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Chapter 4

Influence of Matrix Metalloproteinases MMP-2, -3 and on Age-Related Macular Degeneration Development

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Abstract

Age-related macular degeneration (AMD) is the leading cause of significant and irreversible central visual loss as it affects a small area of the retina, called the macula. However, the pathogenesis of still fairly understood. AMD has a multifactorial etiology, and its development might be influenced by body peculiarities, environmental and genetic factors. Risk factors such as age, gender, cigarette smoking, color of iris, nutrition, body mass index, oxidative stress, and genetic factors (complement factor H gene, Apo E gene, matrix metalloproteinases (MMPs) genes and others) increase probability to develop AMD. Here, we discuss about choroidal neovascularization process, where hypoxia, inflammatory process, and proteolytic enzymes play a main role, but mainly we focus on the family of matrix metalloproteinases (MMPs), especially on MMP -2, -3 and -9, and their impact on AMD development. MMPs belong to a family of proteolytic zinc-containing enzymes, and their mechanism under normal physiological conditions is precisely regulated, but when is dysregulated, MMPs become a cause of various diseases, including and AMD. MMPs are capable of degrading most of the extracellular matrix components, which are important in the remodeling during angiogenesis. Angiogenesis is the main pathological process associated with age-related macular degeneration development. Activated endothelial cells release MMPs which by degrading the basilar membrane allows capillaries to grow beneath the retina and retinal layers. Such capillaries often bleed, more liquids are filtered through the walls, and fibrous tissue grows within. Furthermore, swelling of the retina and impaired vision occur. In this book chapter, we focus on AMD prevalence, risk factors, clinics, diagnostics and influence of MMP-2, -3 and -9 on AMD development.

Keywords: AMD prevalence, risk factors, influence of MMP-2, -3 and -9

1. Introduction

Age-related macular degeneration (AMD) is a multifactorial disorder influenced by interaction between genetic and environmental risk factors. The most important pathogenetic mechanisms which cause AMD are the formation of drusen, hypoxia, local inflammation, and later, neovascularization. The development of neovascularization, mainly induced by retinal hypoxia, is a hallmark of AMD and its blockade has been considered as an inhibition of AMD development. Tissue ischemia leads to an increased secretion of the vascular endothelial growth factor (VEGF) and higher expression of the VEGF receptor 2. Vasodilatation induced by VEGF enhances vascular permeability and protease activity that results in developing and expansion of vascular network of the surrounding tissues and its remodeling [1, 2]. The fragmentation of a basilar membrane and an intracellular connective tissue are essential for the formation of new capillaries. Activated endothelial cells release various enzymes such as matrix metalloproteinases (MMPs) which degrade the basilar membrane, allowing capillaries to grow beneath the retina and between retinal layers. MMPs, which are found in all organisms, are endopeptidases which contain an active site Zn^{2+} and are divided into subfamilies of clans based on evolutionary relationships and structure of the catalytic domain. MMPs comprise a family of currently 25 related, yet distinct vertebrate gene products, of which 24 are found in mammals [3, 4]. MMPs are mainly classified into collagenases (MMP-1, -8, -13), gelatinases (MMP-2, -9), stromelysins (MMP-3, -10), membrane-type MMPs (MMP-14, -15, -16, -17), and others [2, 4].

2. Prevalence of age-related macular degeneration

All researchers agree that AMD is the most common cause of blindness in developed countries [1, 2]. The Lithuanian Medical Social Expertise Commission announced that in 2002, there were 13.8% of blind people due to AMD in Lithuania. The Blind Register Center reported that in Great Britain, nearly 50% of people live with blindness caused by AMD [5]. More than 30% of adults aged 75 years or older have AMD, and in about 6–8% of them, the disease would progress and cause the most severe visual loss [2]. Epidemiological studies in Australia, Europe and North America showed that the prevalence of AMD in the group of 55- to 64-year-old patients is about 0.2% and it increases to 13% in 85 years old patients group [3]. Studies showed that AMD can be diagnosed for people even younger than 40 years old [6]. The prevalence of AMD in a black population was 2.4%, in Spanish—4.2%, in Chinese—4.6%, and in whites—5.4% ($p < 0{,}001$, statistically significant between all groups). The highest prevalence of AMD is determined in 75–84 years old patients group, and it may vary from 7.4% (in a black population) to 15.8% (in white and Chinese populations) ($p = 0.03$). Additionally, it was reported that prevalence of the late AMD in a black population was 0.3%, in a Spanish population—0.2%, in Whites—0.6% and in Chinese population—1.0%. Several studies revealed that AMD was diagnosed in 8.5% of 43–54 year old population, while in patients over 75 years old—37% of AMD cases were diagnosed [7]. It is predicted that from 1980 to 2020, the elderly population in the developed countries and the developing countries will increase by

