

Matrix Metalloproteinase-3 Associated with Severity of Rheumatoid Arthritis: A Meta Analysis

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Abstract: *Our study examined the relationship between the levels of matrix metalloproteinases [MMP]-3 and the pathogenesis of Rheumatoid Arthritis [RA] & among severity of the disease. We employed rigorous inclusion and exclusion criteria in various bibliographic databases to extract published studies relevant to this investigation. The Revman manager version 5.3 & winpepi version 11.65 software was used for the statistical analysis. A total of 329 studies were initially searched, and 6 studies with 652 RA patients and 658 healthy controls were included in this meta-analysis. Results are represented in terms of Odds ratios [ORs] and 95% confidence intervals [CIs] to estimate the association between MMP-3 and RA. There was significant association between MMP-3 and RA. Significance between study heterogeneity existed between mild and severe RA. The meta-analysis results suggested that the levels of MMP-3 were higher in patients with RA than those in the control group. Subgroup analysis according to severity showed that the levels of MMP-3 were higher in severe RA than in mild RA. We systematically investigated the association between MMP-3 and RA susceptibility; however, the results show a correlation among the disease severity & these raised level of MMP-3 associated with the pathogenesis of RA.*

Keywords: Rheumatoid arthritis, Matrix metalloproteinase, Meta-analysis

1. Introduction

Rheumatoid arthritis [RA] is a chronic, serious systemic autoimmune disease that is primarily characterized by multi-joint synovitis. Therefore, comprehensive approaches to assessing joint damage in patients with RA are pivotal for the early diagnosis and treatment of RA in clinical settings.^[1] Rheumatoid arthritis [RA] is characterized by progressive, erosive and symmetrical polyarthritis associated with various extra-articular manifestations and with variable prognosis, significant morbidity, functional damage, disability and increased mortality. It affects approximately 0.5% of the adult population.^[2] The etiology of chronic arthritis has not been identified, but the pathogenesis of RA has become somewhat clear. Early characteristics of rheumatoid joints include neovascularization and proliferation of synovial tissue, eventually resorption of bone is due to the action of osteoclasts. This Overgrown synovium is a major source of proinflammatory cytokines as well as proteinases.^[3]

MMPs are a group of zinc-dependent endopeptidases, which can degrade every component of the extracellular matrix. The MMP family consists of at least 28 members and amino acid sequences of all MMPs have two conserved domains, a catalytic domain and a prodomain, which are important for their substrate specificity. Members of the MMP family have broad substrate specificities, including substrates like gelatinases, collagenases, matrilysins, stromelysins, membrane-type MMPs, and metalloelastase.^[4] Generally, MMPs can degrade any extracellular matrix component, including proteoglycans, vitronectin, fibronectin, laminin, and collagens.

MMP-3 [stromelysin 1] is the most widely studied member in RA which is considered to be the main MMP involved in

cartilage degradation. It has broader substrate specificity with activity against type II, III, IV, IX, X, XI collagens, proteoglycans, fibronectin and laminin. It can also activate other MMPs such as MMP-1, -2, -9 and -13.^[5] They play a central role not only in many physiological processes but also in many diseases. Increased activity, caused by either up-regulation of their expression or down-regulation of their inhibitors. From a development and disease perspective these enzymes are crucial during embryonic growth and reproduction. They have a major role in tumor growth and metastasis. In the synovial joint, MMPs are mainly secreted by fibroblasts, macrophages and chondrocytes. The expression of most MMPs is regulated at the transcription level by growth factors, hormones, and cytokines.^[6]

The environment factor and genetic participates in development of disease RA. Recently, research has focused on the identification of genes that influence the susceptibility of this disorder. Therefore, analysis and identification of new genes associated with RA susceptibility is an important and meaningful challenge.

It has been seen that the serum and synovial fluid levels of MMP-3 are elevated in early and established RA patients, and are associated with diseased activity and /or joint destruction MMPs participate in the maintenance and remodeling of extracellular matrix [ECM] that is important for creating cellular environments. These enzymes have the ability to cleave several constituents of ECM.^[7]

Recently, a variety of studies have examined the relationship of the expression of MMP-3 and the pathogenesis of RA.^[8] ⁹MMP-3 levels have been reported to be either correlated with RA or not related at all to RA.^[10, 11] we therefore conducted a meta-analysis to examine the relationship of MMP-3 expression and the pathogenesis of RA.

