Association of Single nucleotide polymorphism rs1799750 of MMP-1 with Osteoarthritis susceptibility in Indian population

Sagare Aparna[1*], Dandekar Sucheta[2] and Rajyadhykasha Anjali[3]
1. Department of Biochemistry, MGM Medical College, Nerul, Navi Mumbai, INDIA
2. Department of Biochemistry, Eras Medical College, Lucknow, INDIA
3. Department of Medicine, Seth G.S. Medical College and KEM Hospital, Mumbai, INDIA
*aparnaa4s@yahoo.com

Abstract

Degenerative and inflammatory events in osteoarthritis (OA) lead to release Matrix metalloproteases (MMPs). Single nucleotide polymorphism has been observed influencing release of matrix degrading enzymes. Our study aims to determine influence of genetic variants in MMPs and it may develop risk for development of OA. In the present study, we have investigated the genotypic and haplotypic relationships of the MMP-1 and MMP-3 genes among OA patients. PCR-RFLP was used to determine the genotypes of 60 osteoarthritis patients and 60 healthy age matched controls. Fisher's exact test/Chi-square test was used to compare the differences of the investigated SNPs (rs1799750, rs3025058) allele and genotype distribution between osteoarthritis cases and controls. Correlation between each SNP and osteoarthritis risk was assessed under multiple genetic models analysis and haplotype analysis.

Significant difference was observed for the allele and genotype distribution of the SNPs between cases and controls. Analysis of the genetic models (codominant, dominant, overdominant) and haplotype (2G-5A, 1G-5A) block showed significant association with OA susceptibility. Present case-control study is the study among Indian population to explore the potential correlation between MMP-3 polymorphisms and osteoarthritis risk.

Keywords: Osteoarthritis, Single nucleotide polymorphisms (SNPs), MMP-1, MMP-3, Haplotypes.

Introduction

Osteoarthritis (OA) is the most common form of joint disease among musculoskeletal (MSK) disorder and most common cause of disability in elderly. [21] Prevalence of OA was found highest among MSK disorders in India. On an average, 5-7 % females get affected more as compared to males.[10] OA usually affects any population from low to high socioeconomic strata and disease usually begins at later stages of life. OA mainly involves damage to articular cartilage, formation of osteophytes, subchondral bony cysts, thickening of subchondral plate and synovial membrane neovascularization.[16] Acute inflammations to the synovium and release of inflammatory products play important role in pathogenesis of disease. OA led to chronic and subclinical inflammation in disease. Furthermore, genetic predispositions are also the potential risk factors for the initiation and progression of this disease.[3,20]

In OA, the matrix metalloproteinases (MMPs) are a group of zinc-dependent endopeptidases involved in degradation of cartilage like aggrecansess (proteoglycans) and collagenases. (collagen)[1,22]. Synovial fibroblasts, neutrophils and mast cells situated in the synovial membrane further release MMP enzymes which in turn contribute to this cartilage degradation. Studies reported that the releases of inflammatory cytokines in particularly interleukin-1 (IL-1) and tumor necrosis factor-α (TNF- α), influence release of inducing synthesis of proteolytic enzymes by articular chondrocytes[6,9].

The gene of MMP-1 and MMP-3 located on long arm of chromosome 11 covers length of 8 kb and is expressed in variety of the cells such as fibroblasts, chondrocytes, endothelial and epithelial cells and in various tumor cells[11]. Several studies have found overexpression of such proteolytic enzymes MMP-1. MMP-3 provides a molecular mechanism for extensive degradation of ECM[11]. The promoter polymorphism results into variable transcription activity of both these enzymes[15,18]. Whether polymorphisms of the MMP-1 and MMP-3 gene are involved in the pathogenesis of OA, is not clear. Therefore, the objective of the present study was to investigate the potential association between MMP-1 and 3 polymorphisms and OA susceptibility through a case-control study in an Indian population.