186 and 356%, respectively [1]. It was stated that older age is a natural risk factor for development of AMD; thus, blindness might experience increasing numbers in the older population. The World Health Organization predicts that in 2020, in the population over 60 years of age, the number of people with vision loss due to eye and vision-related problems would reach approximately 54 million [1]. Older blind or visually impaired persons are increasing in numbers as the population over the world is growing very rapidly. Today, there are over 6 billion people and by 2020, the population all over the world might reach up to 8 billion. Additionally, this increases a life expectancy of women and men, especially in the developing countries. It is predicted that during next 20 years, the population of people over 60 years of age will double from 400 to 800 million in the world [5].

3. Risk factors for age-related macular degeneration development

Various risk factors (modifiable and non-modifiable) such as smoking, obesity, age, gender, and others are associated with AMD development. Epidemiological studies have shown that genetic predisposition, systemic factors, lifestyle, environmental risk factors, age, and others may play a role in AMD development. However, association of environmental and genetic factors and gene-environment interactions with risk, have been reported to be closely related with the development of AMD [8]. Age is the strongest known risk factor. The older the individual, the higher AMD risk [9–11]. Additionally, a gender has a big impact on AMD development as it was determined that women have a higher risk to develop AMD compared to men [12]. People with blue iris have the higher possibility to develop AMD than people with other iris colors [13]. The meta-analysis of the prospective cohort and cross-sectional studies suggested that darker (brown) iris pigmentation was protective, however, the overall results were not significant [13]. Moreover, ethnicity may influence AMD development. Caucasians are more often diagnosed with AMD than black people. Wong et al. reported a higher prevalence of early and any AMD in Europeans than Asians or Africans as in geographical regions; cases of early and any AMD were less prevalent in Asia than in Europe and North America [11]. Controversial results by different studies have been reported on a risk factor such as sunlight. One study found no association between AMD and sun exposure or related factors except for an association between sunburn prone skin type and geographic atrophy which reached borderline significance [14], while the other study concluded that AMD was probably related to visible radiation, especially blue light [15]. Smoking is another significant and modifiable factor. Many studies have determined the influence of smoking on AMD formation and demonstrated that former and current smokers are inclined to develop AMD at least 5–10 years earlier than nonsmokers [16]. Higher systolic blood pressure, overweight and obesity, and physical exercise duration and frequency are associated with late AMD in women only [17]. The prevalence of AMD is significantly higher in patients with myocardial infarction (MI) than in a simple random sample of the population [18]. It was established that prevalence of early AMD in the random sample was 7.3%, while in MI patients, it was 54.5% ($p < 0.001$). AMD increases more with age in females (3.7 and 10.8% at the age 45–54 and 55–64 years, $p < 0.05$, respectively) while in males, frequency of AMD did not differ significantly

between latter age groups (9.9% vs. 11.6%; $p > 0.05$) [18]. Increased intake of fish reduced the risk of AMD, particularly for two or more servings per week. Dietary omega-3 fatty intake was inversely associated with AMD comparing the highest vs. lowest quartile. Reduction of risk for AMD with higher intake of omega-3 fatty acids was seen primarily among subjects with low levels (below median) of linoleic acid intake, an omega-6 fatty acid [19].

Oxidative stress is believed to be a major mediator of the effect of age as mitochondrial oxidation impairment with aging and oxidative damage is widely observed. Oxidative stress and the production of reactive oxygen species seem to play a pivotal role in AMD pathogenesis [20]. The levels of inflammatory markers, such as serum high-sensitivity C-reactive protein, tumor necrosis factor-α receptor 2, interleukin-6, and soluble vascular cell adhesion molecule-1, in blood, were moderately associated to the 20-year cumulative incidence of early AMD independent of age, smoking status, and other factors [21].

