University of Thessaly, School of Medicine

Postgraduate Program M.Sc.

"Research Methodology in Biomedicine, Biostatistics and Clinical Bioinformatics"

THESIS TITLE

"The role of MMPs in intracranial aneurysms: a field synopsis"

«Η σχέση των μεταλλοπρωτεϊνασών και των ενδοκράνιων ανευρυσμάτων: ποιοτική ανασκόπηση της βιβλιογραφίας»

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ΠΕΡΙΛΗΨΗ

Εισαγωγή: Ενδοκράνια ανευρύσματα (ΕΑ) ονομάζονται οι παθολογικες διατάσεις των ενδοκράνιων αγγείων, με υψηλή επικινδυνότητα σε περίπτωση ρήξης. Οι μεταλλοπρωτεϊνάσες (ΜΜΠ) αποτελούν μια ομάδα πρωτεασών της εξωκυττάριας ουσίας και έχουν σημαντικό ρόλο στην εμφάνιση, εξέλιξη και ρήξη των ΕΑ. Συνεπώς, υπάρχει μεγάλο ενδιαφέρον για την συμβολή τους στην παθοφυσιολογία των ΕΑ και την πρόβλεψη της έκβασης.

Στόχοι: Σκοπός της μελέτης είναι η ανασκόπηση των δημοσιευμένων ερευνών σχετικά με τις ΜΜΠ και τα ΕΑ.

Μέθοδοι: Πραγματοποιήθηκε εκτενής αναζήτηση στο Pubmed και η βιβλιογραφία εξετάστηκε ως προς τη συνάφεια.

Αποτελέσματα: 81 μελέτες συμπεριλήφθηκαν στην ανασκόπηση μετά την εφαρμογή κριτηρίων αποκλεισμού. Υψηλή έκφραση των ΜΜΠ (κυρίως ΜΜΠ-2 και ΜΜΠ-9) έχει παρατηρηθεί σε ιστικά δείγματα ΕΑ, ενώ τα επίπεδα των ΜΜΠ σε ορό και εγκεφαλονωτιαίο υγρό έχουν συσχετισθεί με την ύπαρξη και έκβαση της ρήξης των ΕΑ. Υπάρχουν αμφιλεγόμενα συμπεράσματα σχετικά με τη συσχέτιση μονονουκλεοτιδικών πολυμορφισμών των γονιδίων των ΜΜΠ και των αναστολέων τους και τα ΕΑ. Τέλος, πειραματικά πρότυπα θηλαστικών έχουν χρησιμοποιηθεί για τη μελέτη των ΜΜΠ και φαρμακευτικών σκευασμάτων με ανασταλτική δράση στην εξέλιξη και ρήξη των ΕΑ.

Συμπεράσματα: Η παρούσα μελέτη αποτελεί ποιοτική συστηματική ανασκόπηση της βιβλιογραφίας, με περιορισμούς. Για ποσοτικά αποτελέσματα, μεγαλύτερες μελέτες και μετα-αναλύσεις πρέπει να λάβουν χώρα.

ABSTRACT

Introduction: Intracranial aneurysms (IAs) are pathological dilatations of the cerebrovasculature, hazardous in case of rupture. Matrix metalloproteinases (MMPs) are a proteases family of extracellular matrix, significant in initiation, development and rupture of IAs. Thus, there is great interest concerning their impact on IAs pathophysiology and their role as biomarkers predicting outcome.

Aim: The aim of the current study is to examine the studies published regarding the association between MMPs and IAs.

Methodology: An extended search was performed in Pubmed and the whole literature was examined.

Results: After applying exclusion criteria, 81 studies were included. Elevated MMPs (especially MMP-2 and MMP-9) expression has been noted in aneurysmal tissue, while serum and cerebrospinal fluid levels of MMPs have been associated with occurrence of IAs and outcome after rupture. Correlation between single nucleotide polymorphisms in MMPs and MMPs inhibitors genes and IAs has been controversial. Animal models have been used in order to assess the role of MMPs in IAs and pharmacological agents preventing the development and rupture of experimentally induced IAs.

Conclusions: The present study constitutes a qualitative systematic review of the literature concerning MMPs and IAs, with limitations. For quantitative results, larger scale studies and meta-analysis should be conducted.

INTRODUCTION

Intracranial or cerebral aneurysms (IAs) are pathological focal dilatations of the cerebrovasculature. Based on morphology, IAs are classified as saccular, fusiform and dissecting. Saccular IAs are the most common type and are described as “berry-like” aneurysms, while fusiform (or dolichoectatic) IAs constitute dilatations of the entire vessel wall. Dissecting IAs are formed by blood accumulation within the vessel wall. IAs may be asymptomatic, present with headache, focal neurological deficits or in case of rupture, with sudden onset headache and impaired consciousness. Rupture of an IA leads to intracranial bleeding and subarachnoid hemorrhage (SAH). SAH has a high rate of morbidity and mortality, commonly attributed to delayed cerebral vasospasm that unrarely occurs after rupture. The most dreadful consequence of cerebral vasospasm is delayed cerebral ischemia.

The gold standard for the diagnosis of an unruptured IA is angiography. If rupture occurs, computed tomography assists in the diagnosis of SAH, while post-SAH outcome is monitored with transcranial Doppler and magnetic resonance imaging. There is no approved medication for IAs treatment, except from pharmacological management of hypertension. Surgical clipping or endovascular techniques are necessary for the treatment of IAs, whereas in case of rupture, surgical intervention may be urgent and involve decompression of the subsequent cerebral edema.

The etiology of IAs formation is unknown. Although several genetic and acquired factors seem to attribute to their development and rupture, there is a distinct histological pattern of the cerebral vasculature that enables their initiation. Normal arterial walls present with three distinct layers: the intima, media and adventitia layer. Intima is lined by a layer of endothelial cells. An internal elastic lamina separates the intima from media, providing mechanical support. Smooth muscle cells are seen in the media layer. In intracranial arteries, there is no external elastic lamina between the media and adventitia layer, as it is found in extracranial arteries. Moreover, the adventitia of intracranial arteries is thinner than usual for extracranial arteries of the same size. These histological characteristics make cerebral arteries more prone to IAs.

IAs development has been associated with genetic components (ethnicity, gender and familial history), connective tissue disorders (such as Marfan and Ehlers- Danlos syndromes), substance abuse (alcohol, smoking, cocaine) and vascular diseases, mainly hypertension and atherosclerosis. These etiologic factors are assumed to cause hemodynamic alterations within the cerebrovascular system (Penn, et al., 2011). Hemodynamic and oxidative stress, along with pro-inflammatory genetic predisposition and various environmental hazardous factors, lead to pathological alterations in microvasculature

morphology. Thus, increased concentration of proteases is observed, as inflammatory cells infiltration occurs and endothelial structure and function are disrupted (Amenta, et al., 2015). As a result of the structural remodeling, the arterial wall is focally weakened and dilated, forming the lesion that is called an aneurysm.

