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Research Article

DNA Damage/Repair and Polymorphism of the *hOGG1* Gene in Lymphocytes of AMD Patients

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Oxidative stress is thought to play a role in the pathogenesis of age-related macular degeneration (AMD). We determined the extent of oxidative DNA damage and the kinetics of its removal as well as the genotypes of the Ser326Cys polymorphism of the *hOGG1* gene in lymphocytes of 30 wet AMD patients and 30 controls. Oxidative DNA damage induced by hydrogen peroxide and its repair were evaluated by the comet assay and DNA repair enzymes. We observed a higher extent of endogenous oxidative DNA damage and a lower efficacy of its repair in AMD patients as compared with the controls. We did not find any correlation between the extent of DNA damage and efficacy of DNA repair with genotypes of the Ser326Cys polymorphism. The results obtained suggest that oxidative DNA damage and inefficient DNA repair can be associated with AMD and the variability of the *hOOG1* gene may not contribute to this association.

1. Introduction

Age-related macular degeneration (AMD) is a leading cause of visual impairment and blindness in Western countries among people aged 50 years and older [1]. The prevalence of AMD in various European countries (Norway, Estonia, United Kingdom, France, Italy, Greece, and Spain) was 3.32% with the of geographic atrophic AMD of 1.2%, neovascular AMD 2.3% and bilateral AMD 1.4% [2]. AMD is also a significant health problem in the United States, with a current estimate of about 1.75 million persons with advanced AMD in the general population and about 7.3 million people with early stages of AMD defined by large retinal drusen. It has been projected that by the year 2020, approximately about 2.95 million people will have advanced AMD and an additional 6.4 million white individuals will have the early stages of AMD in at least one eye [3]. AMD prevalence has been rising across the globe. As the average life span of humans continues to increase, especially in the developed countries, the incidence of AMD is expected to nearly double within the next 25 years.

The aetiology of AMD remains elusive because it is a multifactorial disease in which both genetic and environmental factors have been implicated. To date, age, smoking, exposure to light, and diet have been successfully identified [4, 5]. Various studies have indicated a significant genetic contribution to AMD, including those reported a higher occurrence of AMD among monozygotic twins and first-degree relatives than spouses and unrelated individuals [6]. AMD was also associated with several DNA single nucleotide polymorphisms (SNPs) [7–9].

Oxidative stress has been implicated in the pathogenesis of AMD. The retinal pigment epithelium (RPE) cells function in an environment that is rich in endogenous reactive oxygen species (ROS). The activity of RPE cells, the high local oxygen concentration, and the chronic exposure to light contribute to the production of ROS [10–13]. Although multiple physiologic mechanisms protect the RPE from the toxic effects of light and oxidative damage, mounting evidence suggests that chronic exposure to oxidative stress over the long term may damage the RPE and predispose it to the development of AMD. Supporting this theory is the

observation that large drusen, which are deposited under the RPE in patients with macular degeneration, consist of insoluble aggregates of oxidized lipids and proteins derived from the photochemical reactions of visual transduction [14, 15].

In the present paper we checked the correlation between the level of DNA damage measured with the alkaline comet assay and the kinetics of removal of DNA damage induced by hydrogen peroxide in peripheral blood lymphocytes of patients with wet form of AMD and individuals without visual disturbances. Most oxidative damage associated with AMD will occur within postmitotic cells of the retina and will be environmental in origin. We chose peripheral blood lymphocytes as they would be affected by the environmental condition causing oxidative DNA damage in the retina. Moreover, they could provide evidence on inherited defect in DNA damage/repair, which would be enhanced by oxidative stress. Furthermore, metabolic disturbances associated with AMD may affect whole organism [1]. We also correlated the metrics of DNA damage and repair with the genotype of the *hOGG1* gene polymorphism: a C → G transversion at 1245 position producing a Ser → Cys substitution at the codon 326 (the Ser326Cys polymorphism). We chose the *hOGG1* gene due to its central role in the repair of oxidatively damaged DNA. To evaluate the extent of DNA damage, the efficacy of DNA repair and the sensitivity to exogenous mutagens in AMD patients we determined (1) the level of DNA damage measured by alkaline comet assay and oxidative DNA damage and (2) the capacity to remove DNA damage induced by hydrogen peroxide in the peripheral blood lymphocytes of AMD patients and healthy individuals. DNA damage and repair were evaluated by alkaline single cell gel electrophoresis (comet assay). Hydrogen peroxide is a standard agent to induce oxidative DNA damage. In order to assess the role of oxidative DNA damage in AMD patients, we employed two DNA repair enzymes: endonuclease III (Nth) and formamidopyrimidine-DNA glycosylase (Fpg), preferentially recognizing oxidized DNA bases. Nth converts oxidized pyrimidines into strand breaks, which can be detected by the comet assay [16]. Fpg is a glycosylase initiating base excision repair in *E. coli*. It recognizes and removes 7,8-dihydro-8-oxoguanine (8-oxoguanine), the imidazole ring-opened purines, 2,6-diamino-4-hydroxy-5-formamidopyrimidine (Fapy-Gua), and 4,6-diamino-5-formamido-pyrimidine (Fapy-Ade) as well as small amounts of 7,8-dihydro-8-oxoadenine (8-oxoadenine) [17]. The removing of specific modified bases from DNA by this enzyme leads to apurinic or apirymidinic sites, which are subsequently cleaved by its AP-lyase activity, giving a gap in the DNA strand, which can be detected by the comet assay [18].