2. Material and Methods

2.1 Methods

In order to capture all published studies relevant to MMP-3 in pathogenesis of RA. We performed a comprehensive search of all electronic database PUBMED, medline, EBSCO, springer link, Elsevier, National digital library, Knimbus digital library by using Mesh terms Matrix Metalloproteinase, MMP-3, Rheumatoid arthritis, osteoarthritis, serum MMP3, synovial MMP 3, infective synovitis etc. The bibliographies of related studies were examined for relevant articles. Manual search of references from original research or review articles was performed to identify additional studies. No language and time restrictions were applied.

Inclusion Criteria

Full articles are retrieved and evaluated by reading the abstract for their suitability based on following inclusion criteria

- 1) Case control study focuses on MMP-3 in rheumatoid arthritis.
- 2) All patients should clinically diagnose with RA
- 3) Detection method is ELISA complete & sufficient data for estimation of odds ratio and confidence interval 95%
- 4) Studies were included only from year 2000-2017. Additionally similar baseline characteristics of the case group and control group to be determined.

Exclusion criteria

Incomplete data, Studies without control group, improper diagnostic scales for disease diagnosis were excluded from study.

Data extraction

The following data were extracted from eligible studies: the first author's name, year of publication, country of origin, ethnicity of the studied population, total numbers of cases and controls, age, gender, study design and sample size, detection method respectively. The data mainly consisted of disagreements in data extraction were resolved by a discussion among researchers.

Statistical Analysis

OR with their confidence interval has been calculated for each study to assess MMP-3 level in Rheumatoid arthritis. The significance of the pooled OR was determined by the Z-test. [$P \leq 0.05$ was considered representative of statistical significance]. To assess the differences in MMP-3 levels between the case group and control group, a standard mean

difference [SMD] and 95% confidence intervals [95%CI] were calculated with the assistance of fixed- or random-effect models. A Z test was used to assess the significance of the pooled effect size. We utilized the Cochran's Q -statistic [$P < 0.05$ indicates significance] and the I^2 test to assess the heterogeneity among studies^[12][Zintzaras and Ioannidis, 2005b]. The random-effect model was utilized when evidence of significant heterogeneity was obtained [$P < 0.05$ or I^2 test $> 50\%$]; otherwise, the fixed-effect model was applied.^[13,14] A sensitivity analysis was performed to examine the influence of each study on the overall results by removing the study and re-examining the results. In addition, a publication bias was tested with the Egger linear regression test [$P < 0.05$ indicated significance] together with asymmetry funnel plots. An estimate of potential publication bias was carried out by Begg's funnel plot and Egger's regression test.^[15] All of the statistical analyses were conducted by Revman manager version 5.3 & winpepi version 11.65 software. Our study also followed the PRISMA guidelines.^[16]

3. Results

Characteristics of study included

Study selection process is given in Figure No I. Based on our search strategy.

A total of 329 relevant studies were initially identified by an electronic database search and subsequent manual searching. We read the titles and abstracts and excluded 2 studies for duplicity; 7 studies for being letters, reviews, or meta-analyses; 4 studies for not examining humans; and 26 studies for being unrelated. 18 studies for effectiveness of therapy & its influence on MMP level. Furthermore, 17 studies were removed after reading the full text for not being case-control studies or not involving MMP-3 and RA. Additionally, 4 studies were excluded for lacking sufficient information. Finally, a total of 6 case-control studies [Dominique de Seny2013, Hishashi Yamanaka 2000, Jian-Da Ma 2014, Ling Zhou 2017, Martina Skacelova 2017, Takashi Adachi 2013] published between 2000 and 2017 were included in our meta-analysis. These studies included 1330 subjects [652 patients with RA and 658 normal healthy controls]. The baseline characteristics of the studies included are listed in Table No. I.

We have also analyzed MMP-3 level in severity of rheumatoid arthritis. Only 3 studies among 6 had sufficient information with severity of disease. The detection of serum MMP-3 levels were performed by using enzyme linked immunosorbent assay [ELISA] in the all included studies.