Material and Methods

Study subjects: 60 OA patients and 60 healthy controls were enrolled in present study. All subjects were recruited between June 2021 to December 2022 at KEM Hospital, Mumbai. Inclusion and exclusion criteria were as follows: subjects were ethnically homogenous population from Mumbai, Maharashtra. The OA cases were diagnosed as per the criteria of the American College of Rheumatology. Subjects who have other type of arthritis or other joint diseases were excluded from the study. This work was approved by Institutional Ethics committee of Seth G.S. Medical College and KEM Hospital, Mumbai (EC/GOVT03/2018). All the study participants gave written informed consent.

Present study was carried out to determine SNPs of MMP-1 and MMP-3 used to investigate association with OA susceptibility. 10 ml of venous blood samples were collected in EDTA bulb after taking their written consent for extraction of genomic DNA. We used Epigentek mini kit for isolation of DNA from blood. Primers were designed by using NCBI search tool procured from Genie, Bangalore. Post PCR RFLP analysis as per protocol described earlier was applied to observe status of these SNPs in cases and controls[4]. Homozygous and heterozygous status was observed by UV transilluminator.

Statistical Analysis: All statistical analyses were performed using SPSS 26.0 (SPSS, Chicago IL USA). Differences in gender and age between patients with OA and healthy controls were evaluated by Pearson Chi-square test. Deviation from Hardy-Weinberg equilibrium (HWE) of allele frequency of MMP-1 controls was analysed by the exact test. Chi-square test/Fisher's exact test was used to compare the differences of the investigated SNPs allele and genotype distribution between OA cases and controls. After that, the correlation between each SNP and OA risk was assessed under four genetic models: codominant, dominant, recessive and additive model using SNP Stat software.

The odd ratio (OR) and corresponding 95% confidence intervals (CI), calculated by multivariate unconditional logistic regression models with adjustment for gender and age, were used to assess the relationship between each SNP and OA susceptibility. Two-sided P≤0.05 was regarded statistically significant for statistical tests. We also used the SHEs is online version to evaluate and visualize patterns of linkage disequilibrium (LD) and haplotype construction[2,17].

Results

60 OA and 60 controls were enrolled in this study. Significant differences were observed between OA cases and controls in term of age and gender. Table 1 shows that MMP-1 and MMP-3 SNPs were all in HWE in controls. Allele and genotype distribution did not show any statistical significance. Allele and genotypic multivariate analysis with adjustment of gender did not reveal any significant SNP and OA risk. Table 2 suggested no significant differences among the genotypic frequency observed between OA cases and controls in term of gender (P>/=0.050).

Table 3 showed multivariate unconditional logistic regression analysis without adjustment of age and gender. It showed statistically significant correlation between the SNP and OA risk in codominant, dominant, recessive and additive models with a value of P< 0.05. We found that rs 1799750 was associated with an increased OA risk by codominant (genotype "1G/2G", OR = 0.02, 95% CI: 0.01-0.07, P <0.0001) and dominant model analyses ("1G/1G", OR = 0.03, 95% CI: 0.01-0.10, P <0.0001). In over-dominant models (1G/2G OR = 0.03, 95% CI: 0.01-0.09, P = P <0.0001) overall suggestive of 1G/2G status shows association between MMP-1 loci and OA susceptibility.

Model association analyses were performed for MMP-3 SNP by unconditional logistic regression analysis. We found that rs3025058 was not associated with an increased OA risk by codominant, dominant and over-dominant models showing no association between MMP-3 loci and OA susceptibility.

Furthermore, the association between MMP-1 polymorphisms and OA risk was evaluated by haplotype analysis. The MMP-1 linkage disequilibrium (LD) block showed statistically significant linkage between rs1799750 and rs3025058. Haplotype frequency is of more than 0.05 (Table 5). We found that the "2G5A" (adjusted OR=0.169, 95% CI: 0.094-0.303, P< 0.001) and "1G5A" (adjusted OR=17.58, 95% CI: 6.6-46.1, P< 0.001) haplotypes were associated with OA susceptibility compared with "2G6A" haplotype P=0.563.