2. Materials and Methods

2.1. Patients. Blood samples were obtained from patients with wet form of AMD ($n = 30$) and healthy sex- and age-matched individuals ($n = 30$). Medical history was obtained from all subjects. The patients underwent ophthalmic examination including best-corrected visual acuity, intraocular pressure, slit-lamp examination, and fundus examination using noncontact and contact fundus lenses with a slit lamp. Diagnosis of wet form AMD was confirmed by optical coherence tomography (OCT), fluorescein angiography (FA), and in some cases indocyanin green angiography (ICG). OCT evaluated retinal thickness, the presence of subretinal fluid and intraretinal oedema; angiography assessed the anatomical status of the retinal vessels, the presence of choroidal neovascularisaton (CNV) and leakage.

The OCT examinations were performed with Stratus OCT model 3000, software version 4.0. The FA and ICG examinations were completed with a Topcon TRC-50I IX fundus camera with the digital Image Net image system (ver. 2.14; Topcon Co., Tokyo, Japan).

The Local Ethic Committee approved the study and each patient gave a written consent. Neither patients nor controls reported cancer, diabetes, or other disease known or suspected to affect DNA repair.

2.2. Cell Preparation. Blood samples were immediately transported to the laboratory on ice. Peripheral blood lymphocytes (PBL) were isolated by centrifugation in a density gradient of histopaque-1077 (15 minutes, 280 g, 4°C) and suspended in RPMI 1640 medium at $1–3 \times 10^5$ cells per mL.

2.3. Comet Assay. The comet assay was performed at pH >13 essentially according to the procedure of Singh et al. [19] with modifications [16, 18] as described previously [20]. A freshly prepared suspension of the cells in 0.75% LMP agarose dissolved in PBS was spread onto microscope slides precoated with 0.5% NMP agarose. The cells were then lysed for 1 hour at 4°C in a buffer consisting of 2.5 M NaCl, 100 mM EDTA, 1% Triton X-100, 10 mM Tris, pH 10. After the lysis, the slides were placed in an electrophoresis unit, DNA was allowed to unwind for 20 minutes in the electrophoresis solution consisting of 300 mM NaOH and 1 mM EDTA, pH >13. Electrophoresis was conducted at ambient temperature of 4°C (the temperature of the running buffer did not exceed 12°C) for 20 minutes at an electric field strength of 0.73 V/cm (28 mA). The slides were then washed in water, drained and stained with 2 μg/mL DAPI, and covered with cover slips. To prevent additional DNA damage, all the steps described above were conducted under dimmed light or in the dark.

The comets were observed at 200 × magnification in an Eclipse fluorescence microscope (Nikon, Tokyo, Japan) attached to COHU 4910 video camera (Cohu, San Diego, CA, USA) equipped with a UV-1 filter block (an excitation filter of 359 nm and a barrier filter of 461 nm) and connected to a personal computer-based image analysis system Lucia-Comet v. 4.51 (Laboratory Imaging, Praha, Czech Republic). One hundred images were randomly selected from each sample and the percentage of DNA in the tail of comets was measured. All the values in this study were expressed as mean ± S.E.M.

