• Basic Research •

MMP-2 Rs24386 (C-->T) gene polymorphism and the phenotype of age-related macular degeneration

Rasa Liutkeviciene[1,2], Vaiva Lesauskaite[3], Giedre Sinkunaite-Marsalkiene[3], Sandrita Simonyte[3], Reda Zemaitiene[1], Loresa Kriauciuniene[1,2], Dalia Zaliuniene[1]

[1]Department of Ophthalmology, Lithuanian University of Health Sciences, Medical Academy, Kaunas LT-50161, Lithuania
[2]Neuroscience Institute, Lithuanian University of Health Sciences, Medical Academy, Kaunas LT-50161, Lithuania
[3]Intitute of Cardiology, Lithuanian University of Health Sciences, Medical Academy, Kaunas LT-50161, Lithuania
Correspondence to: Sandrita Simonyte. Intitute of Cardiology, Medical Academy, Lithuanian University of Health Sciences, Sukileliu 17, Kaunas LT-50161, Lithuania. sandrita.simonyte@lsmuni.lt
Received: 2016-03-24 Accepted: 2017-06-23

Abstract

• AIM: To examine the *MMP-2 (-1306 C/T)* gene polymorphism and the phenotype characterized by soft and hard drusen of early age-related macular degeneration (AMD) and geographic atrophy of late AMD form.

• METHODS: The study enrolled 850 investigations (290 AMD patients with soft and hard drusen, 34 with geographic atrophy and a random sample of the population *n*=526). Early AMD was classified according to the International Classification and Grading System. For geographic atrophy diagnosis the Age-Related Eye Disease Study classification was used. The potential association with single nucleotide polymorphisms on *MMP-2 Rs243865* was evaluated for all patients, adjusted for age and sex. The genotyping test of *MMP-2 Rs243865 (C-->T)* was conducted using the real-time polymerase chain reaction method.

• RESULTS: *MMP-2 (-1306 C/T) C/C* genotype was more frequently detected in AMD patients with hard drusen than the soft drusen or control group (66.43% *vs* 53.74%, *vs* 54.94%, *P*=0.047). Logistic regression analysis showed that the *MMP-2 (-1306) C/C* genotype increased the likelihood to develop hard drusen in AMD patients (OR=1.7, 95% CI: 1.06-2.74; *P*=0.028). No association between *MMP-2 (-1306 C/T)* gene polymorphism in patients with atrophic AMD and control group was found (54.94%, 37.64%, 7.41% *vs* 50%, 38.24%, 11.76%; *P*=0.6).

• CONCLUSION: The *MMP-2 Rs24386 (C-->T)* polymorphism is found to be associated with the development of hard drusen in patients with AMD.

• KEYWORDS: age-related macular degeneration; phenotype; matrix metalloproteinases; gene polymorphism
DOI:10.18240/ijo.2017.09.03

Citation: Liutkeviciene R, Lesauskaite V, Sinkunaite-Marsalkiene G, Simonyte S, Zemaitiene R, Kriauciuniene L, Zaliuniene D. *MMP-2 Rs24386 (C-->T)* gene polymorphism and the phenotype of age-related macular degeneration. *Int J Ophthalmol* 2017;10(9): 1349-1353

INTRODUCTION

Age-related macular degeneration (AMD) causes significant and irreversible loss of central vision. In developed countries, AMD is the most common cause of visual loss in persons aged 60y and older[1].

Macular degenerative lesions in case of AMD include drusen formation, changes in the retinal pigment epithelium, atrophy of the retinal pigment epithelium and the choroidal choriocapillary layer, lesion of Bruch's membrane, geographic atrophy of the central fovea, exudative AMD with choroidal neovascularization, detachment of the retinal pigment epithelium, or submacular disciform scarring.

Drusen are defined as outgrowth of colloidal material, similar to hyaline, that accumulates in the retina, in Bruch's membrane underlying the retinal pigment epithelium. Drusen formation causes progressive degeneration of the retinal pigment epithelium and photoreceptors[2] by disturbing oxygen metabolism and leading to the degeneration of photoreceptors, while visual function impairment is associated with the quantity of damaged photoreceptors. The fovea, with the largest quantity of photoreceptors, is dominated by cones, whereas in the parafoveal region, surrounding the fovea, rods dominate. In the early stages, mostly photoreceptors in the parafovea are damaged.

Drusen are divided into hard and soft. Hard drusen can induce atrophy of the retinal pigment epithelium and choriocapillary layer. Soft drusen may confluent and cause exudative AMD and can later induce the detachment of the neuroepithelium[3].