Table 1
Gender wise Allelic frequency distribution for MMP-1 in in study subjects

SNP1 allele frequencies (n=120)						
	All subjects		Sex=Female		Sex=Male	
Allele	Count	Proportion	Count	Proportion	Count	Proportion
2G	179	0.75	85	0.69	94	0.81
1G	61	0.25	39	0.31	22	0.19

n= no. of individuals

Table 2
Genotype frequency for MMP-1 in study subjects

SNP1 genotype frequencies (n=120)						
	All subjects		Sex=Female		Sex=Male	
Genotype	Count	Proportion	Count	Proportion	Count	Proportion
1G/1G	9	0.08	6	0.1	3	0.05
2G/1G	43	0.36	27	0.44	16	0.28
2G/2G	68	0.57	29	0.47	39	0.67

n= no. of individuals

Table 3
Multivariate unconditional logistic regression analysis of SNP1 MMP1 association crude analysis

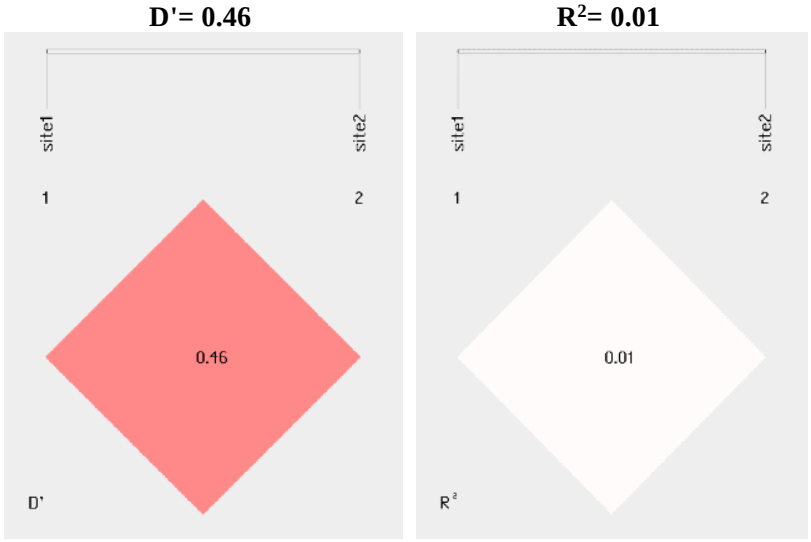
SNP1 association with response status (n=120, crude analysis)							
Model	**Genotype**	**status=OA**	**status=co**	**OR (95% CI)**	**P-value**	**AIC**	**BIC**
Codominant	2G/2G	14 (23.3%)	54 (90%)	1.00	<0.0001*	108.4	116.7
	1G/2G	40 (66.7%)	3 (5%)	**0.02 (0.01-0.07)**			
	1G/1G	6 (10%)	3 (5%)	**0.13 (0.03-0.58)**			
Dominant	2G/2G	14 (23.3%)	54 (90%)	1.00	<0.0001*	110.3	115.9
	1G/2G-1G/1G	46 (76.7%)	6 (10%)	**0.03 (0.01-0.10)**			
Recessive	2G/2G-1G/2G	54 (90%)	57 (95%)	1.00	0.29	169.3	174.8
	1G/1G	6 (10%)	3 (5%)	0.47 (0.11-1.99)			
Over dominant	2G/2G-1G/1G	20 (33.3%)	57 (95%)	1.00	<0.0001*	114	119.5
	1G/2G	40 (66.7%)	3 (5%)	**0.03 (0.01-0.09)**			
Log-additive	---	---	---	**0.08 (0.03-0.20)**	<0.0001*	125	130.6

n= no of individuals OA = cases, Co= control
OR= odds ratio (95% CI) AIC = Akaike information criterion
P statistical significance <0.05 BIC = Bayesian information criterion

Table 4
Multivariate unconditional logistic regression analysis SNP2 MMP-3 association crude analysis