2.4. DNA Damage and Repair. Hydrogen peroxide was added to the suspension of the cells to give a final concentration of 10 μM. Lymphocytes were incubated with hydrogen peroxide for 10 minutes at 4°C. The cells after treatment were washed and resuspended in RPMI 1640 medium. A freshly prepared suspension of the cells in LMP agarose dissolved in PBS was spread onto microscope slides. The slides were processed as described in the Section 2.3.

To examine DNA repair, the cells, after treatment with hydrogen peroxide, were washed and resuspended in a fresh, RPMI 1640 medium preheated to 37°C. Aliquots of the cell suspension were taken immediately "time zero" and at 120 minutes later. Placing the samples in an ice bath stopped DNA repair. The slides were processed as described in the Section 2.3.

We considered a relative difference between the extents of DNA damage at "time zero" and 120 minutes as a measure of the efficacy of DNA repair.

2.5. DNA Repair Enzymes Treatment. A freshly prepared suspension of the cells in LMP agarose dissolved in PBS was spread onto microscope slides. The slides after cell lysis were washed three times (5 minutes, 4°C) in an enzyme buffer containing 40 mM HEPES-KOH, 0.1 M KCl, 0.5 mM EDTA, 0.2 mg/mL bovine serum albumin, pH 8.0. The slides were then drained and incubated for 30 minutes at 37°C with 0.03 μg of Nth or Fpg in this buffer [16, 18]. The control received only the buffer. The slides were processed as described in the Section 2.3. Each value of oxidative DNA modification recognized by these enzymes derives a mean difference between Fpg/Nth-treated and nontreated control from 30 patients with AMD and 30 healthy controls. To check the ability of the enzymes to recognize oxidized DNA bases in our experimental conditions, the cells were incubated with hydrogen peroxide, lysed, and posttreated with Fpg or Nth.

2.6. Genotype Determination. Genomic DNA was prepared from peripheral blood of AMD patients and healthy individuals by using of commercial Blood Mini Kit (AKOR Laboratories, Gdansk, Poland). The genotypes of the Ser326Cys polymorphism of the *hOOG1* gene were determined with the following primers: sense 5′-GTTTTCACTAATGAGCTTGC-3′, antisense 5′-AGTGGTATAATCATGTGGGT-3′. The 200 bp PCR product was digested overnight with 5 U of the restriction enzyme *Sat*I (Fermentas, Vilnius, Lithuania). The Cys allele was digested into 100 bp fragments, whereas the Ser variant remained intact. The PCR products were run on 2.5% agarose gel.

2.7. Data Analysis. The values of the comet assay in this study were expressed as mean ± S.E.M. from two experiments, that is, data from two experiments, 100 measurements each, were pooled and the statistical parameters were calculated. If no significant differences between variations were found, as assessed by Snedecor-Fisher test, the differences between means were evaluated by applying the Student's t test. Otherwise, the Cochran-Cox test was used. Relative difference between extents of DNA damage in time zero and 120 minutes expressed in percentage was used as a measure of the efficacy of DNA repair after treatment with hydrogen peroxide. Distribution of genotypes and alleles between groups was analysed using the χ^2-test. Relationship between genotype and cancer was assessed by the logistic regression. The data were analysed using Statistica package (StatSoft, Tulsa, OK, USA).

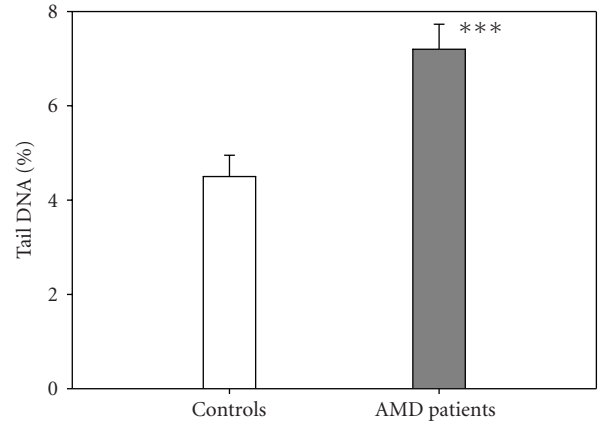

Figure 1: Endogenous DNA damage (DNA strand breaks and alkali-labile sites), measured as the mean comet percentage tail DNA in the alkaline comet assay in human peripheral blood lymphocytes of healthy controls (white bar) and AMD patients (grey bar). Thirty individuals were analysed in either group. The number of cells scored for each individual was 100. The results displayed are the mean of three independent experiments ± S.E.M. $^{***}P < .001$.

