(19)

Association between the 25129A>C polymorphism of the nuclear respiratory factor 2 gene and age-related macular degeneration

Związek między polimorfizmem 25129A>C genu kodującego jądrowy czynnik oddechowy 2 a występowaniem zwyrodnienia plamki związanego z wiekiem

Tomasz Śliwiński[1], Urszula Kołodziejska[2], Jacek P. Szaflik[2], Janusz Błasiak[1], Jerzy Szaflik[2]

1 Department of Molecular Genetics, University of Lodz, Poland
Head: Associate Professor Katarzyna Woźniak, Ph.D.
2 Department of Ophthalmology, Medical University of Warsaw
and Samodzielny Publiczny Szpital Okulistyczny of Warsaw, Poland
Head: Professor Jerzy Szaflik, M.D., Ph.D.

Streszczenie:

Cel: stres oksydacyjny należy do głównych czynników wywołujących zwyrodnienie plamki żółtej związane z wiekiem, charakteryzujące się uszkodzeniem komórek nabłonka barwnikowego siatkówki oraz fotoreceptorów. Komórki nabłonka barwnikowego siatkówki bogate są w mitochondria wytwarzające duże ilości reaktywnych form tlenu, będących produktem aktywności łańcucha oddechowego. Rozkład w aktywności łańcucha może być wywołany przez uwalnianie cytochromu C z mitochondriów do cytoplazmy. Proces ten może być aktywowany przez jądrowy czynnik oddechowy 2 Nrf2, który jest kodowany przez wysoce polimorficzny gen. W badaniach tych przeanalizowano związek między ryzykiem wystąpienia zwyrodnienia plamki związanego z wiekiem a polimorfizmem 25129A>C w genie kodującym jądrowy czynnik oddechowy 2 (rs12594956).

Materiały i metody: genotypy określane były za pomocą metody PCR-RFLP w DNA wyizolowanym z limfocytów krwi obwodowej, pobranej od 281 pacjentów, u których zdiagnozowano zwyrodnienie plamki związane z wiekiem (od 181 chorych na wysiękową postać i od 101 chorych na suchą postać) oraz od 105 osób z grupy kontrolnej.

Wyniki: wykazano słaby związek między genotypem C/C polimorfizmu 25129A>C a występowaniem zwyrodnienia plamki związanego z wiekiem (OR 1.96; p = 0.023). Zaobserwowano silny związek między występowaniem suchej postaci tego schorzenia a genotypem C/C badanego polimorfizmu (OR 2.23; p = 0.018). Dodatkowo wykazano, że genotyp A/C zmniejsza ryzyko występowania zwyrodnienia plamki związanego z wiekiem oraz jego suchej postaci (odpowiednio OR 0.51; p = 0.023 i 0.44; p = 0.018). Potencjalne czynniki ryzyka takie jak wiek, płeć, palenie tytoniu, środowisko zamieszkania (wiejskie bądź miejskie) czy też rodzinny status zwyrodnienia plamki związanego z wiekiem wpływają na zwiększenie ryzyka występowania tego schorzenia, w korelacji z genotypem C/C badanego polimorfizmu (OR 2.52; p = 0.012).

Wnioski: polimorfizm 25129A>C w genie *NRF2* może mieć związek z występowaniem zwyrodnienia plamki związanego z wiekiem.

Słowa kluczowe: mitochondria, reaktywne formy tlenu, polimorfizm genu, gen *NRF2*, zwyrodnienie plamki związane z wiekiem – AMD.

Summary:

Purpose: Oxidative stress belongs to the main factors of pathogenesis of age-related macular degeneration, characterized by the damage to the retinal pigment epithelium cells and photoreceptors. Retinal pigment epithelium cells are rich in mitochondria, producing large amount of reactive oxygen species, which are by-products of the activity of the respiratory chain. The distribution in the activity of the chain may be evoked by the release of cytochrome C from the mitochondrion to the cytoplasm. This process may activated by nuclear respiratory factor 2, Nrf2, which is encoded by a highly polymorphic gene. In this study we examined the association between age-related macular degeneration risk and the 25129A>C polymorphism of the gene encoding nuclear respiratory factor 2 (rs12594956).

Material and methods: Genotypes were determined in DNA from peripheral blood lymphocytes of 281 patients with age-related macular degeneration (181 with wet form of the disease and 101 with its dry form), and 105 controls by PCR-restriction fragment length polymorphism.

Results: A weak association (OR 1.96; p = 0.023) between the C/C genotype of the 25129A>C polymorphism and the occurrence of age-related macular degeneration was found. A stronger association was observed between dry age-related macular degeneration occurrence and the C/C genotype of the polymorphism (OR 2.23; p = 0.018). The A/C genotype decreased the risk of age-related macular degeneration and its dry form (OR 0.51; p = 0.023 and 0.44; p = 0.018, respectively). Potential risk factors such as age, gender, smoking habit, living environment (rural or urban) and family status of age-related macular degeneration increased the risk of AMD associated with the C/C genotype (OR 2.52; p = 0.012).

Conclusions: The 25129A>C polymorphism of the *NRF2* gene may be associated with age-related macular degeneration.