SNP2 association with response status (n=120, crude analysis)							
Model	**Genotype**	**status=OA**	**status=co**	**OR (95% CI)**	**P-value**	**AIC**	**BIC**
Codominant	5A/5A	43 (71.7%)	43 (71.7%)	1.00	0.83	172	180.3
	5A/6A	15 (25%)	16 (26.7%)	1.07 (0.47-2.43)			
	6A/6A	2 (3.3%)	1 (1.7%)	0.50 (0.04-5.72)			
Dominant	5A/5A	43 (71.7%)	43 (71.7%)	1.00	1	170.4	175.9
	5A/6A-6A/6A	17 (28.3%)	17 (28.3%)	1.00 (0.45-2.21)			
Recessive	5A/5A-5A/6A	58 (96.7%)	59 (98.3%)	1.00	0.56	170	175.6
	6A/6A	2 (3.3%)	1 (1.7%)	0.49 (0.04-5.57)			
Over dominant	5A/5A-6A/6A	45 (75%)	44 (73.3%)	1.00	0.83	170.3	175.9
	5A/6A	15 (25%)	16 (26.7%)	1.09 (0.48-2.47)			
Log-additive	---	---	---	0.94 (0.47-1.89)	0.86	170.3	175.9

n= no of individuals OA = cases, Co= control
OR= odds ratio (95% CI) AIC = Akaike information criterion
P statistical significance <0.05 BIC = Bayesian information criterion



D’ R2

Figure 1: Value of D’ and R ^{2} in haploview LD plot

Table 5
Haplotype association response in OA

Haplotype	Case (freq)	Control (freq)	Chi2	Fisher's p	Pearson's p	OR [95% CI]
2G5A	50(0.416)	97(0.808)	38.779	5.79e-10	4.74e-10	0.169 [0.094~0.303]
2G6A	17(0.141)	14(0.116)	0.333	0.7	0.563	1.249 [0.585~2.665]
1G5A	52(0.433)	5(0.041)	50.825	1.63e-13	1.01e-12	17.588 [6.697~46.186]

OR= odds ratio (95% CI) P statistical significance <0.05

Haplotype association response in OA and control is suggestive of haplotypic response among MMP-1 and MMP- 3. Specifically, combinations of 2G- 5A, 1G -5A remain statistically significant with nonsignificant allelic combination 2G- 6A. Linkage disequilibrium analysis of both SNPs shows that Global Chi2 is 54.042, Fisher's p is 6.63e-14 and Pearson's p is 1.84e-12. Value of D' and R 2 are shown in haploview diagram.

Discussion

In the present study, we found significant correlation under the investigated genetic models between variants of MMP-1 and OA susceptibility but MMP- 3 did not show any association with risk of development of OA. Barlas et al[2] in Turkish population found that several SNPs of MMP gene were linked in development and progression of knee OA. Findings of their study reveal that -1607 1G/2G polymorph 2G/2G genotype most frequently occurred than 1G/2G and 1G/1G polymorphism. Panagiotis et al[15] postulated that rs 1799750 polymorphism mediated by release of MMP contributes risk of developing many diseases. They observed no significant association between MMP-1 gene rs1799750 polymorphism and knee OA in a Greek population.

Luo et al[13] studied rs1799750 polymorphism of MMP-1 for Asian population susceptible for the risk of development of temporomandibular joint OA. Study by Yang et al[22] observed lack of association among SNP and knee OA in Chinese population. Several reasons bring about this discrepancy. First, the allele and genotype frequencies of the studied SNPs present regional disparities among the two groups. Secondly, the sample size of many studies may not be large enough to achieve a convincing result. Thirdly, differences in covariates among patients and controls may affect study findings. This mechanism should be verified by doing large number of research.

Many studies did not show role of MMPs and/ or genetic component of these enzymes in pathological mechanism during disease development and progression. So, lot can be done for identifying genetic architecture of MMPs in health and diseases. Both genes of MMP-1 and 3 have been mapped on chromosome no. 11, the long arm in the region of 11q22.3. 260. Distance between the two genes is near about 37.64 kilobases. The phenotypic and genotypic appearance of both genes remains unknown till date.[13]

The biological function of this phenomenon is not much understood. Our observation suggests that MMP-3 SNPs rs3025058 and MMP-1 rs1799750 are located in the same linkage disequilibrium (LD) block (LD plot among two SNPs) and the association remains significantly strong (D'=0.16, R= 0.01). Our study shows a significant linkage disequilibrium among the haplotypes of 2G5A and 1G/5A. Results of Abd Allah et al[1] showed that the haplotype 2G-6A which remains as mutant alleles showing high frequencies in the patients of OA than in the controls. Study by Geng et al[6] suggested that 5A-1G haplotype associated with erosive status and MMP-1 rs1799750 is mainly associated with joint erosion in OA.