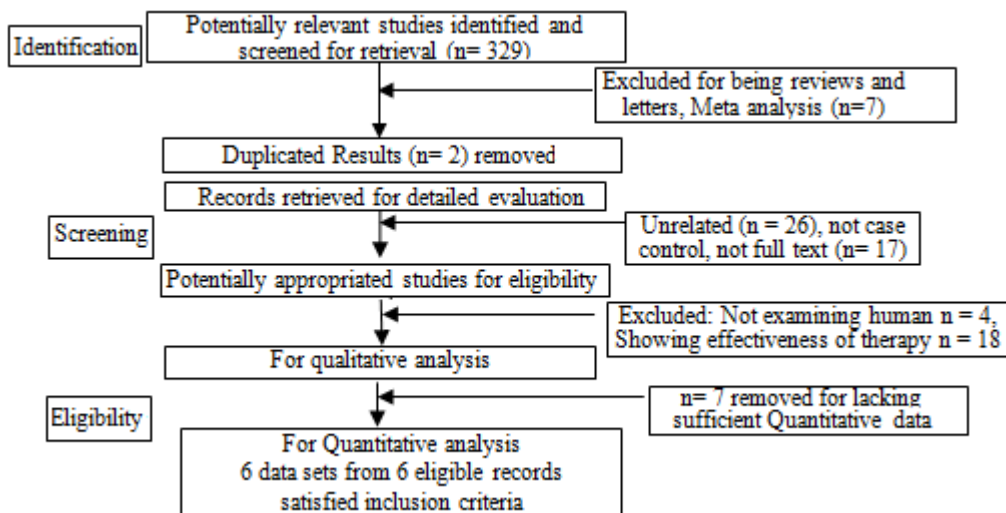


Figure I: PRISMA flow diagram

Table I: Baseline characteristics of all studies in Meta analysis

First Author	Country	Number		Gender		Age	Sample
		RA	Control	RA (F/M)	Control (F/M)		
Dominique de Seny(2013)	Europe	27	35	15/ 12	20/15	50-64	Serum
Hishashi Yamanaka (2000)	Japan	82	267	NA	173/94	23-72	Serum
Jian-Da Ma (2014)	China	62	5	43/82	NA	48-62	Serum
Ling Zhou (2017)	China	151	43	135/16	23/20	22-72	Serum
Martina Skacelova (2017)	Czech Republic Europe	92	26	60/32	13/13	36-66	Serum
Takashi Adachi (2013)	Japan	238	302	200/38	185/34	28-72	Serum

NA- Not available

Table II: Disease characteristics defined by all studies in Meta Analysis

Study	Disease Duration years	ESR	CRP	DAS
Dominique de Seny(2013)	NA	NA	NA	NA
Hishashi Yamanaka (2000)	11.9±7.4	50.1±25.5	2.8±3.2	NA
Jian-Da Ma (2014)	31.5 ± 21	73.5 ± 15 (47-107)	3.9(1.0-5.6)	5.5 (4.6- 6.3)
Ling Zhou (2017)	6.5±2.5	36.5± 7.62	12.40±15.01	3.82 (2.81-5.63)
Martina Skacelova (2017)	15.66±9.04	18.9±15.5	12.2±16.2	3.74± 1.7
Takashi Adachi (2013)	13.9±7.2	27±18.7	0.76±0.87	NA

NA- Not available

Quantitative Synthesis

The random-effect model was utilized to analyze for heterogeneity, which was positive [I2 = 60.9%, Ph = 0.018]. The meta-analysis results suggested that the MMP-3 enzyme levels were markedly and significantly raised in patients with RA than in control [SMD =101.35 95%CI = 93.21 to 109.49, P = 0.00001][Figure II]. Significance between-study heterogeneity existed between RA & control shows results: I²= 99.7 %, p = 0.000; we used both fixed-effect model and random-effect model for comparison. Overall, there was significant association between MMP-3 and RA. The main results of meta-analysis were shown in Table No.II.

Meta analysis also revealed that MMP-3 enzyme level is supposed to be increased as severity of the disease. [SMD =234.56,95%CI = 217.5 to 251.96, P = 0.00001]. Significance between study heterogeneity existed between mild and severe RA I² 98%, P=0.000. We used both fixed-effect model and random-effect model for comparison.

Overall, there was significant association between severity of RA and increased MMP-3 level.