Key words: mitochondria, reactive oxygen species, gene polymorphism, *NRF2* gene, age-related macular degeneration – AMD.

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1. Background

Age-related macular degeneration (AMD) is one of the most common causes of vision loss in the elderly in developed countries, but its pathogenesis still remains unclear. About 11 million people across the globe are affected by AMD, whose incidence increases with age (1, 2). According to data from Western countries, the disease develops among people 50 years old and older (3). Two manifestations of AMD can be distinguished, its dry and wet form. Genetic and environmental risk factors may predispose to the development of AMD (4, 5). The main biological factors associated with AMD pathogenesis are advanced age, female gender, Caucasian ethnicity, hypertension, obesity, cataract and cataract surgery, high-fat diet and chronic sunlight exposure (6–13). To date, tobacco smoking is considered as primary environmental risk factor for AMD (14, 15). Furthermore, family history studies showed, that relatives of AMD patients are at a higher risk of AMD than individuals without family history of this disease (16).

High oxygen concentrations, prolonged exposure to light, and the presence of photosensitizers are factors favoring the generation of reactive oxygen species (ROS) in the macular region. Oxidative stress may play a role for age-related accumulation of lipofuscin in retinal pigment epithelium (RPE), located near the macula (17).

Mitochondria are cytoplasmic organelles that play an import role in the energy production, apoptosis and free radical production. Each of these processes has been implicated in the pathogenesis of AMD (18). Under normal physiologic conditions the release of cytochrome C from mitochondria into the cytosol interrupt the electron flow along the respiratory chain and reduce oxygen to superoxide anion, initiating a cascade of free radicals, that indiscriminately damage biological macromolecules (19). Mitochondria are especially susceptible to damage by ROS because the mitochondrial genome has limited reparative capacity (20). Latest results suggest that the severity of mitochondrial and peroxisomal alterations are different between AMD and normal aging, and the timing of damage to RPE cells may be critical for the development of AMD (21). Moreover, mitochondrial biogenesis, complex assembly, mtDNA replication, transcription, and mitochondrial protein biosynthesis, all require nuclear DNA-encoded factors (22–24). One of these factors is a nuclear respiratory factor 2 (NRF2) protein. NRF2 was discovered as the human homolog of the mouse GA-binding protein (GABP) (25, 26). It is known that the product of the *NRF2* gene (*GABPB1*) regulates several genes encoding mitochondrial proteins, including cytochrome C and cytochrome C oxidase (27–30).

It was shown that the wild type genotype of the 25129A>C polymorphism of the *NRF2* gene (rs12594956), is associated with endurance performance in different population (31). These results support the regulatory role of this gene in the mitochondrial oxygen metabolism. The 25129A>C polymorphism is located on 15q21.2 in an intron region of the *NRF2* gene and it may affect the splicing of the NRF2 splicing. It has a relatively high hetrozygosity and has not been investigated in AMD.

In this work we searched for the association between the 25129A>C polymorphism of the *NRF2* gene and the risk of AMD in a Polish population. We also studied the influence of some risk factors: age, gender, smoking, environment of life and family status of AMD on this association.

2. Material and methods

2.1. Clinical subjects

Peripheral blood samples were obtained from 281 patients with AMD who were examined in the Department of Ophthalmology, Medical University of Warsaw, Poland, and from sex- and age matched individuals with exclusion of AMD (n = 105, controls). 101 patients had dry AMD and the remaining 180 had the wet form of the disease (Table I). Medical history was obtained from all subjects, and no one reported current or previous cancer or any genetic disease. The patients underwent ophthalmic examination, including best-corrected visual acuity, intraocular pressure, slit lamp examination, and fundus examination, performed with a slit lamp equipped with either non-contact or contact fundus lenses. Diagnosis of AMD was confirmed by optical coherence tomography (OCT) and, in some cases, by fluorescein angiography (FA) and indocyanin green angiography (ICG). OCT evaluated retinal thickness, the presence of RPE atrophy, drusen, or subretinal fluid and intraretinal edema; angiography assessed the anatomical status of the retinal vessels, the presence of choroidal neovascularization and leakage. The OCT examinations were performed with Stratus OCT model 3000, software version 4.0 (Oberkochen, Germany). The FA and ICG examinations were completed with a Topcon TRC-50I IX fundus camera equipped with the digital Image Net image system, version 2.14 (Topcon, Tokyo, Japan). Structured questionnaire was used to obtain information from study subjects about smoking habit, living environment and the history of AMD among first-degree relatives. The genetic analyses

Individuals/ Pacjenci	Number/ Liczba	Age (years)/ Wiek/ lata	Gender (females+males)/ Płeć (kobiety+mężczyźni)
All/ Wszyscy badani	386	72.8 ± 9.5	255 F + 131 M
AMD/ Chorzy na AMD	281	72.8 ± 8.4	185 F + 96 M
Dry AMD/ Chorzy na postać suchą AMD	101	74.3 ± 8.7	67 F + 34 M
Wet AMD/ Chorzy na postać wysiękową AMD	180	72.5 ± 8.0	118 F + 62 M
Controls/ Grupa kontrolna	105	71.7 ± 10.2	70 F + 35 M

Tab. I. Characteristics of patients with AMD and individuals with exclusion of AMD (controls; mean ± SD).
Tab. I. Charakterystyka chorych na AMD oraz osób, u których AMD wykluczono (średnia ± odchylenie standardowe).

did not interfere with diagnostic or therapeutic procedures for the subjects. The study was approved by the Bioethics Committee of the Medical University of Warsaw, Poland, and each patient gave a written informed consent.