More recently, the data from the Age-Related Eye Disease Study showed that cataract surgery is safe in the setting of dry AMD and no accelerate progression to advanced sight threatening forms of AMD were observed [22]. There is a probable relationship between cataracts and the aging process, manifesting in cataract formation with partial nuclear sclerosis and AMD. Some researchers found no link between the cloudy lens and AMD, whereas the others have revealed a relationship between the lens nuclear sclerosis and AMD. West et al. and Klein et al. suggested that nuclear sclerosis of the lens than cortical cloudiness is more often observed in the patients with AMD [23–25]. Progression of AMD in the operated eye due to cataract was more commonly observed than in the patients without the intervention. Moreover, late AMD in the operated eyes developed within 5 years after operation [24].

Levels of vitamin D serum are inversely associated with early, but not advanced, AMD. Consistent use versus non-use of vitamin D from supplements was inversely associated with early AMD only in individuals who did not consume milk daily [26]. Increased blood levels of homocysteine are associated with increased risk of AMD [27].

A whole genome study of the patients with AMD has determined that the complement H factor gene haplotype increases the possibility of developing AMD [28]. Gold B et al. have studied two independent cohorts consisting of 900 patients with AMD and 400 control group persons, and genetic lesions of two complement system factors, i.e., the variants of the genetic factor B (BF) (6p21.3) and the second complement factor (C2) (6p21.3) [29]. The gene of apolipoprotein E (Apo E) was found to be associated with development of AMD. Apo E, which codes the plasma protein participating in the metabolism of cholesterol and other lipids [30], is determined in drusen [31, 32]. The second major locus of the risk of AMD development is linked to genes *HTRA1* and *ARMS2* [33]. In 2013, a genome-wide association study identified seven new loci near genes *COL8A1-FILIP1L*, *IER3-DDR1*, *SLC16A8*, *TGFBR1*, *RAD51B*, *ADAMTS9*, and *B3GALTL* [34].

In recent years, the blockage of the neovascularization chain has been considered to inhibit the development of AMD. The vascular endothelial growth factor (VEGF) and the fibroblast growth factor are believed to promote the angiogenesis [35]. Meanwhile, it is inhibited by the pigment epithelial factor, angiostatin, endostatin, and other enzymes. The neovascularization is mainly induced by retinal hypoxia. Tissue ischemia leads to increased secretion of the

VEGF and higher expression of the VEGF R2. Vasodilatation induced by VEGF enhances vascular permeability and protease activity that results in developing and expansion of vascular network of the surrounding tissues and its remodeling [36]. The fragmentation of a basilar membrane and an intracellular connective tissue are essential for the formation of new capillaries. Activated endothelial cells release matrix metalloproteinases which, by degrading the basilar membrane, allow capillaries to grow beneath the retina and between retinal layers, creating favorable conditions for AMD development.