Sensitivity analysis & publication bias

Sensitivity analyses were preformed to assess the stability of the results. The result did not change when a single study involved in the meta-analysis was deleted each time. The shapes of the Begg's funnel plots did not reveal any evidence of obvious asymmetry [Figure No.III]. Meanwhile, the results of Egger's regression test still did not provide any evidence of publication bias [P = 0.523]

A sensitivity analysis revealed that each study in our meta-analysis had no significant influence on the pooled SMDs of protein expression of MMP-3 in the RA and control groups [Figure No.IV]. For the levels of expression of MMP-3, Begg's funnel plots revealed some symmetry, and subsequent Egger's tests demonstrated the absence of publication bias in the studies that were included [MMP-3: P=0.523].

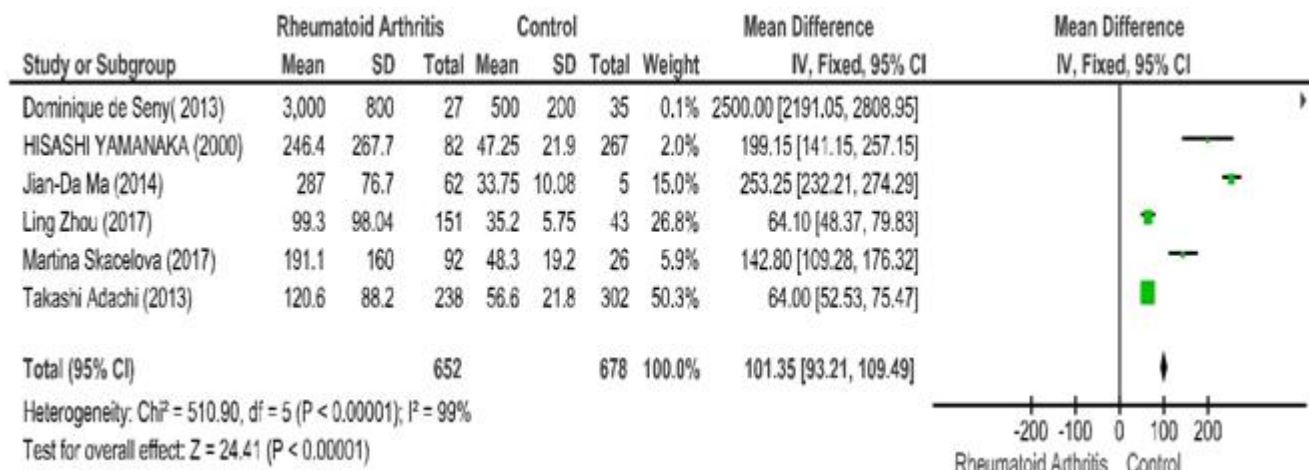


Figure II: Forest plots of the associations between the levels of matrix metalloproteinase MMP- 3 Rheumatoid Arthritis.

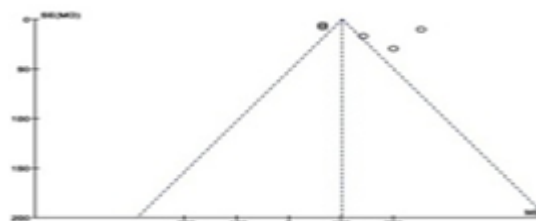


Figure III: Funnel plot to demonstrate the risk of publication biases on the associations between the protein levels MMP-3 and Rheumatoid Arthritis

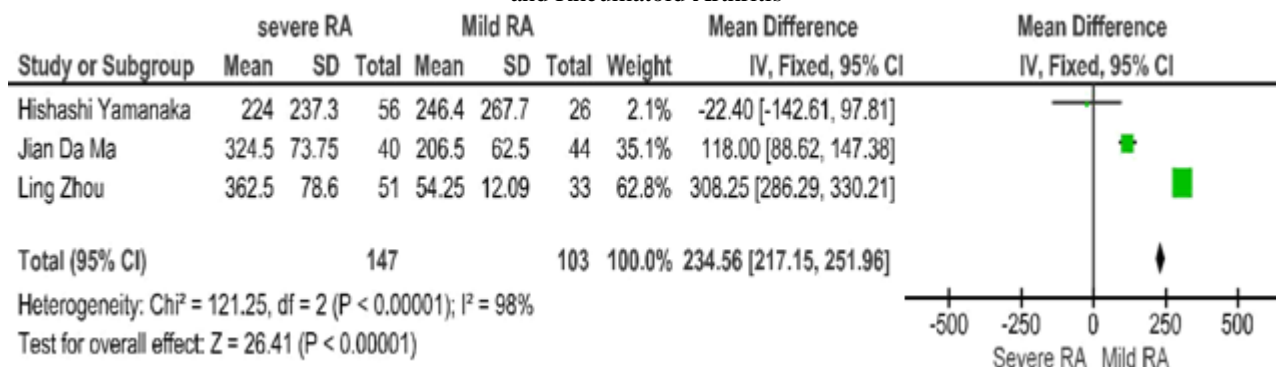


Figure IV: Forest plot of subgroup analysis for levels of MMP-3 in severity of the disease mild & severe RA