Recent studies have demonstrate that angiogenesis is the most important mechanism of AMD development and is associated with important extracellular remodeling involving different proteolytic systems, among which matrix metalloproteinases (MMPs) play an essential role[4]. It has been suggested that the

decrease in MMP-2 activity correlates to drusen formation[5]. In the *MMP-2 (–1306)* gene promoter transcription region, a mutation (rs243865), which causes an increase in promoter activity, has been discovered. The coding gene for MMP-2 is located in 16q13-q21 locus. The C-to-T allelic variation located at nucleotide -1306 disrupts the Sp1-binding site in the promoter region and leads to low transcriptional activity; and when the T allele has a markedly lower promoter activity than the C allele where promoter loses 50% of activity[6].

Assuming the fact that MMP-2 could have an influence on subretinal deposit formation[4-5], we hypothesized that the *MMP-2* gene might be associated with soft/hard or atrophic AMD.

SUBJECTS AND METHODS

Permission to undertake the study was obtained from Kaunas Regional Ethics Committee for Biomedical Research (No BE-2-14). All the subjects provided written, informed consent in accordance with the Declaration of Helsinki. The study was conducted in the Department of Ophthalmology, Hospital of the Lithuanian Health Sciences University and Institute of Cardiology, Medical Academy, Lithuanian University of Health Sciences.

The control group comprised 526 persons, selected from persons participating in the International Health, Alcohol and Psychosocial Factors in Eastern Europe Project[7], the International Countrywide Integrated Non-communicable Disease Intervention Project[8], and the Kaunas Healthy Ageing Study[9].

Of the 400 patients with early AMD, 290 (580 eyes) were recruited into the study according to the inclusion criteria. The early AMD patients and the control persons were matched by age and gender (Table 1). The atrophic AMD group matched the control group only by gender.

Ophthalmological Evaluation Ophthalmological evaluation for all subjects in our study was carried out as described previously[10]. Morphologic fundus changes were classified in our study as follows. Hard drusen were defined as discrete whitish-yellow spots with a diameter of less than 63 μm. Soft distinct drusen were pale-yellow spots with uniform density and discrete edges with a diameter larger than 125 μm. Soft indistinct drusen were pale-yellow spots with decreasing density from the center outwards and fuzzy edges with a diameter greater than 125 μm. The latter two groups were integrated into one group. For the diagnosis of geographic atrophy, the classification system of AMD formulated by the Age Related Eye Disease Study (AREDS) was used[10]. Geographic atrophy was characterized by well seen separate areas of retinal pigment epithelium atrophy with visible choroidal blood vessels, involving the fovea and any of the features of neovascular AMD[11].

Table 1 Demographic characteristics of patients with AMD and reference group subjects

Group	*n*	Age, median (95%CI)	Males, *n* (%)
Early AMD	290	67 (59.94-67.85)[1]	126 (43)[1]
Control	526	68 (60.88-68.32)[1]	210 (40)[1]
P	-	DSI	DSI
Late AMD geographic atrophy	34	73 (69.68-76.85)[2]	12 (35)[1]
P	-	DSS	DSI

[1]DSI: Difference statistically insignificant; [2]DSS: Difference statistically significant.

The fundus images were graded according to the digital analysis method "A Drusen Volume Quantification Method based on a Segmentation algorithm in VIP Image"[12]. Where disagreement occurred between the two graders, the results were adjudicated by a senior retinal specialist (when there was a disagreement rate of over 4.5%, patients were excluded from further research).

Optical coherence tomography (OCT) was performed on all AMD patients, and fluorescence angiography was performed on the patients with suspected exudative stage AMD after the OCT examination.

The exclusion criteria for subjects were as described previously[10]. Exudative AMD was diagnosed in two patients excluding them from further analysis. Five patients were excluded from the study because of suspected inherited macular dystrophies.

The inclusion criteria for patients with AMD were as follows: 1) patients of both genders, with diagnosis of early mild or early intermediate AMD and without other eye disorders, found on detailed ophthalmologic examination; 2) patients with AMD but only with soft and hard drusen classified according to the International Classification and Grading System; 3) patients with the diagnosis of geographic atrophy according to the AREDS classification; 4) participation consent.

DNA Extraction and Genotyping DNA extraction and analysis of *MMP-2 Rs243865 (C-->T)* gene polymorphism were carried out in the Laboratory of Ophthalmology, Institute of Cardiology, Lithuanian University of Health Sciences, as described previously[10].

Statistical Analysis Statistical analysis was performed using the computer program SPSS/W 13.0 (Statistical Package for Social Science for Windows, Inc., Chicago, Illinois, USA). To compare the observed and expected MMP's genotype frequencies Hardy-Weinberg analysis was performed using the χ^2 test in all groups. The distribution of *MMP-2 Rs243865* single-nucleotide polymorphism in the early and atrophic AMD and reference group was compared using the χ^2 test or the Fisher exact test. A difference was considered statistically significant at $P<0.05$.