2.2. DNA preparation

Peripheral blood lymphocytes (PBLs) were isolated by centrifugation in a density gradient of Histopaque-1077 (15 min, 280×g). The pellet containing the PBLs was resuspended in Tris–EDTA buffer, pH 8, to yield about 1–3×10^5 cells/ml. Genomic DNA was extracted from the PBLs by DNA Blood Mini Kit (A & A Biotechnology, Gdansk, Poland). The final samples were kept in Tris–EDTA buffer, pH 8, at -20°C until use.

2.3. Genotype determination

Restriction fragments length polymorphism PCR (RFLP-PCR) was employed to determine the genotypes of the 25129A>C polymorphism. Each 20 μl of the PCR reaction contained 10 ng genomic DNA, 1.25 U *Taq* polymerase (Epicentre, Madison, WI, USA) in 1×PCR buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 11 mM MgCl2, 0.1% gelatine), 1.5 mM $MgCl_2$, 50 mM dNTPs, and 250 nM each primer. Thermal cycling conditions for investigated polymorphism were as follows: initial denaturation step at 95°C for 5 min, 30 cycles at 95°C for 30 sec and 30 sec at the 55°C annealing temperature, and at 72°C for 1 min. The final extension was performed at 72°C for 10 min. The PCR was carried out in a MJ Research, INC thermal cycler; model PTC-100 (Waltham, MA, USA).

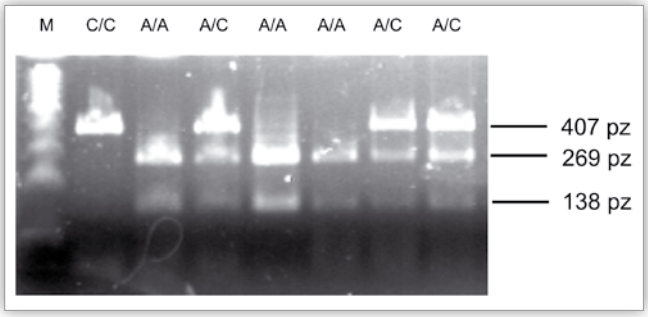

Fig. 1. Genotyping of the 25129A>C polymorphism of the *NRF2* gene by restriction length fragment polymorphism PCR in age-related macular degeneration. A representative analysis of 3% agarose gel electrophoresis with ethidium bromide. Band sizes are indicated on the right of the panel. Lane M, DNA marker 100 bp (Fermentas), lane 10, the C/C homozygote is not cleaved by the *Mfe*I enzyme and remains as a single 407 bp band, lanes 3 and 7 the A/A homozygote is cleaved by *Mfe*I producing 269 bp and 138 bp bands, lanes 1, 2 4–6, 8 and 9, the A/C heterozygote is represented by 3 bands (407, 269 and 138 bp).

Ryc. 1. Genotypowanie polimorfizmu 25129A>C genu *NRF2* w AMD za pomocą metody PCR-RFLP. Przykładowy wynik analizy przeprowadzonej w 3% żelu agarozowym zawierającym bromek etydyny. Wielkości produktów DNA (w parach zasad) zostały pokazane po prawej stronie ryciny. M oznacza marker DNA 100 bp (Fermentas), ścieżka 10. – genotyp C/C nie był rozpoznany przez enzym *Mfe*I, ścieżki 4. i 7. – genotyp A/A był rozpoznawany przez enzym *Mfe*I – w wyniku trawienia dawał dwa pasma o wielkości 269 i 138 pz; ścieżki 1., 2., 4.–6., 8. i 9. – heterozygota A/C była widoczna na żelu w postaci 3 pasm o długościach 407, 269 i 138 pz.

The 25129A>C polymorphism of the *NRF2* gene was determined using the following primers (Sigma-Aldrich, St. Louis, MO, USA):

sense, 5'- TAAAATGAATAAAGGTGGGGGT -3';

antisense, 5'- TAAGAGTGGAAGGGTGGAGAA -3'.

The 407 bp PCR product was digested 3 hours with 2 units of the restriction enzyme *Mfe*I (New England Biolabs, Ipswich, UK). The A allele was digested into 269 and 138 bp fragments whereas the C variant remained intact. The PCR products were separated onto a 3% agarose gel. Figure 1 presents a representative gel obtained after genotyping of this polymorphism. More than 10% of the samples were repeated, and the results were 100% concordant.