MMP-1 polymorphism 1G/2G rs1799750 remains dependent on 5A/6A allele of rs3025058 MMP-3 polymorphism. Our results also suggest that 6A allele remains independent on both 1G and 2G allele. There is significant association of polymorphism of MMP-3 with MMP-1 polymorphic status in OA. Study by Abd-allah et al[1] found 6A/6A genotype of MMP-3 polymorphism was significantly more frequent in patients with OA as compared to controls.

No data or other studies were found till date about MMP-3 (- 1171 5A/6A) polymorphism and susceptibility with OA. Similarly, it was also observed that 2G allele at promoter region of MMP-1 gene increases transcription by 20 folds as compared to 1G allele. Previous studies have also correlated -1607 1G/2G SNP associated with progression of disease, from inflammatory diseases to cancer. Our observation of association among haplotypes is suggestive of proximity of the MMP-1 and MMP-3 genes. Expressions of MMP-1 and MMP3- are mostly synchronized and their promoters may contain some regulatory elements.

MMP-1 polymorphisms are associated with OA risk in an Indian population. Given the fact that identification of candidate genes and SNPs would help to elucidate the molecular pathogenesis of OA and would have the possibility to predict the risk of OA. Further study is still required to validate the potential association in other populations and a larger sample size.

Conclusion

Articular cartilage deterioration is the main event in pathogenesis of OA leading degradation of extracellular matrix (ECM). Furthermore, inflammation in OA results in activation of chondrocytes and neutrophils which in turn produce large amount of matrix-degrading enzymes including MMPs. The increased and aberrant expression of

MMPs during osteoarthritis initiation and progression remains important evidence justifying the association between MMP-1 polymorphisms and OA susceptibility.

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References
1. Abd-Allah S.H., Shalaby S.M., Pasha H.F., El-Shal A.S. and Abou El-Saoud A.M., Variation of matrix metalloproteinase 1 and 3 haplotypes and their serum levels in patients with rheumatoid arthritis and osteoarthritis, *Genet Test Mol Biomarkers*, **16(1)**, 15-20 **(2012)**

2. Barlas I.O. et al, Association of (-1,607) 1G/2G polymorphism of matrix metalloproteinase-1 gene with knee osteoarthritis in the Turkish population, *Rheumatol. Int*., **9(4)**, 383–388 **(2009)**

3. Burger M.C., De Wet H. and Collins M., Matrix metalloproteinase genes on chromosome 11q22 and risk of carpal tunnel syndrome, *Rheumatol Int*., **36(3)**, 413-9 **(2016)**

4. Cheng Y.C. et al, Genome-wide association scan identifies variants near matrix metalloproteinase (MMP) genes on chromosome 11q21-22 strongly associated with serum MMP-1 levels, *Circ Cardiovasc Genet*., **2(4)**, 329–337 **(2009)**

5. Chopra A., Patil J., Billempelly V., Relwani J. and Tandle H.S., WHO-ILAR COPCORD Study. WHO International League of Associations from Rheumatology Community Oriented Program from Control of Rheumatic Diseases, Prevalence of rheumatic diseases in a rural population in western India: a WHO-ILAR COPCORD Study, *J Assoc Physicians India*, **49**, 240-6 **(2001)**

6. Geng R., Xu Y., Hu W. and Zhao H., The association between MMP-1 gene rs1799750 polymorphism and knee osteoarthritis risk, *Biosci Rep*., **38(5)**, BSR20181257 **(2018)**

7. Helmick C.G., Felson D.T., Lawrence R.C., Gabriel S., Hirsch R. and Kwoh C.K., Estimates of the prevalence of arthritis and other rheumatic conditions in the United States, Part I, *Arthritis Rheum*., **58(1)**, 15–25 **(2008)**

8. Heinegard D. and Saxne T., The role of the cartilage matrix in osteoarthritis, *Nat Rev Rheumatol*, **7(1)**, 50-56 **(2011)**

