Associations between *CYP2J2* (-76G>T) rs890293 polymorphism and age-related macular degeneration

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Backgroung. Age-related macular degeneration (AMD) is a disease of the macula, which significantly affects the eyesight and leads to irreversible central vision loss. The etiopathogenesis of AMD is still not absolutely clear. It is thought that age-related macular degeneration has a multifactorial etiology, the development of which may be caused by interrelation of environmental with innate factors, while genetic factors also have an impact. Macular degenerative changes occur due to the formation of drusen, about 40% of which is lipids. As the *CYP2J2* gene is involved in the metabolism of lipids, it was selected for investigation in this study.
Purpose. To determine the relation between early stage and exudative AMD and *CYP2J2* (-76G>T) gene rs890293 polymorphism in a Lithuanian population.
Methods. The study enrolled 204 patients with early AMD, 197 patients with exudative AMD and 198 healthy controls. Samples of DNA from peripheral white blood cells were purified using commercial kits. The genotyping was carried out using a real-time PCR method.
Results. The *CYP2J2* (-76G>T) rs890293 TT genotype in patients with early AMD was statistically significantly less frequent than in the control group: 0% vs. 2.5% (*P*=0.028). There were no significant differences in rs890293 gene polymorphisms between the exudative AMD and control groups. Also, the *CYP2J2* (-76G>T) rs890293 TT genotype was statistically significantly less frequent in older early AMD patients (≥65 years) compared to control group persons (≥65 years): 0% vs. 5.4% (*P*=0.03).
Conclusion. The *CYP2J2* (-76G>T) *TT* genotype may be associated with reduced manifestation of early stage AMD; therefore, a larger sample size is required for further analysis.

Key words: age-related macular degeneration, *rs890293*, gene polymorphisms

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INTRODUCTION

Age-related macular degeneration (AMD) is damage to the macula which causes significant and irreversible central vision loss in aging patients[1]. It is thought that the number of people suffering from AMD will continue to grow, and by 2020 it will have increased up to 196 million, and by 2040 the disease will have damaged visual function of 288 million people[2]. AMD is diagnosed in one third of persons over 75 years. In the beginning of the disease, vision is intact and visual acuity changes are observed only as the disease progresses[1]. While progressing to late stages, such as central geographic atrophy (GA) and choroidal neovascularization (CVN) (ref.[1]), AMD causes visual loss in 6-8% of patients[3]. Thus, patient age has one of the greatest effects on the development of AMD (ref.[4]).

The exact etiopathogenetic mechanisms of AMD are not completely clear. Age-related macular degeneration has a multifactorial etiology with interrelated environmental and genetic factors. Intracellular metabolic processes create conditions for normal molecule transport and synthesis, as well as regulatory processes in the cell. Disorders of metabolism in aging patients disturb physiological processes and may induce oxidative stress. Such alteration of homeostasis implicates the inflammatory system. It is noteworthy that inflammatory processes during AMD are not accompanied by an intense reaction, which usually results in reparation, but lead to chronic inflammation and dysregulation of reparative reactions[5].

AMD involves drusen formation, which gradually degrades the retinal pigment epithelium (RPE) cells. Drusen consist of inactive complement associated inflammatory products, lipoprotein aggregates, oxidized phospholipids, cell debris, oxysterols and Alu RNA deposits. These components may also induce angiogenic factors which stimulate the formation of new blood vessels[4]. Currently, according to the processes of manifestation, there are two main categories of the disease: dry non-neovascular (CNV is absent) and wet neovascular (CNV is present) (ref.[1]).

As it was mentioned before, dysregulation of inflammation and oxidative stress are the key players in the pathogenesis of AMD (ref.[6]). Levels of CYP 450 enzyme (CYP4F2, CYP4F3 and CYP4A) derived oxylipins are elevated during inflammation[7]. These CYP4 family en-