3. Statistical analysis

The allelic frequencies were estimated by gene counting and the genotypes were scored. The χ^2 analysis was used to compare the observed number of genotypes with that expected for a population in a Hardy–Weinberg equilibrium. The χ^2 analysis was also used to test the significance of the differences of observed alleles and genotypes between groups. A logistic regression model was used to calculate the odds ratios (ORs) and 95% confidence intervals (CIs). In all tests p values of less than 0.05 were considered statistically significant. The genotype-associated risk was given by the crude ORs and the *p* value. Additionally, unconditional logistic regression analyses with adjustment for possible confounders (age, gender, smoking habit, environment of life – rural or municipal – and familiar status of AMD) were performed to calculate adjusted ORs and *p* values. Smoking habit were categorized in terms of never smokers and smokers (including former and current). Statistical analysis was performed using STATISTICA 9.0 package (Statsoft, Tulusa, USA).

4. Results

The patients were divided into three groups according to genotypes of the 25129A>C polymorphism of the *NRF2* gene (A/A, A/C, and C/C). The observed genotype frequency of the polymorphism in the control subjects was in agreement with Hardy-Weinberg equilibrium (p 0.612; $\chi^2 = 0.98$). The distribution of the genotypes of the polymorphism differed significantly between the patients with dry AMD and controls ($p < 0.05$).

We observed an association of AMD and its dry form with the C/C genotype of the 25129A>C polymorphism of the *NRF2* gene (Table II and III). The A/C genotype of this polymorphism decreased the risk of AMD and its dry form (Table II and III).

We also investigated relationship between age, gender, smoking habit, environment of life (rural or municipal) and familiar status of AMD and risk of AMD independent of genotype. We compared controls and AMD patients according to these parameters. Male gender, age over 75 years and familiar history of AMD significantly increased the risk of AMD (Table IV).

Next, we examined gene–investigated parameters interactions. To estimate the interaction between these parameters and genotype relative to the risk of AMD we performed analysis with stratification of controls and AMD patients in separate groups dependent of age, gender, smoking habit, environment of life (rural or municipal) and familiar status of AMD. Only matching variables and factors that altered the ORs by ≥ 10% were

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Genotype or allele (polymorphism in bold)/ Genotypy albo allele (polimorfizm oznaczono grubszą czcionką)	Controls/ Grupa kontrolna (n=105)		AMD/ Chorzy na AMD (n=281)		Crude/ Analiza OR (95% CI)	Adjusted[a]/ OR skorygowany (95% CI)
	Number/ Liczba	Frequency/ Częstość	Number/ Liczba	Frequency/ Częstość		
25129A>C						
A/A	27	0.26	48	0.17	Ref.	Ref.
A/C	59	0.56	143	0.51	**0.51 (0.28–0.92) *p* 0.023**	**0.48 (0.26–0.90) *p* 0.021**
C/C	19	0.18	90	0.32	**1.96 (1.09–3.52) *p* 0.023**	**2.06 (1.11–3.83) *p* 0.021**
A	113	0.54	239	0.43	Ref.	Ref.
C	97	0.46	323	0.57	0.64 (0.37–1.13)	0.64 (0.34–1.08)

Data in boldface are statistically significant, Ref. denotes reference group ie. group relative which ORs were calculated.
[a]Adjusted for age, gender, smoking, environment of life and familiar status of AMD/ Dane istotne statystyczne zostały pogrubione. Ref. oznacza grupę odniesienia, względem której obliczano OR. aSkorygowany względem wieku, płci, palenia tytoniu, środowiska zamieszkania i rodzinnego statusu AMD.

Tab. II. Distribution of genotypes, frequency of alleles of the 25129A>C polymorphism of the *NRF2* gene and odds ratio (OR) with 95% confidence interval (95% CI) in patients with age-related macular degeneration (AMD) and individuals without visual disturbances (controls).

Tab. II. Rozkład genotypów, częstość alleli polimorfizmu 25129A>C genu *NRF2* oraz analiza OR z 95% przedziałem ufności u chorych na AMD oraz u osób z grupy kontrolnej.

Genotype or allele (polymorphism in bold)/ Genotypy albo allele (polimorfizm oznaczono grubszą czcionką)	Controls/ Grupa kontrolna (n=105)		AMD/ Chorzy na AMD (n=101)		Crude/ Analiza OR (95% CI)	Adjusted[a]/ OR skorygowany (95% CI)
	Number/ Liczba	Frequency/ Częstość	Number/ Numer	Frequency/ Częstość		
25129A>C						
A/A	27	0.26	18	0.18	Ref.	Ref.
A/C	59	0.56	57	0.56	**0.44 (0.22–0.88) *p* 0.018**	**0.39 (0.18–0.82) *p* 0.012**
C/C	19	0.18	26	0.26	**2.23 (1.13–4.39) *p* 0.018**	**2.52 (1.21–5.24) *p* 0.012**
A	113	0.54	93	0.46	Ref.	Ref.
C	97	0.46	109	0.54	0.50 (0.24–1.02)	0.51 (0.23–1.09)

Data in boldface are statistically significant, Ref. denotes reference group ie. group relative which ORs were calculated.
[a]Adjusted for age, gender, smoking, environment of life and family status of AMD/ Dane istotne statystyczne zostały pogrubione. Ref. oznacza grupę odniesienia, względem której obliczano OR. [a]Skorygowany względem wieku, płci, palenia tytoniu, środowiska zamieszkania i rodzinnego statusu AMD.

