism of the *MUTYH* gene in KC patients and controls are presented in Table II. The observed genotype frequencies were all in agreement with the Hardy-Weinberg equilibrium calculated for the controls and patients ( $p > 0.05$ , data not shown). The difference in the frequency distributions of genotypes of the polymorphism between the cases and controls was not statistically significant ( $p > 0.05$ ). We did not find any correlation between genotypes/alleles of this polymorphism and KC occurrence.

### The c.977C>G polymorphism of the *hOGG1* gene and KC occurrence

Details of genotype and allele frequencies of the c.977C>G polymorphisms of the *hOGG1* gene



**Figure 2.** Detection of the *hOGG1* – c.977C>G (rs1052133) 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 comprising reaction mixture without target DNA

and summary statistics are shown in Table III. There was no difference in the frequency distributions of genotypes of this SNP between patients and controls ( $p > 0.05$ ), and the observed genotype frequencies did not differ significantly from Hardy-Weinberg equilibrium ( $p > 0.05$ ; data not shown). We did not find any correlation between genotypes/alleles of this polymorphism and KC occurrence.

### Gene-gene interaction and KC occurrence

We also investigated the association between the occurrence of KC and combined genotypes of the c.977C>G-*hOGG1* and c.972G>C-*MUTYH* polymorphisms. The distribution of such genotypes is shown in Table IV. We did not find any correlation between combined genotypes of both polymorphisms and the occurrence of KC.

## Discussion

As mentioned in the Introduction, both environmental and genetic factors and the interaction between them are involved in the pathogenesis of KC. 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. Several studies have found a decreased antioxidant capacity in KC corneas [23, 29]. Mitochondrial DNA is particularly prone to oxidative damage compared with its nuclear counterpart because of its close proximity to oxidative phosphorylation (OXPHOS) [59]. It does not contain introns and has a high transcription rate, resulting in a high probability of oxidative modification of the coding region of genes [29]. When mitochondria are damaged, the level of OXPHOS is decreased, but ROS production is usually increased [60]. It has been shown that mitochondrial dysfunction led to altered gene

**Table II.** Distribution of genotypes and alleles of the c.972G>C polymorphism of the *MUTYH* gene and odds ratio (OR) with 95% confidence interval (95% CI) in patients with KC and controls

Genotype/allele	Controls (n = 220)		KC (n = 205)		Crude OR (95% CI)	Value of p	Adjusted OR <sup>a</sup> (95% CI)	Value of p
	Number	Frequency	Number	Frequency				
G/G	142	0.65	136	0.66	1.08 (0.73–1.62)	0.697	1.28 (0.82–2.01)	0.286
G/C	73	0.33	62	0.30	0.87 (0.58–1.32)	0.516	0.73 (0.46–1.16)	0.184
C/C	5	0.02	7	0.04	1.52 (0.48–4.87)	0.481	1.72 (0.41–7.26)	0.464
$\chi^2 = 0.83; p = 0.6601$								
G	357	0.81	334	0.81	1.02 (0.72–1.46)	0.901	1.17 (0.78–1.74)	0.456
C	83	0.19	76	0.19	0.98 (0.69–1.39)	0.901	0.86 (0.57–1.28)	0.456

<sup>a</sup>OR adjusted for co-occurrence of visual impairment, allergies, sex and family history for KC.

**Table III.** Distribution of genotypes and alleles of the c.977C>G polymorphism of the *hOGG1* gene and odds ratio (OR) with 95% confidence interval (95% CI) in patients with KC and controls

Genotype/allele	Controls (n = 220)		KC (n = 205)		Crude OR (95% CI)	Value of p	Adjusted OR <sup>a</sup> (95% CI)	Value of p
	Number	Frequency	Number	Frequency				
C/C	144	0.65	125	0.61	0.83 (0.55–1.22)	0.415	0.83 (0.54–1.29)	0.415
C/G	69	0.31	70	0.34	1.14 (0.76–1.70)	0.541	1.18 (0.75–1.85)	0.483
G/G	7	0.03	10	0.05	1.56 (0.58–4.18)	0.376	1.96 (0.41–3.53)	0.746
$\chi^2 = 1.35; p = 0.5089$								
C	357	0.81	320	0.78	0.83 (0.59–1.16)	0.263	0.86 (0.59–1.25)	0.422
G	83	0.19	90	0.22	1.21 (0.87–1.70)	0.263	1.17 (0.80–1.70)	0.422

<sup>a</sup>OR adjusted for co-occurrence of visual impairment, allergies, sex and family history for KC.