Histology shows that all three layers of aneurysmal arterial wall are abnormal. Endothelial cells are found reduced in the intima, while collagen fibers are increased, throughout the wall. Smooth muscle cells of media are abnormally located and reduced, while inflammatory cell infiltrate into media and adventitia. Lipid deposition is also common in adventitia layer (Austin, et al., 1993). (Figure 1)

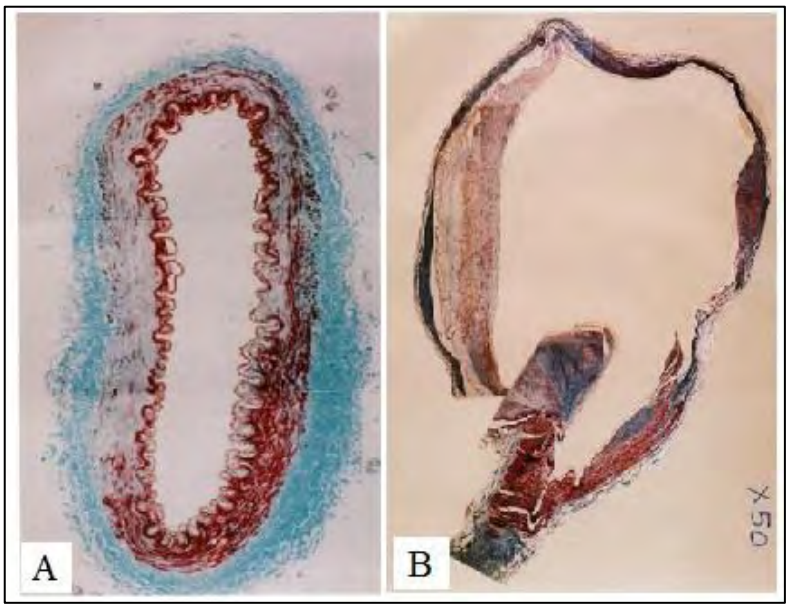

Figure 1. A) Trichrome stain of normal cerebral artery wall. Collagen is shown with green, while smooth muscle cells are red. B) Trichrome stain of an IA, presenting complete loss of architecture. (Austin, et al., 1993)

As presented, proteases play a key role in the development of aneurysms. Matrix metalloproteinases (MMPs) are a protein family that includes over 20 known members (Table 1) and is involved in extracellular matrix remodeling, by degrading macromolecular components of extracellular matrix. Extracellular matrix is a dynamic network of proteins and proteoglycans. MMPs have been linked to many functions, such as signaling, inflammation and angiogenesis. Within the cerebral microvasculature environment, these proteases are produced by smooth muscle cells, macrophages and glial cells (Amenta, et al., 2015). Because of their well-studied proteolytic activity in the extracellular matrix, abnormal MMPs function has been associated with many pathological conditions, including insulting vessels' integrity, thus inducing formation of aneurysms and rupture; a known mechanism in abdominal aortic aneurysms. MMPs seem to also play a key role in the vasospasm that may follow IA rupture and SAH.

Table 1. Members of matrix metalloprotease (MMP) family. (Yong, et al., 2001)

Member	Name	Member	Name
MMP-1	Collagenase 1	**MMP-16**	MT3-MMP
MMP-2	Gelatinase A	**MMP-17**	MT4-MMP
MMP-3	Stromelysin 1	**MMP-18**	Collagenase 4
MMP-7	Matrilysin	**MMP-19**	RAS I 1
MMP-8	Collagenase 2	**MMP-20**	Enamelysin
MMP-9	Gelatinase B	**MMP-21**	Xenopus MMP
MMP-10	Stromelysin 2	**MMP-22**	Chick embryo MMP
MMP-11	Stromelysin 3	**MMP-23**	
MMP-12	Metalloelastase	**MMP-24**	MT5-MMP
MMP-13	Collagenase 3	**MMP-25**	MT6-MMP
MMP-14	MT1-MMP	**MMP-26**	Matrilysin 2/ Endometase
MMP-15	MT2-MMP	**MMP-27**	Human MMP-22
MMP-16	MT3-MMP	**MMP-28**	Epilysin

MMP-4, MMP-5 and MMP-6 were excluded, as it is mentioned to be similar to other MMPs

MMPs are part of a larger protein family that includes structurally related zinc-dependent metalloproteinases, called metzincins. Other subfamilies of the metzincins are ADAMs, bacterial serralysins and the astacins. At the active site of the metzincins, three histidine residues are responsible for binding the zinc ion, while a distinct β-turn is present, delineated by a methionine residue ('met-turn'), playing an important role in protein activity (Yong, et al., 2001). Structurally, MMPs present with three different domains: an amino-terminal propeptide region, an amino-terminal catalytic domain (which contains the zinc-binding site) and a carboxy-terminal domain, which has a high level of similarity to members of the hemopexin family and includes four repeat units. The last two domains are connected via a hinge (Figure 2). MMP-2 and MMP-9 present a unique fibronectin type II-like domain in the catalytic site, while MT-MMPs have a transmembrane domain at the carboxy-terminal part. (Yong, et al., 2001).

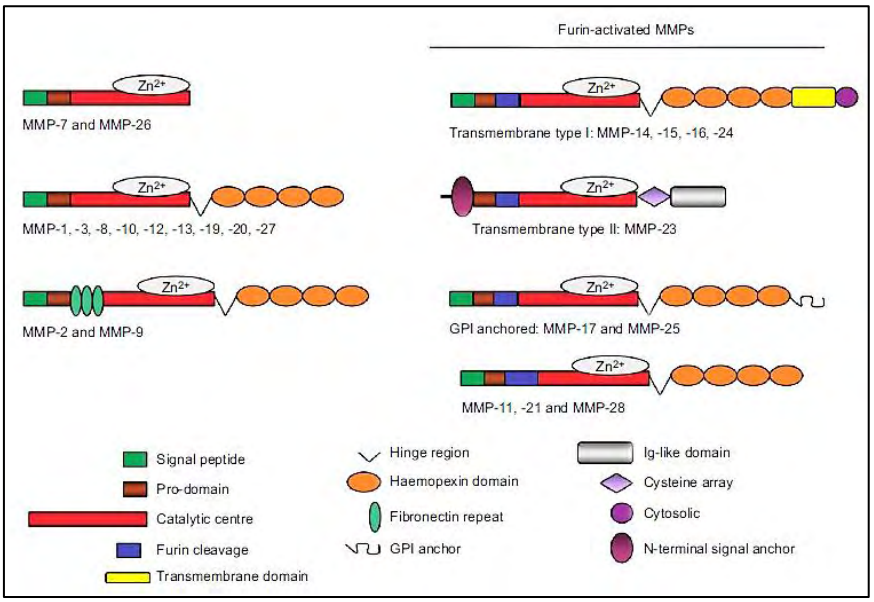

Figure 2. Structure of MMPs. Pro-domain includes "cysteine switch". GPI: glycosylphosphatidylinositol, Ig: immunoglobin (Löffek, et al., 2011)