Tab. III. Distribution of genotypes, frequency of alleles of the 25129A>C polymorphism of the *NRF2* gene and odds ratio (OR) with 95% confidence interval (95% CI) in patients with dry age-related macular degeneration (AMD) and individuals without visual disturbances (controls).

Tab. III. Rozkład genotypów, częstość alleli polimorfizmu 25129A>C genu *NRF2* oraz analiza OR z 95% przedziałem ufności u chorych na AMD oraz u osób z grupy kontrolnej.

Characteristics/ Charakterystyki	Controls, number/ Liczba osób w grupie kontrolnej (%)	AMD patients, number/ Liczba chorych na AMD (%)	OR (95% CI)	p value/ Istotność statystyczna
Age/ Wiek				
Up to 75 years/do 75 lat	77 (73)	150 (53)	Ref.	
Over 75 years/ powyżej 75 lat	28 (27)	131 (47)	**2.40 (1.46–3.92)**	**< 0.001**
Gender/ Płeć				
Female/ Kobiety	82 (78)	185 (66)	Ref.	
Male/ Mężczyźni	23 (22)	96 (34)	**1.85 (1.09–3.12)**	**0.021**
Smoking/ Palenie tytoniu				
Never/ Nigdy	59 (66)	135 (59)	Ref.	
Ever/ Zawsze	30 (34)	94 (41)	1.36 (0.82–2.28)	0.229
Living Environment/ Miejsce zamieszkania				
Rural/ Wieś	33 (37)	50 (30)	Ref.	
Municipal/ Miasto	56 (63)	115 (70)	1.35 (0.78–2.33)	0.272
AMD in family/ Występowanie AMD w rodzinie				
No/ Nie	86 (97)	132 (80)	Ref.	
Yes/ Tak	3 (3)	33 (20)	**7.16 (2.13–24.09)**	**< 0.001**

Data in boldface are statistically significant. Ref. denotes reference group ie. group relative which ORs were calculated/ Dane istotne statystyczne zostały pogrubione. Ref. oznacza grupę odniesienia, względem której obliczano OR.

Tab. IV. AMD association with age, gender, smoking habit, environment of life and familiar status of AMD.

Tab. IV. Związek AMD z wiekiem, płcią, paleniem tytoniu, środowiskiem zamieszkania i rodzinnym statusem AMD.

Genotype or allele (polymorphism in bold)/ Genotypy albo allele (polimorfizm oznaczono grubszą czcionką)	Controls/ Grupa kontrolna (n=105)		AMD/ Chorzy na AMD (n=180)		Crude/ Analiza OR (95% CI)	Adjusted[a]/ OR skorygowany (95% CI)
	Number/ Liczba	Frequency/ Częstość	Number/ Liczba	Frequency/ Częstość		
25129A>C						
A/A	27	0.26	30	0.17	Ref.	Ref.
A/C	59	0.56	86	0.48	0.55 (0.29–1.03)	0.52 (0.27–1.00)
C/C	19	0.18	64	0.35	1.80 (0.97–3.34)	1.93 (0.99–3.74)
A	113	0.54	146	0.41	Ref.	Ref.
C	97	0.46	214	0.59	0.64 (0.36–1.15)	0.61 (0.32–1.17)

Data in boldface are statistically significant, Ref. denotes reference group ie. group relative which ORs were calculated.
[a]Adjusted for age, gender, smoking, environment of life and family status of AMD/ Dane istotne statystyczne zostały pogrubione. Ref. oznacza grupę odniesienia, względem której obliczano OR. [a]Skorygowany względem wieku, płci, palenia tytoniu, środowiska zamieszkania i rodzinnego statusu AMD.

Tab. V. Distribution of genotypes, frequency of alleles of the 25129A>C polymorphism of the *NRF2* gene and odds ratio (OR) with 95% confidence interval (95% CI) in patients with wet age-related macular degeneration (AMD) and individuals without visual disturbances (controls).

Tab. V. Rozkład genotypów, częstość alleli polimorfizmu 25129A>C genu *NRF2* oraz analiza OR z 95% przedziałem ufności u chorych na wysiękową postać AMD oraz u osób z grupy kontrolnej.

considered as risk factor in the final multivariate model. Adjustment for investigated potential confounders indicated that they altered the observed estimates of association between C/C genotype of the 25129A>C polymorphism of the *NRF2* gene and AMD risk (Table V).

We also divided AMD patients into groups depending on the same biological and environmental factors. We did not found any relationship between any group and investigated polymorphism in the single stage OR analysis (data not shown).

5. Discussion

Organs requiring large amounts of energy, such as brain, heart, muscles, and retina, may be dramatically affected by mitochondrial dysfunction (32, 33). Mitochondria are especially sensitive to damage because they are major bioenergetic machinery and source of oxidative stress in cells. Therefore, effective control of mitochondrial biogenesis and turnover is critical for the maintenance of energy production, the prevention of endogenous oxidative stress and promotion of healthy aging (34). Processes in which mitochondria play a central role, including production of cellular energy, free radical production and apoptosis, have been implicated in the pathogenesis of AMD (18).