3. Results

3.1. DNA Damage. The mean extent of endogenous DNA damage measured as the percentage of DNA in comet tail of lymphocytes of AMD patients and controls is displayed in Figure 1. We observed a higher level of endogenous DNA damage in AMD patients than in the controls ($P < .001$).

3.2. Endogenous Oxidative DNA Damage. Figure 2 presents the mean DNA damage measured as percentage tail DNA of lymphocytes from AMD patients and healthy individuals, lysed and posttreated with Nth (Figure 2(a)) or Fpg (Figure 2(b)). Subtracting the values for an enzyme-specific buffer only treatment normalized these results. Therefore, the results indicate solely the DNA base-modification, which are not alkali-labile. There were significant differences ($P < .01$) between the mean extent of oxidative DNA damage recognized by Nth between AMD patients and controls (Figure 2(a)). There were no significant differences ($P > .05$) between the mean extent of oxidative DNA damage recognized by Fpg between AMD patients and controls (Figure 2(b)).

Because high level of oxidative DNA damage may be associated with an impaired DNA repair, individuals with the extent of oxidative DNA damage higher than the mean for respective group were selected for further genotype analysis.

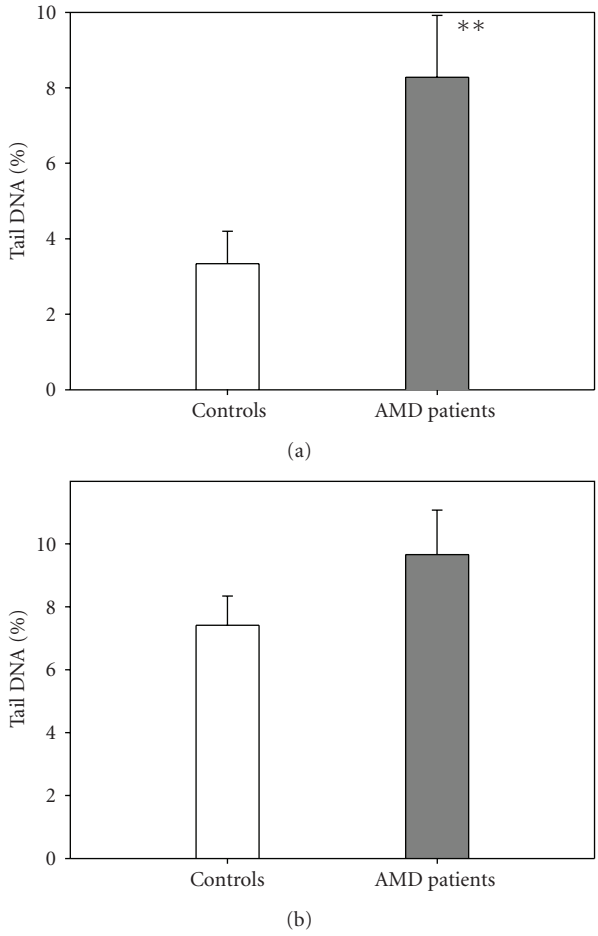

Figure 2: Oxidative DNA damage in peripheral blood lymphocytes of 30 AMD patients (grey bars) and 30 healthy controls (white bars) recognized by Nth (a) or Fpg (b) at 1 μg/mL in the alkaline comet assay. Each bar is a mean difference between Fpg or Nth treated cells and enzyme-nontreated controls. DNA damage was measured as the percentage of DNA in the tail in the alkaline comet assay. The number of cells scored for each individual was 100. The results displayed are the mean of three independent experiments ± S.E.M. $^{**}P < .01$.

3.3. DNA Repair. The mean percentage tail DNA of lymphocytes from AMD patients and controls exposed for 10 minutes to 10 μM hydrogen peroxide immediately after the exposure as well as 120 minutes thereafter is presented in Table 1 along with the efficacy of DNA repair. The "minus" sign indicates that the extent of DNA damage at 120 minutes was greater than at the zero time. The efficacy of DNA repair in AMD patients was significantly ($P < .001$) lower than that observed in the controls. Moreover, the extent of initial DNA damage after hydrogen peroxide treatment was significantly higher in AMD group than in the control ($P < .01$). We assumed that inefficient DNA repair occurred when we did not observe significant decrease in DNA damage after 120 minutes of repair incubation. We did not observe any difference in the mean efficacy of DNA repair between AMD patients (21 subjects with inefficient DNA repair) and control group (17 subjects with inefficient DNA repair). However, the data show that all control samples showed decrease in DNA damage after 120 minutes. 43% of the AMD patients (13 persons) show a further increase in DNA damage at 120 minutes. These results suggest that the efficacy of the repair of oxidative DNA damage in AMD patients was lower than in the controls. We observed considerable differences in the efficacy of DNA repair between individuals enrolled in the study, especially in AMD patients. We speculate that it could be linked with different activity of allelic variants of genes involved in the repair of oxidative DNA lesions. To verify this hypothesis, we selected only individuals with inefficient DNA repair (Table 1) for further genotype analysis.