MMPs regulation takes place at three steps (Figure 3A). **First regulatory step** is at the level of transcription, as most MMPs are expressed after cell activation/stimulation by inflammatory cytokines, growth factors, chemokins, oncogenes and cell–cell or cell–matrix interactions (Yong, et al., 2001). Post-translational modifications provide **the second step** of MMPs regulation. MMPs are secreted as inactive zymogens. Activating factors include the plasminogen– plasmin cascade, as well as other MMPs (Figure 3B) that disrupt the interaction between cysteine and zinc (the so-called 'cysteine switch' mechanism) and then remove the propeptide region for full activation (Van Wart & Birkedal-Hansen, 1990). Zymogens may be activated also by non-proteolytic compounds, such as sulphydryl-reactive agents, denaturants (urea) or heat (Yong, 1999). MT-MMPs are activated during secretion and appear on the cell surface in the active form (Yong, et al., 2001). **Third step of MMPs regulation** is the interaction of active MMPs with tissue inhibitors of metalloproteinases (TIMPs). Four TIMPs have been studied: TIMP-1, TIMP-2, TIMP-3 and TIMP-4. These molecules bind themselves to the catalytic site of MMPs, resulting in their inactivation. However, some TIMPs are involved in the activation process of MMPs (Yong, 1999). Imbalance between MMPs and TIMPs has been accused of excessive collagen and elastin breakdown within the extracellular matrix and arterial wall weakening (Amenta, et al., 2015).

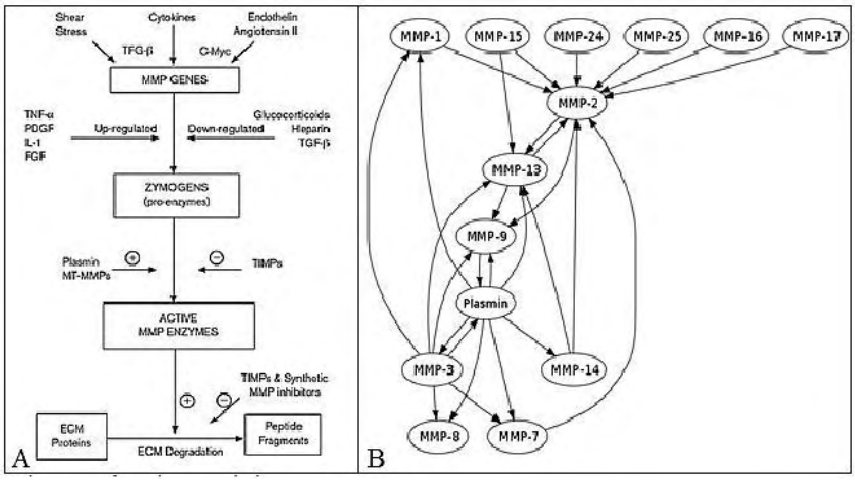

Figure 3. Regulation of MMPs. A) Steps of regulation (Ronco, et al., 2007). B) The activation interaction between MMPs. Author: M. Bauer, from Chakraborti et al. (2003) (Chakraborti, et al., 2003)

There is a great interest in the literature concerning the association between MMPs and the initiation and rupture of IAs. The aim of this study is to provide a qualitative synopsis of the published literature, concerning the impact of MMPs on IAs pathogenesis, development and rupture in human and experimental animal models. For that purpose, a systematic review of the published studies was conducted.

METHODS

The whole literature concerning MMPs and IAs was reviewed by an extended search in PUBMED, using the terms: “intracranial aneurysms”, “cerebral aneurysms”, “subarachnoid hemorrhage”, “matrix metalloproteinases”, “MMPs”, “tissue inhibitors of metalloproteinases” and “TIMP”, combined. Duplicates were removed using the Mendeley® reference management software. After duplicates removal, titles and abstracts of the remaining studies were examined for relativity. Studies that were not written in English language and did not produce an original outcome were excluded. Additionally, studies concerning experimentally induced SAH and common carotid aneurysm induction animal models were also excluded. All other published material was included. Last date of search was 01.09.2017.

RESULTS

Studies included in the systematic review

After the original search in Pubmed library, 850 articles were found, while 13 articles were found manually by other sources. Duplicates were removed and titles/abstracts of the remaining studies were reviewed. Studies with no relevant subject were excluded. This process resulted in the review of 134 full-text manuscripts, from which 53 were excluded based on reasons described in the Methodology.

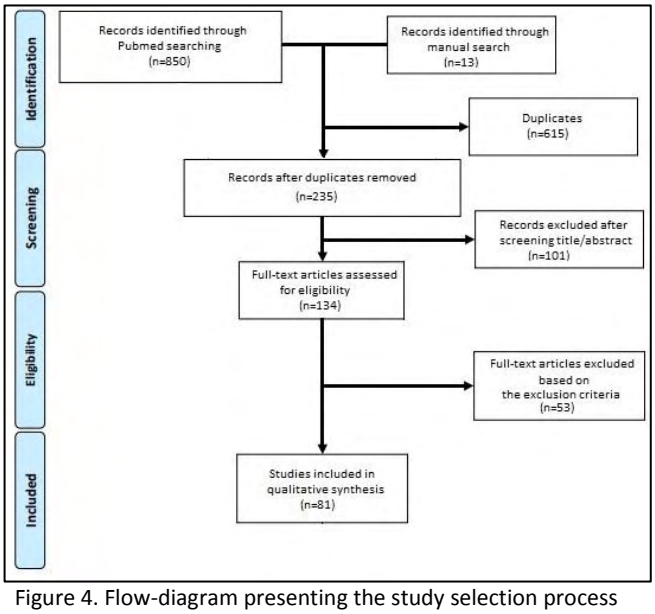

Figure 4. Flow-diagram presenting the study selection process

Wang’s et al. article (1999) with title: *“Analysis of coding sequences for tissue inhibitor of metalloproteinases 1 (TIMP1) and 2 (TIMP2) in patients with aneurysms.”* was excluded as it did not provide information on patients with IAs.