The etiology of AMD is not completely known and this hampers the development of rational therapies. Some aspects of susceptibility to this disease suggest the involvement of genetic factors in its pathogenesis (35). The identification of these factors would be helpful in prevention, diagnosis and therapy of AMD. Because oxidative stress is frequently implicated in the induction and development of AMD, it seems reasonable to assume that genetic variability of components of antioxidant defense system may contribute to this disease. An association between genetic polymorphism of genes encoding antioxidant enzymes and development of AMD was reported (36).

Nuclear DNA-encoded factors are essential for mitochondrial biogenesis and mitochondrial protein biosynthesis (22–24). One of these factors is encoded by the *NRF2* gene and plays a significant role in these processes (37, 38). It is unclear how a mutation located in an intron, can influence gene expression (39, 40). The 25129A>C polymorphism of the *NRF2* gene, may influences the process of canonical or alternative splicing of mRNA, leading to the expression differences and hence interfere with mitochondrial biogenesis (41). That is why we decided to study the variability in the intron of the *NRF2* gene in both forms of AMD. This is the first study showed that the polymorphism of the *NRF2* may be associated with AMD.

We found that the C/C variant of the polymorphism may increase the risk of AMD. We speculate that this polymorphism may lead to an extended activation of the mitochondrial respiratory chain elements and their overproduction in AMD patients. The wild type genotype of this polymorphism was associated with endurance performance in a Chinese population, due to the mitochondrial role of the NRF2 (31). Moreover, a recent study suggested that the A/G genotype of the A/G polymorphism in the intron 3 of the *NRF2* gene (rs7181866) was associated with endurance performance (42). These results supported the regulatory role of this gene in oxygen metabolism.

Epidemiologic studies have suggested that ocular and systemic factors, such as hyperopia, cardiovascular disorders, and some environmental factors as sunlight exposure and tobacco smoking, are risk factors for AMD (43, 44). AMD is caused by environmental factors triggering disease in genetically susceptible subjects (45–49).

In this study we investigated the relationship between biological and environmental factors and risk of AMD independent of genotype. Our study suggests that age, gender and family history of AMD are risk factors in the development of AMD. This is in general agreement with results obtained by other scientist (50–53). In contrast to some previous reports we did not find any association between tobacco smoking and AMD risk (54). It may be underlined by different criteria for classification of controls and patients into groups, because we stratified controls and AMD patients into two groups: never smokers and ever smokers (former and current). We also correlated age, sex, smoking, living environment (rural or municipal) and family status of AMD and *NRF2* polymorphism with AMD risk in a multivariable model. We showed for the first time an interaction between these biological and environmental factors and polymorphic variants of the *NRF2* gene influencing AMD risk.

6. Conclusions

The C/C genotype of the 25129A>C polymorphism of the *NRF2* gene may be associated with enhanced risk of AMD in a Polish population, whereas the A/C genotype of this polymorphism may reduce the risk of the disease. Thus, this poly-

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morphism might be regarded as a risk factor associated with the AMD occurrence. However, expression profiles and biological activities of examined polymorphic variants *in vivo* will provide a better understanding of their role in AMD pathogenesis.

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References:

