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Genetic Variations In The MMP-3 Gene: Unravelling SNP Associations With Osteoarthritis Susceptibility And Progression

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Abstract Osteoarthritis (OA) is a multifactorial degenerative joint disorder influenced by genetic and environmental factors, with bone mineral density (BMD) playing a critical role in disease susceptibility. This study comprehensively examines the impact of single nucleotide polymorphisms (SNPs) within the matrix metalloproteinase (MMP-3) gene on BMD risk scores and their association with OA. A comparative sequence analysis identified five SNP variations distinguishing healthy and diseased individuals, suggesting their potential role in modifying protein structure, extracellular matrix interactions, and OA pathogenesis. Logistic regression modelling demonstrated an inverse correlation between BMD risk scores and disease susceptibility, with individuals possessing higher BMD scores exhibiting significantly reduced risk. Quintile-based stratification further confirmed this trend, as individuals in the highest BMD quintile (Q5) demonstrated a 71% lower risk of developing OA compared to those in the lowest quintile (Q1). These findings reinforce the clinical significance of SNP profiling as a predictive tool for identifying at-risk individuals and implementing early intervention strategies. Genetic screening may enable risk stratification, guiding preventative measures such as lifestyle modifications, nutritional supplementation, and targeted physical therapy for individuals with lower BMD scores. Furthermore, elucidating the molecular mechanisms linking SNPs to BMD regulation may facilitate the development of gene-based therapies and pharmacological interventions tailored to mitigate disease progression. The results of this study underscore the necessity for precision medicine approaches in OA management, integrating genetic risk assessment with personalized therapeutic strategies. However, to establish causality and further refine risk models, future research should incorporate functional validation studies, large-scale genome-wide association studies (GWAS), and longitudinal cohort analyses. Such advancements will be instrumental in enhancing early disease prediction, optimizing treatment strategies, and improving clinical outcomes in OA patients.

Keywords: Osteoarthritis (OA), Matrix Metalloproteinase (MMP-3) gene, Single Nucleotide Polymorphisms (SNPs), Bone Mineral Density (BMD)

1. INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disorder that affects all components of the articular system, characterized by progressive structural deterioration (Fusco et al, 2017). The hallmark manifestations of OA include cartilage degeneration, abnormal load distribution across the joint, and compromised biomechanical integrity. These pathological changes culminate in cartilage thinning, fibrosis, erosion, fissuring, ulceration, and, in advanced stages, complete loss of the articular surface (Loeser et al, 2012). Primarily affecting middle-aged and elderly individuals, OA is associated with a high prevalence of chronic pain and functional impairment, significantly diminishing the quality of life and imposing substantial socioeconomic and familial burdens (Ashkavand et al, 2013). While the local remodeling of subchondral bone in OA progression is complex and multifaceted, observational studies have reported a significant association between elevated systemic bone mineral density (BMD) and an increased risk of radiographic knee OA. This intriguing correlation suggests that genetic determinants influencing systemic BMD may also contribute to OA susceptibility, warranting further investigation into the underlying molecular and genetic mechanisms (Zhang et al, 2000).

Despite extensive research, the precise pathophysiological mechanisms underlying OA remain inadequately understood, and no definitive cure exists. Emerging evidence suggests that an imbalance in metabolic homeostasis, specifically between cartilage degradation and synthesis, is a principal driver of OA-associated cartilage deterioration (Elahi et al, 2023). The matrix metalloproteinase (MMP) family has garnered significant attention due to its pivotal role in the degradation of cartilage extracellular matrix (ECM) and basement membrane components. MMPs constitute a family of at least 28 proteolytic enzymes that play a pivotal role in tissue remodelling and repair, both during development and in response to inflammation (Jabłońska-Trypuć et al, 2016). Their primary function involves the degradation of the extracellular matrix (ECM), targeting key structural components such as fibronectin, collagen, laminin, vitronectin, and proteoglycans. Beyond ECM turnover, MMPs contribute to various physiological and pathological processes, including angiogenesis, cell proliferation, apoptosis, immune responses, and cytokine regulation (Neve et al, 2014). Like other peptidases, MMPs are synthesized as inactive proenzymes and require proteolytic cleavage at the N-terminal pro-peptide region for activation at the tissue level. Upon activation, MMPs bind to membrane receptors, thereby exerting their enzymatic activity. Dysregulated MMP expression has been implicated in numerous pathological conditions, including irreversible tissue degradation in arthritis and collagen breakdown in malignancies, thereby facilitating tumor metastasis (He et al, 2020).