zymes participate in the metabolism of such pro-inflammatory metabolites of arachidonic acid as 20-HETE (20-hydroxyeicosatetraenoic acid) or leukotriene B4 and regulate inflammation. It is noteworthy that 20-HETE has been shown to impair endothelial insulin signaling and to increase vascular endothelial growth factor (VEGF) activity by stimulating nicotinamide adenine dinucleotide phosphate oxidase[7]. VEGF in turn, induces cell proliferation and angiogenesis[8], causes vasodilatation, increases vascular permeability and protease activity in the retina. Such changes enable the development and expansion of the vascular network into surrounding tissues and its remodeling. The fragmentation of basilar membrane and intracellular connective tissue is essential for the formation of new capillaries and development of choroidal neovascularization[9]. Thus, to understand the impact of the enzymes participating in the regulation of inflammation, we determined the frequency of *CYP4F2* (rs2108622) variant in patients with early and exudative AMD. However, our results revealed that this gene polymorphism had no real effect on the development of early AMD and exudative AMD (ref.[10]).

CYP2J2 is another member of the cytochromes, which are common in heart and vessel endothelium. CYP2J2 participates in the metabolism of arachidonic acid and a few of its derivatives[11]. Variants of CYP2J2 have been shown to be associated with coronary artery disease[6] and Alzheimer`s disease[12]. Therefore, we aimed to determine the association of the *CYP2J2* (-76G>T) rs890293 variant participating in bio-active lipid-oxylipin metabolism and inflammation with the development of age-related macular degeneration.

MATERIALS AND METHODS

Permission to conduct the study was obtained from the Ethics Committee for Biomedical Research. The study was done in the Department of Ophthalmology, Hospital of Lithuanian University of Health Sciences (Number BE-2-/13).

Study population and inclusion criteria

All consecutive patients with AMD, who attended the Department of Ophthalmology, Hospital of Lithuanian University of Health Sciences, were asked to take part in the study and undergo an ophthalmological and general examination. The patients who agreed to participate and had no other systemic or non-systemic inflammatory or non-inflammatory pathology during ophthalmological and general examination comprised the study group. A total of 204 patients with a diagnosis of early AMD and 197 patients with exudative AMD were enrolled in the study, based on exclusion criteria. The following subject exclusion criteria were used:

(i) unrelated eye disorders, e.g., high refractive error, cloudy cornea, lens opacity (nuclear, cortical, or posterior subcapsular cataract), except minor opacities, keratitis, acute or chronic uveitis, glaucoma, or diseases of the optic nerve;

(ii) systemic illnesses, e.g., diabetes mellitus, malignant tumors, systemic connective tissue disorders, chronic infectious diseases, or conditions following organ or tissue transplantation;

(iii) ungraded color fundus photographs resulting from obscuration of the ocular optic system or because of poor fundus photograph quality.

The classification system of AMD formulated by the Age-Related Eye Disease Study[13] was used: early AMD consisted of a combination of multiple small drusen and several intermediate (63-124 µm in diameter) drusen or retinal pigment epithelial abnormalities; intermediate AMD was characterized by the presence of extensive intermediate drusen and at least one large (≥125 µm in diameter) druse or geographic atrophy (GA) not involving the centre of the fovea; advanced AMD was characterized by GA involving the fovea and/or any of the features of neovascular AMD. Early and exudative AMD were diagnosed by two ophthalmologists. Optical coherence tomography was performed on all AMD patients. Fluorescein angiograms were performed if necessary.

The control group comprised 198 persons who had no ophthalmologic pathology on examination and who agreed to take part in this study. They matched the patients with early AMD by age and gender (*P*=0.05) (Table 1).

During analysis, the study population was divided into two groups according to their age: younger than 65 years and 65 years and older.

Ophthalmological evaluation

Ophthalmological evaluation for all subjects in the study was carried out as described previously. All the study

Table 1. Demographic characteristics of the study population.

Characteristic	Group			*P*
	Early AMD n=204	Exudative AMD n=197	Control n=198	
Men, n (%)	54 (26.5)	63 (31.98)	44 (22.2)	0.09
Women, n (%)	150 (73.5)	134 (68.02)	154 (77.8)	
Age, mean (SD)	68.54 (10.44)	69.53 (8.17)	68.15 (11.81)	0.519
Age groups				
<65 years	83 (40.7)	54 (27.6)	142 (71.7)	
≥65 years	121 (59.3)	142 (72.4)	56 (28.3)	

patients were evaluated by slit-lamp biomicroscopy to assess corneal and lenticular transparency. Classification and grading of lens opacities was performed according to the Lens Opacities Classification System III. During examination, intraocular pressure was measured. Pupils were dilated with tropicamide 1%, after which fundoscopy using a direct monocular ophthalmoscope and slit-lamp biomicroscopy with a double aspheric lens of +78 diopters were performed. Results of eye examinations were recorded on specially standardized forms. For detailed analysis of the macula, stereoscopic color fundus photographs of the macula, centered at 45° and 30° to the fovea, were obtained with a Visucam NM Digital camera (Carl Zeiss Meditec AG, Germany).