Human studies on IAs and MMPs

Many studies have been published concerning the association between IAs and MMPs. Multiple histological and molecular techniques have been used to assess the expression and activity of MMPs in tissue samples acquired during surgery, serum and cerebrospinal fluid (CSF), such as zymography, immunohistology, RT-PCR and western blotting, while the impact of genetic component has recently been investigated. One of the first published studies that associated IAs formation and rupture with excessive catabolism of extracellular matrix macromolecular components was written by Chyatte&Lewis in 1997. The authors concluded that there was a statistically significant threefold increase of native serum gelatinase activity in patients with IAs compared to control (Chyatte & Lewis, 1997). Approximately one year later, the same laboratory identified the increased serum gelatinase of a subgroup of IA patients as pro-MMP-2 (Todor, et al., 1998). Also in 1997, Kim et al. reported elevated expression of MMP-9 and TIMP in IAs tissue, compared to normal arteries, but no elevation of MMP-9 plasma concentration (Kim, et al., 1997). Significantly elevated concentration of serum elastase and increased IAs tissue activity of elastases and collagenases has also been mentioned in cases of ruptured IAs compared to unruptured IAs and control (Gaetani, et al., 1999)

In another study, MMP-2 was found focally expressed in more than half of the IAs studied, while rarely found in normal arterial tissue. Additionally, MMP-9 was expressed in both normal and IAs arterial tissue, however its expression was focally increased in some IAs. MMP-14 (MT1-MMP) was solely found in IAs tissue (Bruno, et al., 1998). Plasmin was also found focally expressed only in IAs; both MMP-14 and plasmin are known to upregulate the MMPs transcription, entering in a cascade of MMPs activation and active vessel remodeling. Elevated expression in IAs tissue and association between MMP-2 and NF-κ B factor (Cheng & Wang, 2013), as well as between MMP-2, MMP-9 and osteonectin (Li, et al., 2013) has also been mentioned in the literature, implying the multiplicity of MMPs activation pathways. Additionally, the association between atherosclerosis, IAs and MMPs expression profile has been studied, indicating that although both MMP-2 and MMP-9 expression was found elevated in IAs tissue, only MMP-2 was detected in non- atherosclerotic IAs (Caird, et al., 2006).

Two studies have been published, noting MMP-16 (MT3-MMP) (Li, et al., 2009) and TIMP-4 (Li, et al., 2009) significant increased and decreased expression in unruptured IAs tissue compared to control, respectively, while MMP-1 expression was examined in the study of Ameku et al. (2016) in relation to IAs in patients with autosomal dominant polycystic disease. MMP-1 expression was found elevated in endothelia, but decreased in smooth muscle cells derived from skin fibroblasts induced pluripotent stem cells. Serum MMP-1 levels were also significantly elevated in nephrology patients with IAs (Ameku, et al., 2016). Elevated MMP-1 expression in IA tissue has been mentioned in a case report of

an IA located at the non-branching part of the distal middle cerebral artery, too, along with increased expression of MMP-2 and MMP-9 compared to the parent artery, while no MMP-8 expression was detected in either arterial tissue (Takemura, et al., 2010). Finally, a strong correlation of low MMP-3 serum levels, but no association between plasma levels of MMP-2 and MMP-9, with unruptured fusiform IAs was described by Pico et al. (2010). The authors also noted a negative correlation between basilar artery diameter and MMP-3 plasma levels (Pico, et al., 2010).

Ruptured IAs seem to present different expression of MMPs and TIMPs than unruptured IA. In the study of Jin et al. (2007), it was observed that MMP-2 and MMP-9, along with TIMP-1, TIMP-2 and TIMP-3, presented elevated expression within ruptured versus unruptured IA and mainly found in the intima layer and extracellular matrix. The ratios between MMP-9 to TIMP-2 and MMP-2 to TIMP-1, TIMP-2 and TIMP-3 were elevated in ruptured IAs. The authors also observed elevated MMP-2 and MMP-9 serum concentrations after IAs rupture, while they concluded that the upregulation of TIMPs could be an adaptive reaction to MMPs elevated expression in the extracellular matrix (Jin , et al., 2007). In other studies, increase of TIMP-1 mRNA was found in ruptured and unruptured IA tissue versus control (Ohkuma, et al., 2003), whereas downregulation of TIMP-3 and upregulation of MMP-2 and MMP-9 has been noted by Marchese et al (2010) (Marchese, et al., 2010). In the meta-analysis of Roder et al. (2012) on whole-genome microarray gene expression studies (Krischek, et al., 2008; Marchese, et al., 2010; Pera, et al., 2010), it was concluded that the expression of TIMP-4 was downregulated in IAs patients, compared to control (Roder, et al., 2012).

In patients presenting with ruptured IAs, higher tissue expression and serum MMP-9 and neutrophil gelatinase-associated lipocalin levels were also found compared to unruptured IA cases in the small study of Serra et al. (2014) (Serra, et al., 2014). Additionally, significantly increased tissue expression of MMP-9 in ruptured versus unruptured IAs, along with decreased expression of a particularly interesting Cys-His-rich protein has been mentioned in the literature (Peng, et al., 2016). In a ruptured dissecting IA case, markedly increased expression of MMP-2, MMP-9 and TIMP-2 was observed in the ruptured tissue, as opposed to weak expression noted in the unruptured dissecting IA of the same patient (Saito, et al., 2010), while in another case report of multiple ruptured IAs, no MMP-2 or MMP-9 expression was observed (Peters, et al., 2001). Finally, 7-fold increase of MMP-13 expression was found in ruptured IA tissue compared to normal in a miRNA microarray study (Bekelis, et al., 2016).

Human studies on SAH and MMPs

MMPs levels in serum and CSF have been widely studied in the literature as potential biomarkers for IAs outcome. In 2002, McGIrt et al. published a cohort study including 35 patients presenting with aneurysmal SAH, 7 patients with unruptured IAs and a third group of 42 patients with focal ischemic stroke. Patients with SAH were monitored with transcranial Doppler and blood samples were obtained until cerebral vasospasm occurred. Serum MMP-9 concentrations were measured, along with serum von Willebrand and vascular endothelial growth factors during the pre-vasospasm period. 57% of the SAH patients presented vasospasm. Mean serum MMP-9 levels were significantly elevated after the second day of the bleeding in the pre-vasospasm SAH group, compared to the rest SAH patients and the stroke group, presenting peak value three days before the vasospasm occurred. Interestingly, the authors also mentioned that serum MMP-9 levels were not significantly different among patients with SAH and unruptured IA. Their findings suggested that MMP-9, as well as serum von Willebrand and vascular endothelial growth factors, may be candidates for predicting vasospasm (McGirt, et al., 2002).