1. Sickenberg M.: *Early detection, diagnosis and management of choroidal neovascularization in age-related macular degeneration: the role of ophthalmologists*. Ophthalmologica 2001; 215: 247–253.
2. Freund K.B., Yannuzzi L.A., Sorenson J.A.: *Age-related macular degeneration and choroidal neovascularization*. Am. J. Ophthalmol. 1993; 115: 786–791.
3. Wozniak K., Szaflik J.P., Zaras M., Sklodowska A., Janik-Papis K., Poplawski T.R. i wsp.: *DNA damage/repair and polymorphism of the hOGG1 gene in lymphocytes of AMD patients.* J. Biomed. Biotechnol. 2009; 827562: 1–9.
4. Wang J., Klein R., Smith W., Klein B.E., Tomany S., Mitchell P.: *Cataract surgery and the 5-year incidence of late-stage age-related maculopathy pooled findings from the Beaver Dam and Blue Mountains eye studies.* Ophthalmology 2003; 110: 1960–1967.
5. Klaver C.C., Wolfs R.C., Vingerling J.R., Hofman A., de Jong P.T.: *Age-specific prevalence and causes of blindness and visual impairment in an older population. The Rotterdam Study.* Arch. Ophthalmol. 1998; 116: 653–658.
6. Tomany S.C., Wang J.J., van Leeuwen R., Klein R., Mitchell P., Vingerling J.R. i wsp.: *Risk factors for incident age-related macular degeneration: pooled findings from 3 continents.* Ophtalmology 2004; 11: 1280–1287.
7. Busch H., Vinding T., la Cour M., Jensen G.B., Prause J.U., Nielsen N.V.: *Risk factors for age-related maculopathy in 14-year follow-up study: the Copenhagen City Eye Study.* Acta Ophtalmol. Scand. 2005; 83: 409–418.
8. Frank R.N., Puklin J.E., Stock C., Canter L.A.: *Race, iris colour, and age-related macular degeneration.* Trans. Am. Ophtalmol. Soc. 2000; 98: 109–117.
9. Klein R., Klein B.E., Tomany S.C., Cruickshanks K.J.: *The association of cardiovascular disease with the long-term incidence of age-related maculopathy: the Beaver Dam Eye Study.* Ophtalmology 2003; 110: 636–643.
10. Clemons T.E., Milton R.C., Klein R., Seddon J.M., Ferris F.L.: *Risk factors for the incidence of advanced age-related macular degeneration in the Age-Related Disease Study (AREDS): AREDS report No. 19.* Ophtalmology 2005; 112: 533–539.
11. Klein B.E., Klein R., Lee K.E.: *Incidence of age-related cataract: the Beaver Dam Eye Study.* Ophtalmology 1998; 116: 219–225.
12. San Giovanni J.P., Chew E.Y., Clemons T.E., Davis M.D., Ferris F.L., Gensler G.R. i wsp. and Age-Related Eye Disease Study Research Group. *The relationship of dietary lipid intake and age-related macular degeneration in a case-control study.* AREDS report No. 20. Arch. Ophtalmol. 2007; 125: 671–679.
13. Vojnicovic B., Njiric S., Coklo M., Spanjol J.: *Ultraviolet sun radiation and incidence of age-related macular degeneration on Croatian Island Rab.* Coll. Antropol. 2007; Suppl 1: 43–44.
14. Wang J.J., Ross R.J., Tuo J., Burlutsky G., Tan A.G., Chan C.C. i wsp.: *The LOC387715 polymorhism, inflammatory markers, smoking, and age-related macular degeneration. A population--based case-control study*. Ophtalmology 2008; 115: 693–699.
15. Seddon J.M., George S., Rosner B.: *Cigarette smoking, fish consumption, omega-3 fatty acid intake, and associations with age-related macular degeneration: the US Twin Study of Age--Related Macular Degeneration.* Arch. Ophtalmol. 2006; 124: 995–1001.
16. Scholl H.P., Fleckenstein M., Charbel Issa P., Keilhauer C., Holz F.G., Weber B.H.: *An update on the genetics of age-related macular degeneration.* Mol. Vis. 2007; 13: 196–205.
17. Brunk U.T., Terman A.: *Lipofuscin: mechanisms of age-related accumulation and influence on cell function.* Free Radic. Biol. Med. 2002; 33: 611–619.
18. Ohia S.E., Opere C.A., Leday A.M.: *Pharmacological consequences ooxidative stress in ocular tissues*. Mutat. Res. 2005; 579(1–2): 22–36.
19. Beatty S., Koh H., Phil M., Henson D., Boulton M.: *The role of oxidative stress in the pathogenesis of age-related macular degeneration.* Surv. Ophthalmol. 2000; 45(2): 115–134.
20. Wallace D.C.: *Mitochondrial diseases in man and mouse*. Science 1999; 283: 1482–1488.
21. Feher J., Kovacs I., Artico M., Cavallotti C., Papale A., Balacco Gabrieli C.: *Mitochondrial alterations of retinal pigment epithelium in age-related macular degeneration.* Neurobiol. Aging. 2006 Jul; 27(7): 983–993.
22. Spinazzola A., Zeviani M.: *Disorders of nuclear–mitochondrial intergenomic signaling.* Gene 2005; 354: 162–168.
23. Pagliarini D.J., Calvo S.E., Chang B. i wsp.: *A mitochondrial protein compendium elucidates complex I disease biology.* Cell 2008; 134: 112–123.
24. Koene S., Smeitink J.: *Mitochondrial medicine: entering the era of treatment.* J. Intern. Med. 2009; 265: 193–209.
25. Virbasius J.V., Scarpulla R.C.: *Activation of the human mitochondrial transcription factor A gene by nuclear respiratory factors: a potential regulatory link between nuclear and mitochondrial gene expression in organelle biogenesis.* Proc. Natl. Acad. Sci. USA 1994; 91: 1309–1313.
26. Virbasius J.V., Virbasius C.A., Scarpulla R.C.: *Identity of GABP with NRF-2, a multisubunit activator of cytochrome oxidase expression, reveals a cellular role for an ETS domain activator of viral promoters.* Genes. Dev. 1993; 7: 380–392.
27. Gleyzer N., Vercauteren K., Scarpulla R.C.: *Control of mitochondrial transcription specificity factors (TFB1M and TFB2M) by nuclear respiratory factors (NRF-1 and NRF-2) and PGC-1 family coactivators.* Mol. Cell. Biol. 2005; 25: 1354–1366.
28. Virbasius J.V., Scarpulla R.C.: *Transcriptional activation through ETS domain binding sites in the cytochrome c oxidase subunit IV gene.* Mol. Cell. Biol. 1991; 11: 5631–5638.
29. Evans M.J., Scarpulla R.C.: *Both upstream and intron sequence elements are required for elevated expression of the rat somatic cytochrome c gene in COS-1 cells.* Mol. Cell. Biol. 1988; 8: 35–41.
30. Evans M.J., Scarpulla R.C.: *Interaction of nuclear factors with multiple sites in the somatic cytochrome c promoter. Characterization of upstream NRF-1, ATF and intron Spl recognition sites.* Biol. Chem. 1989; 264: 14361–14368.