DNA extraction and genotyping

DNA extraction and identification of the *CYP2J2* (-76G>T) rs890293 variant was carried out in the Laboratory of Ophthalmology, Neuroscience Institute, Lithuanian University of Health Sciences. DNA was extracted from 200 µL of venous blood (white blood cells) using a DNA purification kit based on the magnetic beads method (MagJET Genomic DNA Kit, Thermo Fisher Scientific, Lithuania) or the silica-based membrane technology utilizing a genomic DNA extraction kit (GeneJET Genomic DNA Purification Kit, Thermo Fisher Scientific, Lithuania), according to the manufacturer's recommendations. The genotyping of *CYP2J2* (-76G>T) rs890293 was carried out using the real-time polymerase chain reaction (PCR) method with a Rotor-Gene Q real-time PCR quantification system (Qiagen, USA). The single-nucleotide polymorphism *CYP2J2* (-76G>T) rs890293 was determined using TaqMan® Drug Metabolism assay (Applied Biosystems, USA), according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using the SPSS/W 20.0 software (Statistical Package for the Social Sciences for Windows, Inc., Chicago, Illinois, USA). Data are expressed as absolute numbers with percentages. Frequencies of genotypes are expressed in percentages.

The distribution of SNP rs890293 in the early and exudative AMD and control groups was compared using the χ^2 test or the Fisher exact test. Binomial logistic regression analysis was performed to estimate the impact of genotype on early and exudative AMD development. Odds ratios and 95% confidence intervals are presented. The selection of the best genetic model was based on the Akaike Information Criterion (AIC), therefore, the best genetic models were those with the lowest AIC values.

The relative risks of SNP genotypes and alleles were studied using co-dominant, dominant, recessive and over-dominant models that led to a comparison between wild type versus heterozygous and wild type and homozygous in the co-dominant model, wild type versus heterozygous + homozygous in the dominant model, heterozygous versus wild type + homozygous and wild type + heterozygous versus homozygous in the recessive model, respectively. The relative risks for each additional copy of allele in selected SNP were studied using the additive model.

Analysis was stratified by age and gender to evaluate the genetic risk for specific groups where differences in the genotype distribution were found.

Differences were considered statistically significant when $P<0.05$.

RESULTS

Females made up 73.5% (n=150) of the early AMD group, 68.02 % (n=134) of the exudative AMD and 77.8% (n=154) of the control group (P=0.09). The mean age of patients with early AMD, exudative AMD and of control subjects was 68.54 years (range of 50 to 92), 69.53 years (range of 50 to 91) and 68.15 years (range of 50 to 90), respectively (P=0.519).

Analysis showed significant differences in the rs890293 genotype distribution between patients with early AMD and healthy subjects: the T/T variant was not

Table 2. Frequency of *CYP2J2* (-76G>T) rs890293 genotype in patients with early and exudative age-related macular degeneration (AMD) and in control individuals.

Gene/marker	Genotype/ Allele	Early AMD n (%) (n=204)	Control n (%) (n=198)	P	Exudative AMD n (%) (n=197)	Control n (%) (n=198)	P
CYP2J2	Genotype						
(-76G>T) rs890293	G/G	184 (90.2)	180 (90.9)	0.866	173 (87.8)	180 (90.9)	0.319
	G/T	20 (9.8)	13 (6.6)	0.277	23 (11.7)	13 (6.6)	0.078
	T/T	**0 (0)**	**5 (2.5)**	**0.028***	1 (0.5)	5 (2.5)	0.101
	Total	204 (100)	198 (100)		197 (100)	198 (100)	
	Allele						
	G	388 (95.1)	373 (94.2)	0.568	369 (93.65)	373 (94.2)	0.752
	T	20 (4.9)	23 (5.8)		25 (6.35)	23 (5.8)	

*T/T genotype versus G/T+G/G genotypes

Table 3. Frequency of *CYP2J2* (-76G>T) rs890293 genotypes in patients with early age-related macular degeneration (AMD) and control individuals by age.