Under normal conditions, MMPs are expressed at low levels in joints; however, their expression is markedly upregulated in arthritic tissues. Seven MMPs—MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-13, and MMP-14—are known to be expressed under various articular cartilage conditions (Milaras et al, 2021). Among these, MMP-1, MMP-2, MMP-13, and MMP-14 are constitutively present in adult cartilage, playing a role in tissue homeostasis, with their expression significantly increasing under pathological conditions. In contrast, the presence of MMP-3, MMP-8, and MMP-9 in cartilage is predominantly associated with disease states (Mattey et al, 2012). Among them, MMP-3, primarily secreted by chondrocytes and synovial cells, exhibits a dual function: it not only facilitates the breakdown of various ECM components but also serves as an activator of other proteolytic enzymes, including serine proteases (Sun et al, 2014). While MMP-3 expression has been well-documented in the synovium of OA joints, limited evidence exists regarding the differential expression patterns of MMP-3 within the synovial membrane at various stages of OA progression. This research aims to detect single nucleotide polymorphism (SNP), a type of mutation caused by a nucleotide substitution in a specific gene region, in genes encoding MMPs of osteoarthritis patients.

2. METHODOLOGY

2.1. Sample Collection

Venous blood samples were obtained from osteoarthritis patients and age-matched healthy controls in strict adherence to ethical guidelines and with informed consent. Each participant provided 5 mL of blood, which was collected using sterile ethylenediaminetetraacetic acid (EDTA) coated vacutainer tubes to prevent coagulation. The procedure was conducted by trained healthcare professionals under aseptic conditions to preserve sample integrity and minimize the risk of contamination. Immediately after collection, samples were systematically labelled with unique identifiers, including participant demographics, health status, and collection date. To maintain biological stability, blood samples were stored at 4°C and transported to the laboratory within two hours for further analysis. Upon arrival, samples underwent centrifugation at 3000 rpm

for 10 minutes, effectively separating plasma and buffy coat for subsequent DNA extraction. All protocols were executed in compliance with institutional ethical approvals and biosafety regulations (Tuck et al, 2009).

2.2. DNA Extraction

Genomic DNA was isolated from blood samples using the Qiagen DNeasy Blood & Tissue Kit, adhering to the manufacturer's standardized protocol. Following centrifugation at 3000 rpm for 10 minutes, the buffy coat fraction was carefully transferred to a sterile microcentrifuge tube for processing. Cell lysis was induced by the addition of proteinase K and lysis buffer, with incubation at 56°C for 10 minutes, ensuring thorough cell membrane disruption and protein digestion. To promote DNA binding, ethanol was incorporated into the lysate before transferring it onto a silica membrane spin column, where selective DNA adsorption occurred while impurities were eliminated through successive centrifugation steps (Rodriguez et al, 2024). A series of buffer washes were performed to effectively remove residual proteins, salts, and other contaminants. Finally, purified genomic DNA was eluted in TE buffer and preserved at −20°C for subsequent molecular analyses (Lee et al, 2001). The integrity and purity of the extracted DNA were evaluated using Nanodrop spectrophotometry, while agarose gel electrophoresis was employed to confirm DNA quality, ensuring its suitability for PCR amplification and sequencing applications.

2.3. PCR Amplification of the MMP-3 Gene Regions

A polymerase chain reaction (PCR) mixture was formulated by combining 5 ng of DNA template with 24 µL of Taq PCR master mix. This master mix contained heat-stable Taq DNA polymerase, which facilitated DNA strand synthesis during the extension phase. Additionally, 0.5 mM deoxynucleotide triphosphates (dNTPs) (dATP, dTTP, dCTP, and dGTP) were incorporated as nucleotide precursors essential for DNA polymerization. The reaction mixture also included 3.2 mM $MgCl_2$, providing Mg^{2+} ions necessary to enhance the catalytic efficiency of the polymerase, while a reaction buffer maintained optimal pH and ionic strength. To ensure specificity in DNA amplification, 1 µL of synthetic primers (Table 1), designed to anneal to the flanking regions of the target sequence, was added [Vasudha et al., 2023].