Afterwards, several studies have been published, concerning serum MMPs levels and SAH in humans. Horstmann et al. (2006) studied patients presenting with SAH and serum MMP-2 and MMP-9 levels. MMP-9 levels were observed significantly elevated until day 12, whereas MMP-2 levels appeared decreased, compared to healthy control. Nevertheless, only 73% of the studied patients presented with aneurysmal IA (Horstmann, et al., 2006). In a recent study, serum MMP-9 levels were found elevated in SAH patients, as well as in cases of vasospasm, compared to patients with unruptured IAs, within the first two weeks after the bleeding; however the study also included patients with multifactorial SAH and the results were not statistically significant (Akpinar, et al., 2016).

Another study investigated the concentration of MMP-9 in serum and in CSF of patients with SAH and in need of an external ventricular drain, up to 14 days after the bleeding. Patients with traumatic SAH, central nervous system malignancies, infection or systematic disease were excluded. It was concluded that early elevation of MMP-9 levels in both serum and cerebrospinal fluid were strongly associated with poor 3-month outcome, but not with cerebral vasospasm. Additionally, the authors reported that early high blood neutrophil count is correlated with both vasospasm and poor outcome (Chou, et al., 2011i). In the same year, decreased concentrations of anti-inflammatory plasma-type gelsolin in blood and cerebrospinal fluid, along with elevated levels of MMP-9, were reported in patients with SAH compared to control (Chou, et al., 2011ii). In patients of aneurysmal SAH, correlation of pro-MMP-9 concentrations in cerebral extracellular matrix with clinical severity (Sarrafzadeh, et al., 2012) and markedly elevated levels of MMP-9 in cerebral microdialysate during early neuromonitoring has also documented in the literature (Helbok, et al., 2015), whereas in a recent observational study of

aneurysmal SAH patients, MMP-9 CSF levels at 24 hours after SAH have been found to provide high sensitivity and specificity in predicting delayed cerebral ischemia (Triglia, et al., 2016).

Significant elevation and association of serum MMP-9 levels with cerebral vasospasm and ischemia, but not with 6-months outcome in cases of aneurysmal SAH, compared to healthy control, was reported in the study of Fischer et al. (2013). The authors also studied the effect of TIMP-1 and TIMP-3 levels, as well as MMP-3, a known MMP-9 activator (Ramos-DeSimone, et al., 1999). Along with elevated serum MMP-9 levels, TIMP-1 levels were found significantly elevated in SAH patients, as opposed to MMP-3 and TIMP-3, which were decreased in the first days after SAH. MMP-3 levels were also elevated (but lower than in control) in cases of vasospasm. TIMP-1 and TIMP-3 were not associated with vasospasm or poor outcome (Fischer, et al., 2013). On the contrary, no association between serum MMP-9 levels and vasospasm was supported in the study of Lago et al. (2015). The authors studied patients with SAH (approximately 66% aneurysmal) and examined the association between MMP-9 serum levels, vasospasm and delayed cerebral ischemia, using magnetic resonance imaging. It was concluded that MMP-9 levels were elevated compared to healthy volunteers, whereas there was no correlation between MMP-9 levels and vasospasm or poor outcome (Lago, et al., 2015).

Single nucleotide polymorphisms (SNPs) of MMPs genes and IAs

Several genetic association studies investigated the potential correlation between known SNPs of MMPs genes and IAs (Table 2), with controversial results. Concerning the MMP-9 gene, $(CA)_{23}$ microsatellite polymorphism (Peters, et al., 1999) and SNP 7476 C/T of the non-coding area (Pannu, et al., 2006) were significantly associated with IAs in separate studies. However, these findings were not confirmed by other researchers (Yoon, et al., 1999; Zhang, et al., 2001; Krex, et al., 2004; Pannu, et al., 2006; Olsson, et al., 2012). Additionally, MMP-9 1562 C/T SNP prevalence was not found significantly different within patients with ruptured or unruptured IAs (Zhang, et al., 2001; Krex, et al., 2004; Pannu, et al., 2006; Szczudlik & Borratyńska, 2010; Alg, et al., 2013).

Two SNPs of MMP-2 gene have been found significantly associated with male IAs patients in a Japanese population (Low, et al., 2011), although opposite results came from Caucasian studies (Pannu, et al., 2006; Olsson, et al., 2012) and meta-analysis (Alg, et al., 2013). In addition, strong correlation between MMP-3 5A/6A SNP and fusiform IAs has been mentioned (Pico, et al., 2010), but no other study (Yoon, et al., 1999; Zhang, et al., 2001 or meta-analysis (McColgan, et al., 2010; Alg, et al., 2013) confirmed this finding in saccular or ruptured IAs. Finally, although scarcely studied, none of the studied MMP-1, MMP-12 and TIMP- 1 to -3 SNPs was found associated with occurrence of IAs.

Table 2. Studies on MMPs&TIMPs polymorphisms and patients with intracranial aneurysms (IA) or aneurysmal SAH (aSAH). (pos/neg: significant positive association / no association)