Table 2 *MMP-2 (–1306 C/T)* genotype frequency in patients with AMD with soft and hard drusen, in geographic atrophy and in the control group *n* (%)

Gene	Genotype/allele	Control group *n*=526	P_{HWE}	AMD group (soft drusen) *n*=147	P_{HWE}	AMD group (hard drusen) *n*=143	P_{HWE}	AMD group (geographic atrophy) *n*=34	P_{HWE}
MMP-2 (–1306) Rs2285053	Genotype								
	C/C	289 (54.94)[1]	0.4	79 (53.74)[2]	0.22	95 (66.43)[1,2]	0.8	17 (50.00)	0.54
	C/T	198 (37.64)		56 (38.10)		43 (30.07)		13 (38.24)	
	T/T	39 (7.41)		12 (8.16)		5 (3.50)		4 (11.76)	
	Allele								
	C	0.72		0.7		0.81		0.69	
	T	0.28		0.3		0.19		0.31	

P_{HWE}: Significance level by Hardy-Weinberg equilibrium. [1]P=0.017; [2]P=0.03.

RESULTS

A total of 190 patients with early AMD and 34 patients with atrophic AMD were enrolled in this study following to the inclusion and exclusion criteria. The control comprised 526 persons. The age of the patients with early AMD ranged from 50 to 93y (median 67); the age of atrophic AMD group varied from 50 to 93y (median 73). The age of the control patients ranged from 50 to 93y (median 68) (Table 1).

The genotyping of *MMP-2 (–1306) C/T* was performed on patients with soft and hard drusen in early AMD and control group (Table 2). The distribution of the analyzed *MMP* genotypes and allele frequencies in patients with AMD with soft and hard drusen and in the control group matched the Hardy-Weinberg equilibrium.

AMD patients with hard drusen had *MMP-2 (–1306) C/C* genotype detected more frequently than the control group and those with soft drusen (66.43% *vs* 54.94% and 53.74%, P=0.017 and P=0.03, respectively) (Table 2). Logistic regression analysis revealed increased likelihood of developing hard drusen in AMD patients with *MMP-2 (–1306) C/C* genotype (OR=1.7, 95%CI: 1.06-2.74; P=0.028).

Analysis of *MMP-2 Rs24386 (C-->T)* gene polymorphism did not reveal any differences in the distribution of genotype (*T/T*, *C/T*, and *C/C*) between men with soft and hard drusen (14.06%, 35.94%, and 50% *vs* 4.84%, 29.03%, and 66.13%, P=0.95, respectively) and between women with soft and hard drusen (3.61%, 39.76%, and 56.63% *vs* 2.47%, 30.86%, and 66.67%, P=0.41).

To reduce the possibility of type I error due to multiple testing, we employed the Bonferroni correction, and a P value >0.05 (since we analyzed 4 different groups) was considered to be statistically insignificant.

There were no statistical significant differences in distribution of *MMP-2 (–1306 C/T)* genotypes comparing atrophic AMD with soft and hard drusen, and control group as well (Table 2).

DISCUSSION

Studies analyzing the influence of MMP-2 on AMD development are scarce. Some studies analyzed the MMP-2 concentration in the blood[13], MMP-2 expression[14] and *MMP-2* polymorphism in different promoter regions[15-16] and results of these studies are inconsistent. However, to our knowledge, there are no studies analyzing associations between *MMP-2* gene polymorphism and AMD phenotype. Price *et al*[6] determined a mutation which causes an increase in promoter activity. The C-to-T allelic variation located at nucleotide -1306 disrupts the Sp1-binding site in the promoter region leading to significantly lower transcriptional activity; therefore, T allele has a markedly lower promoter activity than the C allele, where promoter loses 50% of activity of the gene. So it was hypothesized that *C/C* genotype, which causes higher gene expression, might participate in exudative AMD development, and *T/T* genotype, which causes lower expression, might participate in geographic atrophy AMD development. Mostly, exudative AMD develops from soft drusen, and geographic atrophy from hard drusen. These results demonstrate that *MMP-2 (–1306) C/C* genotype was more frequent in AMD patients with hard drusen than the control group (66.43% *vs* 54.94%, P=0.017), and in AMD patients with hard drusen than soft drusen (66.43% *vs* 53.74%, P=0.03). Logistic regression analysis revealed that the *MMP-2 (–1306) C/C* genotype was associated with 1.7-fold greater possibility to develop hard drusen in AMD patients. These results proved that *MMP-2 (-1306 C/T)* gene polymorphism *C/C* genotype was associated with AMD phenotype with hard drusen. Our study supports the role of matrix MMPs and their polymorphisms in hard drusen formation, and we are in agreement with Hyman *et al*[5] which suggested that MMP-2 activity correlates to the increase in collagen deposition and, potentially, subretinal deposit formation.