Gene/marker	Genotype/ Allele	<65 years			≥65 years		
		Early AMD group n (%)	Control group n (%)	*P*	Early AMD group n (%)	Control group n (%)	*P* value
CYP2J2 (-76G>T) rs890293	Genotype						
	G/G	75 (90.4)	132 (93)	0.611	109 (90.1)	48 (85.7)	0.446
	G/T	8 (9.6)	8 (5.6)	0.289	12 (9.9)	5 (8.9)	1.000
	T/T	0 (0)	2 (1.4)	0.532	**0 (0)**	**3 (5.4)**	**0.030**
	Allele						
	G	158 (95.2)	272 (95.8)	0.768	230 (95.0)	101 (90.2)	0.084
	T	8 (4.8)	12 (4.2)		12 (5.0)	11 (9.8)	

Table 4. Frequency of *CYP2J2* (-76G>T) rs890293 genotypes in patients with exudative age-related macular degeneration (AMD) and control individuals by age.

Gene/marker	Genotype/ Allele	<65 years			≥65 years		
		Exudative AMD group n (%)	Control group n (%)	*P*	Exudative AMD group n (%)	Control group n (%)	*P*
CYP2J2 (-76G>T) rs890293	Genotype						
	G/G	51 (94.4)	132 (93.0)	0.707	122 (85.3)	48 (85.7)	0.943
	G/T	3 (5.6)	8 (5.6)	0.939	20 (14.0)	5 (8.9)	0.333
	T/T	0 (0)	2 (1.4)	1.000	1 (0.7)	3 (5.4)	**0.035**
	Allele						
	G	108 (97.3)	272 (95.8)	0.477	264 (92.3)	101 (90.2)	0.489
	T	3 (2.7)	12 (4.2)		22 (7.7)	11 (9.8)	

Table 5. Comparison of *CYP2J2* rs890293 genotype distribution in control groups of other studies.

	Study	Country	GG (%)	GT (%)	TT (%)	Total (n)	Ref
1.	Our study	Lithuania	90.9	6.6	2.5	198	
2.	Yan et al.	China	95.7	4.3		351	36
3.	Zhu et al.	China	90.33	9.67	0.00	455	37
4.	Ali et al.	Egypt	87.6	10.7	1.6	429	38

observed in the early AMD group, while in healthy controls it was observed in 5 participants (0% in the early AMD vs. 2.5% in the control group, *P*=0.028) (Table 2).

Additionally, we performed binomial logistic regression analysis of patients with AMD and control individuals, but it did not reveal any significant results (data not shown).

The distribution of *CYP2J2* rs890293 genotypes and alleles in individuals with early and exudative AMD and control subjects was analyzed in groups by age (Table 3 and 4). The same significance of results was found in the older age group (≥65 years). The rs890293 T/T variant was not observed in patients with early AMD, while it was observed in 3 subjects of the control group (0% in the early AMD group vs. 5.4% in the control group, *P*=0.030) (Table 3). In the exudative AMD group, only one older patient with the rs890293 T/T variant was found, while it was observed in 3 subjects of the control group (0.7% in the exudative AMD group vs. 5.4% in the control group, *P*=0.035) (Table 4).

Binomial logistic regression analysis of the rs890293 genotype polymorphism in groups by age did not show any statistical significance (data not shown).

The *CYP2J2* rs890293 genotype analysis by gender was performed but it did not reveal any significant results (data not shown).