3.4. Genotype Analysis. There were no significant differences between the distributions of genotypes of the Ser326Cys polymorphism of the *hOGG1* gene and the frequency of the Ser and Cys alleles for AMD patients and controls with high level of endogenous oxidative DNA damage (Table 2). There also were no significant differences between the distributions of the genotypes and frequencies of the alleles of the Ser326Cys polymorphism for AMD patients and controls with impaired DNA repair after hydrogen peroxide treatment (Table 3).

4. Discussion

Oxidative damage to RPE cells and photoreceptors has been implicated in the pathogenesis of AMD. This kind of cellular stress induces various types of DNA damage, like base modifications, DNA breaks, and alkali-labile sites. They are mainly removed by base excision repair (BER) pathway. BER removes modified base by specific glycosylases, which can be assisted by endonucleases. The next steps are abasic site priming, gap filling, and ligation. 8-Hydroxydeoxyguanosine (8-OH-Gua) glycosylase 1 (*hOGG1*) is the primary enzyme for the repair of 8-OH-Gua in human cells [21]. The presence of 8-OH-Gua residues in DNA leads to a GC → TA transversion, unless it is repaired before DNA replication. For this reason, the presence of 8-OH-Gua in DNA may lead to mutagenesis and the level of 8-OH-Gua is commonly used as a biomarker of oxidative DNA damage [22].

A growing body of evidence suggests that mitochondrial dysfunction may be associated with AMD and proposes a specific pathophysiological mechanisms involving mtDNA oxidative damage, altered mitochondrial translation, import of nuclear-encoded proteins and ATP synthase activity as an explanation of this association [23–25]. Unfortunately, data on the involvement of DNA repair in AMD pathogenesis are scare. There are a few reports focusing on the mitochondrial DNA. These reports suggest increased mitochondrial DNA damage and downregulation of mitochondrial DNA repair in RPE cells and choroid. An increased level of 8-OHdG and downregulation of base excision glycosylases in aged rodent RPE and choroid as compared with young controls was also reported [23]. The recent data indicate also that the altered function of the putative mitochondrial protein LOC387715/ARMS2 by a 69A>S substitution strongly

Table 1: Repair of hydrogen peroxide-induced DNA damage in 30 AMD patients and 30 healthy controls. DNA damage for each treatment was analyzed as the percentage of DNA in the tail of 100 comets; mean ± S.E.M. The number of cells scored in each treatment (patient) was 100. The efficacy of DNA repair was calculated by comparing the extent of DNA damage immediately and 120 minutes after hydrogen peroxide treatment. The subjects with no significant repair are presented bold.