We hypothesized that *T/T* genotype, which causes lower expression, might be associated with hard drusen development; however, the results were opposite. Maybe it can be explained by drusen phenotype changing over time as the disease progresses, thereby confounding the phenotype and stage of the disease.

To detect the phenotypical influence of genetic polymorphisms a greater number of patients may be needed.

To our knowledge, currently there are only three studies analyzing *MMP-2* (*–1306*) *C/T* gene influence on AMD development[10,15-16] but not to AMD phenotype. Two studies found no association between AMD[15-16]. Seitzman *et al*[15] analyzed *MMP-2 (-1306) C/T* gene polymorphism in females with AMD and did not find any association between *MMP-2* and early or late stage AMD in older women. Ortak *et al*[16] also analyzed genotype distributions and allelic frequencies of *MMP2 (-1306 C>T)*. They did not find significant differences in either genotype distribution or allelic frequencies of *MMP2 (-1306 C>T)* among the patients with dry AMD, wet AMD and the control group[13]. However, the latter study did not analyze *MMP2 (-1306 C>T)* association with AMD phenotype, but analyzed the association between dry AMD, wet AMD and the control group[16], and, is in disagreement with our study, we found association with *MMP-2 (-1306) C/C* genotype and hard drusen. We hypothesized that this genotype might be associated with only hard drusen development but not with exudative or atrophic AMD, because others studies did not find any association with this genotype and latest forms of AMD[15-16]. The third study found a significantly more frequent distribution of the *C/C* and *C/T* genotypes in the patients with AMD younger than 65y and those aged ≥65y, respectively. Moreover, the AMD women aged <65y had the *C/C* genotype significantly more frequently than the controls of that age[10]. There are studies analyzing MMP-2 concentration in blood[13,17], and the choroidal neovascular membrane[18], or another genotype (*MMP-2, rs2287074*)[15]. In short, these studies found no association between circulating MMP-2 and AMD development, although low expression of MMP-2 in the corneal neovascularization (CNV) membrane of mice and Bruch's membrane-choroid preparations in human donors eyes was detected in the other studies[14].

Summarizing gene polymorphism data on *MMP-2 (-1306) C/T* gene polymorphism, it was found to have a significant part in the development of hard drusen in patients with early AMD.

Further advances in research about the MMP genotype-phenotype correlation in AMD may prove valuable for better understanding of the genetic influence on the pathogenesis of AMD, developing new prophylactic and therapeutic measures, and, ultimately, careing for patients in a more cost-effective individualized, way.

In conclusion, the *MMP-2 Rs24386 (C-->T)* polymorphism was found to be associated with the development of hard drusen in patients with AMD but no association was found in the group of geographic atrophy.

This study had strengths. To our knowledge, this is the first study to have looked at the association between *MMP-2 (-1306 C/T)* genotype and phenotype characterized by soft and hard drusen and geographic atrophy.

This study had several limitations. In future, these results need to be replicated in larger studies, with bigger sample sizes in particular, to confirm the association with late AMD and to determine whether the association might differ among wet AMD and early AMD, and to include classification into 12 subgroups based upon the International Classification and Grading System. The second of our study limitations is absence of fundus autofluorescence (FAF) imaging, because fundus autofluorescence is valuable for AMD progression detecting. It is known that fundus autofluorescence imaging allows *in vivo* analysis of the dynamics of accumulation and degradation of lipofuscin in the retinal pigment epithelium (RPE) in eyes with AMD and documentation of metabolic activity of the RPE[19]. Domalpally *et al*[20] reported that geographic atrophy may be detected earlier by the use of FAF images. Progression of geographic atriphy area is comparable between color photographs and FAF images, but evaluating involvement of the center of the macula may differ, probably because of macular pigmentation blocking autofluorescence. FAF is relevant for imaging in diagnosis and monitoring of inherited retinal dystrophies, early AMD, geographic atrophy and central serous chorioretinopathy[21].

In conclusion, the *MMP-2 Rs24386 (C-->T)* polymorphism was found to be associated with the development of hard drusen in patients with AMD and logistic regression analysis revealed that *MMP-2 (–1306) C/C* genotype increased the likelihood of developing hard drusen in AMD patients (OR=1.7, 95% CI: 1.06-2.74; *P*=0.028) but no association was found in the group of geographic atrophy.

ACKNOWLEDGEMENTS

Foundation: Supported by Lithuanian Science Council (No. MIP-10330).

Conflicts of Interest: Liutkeviciene R, None; **Lesauskaite V,** None; **Sinkunaite-Marsalkiene G,** None; **Simonyte S,** None; **Zemaitiene R,** None; **Kriauciuniene L,** None; **Zaliuniene D,** None.

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