4. Age-related macular degeneration clinics and diagnostics

During the initial stage of AMD, first symptoms of the disease include blurred or fuzzy vision. Patients complain that in Amsler grid of straight horizontal and vertical lines appear wavy, blurred or distorted or boxes in the grid look square and in the different size. Later, in the center of the visual field appears dark area which interferes with vision [37–39]. First symptom of the dry AMD is blurred vision which later would transform into central scotoma, e.g., a black spot in the center of the vision field. This is especially noticeable while reading or looking at objects closely. In the beginning, the disease is asymptomatic and changes in the eye fundus would not cause any complains to the patient. A dry form of AMD usually advances slowly and it takes long months to notice the changes of vision. Sometimes dry form of AMD shifts to the wet form. An exudative AMD is associated with sudden loss of vision, sometimes in a few days' time. Up to 90% of all exudative AMD cases would lead to the total blindness. The Amsler grid test is a simple test which is used at home to check whether lines look wavy or distorted, or areas of the visual field are missing. If any of these changes are detected, the ophthalmologist should be contacted immediately. Examination of retina's central part (the macula) is performed by using the Amsler grid. Each eye is tested separate, and the patient holds the Amsler's grid approximately 40 cm from his eyes and looks to a spot in the middle of a standard grid. It is observed whether there are no wavy or invisible places, and crooked or patchy thickness lines. If a patient sees straight lines, the test is evaluated as negative. Positive test usually is confirmed for patients with the advanced AMD; thus if any complains occur, the ophthalmologist should be contacted and treatment should be started. A detailed anamnesis is needed for an accurate diagnosis of AMD. It would help to identify a cause of a condition and risk factors which may influence the AMD development. An ordinary eye examination starts from an evaluation of eyesight sharpness which abroad is evaluated by using a chart of an Early Treatment Diabetic Retinopathy Study (ETDRS), while in Lithuania—the Snellen chart. ETDRS is an accurate (in case of low visual acuity) with a small error, objective, vigorously scientific method [40]. It is important constantly to check visual acuity for a comparison of previous checkup results. Various diagnostic ophthalmological methods are used to diagnose AMD: perimetry, Amsler's grid testing, functional acuity contrast sensitivity, direct and indirect ophthalmoscopy, scanning laser ophthalmoscopy, color fundus photography with blue and red light filters, fluorescein angiography or with indocyanine green and histological research and electronic biomicroscopy, optical coherent tomography. After performance of color photograph of eye fundus, the progression of AMD should be followed (**Figure 1(a)** (a hard drusen in right eye fundus) and **(b)** (a soft drusen in the left eye fundus)). AMD diagnosis

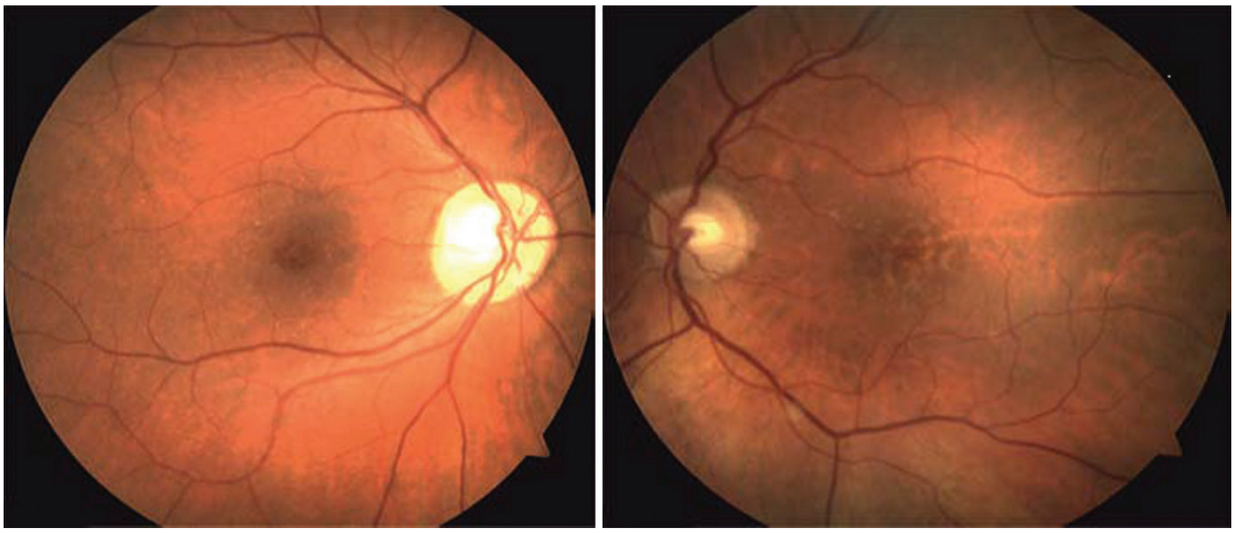

Figure 1. (a) Hard drusen in the right eye fundus and (b) soft drusen in the left eye fundus.

is confirmed after evaluation of clinical symptoms, (and patient's complains) and examination of eye fundus after pupil dilatation with mydriatics (specific changes are checked for in the central retina part). Amsler's grid test and a perimetry (visual field test) are performed to evaluate central eyesight changes. Fluorescein angiography shows a presence of abnormal new blood vessels. The latest method for the confirmation of AMD diagnosis is optical coherent tomography (OCT). This is a non-invasive method, allowing to get good resolution retina photography *in vivo*, to monitor the dynamics of diseases of retina and facilitating diagnostics [41]. OCT provide quantitative and qualitative information on retina condition. Today, OCT is one of additional tools besides eye fundus photography and fluorescence angiography. However, it is step-by-step used instead of previously mentioned methods, especially in monitoring of diseases dynamics during treatment and for determination of retina disease stage [40].