**Table IV.** Distribution of combined genotypes of the c.977C>G polymorphism of *hOGG1* and c.972G>C polymorphism of *MUTYH* and odds ratio (OR) with 95% confidence interval (95% CI) in patients with KC and controls

Genotype/allele	Controls (n = 220)		KC (n = 205)		Crude OR (95% CI)	Value of p	Adjusted OR <sup>a</sup> (95% CI)	Value of p
	Number	Frequency	Number	Frequency				
G/G-C/C	91	0.41	80	0.39	0.91 (0.62–1.34)	0.623	0.99 (0.64–1.52)	0.952
G/G-C/G	46	0.21	51	0.25	1.25 (0.80–1.97)	0.330	1.45 (0.87–2.41)	0.156
G/G-G/G	5	0.02	5	0.02	1.08 (0.31–3.77)	0.910	0.75 (0.19–3.05)	0.688
G/C-C/C	48	0.22	43	0.21	0.95 (0.60–1.51)	0.832	0.81 (0.49–1.35)	0.423
G/C-C/G	23	0.10	14	0.07	0.63 (0.31–1.26)	0.188	0.55 (0.25–1.22)	0.141
G/C-G/G	2	0.01	5	0.02	2.73 (0.52–14.20)	0.234	2.27 (0.40–12.94)	0.355
C/C-C/C	5	0.02	2	0.01	0.42 (0.08–2.21)	0.308	0.76 (0.11–5.41)	0.789
C/C-C/G	0	–	5	0.02	–	–	–	–
C/C-G/G	0	–	0	–	–	–	–	–

<sup>a</sup>OR adjusted for co-occurrence of visual impairment, allergies, sex and family history for KC.

expression, apoptosis and loss of cell viability [61–63]. It was shown that human KC corneas displayed increased levels of mtDNA deletions and point mutations compared with age-matched normal corneas [29]. Furthermore, it was found that sequence variations in the mitochondrial complex I were the main cause of elevated ROS production [64]. Taking into account these facts, antioxidant-directed therapy may be considered in KC patients to minimize ROS-related damage.

Although KC is recognized as a multifactorial disease, genetic factors may play an essential role in its development [40, 65–68], which was confirmed by segregation analyses, genetic epidemiological data and gene mapping studies. Several gene loci/chromosomal regions for familial KC have been mapped by genome-wide linkage analysis, including 16q22.3-q23.1 [69], 3p14-q13 [17], 2p24 [70], 1p36.23-36.21 [71], 5q14.3-q21.1 [19], 5q21.2, 5q32-q33 [72], 8q13.1-q21.11 [71], 9q34 [18], 14q11.2 [72], 14q24.3 [73], 15q2.32 [72], 15q22.33-q24.2 [16], 17p13 [74] and 20q12 [75]. The first gene to be causally linked with KC was the visual system homeobox 1 (*VSX1*) gene [41]. It is located on 20p11-q11 [76, 77] and plays a role in craniofacial and ocular development. *VSX1* is a member of the paired-like homeodomain transcription factors (TFs), which regulate expression of the cone opsin genes during embryonic development [78, 79]. Although *VSX1* plays a role in the development of retinal bipolar interneurons, several studies did not confirm its expression in the mouse and human corneas [41, 80]. In addition, a mouse model with the loss of *VSX1* function did not support its role in the cornea [79, 81]. It was shown that two missense mutations in *VSX1*, R166W and L159M, were associated with KC [41]. However, a similar number of studies did not confirm the presence of these mutations in KC patients [82–85], suggesting that *VSX1* might not play a major role and other genetic factors exert a more powerful effect on the development of KC. These conflicting results may be partly explained by low frequency of changes, ethnic variation, and increasing evidence that KC is likely a multifactorial and polygenic disease [86, 87].