4. Discussion

Rheumatoid arthritis [RA] is an autoimmune inflammatory disease with unknown etiology. It is characterized by synovial inflammation & hyperplasia, autoantibody production, cartilage and bone destruction. The clinical manifestations and outcomes of RA range from mild to severe polyarthritis with progressive destruction of cartilage and bone. Much of the destruction in RA is mediated by abnormal release of matrix metalloproteinase [MMPs] in synovium stimulated by persistent inflammation.^[17] MMP-3 [stromelysin 1] is considered to be the main MMP involved in cartilage degradation.

We conducted a comprehensive meta-analysis of selected published studies to investigate the association between the levels of MMP-3 in pathogenesis of RA. Our major finding was that the increased levels of MMP-3 in RA might accelerate the pathogenesis of the disease, and this relationship has been neglected clinically. Our study also reveals association of MMP 3 with disease severity. Few of earlier work shows that joint damage in RA are a result of

active synovitis also inflammatory markers can also be predictors of joint destruction. The CRP level, ESR, and their time-integrated values have been reported to predict the progression of joint destruction in early RA [^{18, 19, 20}]. In this study, the concentration of serum MMP-3 showed a positive correlation with the CRP level and the ESR in both early and long-term RA.

Kazuko Shiozawa et al suggested that serum MMP-3 levels alone are a crude reflection of disease progression [²¹], they presumably reflect the levels of other joint-destroying MMPs as well, as for example shown in the collagen-induced arthritis model in mice.^[22] These may explain serum MMP-3 determined by ROC curve analysis is negatively correlated with later radiographic evidence of progression in the study.

It has also been observed by group of researchers that effect of treatment and decrease in serum MMP-3 level. MMP-3 is a marker that reflects inflammation of the synovium, and is thus directly linked to disease activity.

Since MMP-3 level is known to decrease after effective treatment, which reflects treatment response. Another study found that continuously elevated serum MMP-3 may predict evident progression of radiographic damage over 1 year in patients treated with various DMARDs using a treat-to-target protocol.^[23]

But there are also evidences with contradictory results Serum MMP-3 was significantly elevated both in patients with early RA and in those with long-term RA compared with the healthy controls.^[24] Thus, elevation of the serum MMP-3 level is closely associated with RA, but it would not be an early marker in the course of RA progression. Indeed serum MMP-3 levels were also elevated in advanced RA with severe joint damage.

Conversely, no significant difference in serum MMP-3 levels was observed between RA patients classified as high or low eroders, nor did SF MMP-3 levels correlate with the Larsen score.^[25]

In one of the study, they have found an additive effect of the 6A allele on increasing level of serum MMP-3, no significant effect of the polymorphism was found on the disease activity or severity of RA in Japanese population.^[26] These serum MMP profiles can be used to distinguish advanced OA & RA from early OA & control, and even between synovial fluid samples from OA and RA joints. Although this methodology cannot be used for the diagnosis of early OA, high throughput multiplex technology of MMPs and TIMPs in synovial fluid may be useful in determining the severity of the disease state.^[27] MMP profile might also quantify the response of individuals to disease interventions.

MMP-3 production is increased in joint synovitis and is continually produced even in noninflamed. This is one reason why our marker correlated well with disease activity. In addition, MMP-3 expression is relatively restricted within the joint and partly leaks into the serum. Thus, it might be possible to directly determine inflammation at local lesions without an effect of any other influences.

There were a few limitations in the present meta-analysis. First, lack of confidence in the overall results due to the sample sizes in the enrolled studies was comparatively small. Secondly, the absence of some data in the published studies that were examined may limit the validity of our results. Thus, together with the retrieval and manual selection method, these limitations may have impacted the accuracy of the results by likely missing the more detailed studies. Our meta-analysis included data from Caucasian and Asian, thus our study should be optimized by larger scale of populations. Lack of the original data of available studies limited our further evaluation of potential interactions, such as age, gender, environmental factors, and DAS&VAS scores. Fourth, due to different assessment methods of joints destruction, we did not investigate the association between MMP-3 level and RA severity of all included studies.