31. Eynon N., Alves A.J., Sagiv M., Yamin C., Sagiv M., Meckel Y.: *Interaction between SNPs in the NRF2 gene and elite endurance performance.* Physiol. Genomics 2010; 41: 78–81.
32. Green D.R., Reed J.C.: *Mitochondria and apoptosis.* Science. 1998; 281(5381): 1309–1312.
33. Schapira A.H.: *Mitochondrial disease.* Lancet 2006; 368: 70–82.
34. López-Lluch G., Irusta P.M., Navas P., de Cabo R.: *Mitochondrial biogenesis and healthy aging.* Exp. Gerontol. 2008 Sep; 43(9): 813–819.
35. Rosenfeld P.J., Gorin M.B.: *Age-related macular degeneration.* Genetics. 1999. In: Berger J.W., Fine S.L., Maguire M.G., editors. St Louis: Mosby, 69–80.
36. Kimura K., Isashiki Y., Sonoda S., Kakiuchi-Matsumoto T., Ohba N.: *Genetic association of manganese superoxide dismutase with exudative age-related macular degeneration.* Am. J. Ophthalmol. 2000; 130: 769–773.
37. Kelly D.P., Scarpulla R.C.: *Transcriptional regulatory circuits controlling mitochondrial biogenesis and function.* Genes. Dev. 2004; 18: 357–368.
38. Scarpulla RC.: *Nuclear activators and coactivators in mammalian mitochondrial biogenesis.* Biochim. Biophys. Acta 2002; 1576: 1–14.
39. Saxena R., Voight B.F., Lyssenko V., Burtt N.P., de Bakker P.I., Chen H. i wsp.: *Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels.* Science 2007; 316: 1331–1336.
40. Mercado P.A., Ayala Y.M., Romano M., Buratti E., Baralle F.E.: *Depletion of TDP 43 overrides the need for exonic and intronic splicing enhancers in the human apoA-II gene.* Nucleic. Acids. Res. 2005; 33: 6000–6010.
41. He Z., Hu Y., Feng L., Lu Y., Liu G., Xi Y. i wsp.: *NRF2 genotype improves endurance capacity in response to training.* Int. J. Sports. Med. 2007; 28: 717–721.
42. Eynon N., Sagiv M., Meckel Y., Duarte J.A., Alves A.J., Yamin C. i wsp.: *NRF2 intron 3 A/G polymorphism is associated with endurance athletes' status.* J. Appl. Physiol. 2009; 107: 76–79.
43. Bressler N.M., Bressler S.B., Fine S.L.: *Age-related macular degeneration.* Surv. Ophthalmol. 1988; 30: 375–413.
44. Hawkins B.S., Bird A., Klein R., West S.K.: *Epidemiology of age-related macular degeneration.* Mol. Vis. 1999; 5: 26.
45. Piguet B., Wells J.A., Palmvang I.B., Wormald R., Chisholm I.H., Bird A.C.: *Age-related Bruch's membrane change: a clinical study of the relative role of heredity and environment.* Br. J. Ophthalmol. 1993; 77: 400–403.
46. Hyman L.G., Lilienfeld A.M., Ferris F.L.D., Fine S.L.: *Senile macular degeneration: a case-control study.* Am. J. Epidemiol. 1983; 118: 213–227.
47. Seddon J.M., Ajani U.A., Mitchell B.D.: *Familial aggregation of age-related maculopathy*. Am. J. Ophthalmol. 1997; 123: 199–206.
48. Silvestri G., Johnston P.B., Hughes A.E.: *Is genetic predisposition an important risk factor in age-related macular degeneration?* Eye 1994; 8: 564–568.
49. Klaver C.C., Wolfs R.C., Assink J.J., van Duijn C.M., Hofman A., de Jong P.T.: *Genetic risk of age-related maculopathy. Population-based familial aggregation study.* Arch. Ophthalmol. 1998; 116: 1646–1651.
50. Smith W., Mitchell P.: *Family history and age-related maculopathy: the Blue Mountains Eye Study.* Aust. N. Z. J. Ophthalmol. 1998; 26: 203–206.
51. Hyman L., Neborsky R.: *Risk factors for age-related macular degeneration: an update*. Curr. Opin. Ophthalmol. 2002; 13: 171––175.
52. Tomany S.C., Wang J.J., van Leeuwen R., Klein R., Mitchell P., Vingerling J.R. i wsp.: *Risk factors for incident age-related macular degeneration: pooled findings from 3 continents.* Ophthalmology 2004; 11: 1280–1287.
53. Busch H., Vinding T., la Cour M., Jensen G.B., Prause J.U., Nielsen N.V.: *Risk factors for age-related maculopathy in 14- year follow-up study: the Copenhagen City Eye Study.* Acta Ophthalmol. Scand. 2005; 83: 409–418.
54. Dhubhghaill S.S., Cahill M.T., Campbell M., Cassidy L., Humphries M.M., Humphries P.: *The pathopysiology of cigarette smoking and age-related macular degeneration.* Adv. Exp. Med. Biol. 2010; 664: 437–446.

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Reprint requests to (Adres do korespondencji):
Associate Professor Tomasz Śliwiński, Ph.D.
University of Lodz, Faculty of Biology and Environmental Protection, Department of Molecular Genetics
Pomorska 141/143
Lodz 90-236, Poland
e-mail: tomsliw@biol.uni.lodz.pl

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