9. Horton D.B., Scott F.I., Haynes K., Putt M.E., Rose C.D., Lewis J.D. and Strom B.L., Antibiotic Exposure and Juvenile Idiopathic Arthritis: A Case-Control Study, *Pediatrics*, **136(2)**, e333-43 **(2015)**

10. Jun-Jie Chen, Jie-Feng Huang, Wen-Xi Du and Pei-Jain Tong, Expression and significance of MMP-3 in synovium of knee joint at different stage in osteoarthritic patients, *Asian Pacific Journal of Tropical Medicine*, **7(4)**, 297-300 **(2014)**

11. Li Z., Zhang Z., He Z., Tang W., Li T., Zeng Z., He L. and Shi Y., A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis.bio-x.cn), *Cell Res*., **19(4)**, 519-23 **(2009)**

12. Loughlin J., Genetic contribution to osteoarthritis development: current state of evidence, *Curr Opin Rheumatol*., **27**, 284-288 **(2015)**

13. Luo S., Deng M., Long X., Li J., Xu L. and Fang W., Association between polymorphism of MMP-1 promoter and the susceptibility to anterior disc displacement and temporomandibular joint osteoarthritis, *Arch Oral Biol*., **60(11)**, 1675–1z680 **(2015)**

14. Masuhara K., Nakai T., Yamaguchi K., Yamasaki S. and Sasaguri Y., Significant increases in serum and plasma concentrations of matrix metalloproteinases 3 and 9 in patients with rapidly destructive osteoarthritis of the hip, *Arthritis Rheum*., **46(10)**, 2625-31 **(2002)**

15. Panagiotis Lepetsos, Andreas Pampanos, Emmanouil Kanavakis, Maria Tzetis, Dimitrios Korres, Athanasios G., Papavassiliou A.G. and Efstathopoulos N., Association of MMP-1 – 16071G/ 2G polymorphism with primary knee osteoarthritis in the Greek population, *Journal of Orthopedic Research*, **32(9)**, 1155-1160 **(2014)**

16. Rodriguez-Fontenla C. and Gonzalez A., Genetics of osteoarthritis, *Reumatol Clin*, **11**, 33-40 **(2015)**

17. Sagare Aparna, Dandekar Sucheta and Rajadhyaksha Anjali, Association of Promoter Polymorphism of MMP-1 rs1799750 and MMP-3 rs3025058 with circulating levels of MMP-1 and MMP-3 in patients with Rheumatoid Arthritis, *Res. J. Biotech*., **17(3)**, 111-118 **(2022)**

18. Sarzi-Puttini P., Cimmino M.A., Scarpa R., Caporali R., Parazzini F., Zaninelli A., Atzeni F. and Canesi B., Osteoarthritis: An Overview of the Disease and Its Treatment Strategies, *Arthritis and Rheumatism*, **35(1)**, 1-10 **(2005)**

19. Solé X., Guinó E., Valls J., Iniesta R. and Moreno V., SNP Stats: a web tool for the analysis of association studies, *Bioinformatics*, **22(15)**, 1928-9 **(2006)**

20. Souslova V., Townsend P.A., Mann J., van der Loos C.M., Motterle A., D'Acquisto F., Mann D.A. and Ye S., Allele-specific regulation of matrix metalloproteinase-3 gene by transcription factor NFkappaB, *PLoS One*, **255(3)**, e9902 **(2010)**

21. Tetlow L.C., Adlam D.J. and Woolley D.E., Matrix metalloproteinase and proinflammatory cytokine production by chondrocytes of human osteoarthritic cartilage: Associations with degenerative changes, *Arthritis & Rheumatism*, **44(3)**, 585-594 **(2001)**

22. Yang H.Y., Chuang S.Y., Fang W.H., Huang G.S., Wang C.C., Huang Y.Y., Chu M.Y., Lin C., Su W., Chen C.Y., Yang Y.T. and Su S.L., Effect of RAGE polymorphisms on susceptibility to and severity of osteoarthritis in a Han Chinese population: a case-control study, *Genet Mol Res*, **14(3)**, 11362-70 **(2015)**.

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