Year	Authors	Population	IA/aSAH	Controls	MMP	SNPs	result
1999	Peters et al.	European white	76 IA	93	MMP-9	microsatellite (CA)n	**pos**
1999	Yoon et al.	Finish	57 IA	174	MMP-3	5A/6A	neg
			with family history		MMP-9	A1-A11 including (CA)n microsatellite	neg
2001	Zhang et al.	European white	72 aSAH	158	MMP-1	1G/2G	neg
					MMP-3	5A/6A	neg
					MMP-9	1562 C/T (rs3918248)	neg
						microsatellite	neg
					MMP-12	-82 A/G	neg
2003	Krex et al.	European white	44 IA	44	TIMP-1	19 C/T	neg
						261 C/T	neg
						372 T/C	neg
				41	TIMP-2	621 C/T	neg
						596 A/C	neg
						261 G/A	neg
						303 G/A	neg
				40	TIMP-3	249 T/C	neg
						261 C/T	neg
2004	Krex et al.	Caucasian	40+40 IA	44+40	MMP-9	rs3918248	neg
						rs2274755	neg
						rs17576	neg
						rs3918256	neg
						rs2250889	neg
						rs13969	neg
						rs2274756	neg
						rs13925	neg
						rs20544	neg
						rs9509	neg
2006	Pannu et al.	Caucasian	125 sporadic IA, 69% ruptured	234	MMP-2	1306 C/T (rs243865)	neg
						3307 G/A	neg
						6447 G/C	neg
						10910 C/T	neg
					MMP-9	1562 C/T(rs3918248)	neg
						(CA) microsatellie	neg
						1977 C/T	neg
						7476 C/T (rs20544)	**pos**
2010	Szczudlik & Borratyńska	Polish	211 aSAH 789 other	766	MMP-9	1562 C/T (rs3918248)	neg
2010	Pico et al.*	Caucasian	49 fusiform IA	378	MMP-3	5A/6A	**pos**
2011	Low et al.*	Japanese	2050 IA	1835	MMP-2	rs243865 C/T	**pos****
						rs243847	**pos (**male)
						rs17859859	neg
						rs1132896	neg
					MMP-9	rs3918242	neg
						plus two SNPs, missing info	neg
					MMP-12	rs2276109	neg
					TIMP-1	*one SNP, missing info*	neg
					TIMP-2	rs2277698	neg
						rs2009196	neg
						rs8179090	neg
					TIMP-3	rs5749511	neg
						rs2234921	neg
2012	Olsson et al.	Sweden (?)	183 aSAH	366	MMP-2	rs243864	neg
						rs865094	neg
						rs12934241	neg
						rs243847	neg
						rs2287074	neg
						rs1163996	neg
						rs11541998	neg
						rs7201	neg
					MMP-9	rs17576	neg
						rs2236416	neg
						rs20544	neg
						rs3918256	neg
						rs3787268	neg

* genotype frequencies not provided **male, adjusted for age/hypertension/smoking

Animal models and studies on IAs and MMPs

The first report of an animal model with induced IAs was described by Nagata et al. in 1980, using **rats**. After anesthesia, the left common carotid artery and the posterior branches of both renal arteries were ligated. The animals were fed a special diet, including 8% sodium chloride and 0.12% *aminopropionitrile*, which is an inhibitor of lysyl oxidase that catalyzes the cross-linking of elastin and collagen. The sodium chloride intake, along with the renal arteries iatrogenic occlusion, provided the necessary conditions for systemic hypertension. Common carotid artery ligation and *aminopropionitrile* led to the necessary hemodynamic and microvasculature changes that made the cerebral vasculature prone to IAs formation (Nagata, et al., 1980). Several studies have been published since using this model in order to study the association between MMPs and IAs.

In 2007, Aoki et al. used the aforementioned animal model in order to study the association between MMP-2 and MMP-9 with IAs development. The authors concluded that macrophages infiltration was gradually increasing within three months from IA induction and at three months, both MMP-2 and MMP-9 were elevated within IAs tissue. Sources of MMP-2 and MMP-9 were macrophages and secondly smooth muscle cells. MMP-2 mRNA was constantly elevated within the studied time, whereas MMP-9 mRNA was not detected within the first month but increased later. In situ zymography revealed that gelatinase activity was prominent in the aneurysmal neck at four months. Finally, the authors tested the efficacy of *tolyslam*, a competitive inhibitor of MMP-2, MMP-9 and MMP-12 on IAs formation. Although IAs formation was the same at four months, the incidence of advanced IAs was significantly lower in the *tolyslam* group (Aoki, et al., 2007i).

In the same year, Aoki et al. published a second study using the Nagata et al. rodent model in order to investigate the role of TIMP-1 and TIMP-2 in IAs formation. One month after surgery, early changes leading to IAs initiation were obvious in more than half the specimens; TIMP-1 and TIMP-2 expression was already elevated in the affected regions. Three months later, advanced IAs were seen in the majority of specimens, however interestingly, TIMP-1 and TIMP-2 expression pattern was the same. The main source of TIMP-1 and TIMP-2 expression was smooth muscle cells, while expression was also evident from macrophages and endothelial cells. No expression was noted on normal arterial walls. MMP-2 and MMP-9 expression was found gradually elevated over the three months period. Thus, the ratio of MMP-9 to TIMP-1 and MMP-2 to TIMP-2 was significantly increased over time. The ratio of MMP-9 and MMP-2 to TIMP-3 was unchanged (Aoki, et al., 2007ii).

Moreover, the authors tested normal, TIMP-1 and TIMP-2 knock-out (KO) mice, although they ligated the posterior branches of renal arteries bilaterally a week after the common carotid artery ligation and

studied the animals five months after IAs formation. TIMP KO mice presented a higher incidence of IAs formation than control. TIMP-1, TIMP-2, MMP-2 and MMP-9 expression was observed in IAs tissue of wild type mice. Zymography showed increased activity of MMP-2 in the TIMP-2 KO mice and elevated MMP-9 activity in the TIMP-1 KO mice, compared to the control; however, MMP-2 and MMP-9 mRNA expression were strangely decreased in the TIMP-2 and TIMP-1 KO mice, respectively. The authors concluded that this may indicate the existence of a positive feedback mechanism of the TIMPs to the MMPs transcriptional regulation (Aoki, et al., 2007ii).

More recently, a modification in the aforementioned rat model was introduced by Miyamoto et al. (2016). Along with the ligation of the left common carotid artery and the posterior branches of the renal arteries bilaterally, the right pterygopalatine and external carotid artery were also ligated in a group of rats. The results showed that in the test group, there was a higher number and rupture rate of IAs located at the posterior and anterior Willis circle arteries, compared to the control, partly attributable to a site-specific elevation of MMP-9 expression caused by altered hemodynamics (Miyamoto, et al., 2017).

The hypothesis that drugs with inhibitory effect on MMPs activity may reduce the incidence of IAs has also been studied on Nagata et al. rodent model (Nagata, et al., 1980). Kaufmann et al. (2006) tested the effect of *doxycycline*, an MMPs antagonist used commonly as antibiotic, however no significant difference in IAs incidence was observed within a year of follow-up (Kaufmann, et al., 2006). In another study, *nifedipine*, a dihydropyridine based calcium antagonist used as an anti-hypertensive, was found efficient in decreasing IAs development, macrophages infiltration into the IA wall and MMP-2 activity and expression (Aoki, et al., 2008). Within a short period of time, the same authors published their results on efficacy of *simvastatin* and *pitavastatin* on IAs progression and MMPs expression; they concluded that both statins may inhibit the progression of IAs development, reducing significantly the expression and activity of MMP-2 and MMP-9, that were elevated compared to control before the treatment (Aoki, et al., 2008; Aoki, et al., 2009).