5. Diagnostics

5.1. Impact of metalloproteinase structure, activity, and mutation on activity regulation of matrix metalloproteinases (MMP-2, MMP-3, and MMP-9)

The structure of all MMPs identified is similar (**Figure 1**). The main components of MMP molecule are:

- signal sequence, which is important for MMPs release from cell propeptide, to inactivate a signal sequence of *MMP*;
- catalytic metalloproteinase domain, which include Zn^{2+} ion, essential for enzyme activity;
- axial peptide, which connects catalytic domain with hemopexin domain; and
- hemopexin domain, which determines MMPs possibility to cleave an appropriate substrate [42, 43].

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Expression of most MMPs in tissues under normal conditions is low and it is induced when remodeling of extracellular matrix (ECM) is required. Various factors might induce MMPs production: cytokines, growth factors, physical stress, cell-extracellular matrix and cell-cell interaction. Westermarck et al. found that there are four mechanisms of action of matrix metalloproteinases:

1. MMPs may affect cell migration by changing the cells from an adhesive to non-adhesive phenotype and by degrading the ECM;
2. MMPs may alter ECM microenvironment leading to cell proliferation, apoptosis, or morphogenesis;
3. MMPs may modulate the activity of biologically active molecules such as growth factor or growth factor receptors by cleaving them or releasing them from the ECM; and
4. MMPs may alter the balance of protease activity by cleaving the enzymes or their inhibitors [44].

Activation of MMPs expression could be caused by various gene polymorphisms in promoter region, when a binding place of transcription factors or other regulating elements is disrupted. *MMP* polymorphisms can be caused by nucleotide changes within promoter region by insertions, substitutions or microsatellite instability [45]. Around 90% of cases, a single nucleotide polymorphism is determined where one of the basic changes appears in DNA strain [46]. However, several allele polymorphisms can be determined in *MMP* gene promoter regions. Most parts of detected polymorphisms are not biologically active. Only a small part of polymorphisms which changes gene transcription intensity is biologically active; therefore, they may have an impact on genetic predisposition to certain diseases [45]. A common variant in the promoter region of the human matrix metalloproteinase-3 (*MMP-3*) gene with 1 allele having a run of 5 adenines (5A) and the other having 6 adenines (6A) has an impact on gene expression. *MMP-3* gene is located in chromosome 11 11q22.2-11q22.3 region. Insertion of one adenine (A) in -1171 base-pair position of *MMP-3* promoter caused 6 adenines (6A) formation instead of 5 adenines (5A). It was shown that 6A allele has a higher binding affinity to ZBP-89 transcription factor, which decreases promoter transcription activity and certain gene expression [47]. *In vitro* methods showed that 5A allele has a higher activity and effect on gene expression compared to 6A allele [48]. *Ex vivo* method showed that *MMP-3* mRNA and protein activity depends on genotype: 5A/5A shows the highest activity, 5A/6A—the middle activity and the lowest activity shows 6A/6A genotype [47, 48]. A mutation (NCBI SNP identification no. rs2285053) which causes an increase in promoter activity was determined in the *MMP-2* (-735) gene promoter transcription region. *MMP-2* gene is located in 16q13-q21 region. The C to T allelic variation located at nucleotide -735 disrupts the Sp1-binding site in promoter region and significantly leads to a low transcriptional activity; therefore, T allele has a markedly lower promoter activity than the C allele [49]. In addition, another C to T allelic variation located in *MMP-2* at nucleotide -1306 (NCBI SNP identification no. rs243865) disrupts the SP1-binding site of transcription factor in promoter region. It is a similar effect as it happens for *MMP-2* (-735) gene promoter transcription region mutation [50], where promoter loses 50% activity [51]. A transition of C to T at the 1562 base-pair position upstream of the