No	Age (yrs)/Sex	Control DNA damage ± S.E.M. 0′	120′	Efficacy of DNA repair (%)	P	No	Age (yrs)/Sex	AMD DNA damage ± S.E.M. 0′	120′	Efficacy of DNA repair (%)	P
1	**72/W**	**12.19 ± 4.13**	**5.62 ± 2.34**	**53.90**	**>.05**	1	72/M	7.30 ± 0.88	1.29 ± 0.27	82.33	< .001
2	60/M	15.73 ± 2.12	5.84 ± 1.23	62.87	< .001	2	60/M	6.43 ± 0.62	1.44 ± 0.19	77.61	< .001
3	81/W	15.89 ± 2.09	9.02 ± 1.42	43.24	< .01	**3**	**61/M**	**4.38 ± 0.50**	**6.66 ± 0.84**	**−52.05**	**< .05**
4	**77/W**	**15.99 ± 2.87**	**8.95 ± 2.15**	**44.03**	**> .05**	4	64/W	4.85 ± 0.50	2.00 ± 0.35	58.76	< .001
5	**59/W**	**12.49 ± 1.90**	**5.60 ± 2.99**	**55.17**	**> .05**	**5**	**65/M**	**3.73 ± 0.41**	**6.16 ± 1.07**	**−65.15**	**< .05**
6	64/W	9.78 ± 1.24	4.48 ± 2.12	54.19	< .05	6	82/W	3.66 ± 0.38	0.83 ± 0.17	77.32	< .001
7	80/W	20.01 ± 2.30	9.34 ± 1.57	53.32	< .001	**7**	**59/M**	**7.59 ± 1.37**	**6.67 ± 1.37**	**12.13**	**> .05**
8	60/M	12.51 ± 1.38	4.17 ± 0.49	66.67	< .001	8	70/M	17.57 ± 1.41	6.66 ± 2.20	62.10	< .001
9	**73/M**	**6.89 ± 1.73**	**3.26 ± 0.74**	**52.69**	**> .05**	**9**	**78/W**	**12.37 ± 1.92**	**10.48 ± 1.97**	**15.28**	**> .05**
10	**72/W**	**6.73 ± 0.88**	**4.26 ± 0.98**	**36.70**	**> .05**	**10**	**79/W**	**2.51 ± 0.40**	**4.04 ± 0.38**	**−60.96**	**< .05**
11	**66/M**	**11.18 ± 2.17**	**6.21 ± 1.53**	**44.46**	**> .05**	**11**	**73/W**	**6.26 ± 0.99**	**8.33 ± 1.54**	**−33.07**	**> .05**
12	**80/W**	**13.92 ± 4.11**	**6.47 ± 2.21**	**53.52**	**> .05**	**12**	**60/W**	**3.19 ± 0.45**	**4.46 ± 0.87**	**−39.81**	**> .05**
13	60/M	8.95 ± 1.46	2.04 ± 0.40	77.21	< .001	**13**	**63/W**	**6.81 ± 1.14**	**3.53 ± 1.93**	**48.17**	**> .05**
14	**79/W**	**10.11 ± 1.80**	**8.76 ± 2.04**	**13.35**	**> .05**	**14**	**73/W**	**3.81 ± 0.61**	**3.24 ± 0.39**	**14.96**	**> .05**
15	64/W	14.52 ± 1.36	6.13 ± 0.92	57.78	< .001	15	70/M	7.41 ± 0.64	5.14 ± 0.56	30.64	< .05
16	**75/M**	**4.12 ± 0.65**	**3.63 ± 0.58**	**11.89**	**> .05**	16	74/W	9.40 ± 1.56	3.15 ± 0.49	66.49	< .001
17	73/W	14.63 ± 1.38	5.19 ± 0.67	64.53	< .001	**17**	**75/W**	**9.59 ± 1.58**	**12.53 ± 1.81**	**−30.66**	**> .05**
18	**69/M**	**18.29 ± 1.28**	**6.60 ± 1.12**	**63.92**	**> .05**	**18**	**64/M**	**9.35 ± 1.36**	**7.56 ± 0.97**	**19.15**	**> .05**
19	**66/M**	**17.46 ± 4.25**	**8.80 ± 1.58**	**49.60**	**> .05**	**19**	**77/W**	**2.14 ± 0.23**	**2.48 ± 0.48**	**−15.89**	**> .05**
20	**77/W**	**18.19 ± 4.16**	**8.63 ± 3.20**	**52.56**	**> .05**	**20**	**62/W**	**12.00 ± 1.32**	**14.58 ± 0.85**	**−21.50**	**> .05**
21	**75/M**	**9.55 ± 1.07**	**7.05 ± 2.58**	**26.18**	**> .05**	**21**	**69/M**	**10.11 ± 0.80**	**17.34 ± 0.89**	**−71.51**	**< .001**
22	**64/M**	**11.31 ± 2.09**	**9.35 ± 2.50**	**17.33**	**> .05**	22	74/M	34.88 ± 2.65	26.54 ± 1.43	23.91	< .01
23	70/W	13.53 ± 2.18	7.91 ± 1.52	41.54	< .05	**23**	**72/M**	**8.63 ± 0.89**	**18.09 ± 1.23**	**−109.62**	**< .001**
24	**63/W**	**14.39 ± 3.60**	**7.41 ± 1.18**	**48.51**	**> .05**	**24**	**80/W**	**34.05 ± 4.39**	**25.75 ± 2.97**	**24.38**	**> .05**
25	71/M	12.32 ± 2.12	5.31 ± 1.72	56.90	< .05	25	80/W	52.14 ± 3.41	19.01 ± 1.71	63.54	< .001
26	72/W	8.37 ± 0.87	3.67 ± 0.33	56.16	< .001	**26**	**69/W**	**12.71 ± 1.20**	**21.93 ± 2.30**	**−72.54**	**< .001**
27	70/M	12.32 ± 2.12	5.31 ± 1.72	56.90	< .05	**27**	**72/M**	**5.14 ± 0.46**	**8.13 ± 0.77**	**−58.17**	**< .01**
28	**71/M**	**14.39 ± 2.60**	**7.41 ± 1.28**	**48.51**	**> .05**	**28**	**69/M**	**17.02 ± 1.59**	**23.14 ± 2.03**	**−35.96**	**< .05**
29	73/M	12.53 ± 2.17	7.91 ± 1.22	36.87	< .05	**29**	**73/W**	**18.22 ± 2.75**	**15.72 ± 1.13**	**13.72**	**> .05**
30	**70/W**	**11.31 ± 2.19**	**9.35 ± 2.25**	**17.33**	**> .05**	**30**	**66/M**	**13.48 ± 1.59**	**12.16 ± 1.82**	**9.79**	**>.05**
Mean	70 ± 6	12.65 ± 0.66	6.46 ± 0.37	47.26 ± 2.92		Mean	70 ± 7	11.56 ± 1.98	9.97 ± 1.39	1.11 ± 9. 56	