The important role of mast cells in IAs development was shown in the study of Ishibashi et al. (2010), as treatment with *tranilast,* an agent that prevents mast cells degranulation, attenuated the increase in MMP-2 and MMP-9 expression (Ishibashi, et al., 2010). A couple of years later, the same authors used *imidapril* as an MMP-9 inhibitor to test the effect on IAs formation. The authors concluded that *imidapril* suppressed IAs formation and development, by inhibiting the gelatinolytic activity of MMP-9. Indeed, although MMP-9 expression remained unchanged, in vitro zymography showed that *imidapril* could dosedependently inhibit MMP-9 activity. Interestingly, MMP-2 expression was not found elevated in IAs tissue (Ishibashi, et al., 2012).

Erythopoietin has been also found to reduce IAs size and development by reducing MMP-2 and MMP-9 elevated levels (Xu, et al., 2011). Erythropoietin could increase endothelial progenitor cells; thus, Li et al. (2014) used *endothelial colony forming cells* transfusion and examined IAs development and MMPs. The authors concluded that MM-2 and MMP-9 were upregulated, while TIMP-1 was downregulated in IAs tissue and transfusion of *endothelial colony forming cells* reversed these findings and attenuated IAs degeneration (Li, et al., 2014). *Ibudilast* and *tumor necrosis factor-a inhibitor* have also been noted to suppress the development of IAs by reducing the upregulation of MMP-9 and other inflammation-related molecules (Yagi, et al., 2010; Yokoi, et al., 2014). Finally, Li et al. (2015) tested the anti-inflammatory effect of *aspirin* on IAs development, concluding that MMP-2 and MMP-9 expression was significantly higher in IAs walls compared to normal and significantly decreased when *aspirin* was taken, along with the size of IAs (Li, et al., 2015).

A new animal model was introduced by Nuki et al. in 2009. By combining angiotensin-II infusion and elastase injection into the cerebrospinal fluid of **mice**, IAs formation is achieved within two weeks. Elastase is used in order to provoke disorganization of the cerebrovascular elastic lamina, while systemic hypertension, caused by angiotensin-II infusion, provides the essential hemodynamic insult. After multiple experiments with normal, MMP-9 and MMP-2 KO mice, the authors reported that IAs incidence in wild type mice was 70%. Elevated activity of MMPs was observed in IAs tissue, unlike normal arteries. Moreover, as opposed to MMP-2 KO mice, MMP-9 KO mice presented significantly lower IAs incidence compared to control. Finally, treatment with *doxycycline* reduced the IAs incidence significantly to 10%. These results show the potent protective role of *doxycycline*, as well as MMP-9 impact on IAs formation (Nuki, et al., 2009).

The same team of authors, some years later, published a second study, examining the impact of MMP-12 in IAs formation. Using their introduced angiotensin II-elastase model on wild type and MMP-12 KO mice, they concluded that the incidence of IAs formation was not significantly different between the two groups, thus implying that MMP-12 may not be implicated in the IA development process. The study also proved the important role of macrophages in the formation of IAs, using clodronate liposome-induced macrophage depletion and monocyte chemotactic protein -1 KO mice (Kanematsu, et al., 2011). The role of myeloperoxidase on IA formation has also been studied on IAs patients that underwent IA coiling and myeloperoxidase KO mice. The researchers reported that in humans, myeloperoxidase concentration was almost 3 times higher in the blood drawn from the IA site than in remote vessels, while IAs incidence was significantly lower in the myeloperoxidase KO group compared to control; MMP-9 and MMP-8 genes were down-regulated, as well as MMP-3 and MMP-13 genes (Chu, et al., 2015), which are found to be activators of MMP-9 (Vempati , et al., 2007). In another

study, the impact of lymphocytes in IA initiation and rupture was investigated, using a slightly modified wild type and lymphocyte deprived mice model. It was concluded that in the lymphocyte deprived mice, MMP-2 and MMP-9 expression, along with IAs number and rupture incidence, was significantly reduced (Sawyer, et al., 2016).

In the study of Peña Silva et al. (2014), a series of experiments were performed using the angiotensin II-elastase model (Nuki, et al., 2009) versus sham operated mice and Mas-receptor KO mice. Moreover, angiotensin 1-7, a functional antagonist of angiotensin II which binds to the Mas receptor, was used as intervention. The authors noted the increased expression of MMP-9, MMP-2 and TIMP-1 in IA tissue versus control, while co-infusion with angiotensin 1-7 drastically reduced the increase in MMP-9 and the incidence of SAH and mortality. No difference was observed with angiotensin 1-7 treatment on Mas-receptor KO mice (Peña Silva, et al., 2014).

Finally, the hypothesis of pharmacological stabilization of IAs was tested on the angiotensin II-elastase model (Nuki, et al., 2009). *Doxycycline*, *minocycline* and *selective inhibitors of MMP-2 and MMP-9* were used in order to assess their effect on IA rupture. The authors stated that there was elevated gelatinase activity in IAs tissue and they concluded that treatment with *doxycycline* and *minocycline* reduced significantly the incidence of IA rupture, as opposed to treatment with *selective inhibitors of MMPs* (Makino, et al., 2012).

In a **rabbit** model, IAs formation was induced by ligation of both common carotid arteries and subsequent increase in of the blood flow in the basilar artery. This shortly resulted in the appearance of aneurysmal alterations in the basilar artery (Gao, et al., 2008). Using this model, the impact of smooth muscle cells on induced IAs formation was studied (Kolega, et al., 2011; Mandelbaum , et al., 2013). It was concluded that smooth muscle cells play an important role in the initiation of IAs in response to hemodynamic insult, producing MMPs (especially MMP-2 and MMP-9) that promote early disruption of internal elastic lamina and IAs initiation, whereas macrophages did not seem essential in IAs initiation. In addition, when *doxycycline* was used, IAs formation and size was lower (Mandelbaum , et al., 2013). Finally, the injurious role of nitric oxide synthase and superoxide in IAs initiation, via MMP regulation, was documented by Liaw et al. (2014), as MMP-2 and MMP-9 expression was elevated in IAs tissue versus control and associated with nitric oxide synthase inhibition. (Liaw, et al., 2014)

CONCLUSIONS

Even though the prevalence of IAs in population is low, the potential complications in case of rupture are of high significance. Because of the fact that IAs are commonly asymptomatic, the diagnosis unrarely takes place before rupture. SAH may lead to disability and death and has a disproportionately large socioeconomic effect. Thus, there is great interest in investigating pathophysiological and etiologic factors, in order to establish screening tests for early diagnosis and treatment in asymptomatic cases, as well as predictive tests for outcome after bleeding occurs.