DISCUSSION

Our study revealed that the *CYP2J2* (-76G > T) rs890293 TT genotype was significantly less common in patients with AMD, compared to the control group (*P*=0.028), and TT polymorphism was significantly less common in older early AMD patients (≥65 years), compared to healthy controls (*P*=0.03). Potentially, the *CYP2J2* (-76G > T) polymorphism rs890293 TT could be a protective genotype. As we know, the T allele can lead to the loss of transcription factor Sp1 binding site and diminished *CYP2J2* gene expression, thus setting a

lower number of metabolites in vivo[14,15]. There were no statistically significant differences in the rs890293 variant distribution between the exudative AMD and control groups. Till now, to our knowledge, there have been no studies investigating the same genetic polymorphism in patients with early or late AMD. There are only two studies analyzing other polymorphisms of the same gene in patients with AMD. Another study has analyzed CYP2C19 (G681A) rs4244285 and CYP1A2 (-163C>A) rs762551 gene polymorphisms in patients with early AMD. The study showed that the CYP1A2 (-163C>A) rs762551 C/C genotype was associated with an increased risk of AMD, and the CYP2C19 (G681A) rs4244285 was not associated with early AMD (ref.[16]). Yet another study has analyzed CYP4F2 rs2108622 gene polymorphism in patients with early and exudative AMD and its results revealed that this gene polymorphism had no major effect on the development of early AMD and exudative AMD, but T/T genotype was more frequent in males with exudative AMD compared to females and less frequently present in exudative AMD females compared to healthy females[17].

However, researchers are looking for this polymorphism's relations with other diseases. Yan and colleagues found that *CYP2J2* rs890293 GT + TT genotype is associated with a higher risk of late stage manifestation of Alzheimer's disease[18]. It has also been established that the *CYP2J2* gene polymorphism rs890293 is associated with atherosclerosis, ischemic heart disease, and myocardial infarction[14,19,20]. It is also considered that lipids and genes responsible for their metabolism in these diseases may have links with AMD pathogenesis[21,22]. Butt and collaborating researchers have analyzed the conducted studies and found the results contradictory[23]. Some studies have shown that an increased high-density lipoprotein (HDL) concentration increases the risk of developing AMD (ref.[23,24]), while others asserted that HDL reduces AMD development[25,26]. Yet some other studies have not found any links between HDL concentration in blood and AMD (ref.[27,28]).

Some scientists have confirmed an association of the increased total cholesterol levels in blood with exudative AMD (ref.[29-33]) and some have not[25-27,34].

Cougnard-Grégoire et al. have confirmed the gene polymorphisms associated with AMD: ApoE2, ApoE4, CFH Y420H, ARMS2 A69S, LIPC, LIPC, LPL, ABCA1, and CETP (ref.[35]).

To our knowledge, there are no other studies of AMD and the gene *CYP2J2* (-76G>T) rs890293 polymorphism relation, which we chose to investigate. Therefore, we compared healthy people's gene polymorphism frequencies with other authors' studies. The results of other studies are quite similar to our research. The comparison is presented in Table 5.

Huacheng Yan et al. have explored the relationship between the *CYP2J2* rs890293 polymorphism and Alzheimer's disease. The provided data showed distribution of genotypes in the control group as follows: GG 95.7%, GT+TT 4.3%. Individuals of a Chinese population selected randomly were enrolled as the control group in the study of Huacheng Yan et al.[36]. The frequencies of genotype polymorphism in their control group were similar to our research.

We also compared the results of genotype frequencies in the control groups of our study and Qing Zhu et al. study, where a Chinese population was enrolled as the control group[37]. Our control group genotype frequencies relatively matched the research results of Qing Zhu et al.

Ahmed Ali et al. have analyzed the association between *CYP2J2* rs890293 polymorphism and arterial hypertension[38]. We compared the genotype frequency in their control group[38] with our results: GG 87.6%, GT 10.7%, TT 1.6% vs. GG 90.9%, GT 6.6%, 2.6%.

One of the major issues with our study is a relatively small sample size.

It is obvious that the TT genotype is very rare not only in our study[36,38,39]. Consequently, further studies with a larger sample size are needed in order to ascertain the role of rs890293 polymorphism in pathogenesis of AMD.

CONCLUSION

Our study determined that the CYP2J2 (-76G>T) rs890293 TT genotype was statistically less common in patients with early AMD, comparing to the control group: 0% vs. 2.5% (*P*=0.028) and also less common in older (≥65 years) early AMD patients, comparing to healthy individuals (≥65 years): 0% vs. 5.4% (*P*=0.03). The study should be repeated by investigating a larger sample group.

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