Besides *VSX1*, several other candidate genes for KC have been suggested, including superoxide dismutase 1 (*SOD1*) [42], lipoxygenase (*LOX*) [88], transforming growth factor  $\beta$ 1 (*TGFB1*) [43], human leukocyte antigen (*HLA*) [89], mitochondrial complex 1 genes [64], interleukin 1 $\beta$  (*IL1B*) [90], collagen type IV,  $\alpha$ 3 and collagen type IV (*COL4A3*),  $\alpha$ 4 (*COL4A4*) [46], and calpastatin (*CAST*) [91]. Gajecka *et al.* [65] identified a novel locus for familial KC at 13q32, by using GeneChip Mapping to genotype 10 affected and 11 unaffected individuals from a large Ecuadorian KC (KTCN-014) family. In a subsequent study [44], they revealed that the c.2262A>C

(p.Gln754His) mutation in the *DOCK9* gene, located at 13q32, may contribute to the KC phenotype in that family. In a recent study, an association of KC with the hepatocyte growth factor (*HGF*) gene [92] and microRNA (*MIR184*) [93] gene was recognized. Other studies have indicated that the rs4954218 SNP near the *RAB3GAP1* gene (located at 2q21.3), coding for Rab3 GTPase-activating protein subunit 1, suggests a new susceptibility locus for KC [94].

In the present study, we investigated whether polymorphisms of the *MUTYH* gene (c.972G>C) and the *hOGG1* gene (c.977C>G) are associated with KC. Both SNPs are located in the coding regions of these genes and change amino acids in their products. These changes can directly affect protein function, and therefore they can have serious phenotypic consequences [95, 96]. The c.977G>C polymorphism causes change of serine to cysteine at the 326 codon, whereas c.972C>G polymorphism is associated with substitution of glutamine instead histidine at the 324 codon (Gln324His) of the *MUTYH* protein. Genetic polymorphisms in DNA repair genes can cause inter-individual difference in preventing mutagenesis induced by DNA damage [47]. The polymorphisms under study may result in changes in the levels and the activities of the proteins they affect, which can lead to reduced protection against oxidative stress [50, 97, 98].

The c.972G>C polymorphism of the *MUTYH* gene has been implicated in functional changes in the DNA damage response pathway – it was found that the activity of the SNP c.972G>C *MUTYH* enzyme was decreased by 36% compared with the wild type protein [50]. Recent studies have demonstrated that the G/G genotype increased the risk of end-stage renal disease [99], while the C/C genotype increased the risk of rectal cancer [100]. In this study, we did not find any correlation between genotypes/alleles of this polymorphism and the occurrence of KC.

The c.977C>G (p.Ser326Cys) polymorphism is the most commonly studied *hOGG1* gene variation. The 326Cys allele was less effective in preventing G:C to T:A transversion in *E. coli* mutM-mutY mutants than the 326Ser allele, indicating that 326Cys had a lower ability to remove 8-OH-G in DNA [97, 98]. In addition, the allele frequency of 326Cys was markedly different among different ethnic groups [97, 101, 102]. In the Japanese population, about 25% of individuals are homozygous at codon 326 (Cys/Cys), but the frequency of this allele seems to be lower in the Caucasian population [48]. It was demonstrated that the 326Cys allele was positively correlated with an increased risk of lung, oesophagus, prostate and a subset of stomach cancers [47, 52, 103, 104]. In our previous study we found that the C/G genotype and the G allele significantly increased the

risk of another eye disease – age-related macular degeneration (AMD) [105]. On the other hand, the C/C genotype and the C allele were positively correlated with a decreased risk of this disease. In the present study, we found that this polymorphism was not associated with occurrence of KC, but it should be stressed that our results are limited to a small Polish subpopulation and can be extrapolated at most to the European populace.

In conclusion, the c.972G>C polymorphism of the *MUTYH* gene and the c.977C>G polymorphism of the *hOGG1* gene may not be associated with keratoconus in the Polish population. Further replication studies performed on other ethnic populations are needed to verify this conclusion.

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### Conflict of interest

The authors declare no conflict of interest.

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