Intrinsic characteristics of cerebral vasculature and pathological conditions, mainly hypertension and substance abuse, enable IAs formation and rupture. Hemodynamic changes, inflammation and oxidative stress coordinate the acquired disorganization of microvasculature morphology, leading to disruption of the vessel's internal elastic lamina and focal dilatation, while persistent insult leads to IAs rupture and SAH. MMPs and a whole activation signaling network play a key role during the initiation and rupture of IAs. MMPs are family of zinc-dependent proteases with tight regulation, multiple functions and well-known participation in many conditions of the central nervous system, including IAs. Therefore, many studies have been conducted examining the MMPs and TIMPs altered expression in IA tissue, blood and CSF of patients presenting with IAs, along with genetic associations of SNPs in MMPs and TIMPs genes with IAs occurrence and rupture. The current study was performed in order to assess the published literature correlating MMPs and IAs.

Using histological and molecular techniques, elevated expression of gelatinases MMP-9 and MMP-2 has been reported in many studies of IAs tissue, indicating their role in IAs initiation and rupture (Kim, et al., 1997; Bruno, et al., 1998). In particular, MMP-9 expression and activity was found increased in the IAs tissue, compared to control, presenting altered expression pattern (Bruno, et al., 1998) and association with atherosclerosis (Caird, et al., 2006). Association of MMP-2 and MMP-9 expression with other molecules, such as the plasmin, NF-κ B factor, osteonectin and other MMPs indicate the complex regulation signaling that takes part in the IAs development (Bruno, et al., 1998; Cheng & Wang, 2013; Li, et al., 2013). Elevated expression of MMP-2 and MMP-9 and altered expression of TIMPs has been associated with IAs rupture (Jin , et al., 2007; Marchese, et al., 2010; Saito, et al., 2010; Roder, et al., 2012; Peng, et al., 2016); this imbalance between MMPs and TIMPs could be an adaptive reaction to the increased proteolytic activity of MMPs (Jin , et al., 2007). Other MMPs mentioned to have elevated expression in IAs tissue are MMP-1, MMP-14, MMP-13 and MMP-16 (Bruno, et al., 1998; Li, et al., 2009; Takemura, et al., 2010; Ameku, et al., 2016; Bekelis, et al., 2016).

Significant elevation of serum levels of MMP-2 and MMP-9 in patients with unruptured saccular IAs compared to control has been suggested in the literature (Chyatte & Lewis, 1997; Todor, et al., 1998; Gaetani, et al., 1999; Jin , et al., 2007; Serra, et al., 2014), while no association between MMP-2 and MMP-9 but strong correlation of low MMP-3 serum levels and fusiform IAs has been also described (Pico, et al., 2010). These findings reinforce the hypothesis that MMPs levels may be used for predicting the presence of IAs in asymptomatic patients; however larger scale studies should be conducted in order to assess the possibility of an MMP-2 and MMP-9 screening test.

Moreover, MMPs concentration in serum and CSF of patients with SAH has been examined based on the hypothesis that MMP levels may serve as a biomarker for predicting vasospasm, delayed cerebral ischemia and outcome. MMP-9 serum and CSF levels have been found significantly elevated in most studies of SAH compared to unruptured IAs patients or healthy individuals (Horstmann, et al., 2006; Chou, et al., 2011; Chou, et al., 2011; Fischer, et al., 2013; Triglia, et al., 2016), whereas the results on the association between MMP-9 levels and cerebral vasospasm development have been controversial (McGirt, et al., 2002; Chou, et al., 2011; Fischer, et al., 2013; Sarrafzadeh, et al., 2012; Lago, et al., 2015). Finally, MMP-9 serum levels have been proposed to predict long-term outcome after SAH (McGirt, et al., 2002; Triglia, et al., 2016), but other authors dispute this hypothesis (Fischer, et al., 2013; Lago, et al., 2015).

In addition, genetic association studies have not yet succeeded in enlightening the genetic component of MMPs and TIMPs association with IAs occurrence. In 2013, a comprehensive review and meta-analysis including all genetic association studies correlating SNPs to sporadic IAs was published (Alg, et al., 2013); however, no correlation between MMPs genes and IAs was proved. As it is described in Table 2, only a few separate studies provided positive results on SNPs correlation with IAs, regarding a microsatellite polymorphism in MMP-9, two SNPs in MMP-2 gene and one SNP in MMP-3 promoter region, while opposite results have been published in various studies for all of the above genetic loci (Peters, et al., 2001; Pannu, et al., 2006; Pico, et al., 2010; Low, et al., 2011). Thus, there is no distinct SNP in MMPs genes that may play a significant role in IAs formation and rupture.

Experiments on animal models of acquired IAs are common in the literature, as they provide some insights to pathophysiological mechanisms and treatment options, overcoming the barriers of studies on humans. For each animal model, the basic principles are the same: hemodynamic changes due to hypertension and disorganization of the microvasculature environment (Nagata, et al., 1980; Gao, et al., 2008; Nuki, et al., 2009). The short incubation time and the opportunity of interventions are the main advantages of animal studies. Many agents have been shown promising against IAs formation in the literature; amongst them, the effects of doxycycline, statins, anti-hypertension and anti-inflammation medication are of great interest, as they may provide pharmacological stabilization of IAs

(Nuki, et al., 2009; Ishibashi, et al., 2010; Yagi, et al., 2010; Xu, et al., 2011; Ishibashi, et al., 2012; Makino, et al., 2012; Mandelbaum , et al., 2013; Li, et al., 2014; Peña Silva, et al., 2014; Yokoi, et al., 2014; Li, et al., 2015). Finally, several studies have been published, examining the association between MMPs and experimental SAH in animal models. However, experimental SAH is mainly provoked by injection of autologous blood into the animals' cranium, although in some cases, SAH was induced by endovascular perforation of a vessel. Those studies were excluded from the current review, as no IA formation was induced. Recently, a modified rat model was suggested, presenting high incidence of IAs rupture and SAH (Miyamoto, et al., 2017), allowing investigators to study aneurysmal SAH.

The current study constitutes a qualitative systematic review of the literature concerning the impact of MMPs on IAs initiation, development and rupture. However, due to the heterogeneity of the published studies, no quantitative data are provided, limiting the impact of the results. As described, there are controversial findings regarding the role of MMPs on IAs formation and complications of rupture. Therefore, larger scale studies and meta-analysis should take place in order to assess the mechanisms implicating MMPs and IAs, as well as the potential role of MMPs levels as biomarkers for early diagnosis of IAs presence, rupture and outcome.

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