transcription initiation site (-1562 C/T) of *MMP-9* (NCBI SNP identification no. rs3918242) has shown to have an effect on promoter activity. Transition of C nucleotide to T nucleotide causes more difficulties for nucleic protein complex bind to DNA strain in the presence of T allele. It was determined that once C allele mutates to T allele, a promoter activity increases 1.5 times [52]. *MMP-9* gene is located in 20q11.2-q13.1 region. Matrix metalloproteinases are involved in vascular remodeling, and these appear to be active agents degrading extracellular matrix proteins. Their expression in transcription level depends on gene promoter mutations and various transcription factors.

6. Expression of matrix metalloproteinases in human retina and choroid

Bruch's membrane is a pentalaminated extracellular matrix allowing bidirectional diffusion pathways between the retinal pigment epithelium and the choroidal blood supply. Aging is associated with progressive thickening of retina due to deposition of matrix components and membranous debris rich in lipids. A consequence of the aging process is an exponential decline in the hydraulic conductivity of Bruch's membrane [53]. Hemato-retinal barrier might be disrupted only when lesion in Bruch's membrane or in retinal pigment epithelium occurs. Li et al. found that MMP-3, and MMP-2 and -9 were present in human Bruch's membrane, and that the level of the two inactive gelatinases increased with the age of the donor. Regional differences were apparent in the levels of the two gelatinases. The level of MMP-9 remained invariant, while MMP-2 was lower in the macular region than in the periphery [54]. Given that the thickness of Bruch's membrane increases with age and that of choroid decreases, the observed increase in MMP levels is likely to occur mainly in Bruch's membrane. Cultured retinal pigment epithelium (RPE) cells have been reported to synthesize and secrete MMP-1, -2, -3, and -9, and TIMPs as well. The origin of the various MMPs found in Bruch's membrane and choroid remains unknown. The three potential sources are: (1) RPE cells, (2) choroidal cells, and (3) plasma in the choroidal vessels [55, 56].

There are two pathways whereby these enzymes may be incorporated into Bruch's membrane. First, the enzymes may be released from plasma, RPE, and/or choroidal cells and then diffuse into Bruch's membrane. This is certainly a possibility for the smaller molecular weight forms such as MMP-1 (52 kDa), MMP-2 (65 kDa), and MMP-3 (57 kDa), because the molecular weight exclusion limit for Bruch's membrane is approximately 65–75 kDa. Second, the release of MMPs may be coincident with the synthesis of structural components of Bruch's membrane and, therefore, may be incorporated passively into the ECM of Bruch's membrane. Such a pathway would allow an incorporation of higher molecular weight enzymes such as MMP-9 [57].

MMP-1, MMP-2, MMP-3, and MMP-9 expressions are regulated by various ways such as transcription level, activation of latent MMPs, and inhibition of MMP activity by tissue inhibitors of metalloproteinases (TIMPs) [58]. TIMPs are known as natural tissue inhibitors, which regulate active and non-active balance of MMP forms. MMPs are initially expressed in an enzymatically inactive state due to the interaction of a cysteine residue of the pro-domain with the zinc ion of the catalytic site. Only after disruption of this interaction by a mechanism called

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cysteine switch, which is usually mediated by proteolytic removal of the pro-domain or chemical modification of the cysteine residue, the enzyme becomes proteolytically active. Choroidal neovascularization (CNV) is associated with an upregulation of MMP-9 at the transcriptional level and an activation of pro-MMP-2 by MT1-MMP. This process might be blocked by physiological/natural (TIMP-1 and TIMP-2) and also synthetic inhibitors [59].

MMPs are thought to play a key role during the early phases of choroidal neovascularization. The synthetic inhibitor interacting preferentially with MMP-2, MMP-9, and MT1-MMP (MMP-14) is more efficient comparing to a broad-spectrum synthetic inhibitor in case of choroidal neovascularization. MMPs might have contrary functions—induce or block choroidal neovascularization development [59].