Although we conducted sensitivity analysis, the heterogeneity was still observed. Finally, some inevitable publication bias may exist in the results, although neither the

Begg's funnel plots nor Egger's regression test indicated obvious publication bias in our meta-analysis.

References

- [1] Hishahsi Yamanaka, Yuko Matsuda, Michi Tanaka, Wako Sendo, Hiroshi Nakajima, Atsuo Taniguchi et al. Serum matrix Metalloproteinase - 3 as a predictor of the degree of joint destruction during the six months after measurement, In patients with early Rheumatoid Arthritis. *Arthritis and Rheumatism*. 2000; 43[4]:852-8.
- [2] Martina Skacelovaa, Zuzana Hermanovab, Pavel Horaka, Ahmed Kazia, Katerina Langovac . Higher levels of matrix metalloproteinase-3 in patients with RA reflect disease activity and structural damage. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*. 2017; 161[3]:296-302.
- [3] Takashi Adachi, Naoki Hattori, Takashi Ishihara, Hirokazu Iida, Takanori Saito, Shigeo Miyashima et al. Possible involvement of matrix metalloproteinase-3 in the pathogenesis of macroprolactinaemia in some patients with rheumatoid arthritis. *European Journal of Endocrinology*. 2013; 169: 203–209.
- [4] Stefanie Hemmann, Jürgen Graf, Martin Roderfeld, Elke Roeb. Expression of MMPs and TIMPs in liver fibrosis – a systematic review with special emphasis on anti-fibrotic strategies. *Journal of Hepatology*. 2007; 46: 955- 975.
- [5] Yasuo Yoshihara, Hiroyuki Nakamura, Ken'ichi Obata, Harumoto Yamada, Taro Hayakawa, Kyosuke Fujikawa et al. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in synovial fluids from patients with rheumatoid arthritis or osteoarthritis. *Ann Rheum Dis*. 2000; 59:455–461.
- [6] MG Kazantseva, NA Hung, J Highton and PA Hessian. MMP expression in rheumatoid inflammation: the rs11568818 polymorphism is associated with MMP-7 expression at an extra-articular site. *Genes and Immunity*. 2013; 14:162–169.
- [7] Mahmood M. T. M. Ally, Bridget Hodkinson, Pieter W. A. Meyer, Eustasius Musenge, Mohammed Tikly, and Ronald Anderson. Serum Matrix Metalloproteinase-3 in Comparison with Acute Phase Proteins as a Marker of Disease Activity and Radiographic Damage in Early Rheumatoid Arthritis. *Mediators of Inflammation*. 2013; Article ID 183653, 6 pages.
- [8] Baseline serum MMP-3 levels in patients with Rheumatoid Arthritis are still independently predictive of radiographic progression in a longitudinal observational cohort at 8 years follow up. Houseman M1, Potter C, Marshall N, Lakey R, Cawston T, Griffiths I, Young-Min S, Isaacs JD. *Arthritis Res Ther*. 2012 ;14[1]:R30. doi: 10.1186/ar3734.
- [9] Shu Sun, Anne-Christine Bay-Jensen, Morten A Karsdal, Anne Sofie Siebuhr, Qinlong Zheng, et al. The active form of MMP-3 is a marker of synovial inflammation and cartilage turnover in inflammatory joint diseases. *BMC Musculoskeletal Disorders*. 2014; 15:93. <https://doi.org/10.1186/1471-2474-15-93>.
- [10] Xue M, McKelvey K, Shen K, Minhas N, March L, Park S, Y. Jacson C, J. Endogenous MMP-9 and not MMP-2 promotes rheumatoid synovial fibroblast