Table 2: The allele and genotype frequencies and odds ratio (OR) of the Ser326Cys polymorphism of the *hOGG1* gene in AMD patients and controls with the extent of endogenous oxidative DNA damage higher than the mean for respective group.

Genotype or allele	Controls ($n = 20$)		AMD patients ($n = 14$)		OR (95% PU)
	Number	Frequency	Number	Frequency	
Ser/Ser	11	0.55	10	0.71	2.05 (0.48–8.78)
Ser/Cys	9	0.45	4	0.29	0.49 (0.11–2.09)
Cys/Cys	0	—	0	—	—
Ser	31	0.77	24	0.86	1.74 (0.48–6.35)
Cys	9	0.23	4	0.14	0.57 (0.16–2.09)

Table 3: The allele and genotype frequencies and odds ratio (OR) of the Ser326Cys polymorphism of the *hOGG1* gene in AMD patients with inefficient repair of DNA lesions induced by hydrogen peroxide. Inefficient DNA repair occured when we did not observe a significant decrease in the extent of DNA damage after 120 minutes of repair incubation.

Genotype or allele	Controls ($n = 17$)		AMD patients ($n = 21$)		OR (95% PU)
	Number	Frequency	Number	Frequency	
Ser/Ser	9	0.53	11	0.52	0.98 (0.27–3.52)
Ser/Cys	8	0.47	10	0.48	1.02 (0.28–3.68)
Cys/Cys	0	—	0	—	—
Ser	26	0.76	32	0.76	0.98 (0.34–2.85)
Cys	8	0.24	10	0.24	1.02 (0.35–2.94)

enhances the susceptibility to aging-associated degeneration of macular photoreceptors [26].

The number of patients we analyzed may not seem to be impressive as compared with epidemiological studies assessing a risk linked with particular genotype/phenotype. The primary goal of our study was to search for a correlation between genotype and phenotype, although we calculated the odds ratio, because it is a standard procedure in polymorphism study. Such studies with comet assay are typically performed on a population of several dozen individuals and very exceptionally this number exceeds one hundred.

Although oxidative stress is usually linked with the dry AMD and so are the disturbances in the DNA repair machinery, our results suggest extending this point of view to wet AMD. They may also suggest a closer association between the two forms of the disease.

Our study was performed on peripheral blood lymphocytes. They should have been performed on the retina cells, but these cannot be obtained from live AMD patients as easily as the lymphocytes. Lymphocytes are easily accessible and their genetic constitution with the regards of DNA repair processes reflects that of the retina cells. There is no doubt that AMD is not only an ocular disease. However, we found a decreased efficacy of DNA repair in peripheral blood lymphocytes of AMD patients, but we do not have any solid evidence that this effect was a consequence of AMD. This is rather an open question. We hypothesize that AMD may be a result of a decreased capability of every cell of an organism to repair DNA damage and increased exposure of ocular cells to etiological agents, like UV radiation, inducing DNA damage in these cells. On the other hand, we cannot exclude the possibility that the observed decrease in the efficacy of DNA repair may be a consequence of general metabolic disturbance associated with AMD. We think that the results we obtained suggest that there may be an interplay between disturbances in the general state of an organism, manifested by decreased efficacy of DNA repair and local (ocular) changes evoked by genetic and environmental factors.