7. Matrix metalloproteinases (MMP-2, -3, and -9) association with age-related macular degeneration

Studies on the morphogenesis of AMD draw attention to the role of MMPs. These studies have confirmed that ECM dysmetabolism plays an important role in the pathogenesis of AMD [60, 61] and metabolism of the ECM is closely regulated by MMPs [62]. The pathogenesis of age-related macular degeneration is mostly focused on MMP-2 and MMP-9, due to their ability to split gelatin *in vitro.*

There are no many studies analyzing MMP-2 influence on AMD development. Some studies analyzed MMP-2 concentration in the blood, some MMP-2 expression, and some analyzed genes' polymorphism in different promoters' regions. To our knowledge, currently there are only two studies analyzing *MMP-2* gene (-1306) C/T polymorphism influence on AMD development [63, 64]. The study done by Seitzman et al. analyzed *MMP-2* (-1306) C/T gene polymorphism in females with AMD, where association between *MMP-2* and early or late AMD in older women was not found [63]. The following study done by Ortak et al. also analyzed genotype distributions and allelic frequencies of *MMP2* (-1306C > T). No significant differences in either genotype distribution or allelic frequencies of *MMP2* (-1306C > T) were found among the patients with dry AMD, wet AMD, and control group [64]. An allele of *MMP-2* rs2287074 was less prevalent in subjects with late AMD than in those with early or no AMD ($p = 0.01$) [63]. The third study also proved that analysis of *MMP-2* (-1306 C/T) gene polymorphism has not revealed any differences in the genotype distribution between patients with early AMD and reference group subjects when analyzed in overall groups, but *MMP-2* gene C/C genotype was more frequent in AMD patients younger than 65 years comparing to AMD group ≥65 years (67.21% vs. 49.37%, $p = 0.039$), and C/T genotype was more frequent in AMD patients ≥65 years comparing to AMD patients <65 years (26.23% vs. 44.3%, $p = 0.033$) [65]. MMP-2 expression in experimental models [66] and in Bruch's membrane-choroid preparations in human donors eyes with AMD diagnosis also were analyzed [67]. Berglin et al. detected low expression of MMP-2 in choroidal neovascularization membrane of mice [66]. In consistent, other scientists group also found a significant reduction in the development of laser-induced CNV in MMP-2 knockout mice [68]. Hussain et al. demonstrated that the total level of active

MMP-2 was significantly reduced in Bruch's membrane-choroid preparations of human donor eyes with AMD [67]. As positive association between MMP-2 expression and choroidal neovascularization was observed, in contrary, a potentially protective role of MMP-2 in dry AMD was suggested. As noted above, estrogen depletion in ovariectomized mice resulted in a loss of MMP-2 expression and subsequent changes associated with dry AMD, such as sub-RPE deposit formation and Bruch's membrane thickening occurred [69]. In others two studies [33, 70], there were no differences in MMP-2 concentration found between AMD and control group. MMP-2 levels in human plasma among healthy individuals, AMD patients, and exudative AMD patients gave a confirmation that the mean concentration of MMP-2 in the early and neovascular AMD was not significantly different from that of the control group [70].

MMP-3 is a key member of the MMPs family and plays a central role in the physiological and pathological events associated with connective tissue metabolism and remodeling [71, 72]. Only few studies have been conducted to clarify if MMP-3 has an influence on retinal vascular remodeling and stiffening, and plays a role in the development of AMD. Literature data concerning MMP-3 effect on AMD are scarce and inconsistent. Some results reveal a possible MMP-3 effect on AMD pathogenesis [73], and at the same time are in conflict with controversial data from the other study assuming that MMP-3 expression did not play a role on AMD development [74]. The study analyzing *MMP-3* gene polymorphism on age-related macular degeneration development in patients with myocardial infarction was carried as well. The study results revealed that *MMP-3* gene polymorphism did not have any predominant effect on the development of AMD in patients with myocardial infarction [75]. German study showed that MMP-3 expression in the retinal pigment epithelium was induced by oxidative stress. It is known that oxidative stress is one of the risk factors for the development of AMD, and it is possible that MMP-3 might affect the development of AMD in this way [73]. Swedish researchers conducted a study where the expression of several MMPs, including MMP-3, was analyzed, but no data suggesting MMP-3 involvement in the development of AMD were found [75].