- survival, inflammation and cartilage degradation. *Rheumatology* 2014; 53[12]: 2270-2279. doi:10.1093/rheumatology/keu254.
- [11] M. D. Posthumus P. C. Limburg J. Westra H. A. Cats R. E. Stewart M. A. van Leeuwen M. et al. Serum levels of matrix metalloproteinase-3 in relation to the development of radiological damage in patients with early rheumatoid arthritis. *Rheumatology* 1999;38[11]:1081-1087.
- [12] Zintzaras E and Ioannidis JP [2005a]. Heterogeneity testing in meta-analysis of genome searches. *Genet. Epidemiol.* 2005;28: 123-137.
- [13] Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; 327:557-560.
- [14] Zintzaras E and Ioannidis JP. HEGESMA: genome search meta-analysis and heterogeneity testing. *Bioinformatics* 2005;21[18] : 3672-3673.
- [15] Egger M, Davey Smith G, Schneider M, Minder C: Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; 315:629-634.
- [16] The PRISMA statement <http://www.prisma-statement.org/documents/PRISMA%202009%20flow%20diagram.pdf>.
- [17] G.Q. Zeng, A.B. Chen, W. Li, J.H. Song and C.Y. Gao. High MMP-1, MMP-2, and MMP-9 protein levels in osteoarthritis. *Mol. Res.* 2015; 14 [4]: 14811-14822.
- [18] Zhitao Feng, Guochao He Zhuanghong Chen, Zhengzhi Wu and Juan Li. Lack of association of matrix metalloproteinase-3 gene polymorphism with susceptibility to rheumatoid arthritis: a meta-analysis. *BMC Musculoskeletal Disorders* 2014;15:376.
- [19] Jian-Da Ma, Jing-Jing Zhou, Dong-Hui Zheng, Le-Feng Chen, Ying-Qian Mo, Xiu-ning Wei et al. Serum Matrix Metalloproteinase-3 as a Noninvasive Biomarker of Histological Synovitis for Diagnosis of Rheumatoid Arthritis. Publishing Corporation Mediators of Inflammation. 2000; 4 :852-858.
- [20] Masaru Takeshita, Atsushi Kuno, Katsuya Suzuki, Atsushi Matsuda, Hiroko Shimazaki, Tomomi Nakagawa et al. Alteration of matrix metalloproteinase-3 O-glycan structure as a biomarker for disease activity of rheumatoid arthritis. *Arthritis Research & Therapy*. 2016; 18[1]:112.
- [21] Kazuko Shiozawa, Takashi Yamane, Miki Murata, Ryosuke Yoshihara, Ken Tsumiyama, Shigeaki Imura et al. MMP-3 as a predictor for structural remission in RA patients treated with MTX monotherapy. *Arthritis Research & Therapy*. 2016;18:55-60.
- [22] Ju Hee Ryu, Aeju Lee, Jun-Uk Chu, Heebeom Koo, Chang-Yong Ko, Han Sung Kim Soo et al. Early diagnosis of arthritis in mice with collagen-induced arthritis, using a fluorogenic matrix metalloproteinase 3-specific polymeric probe. *Arthritis & Rheumatism*. 2011; 63[12]:3824-3832.
- [23] Tokai N, Yoshida, Kotani, Yoshikawa, Kimura Y, Fujiki Y, Matsumura Y, Takeuchi T, Makino S, Arawaka S. Serum matrix metalloproteinase 3 levels are associated with an effect of iguratimod as add-on therapy to biological DMARDs in patients with rheumatoid arthritis. *PLoS One*. 2018 Aug 23;13[8]:e0202601.
- [24] Heard BJ, Martin L, Rattner JB, Frank CB. Matrix metalloproteinase protein expression profiles cannot distinguish between normal and early osteoarthritic synovial fluid. *BMC Musculoskeletal Disord.* 2012, 13:126.
- [25] A So A M Chamot, V Péclat J C Gerster. Serum MMP-3 in rheumatoid arthritis: correlation with systemic inflammation but not with erosive status. *Rheumatology*, 1999; 38[5]: 407-410.
- [26] S. Tsukahara M. Shinozaki K. Ikari T. Mochizuki E. Inoue T. Tomatsu M. Hara H. Yamanaka N. Kamatani S. Momohara. Effect of matrix metalloproteinase-3 functional SNP on serum matrix metalloproteinase-3 level and outcome measures in Japanese RA patients. *Rheumatology*. 2008;47:41-44.
- [27] C. Ribbens B. Andre O. Kaye M.J. Kaiser V. Bonnet J.M. Jaspard D. de Groote N. Franchimont M. G. Malaise et al. Synovial fluid matrix metalloproteinase 3 levels are increased in inflammatory arthritides whether erosive or not. *Rheumatology*. 2000; 39: 1357-65.