We used the comet assay to study DNA damage and repair. This technique is a versatile and sensitive method for measuring DNA damage such as single- and double-strand breaks as well as alkali-labile sites in DNA. It is a valuable tool in population monitoring, for example, in assessing the role of oxidative stress in human disease and in monitoring the effects of dietary antioxidants. A simple modification allows the measurement of DNA repair. In combination with the analysis of polymorphisms in relevant genes, comet assay may provide important information on the interaction between genetic variation and environmental factors in the maintaining genome stability [27].

Although the increase in the tail DNA in AMD patients as compared with the controls was statistically significant it should not be a priori considered as biologically or medically relevant. However, because AMD may be considered as a multifactorial disease, this increase may significantly contribute to the disease.

The efficacy of DNA repair may be affected by variation in DNA repair genes. Several types of genetic polymorphisms can be found within the human genome, such as repeat polymorphisms, insertions, and deletions. However, most DNA sequences variation in human populations is in the form of SNPs [28]. SNPs can be defined as persistent substitutions of a single base with a frequency of more than 1% in at least one population. Recently, it has been demonstrated the potential role of SNPs in AMD and other age-related diseases [29]. Three SNPs in the *MnSOD, MEHE,* and *Paraoxonase* genes related to oxidative stress

have previously been reported in association with AMD in Japanese populations [30, 31].

It seems that the *hOGG1* gene is a good candidate to study DNA repair genes, which can be associated with AMD. This gene is expressed in multiple alternatively spliced isoforms and is highly polymorphic [32]. *OGG1-type 1a* of *hOGG1* gene is constitutively expressed in cancerous and noncancerous human cells as nuclear form of protein [33]. The protein level of *hOGG1* decreases in aged RPE and choroids cells [23]. A C → G transversion at 1245 position in the exon 7 of the *hOGG1* gene results in an amino acid substitution from serine to cysteine in the codon 326. There are contradictory results of studies on the role of this polymorphism in the catalytic activity of *hOGG1* protein, but it was shown that the Ser326 allele exhibited higher enzymatic activity than the Cys326 variant in an in vitro *E. coli* complementation assay [34]. Several studies have suggested that Cys326 type allele may be associated with the increased risk for esophageal [35], otolaryngeal [36], lung [37], stomach [38], and prostate cancers [39].

In our studies we did not find significant differences between the distributions of Ser326Cys polymorphism of *hOGG1* gene and the frequency of the Ser and Cys alleles for AMD patients and controls with high level of endogenous oxidative DNA damage and impaired DNA repair after hydrogen peroxide treatment. This is not surprising due to a limited number of patients enrolled in our study and a high variability in the DNA repair rate between them. A lack of association between the Ser326Cys polymorphism and AMD was also demonstrated in other research [40]. However, further studies are needed to confirm the lack of association between the Ser326Cys polymorphism and AMD.

Genetic variability of the genes involved in the expression of the *hOGG1* gene may also contribute to the efficacy of DNA repair. The Cockayne syndrome B (*CSB*) gene, also called *ERCC6*, collaborates with *hOGG1* to carry out preferential DNA repair in eukaryotes [41, 42]. This gene also plays a role in the maintenance of an efficient expression of the *hOGG1* gene. It was shown that the G allele of the C-6530C polymorphism of the *ERCC6* gene could be associated with a risk of AMD development and possibly interacted with an SNP in the *CFH* gene (complement factor H gene) to influence AMD susceptibility [7]. Recently, several studies have also shown a strong association of *CFH* SNPs with AMD [43–45]. It was revealed that *ERCC6* C-6530G, which is located in the regulatory region of the gene, upregulated the transcript and protein expression. These data support the hypothesis that DNA repair mechanisms may play a role in AMD pathogenesis.

5. Conclusions

Our results suggest that endogenous oxidative DNA damage and low efficacy of DNA repair can be associated with the occurrence of AMD in wet form. High level of oxidative DNA damage and impaired repair of such damage may not be associated with the variants of the Ser326Cys polymorphism of the *hOGG1* gene.

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