The studies analyzing an association between MMP-9 and AMD are inconsistent as well. In a few studies, a reduction in MMP-9 was found in choroidal neovascular membranes [76] and in serum [33], while other studies showed an increase in MMP-9 in the aqueous humor [77], plasma [78], and choroidal neovascular membranes [79]. To our knowledge, only one study done by Fiotti et al. revealed the influence of the MMP-9 genotype, which causes greater gene expression on AMD [79]. This study found a relationship between the length of *MMP-9* gene promoter microsatellites and choroidal neovascularization in AMD patients. It has been determined that carriers of one allele with 22 repeats have more than double the risk of AMD. This polymorphism does not cause the disease but increases the MMP-9 expression leading to increased vascular permeability and choroidal neovascularization [80]. No difference between the major AMD risk factors (gender, age, diabetes mellitus, cigarette smoking, and dyslipidemia) and *MMP-9* polymorphism was found. The logistic regression analysis showed that the status of carrier of a microsatellite 22 repeats was the only variable entering into the equation ($p = 0.011$). The only one association was high body mass index value which is linked to a higher risk of developing AMD [80]. The number of cytosine-adenine (CA) sequences in the *MMP-9* gene promoter region was found to determine the transcription activity [45]. Studies with mice

mesangial cells have shown that 24 repeats of (CA) sequences in the *MMP-9* gene promoter region result in up to 20 times higher MMP-9 expression compared with 20 repeats of (CA) sequence [81]. Steen et al. suggests that MMP-2 and MMP-9 may be cooperatively involved in the progressive growth of choroidal neovascular membranes in AMD [74]. Lambert et al. demonstrated a significant reduction in the development of laser-induced choroidal neovascularization in MMP-9 knockout mice suggesting that MMP-9 may be important in the pathogenesis of AMD [76], and in Bruch's membrane-choroid preparations from donor eyes, the total level of active MMP-9 was significantly reduced too [67]. Interestingly, a recent study reported that MMP-9 was significantly elevated in the aqueous humor of patients with neovascular AMD [77] and in the plasma in AMD and CNV groups [70]. Zeng et al. showed different results and demonstrated no relationship between the increased levels of circulating MMP-9 and AMD [33]. Chau et al. found opposite results and proved that the mean plasma levels of MMP-2 were not significantly different in the three groups but, the mean plasma MMP-9 levels were significantly higher in AMD and CNV groups compared to that of the control group (265 ± 134, 659 ± 315, and 740 ± 494 ng/mL ($p = 0.008$)) [78]. To our knowledge, there is only one study analyzing the impact of *MMP-2, MMP-3*, and *MMP-9* genes polymorphism on the development of early AMD. This study proved that the frequency of the *MMP-2* (-735) C/T and *MMP-3* (-1171) 5A/6A genotypes did not differ significantly between the patients with early AMD and the control group, while the *MMP-9* (-1562) C/C genotype was more frequently detected in patients with AMD than the control group (73.7% vs. 64.6%, $p = 0.048$). The logistic regression analysis showed that the *MMP-9* (-1562) C/C genotype increased the likelihood to develop early AMD (OR = 1.51, 95% CI: 1.01–2.21; $p = 0.046$). After the subdivision into the groups by age, a significant difference only in the frequency of the *MMP-9* (-1562) C/C genotype was found comparing the AMD patients and the control group younger than 65 years (79.7% vs. 66.4%, $p = 0.039$) [65].

8. Conclusions

Age-related macular degeneration is a multifactorial disorder. Alteration of matrix metalloproteinases plays a very important role in AMD pathogenesis, especially in the early phases of choroidal neovascularization. During pathological process, MMP-2, MMP-3, and MMP-9 are present in human Bruch's membrane and RPE at different level and position, and are involved in the inflammatory process. MMP-2, MMP-3, and MMP-9 expressions are regulated by various ways: a transcription, activation of latent MMPs, and inhibition of MMP activity by tissue inhibitors of metalloproteinases. However, knowledge on MMP-2, MMP-3, and MMP-9 action in AMD pathogenesis is still controversial, therefore further research is necessary.

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