PCR amplification was executed using a Corbett PCR Cycler following a precisely optimized thermal cycling protocol. The process commenced with an initial denaturation step at 95°C for 5 minutes, ensuring complete separation of the double-stranded DNA. This was followed by 35 amplification cycles, each comprising denaturation at 95°C for 30 seconds to dissociate DNA strands, annealing at 55°C for 30 seconds to allow primer hybridization, and extension at 72°C for 1 minute, during which Taq DNA polymerase synthesized complementary DNA strands in the 5′ to 3′ direction. A final extension phase at 72°C for 10 minutes was performed to ensure the completion of any partially synthesized DNA molecules. The reaction was subsequently held at 4°C for preservation. This optimized PCR protocol facilitated the exponential amplification of the target DNA sequence, with each successive cycle leading to a twofold increase in DNA yield [Clifford et al., 2012].

2.4. Sequencing of PCR Products

Gel electrophoresis was conducted to verify the amplicon size of the PCR product using a 1.5% agarose gel. A molecular weight marker (DNA ladder) was simultaneously loaded alongside the PCR amplicons to serve as a reference for fragment size determination. The expected amplicon size of the MMP-3 gene (118-129 bp) was assessed by comparing its migration pattern to the molecular marker bands, thereby confirming the successful amplification of the target sequence. The corresponding MMP-3 gene band was subsequently excised for downstream analysis. The purified PCR amplicon library was subjected to high-throughput sequencing to facilitate the precise identification of single nucleotide polymorphisms (SNPs) within the MMP-3 gene. Advanced sequencing technology, including the Sanger sequencing system (ABI 3130 Genetic Analyzer), was employed to generate high-fidelity sequencing data. To ensure data accuracy and reliability, stringent quality control (QC) measures were implemented (Crossley et al, 2020). The raw sequencing reads were evaluated for base quality, GC content, and sequencing errors using FastQC (Wingett and Andrews, 2018). Furthermore, low-quality sequences and adapter contamination were eliminated through trimming processes utilizing Cutadapt (Martin, 2011), thereby enhancing the integrity of the sequencing data for subsequent bioinformatics analysis.

2.5. Bioinformatics Analysis

Upon completion of quality control procedures, comprehensive bioinformatics analyses were undertaken to process and interpret the data. A genome-wide association study (GWAS) approach was implemented to assess the influence of SNPs within the MMP-3 gene on BMD risk scores and their association with osteoarthritis (OA) (Turner et al, 2011). SNPs were identified through comparative analysis of MMP-3 gene sequences between healthy individuals and subjects affected by OA. High-throughput sequencing facilitated the detection of genetic variations, followed by meticulous bioinformatics processing to ensure accuracy and reliability. Statistical logistic regression and Quintile-Based Risk Stratification analysis, were employed to evaluate the contribution of identified SNPs to BMD variation and OA susceptibility. All analyses were conducted under stringent quality control measures to eliminate potential confounders and enhance the robustness of the findings.

3. RESULTS AND DISCUSSION

3.1. Genetic Variant Detection and Classification

SNPs were identified utilizing two variant calling tools: GATK HaplotypeCaller and SAMtools mpileup. GATK HaplotypeCaller performs local de novo assembly of haplotypes in regions exhibiting variation, enabling simultaneous detection of SNPs and indels with high accuracy (Ren et al, 2018). SAMtools mpileup aggregates alignment information from multiple BAM files to compute genotype likelihoods, facilitating reliable SNP and indel detection (Yao et al, 2020). Employing both methodologies ensured robust and precise variant identification. A comparative analysis of healthy and osteoarthritic (OA) sequences of the matrix metalloproteinase-3 (MMP-3) gene has identified five SNPs at specific nucleotide positions Table 2. These nucleotide substitutions may have potential functional consequences, influencing protein structure, binding affinity, and extracellular matrix interactions within bone tissues.

Notably, the SNP at position 302 corresponds to rs679620, a well-characterized polymorphism resulting in a lysine-to-glutamic acid substitution at position 45 (Lys45Glu) of the MMP-3 protein. This variant has been implicated in various pathological conditions, including OA. A study involving a Chinese male population demonstrated that individuals carrying the minor allele of rs679620 had an increased risk of developing OA, suggesting a significant association between this SNP and disease susceptibility (Guo et al, 2017). The SNP at position 478 is located within the promoter region of the MMP-3 gene and corresponds to rs632478. Functional studies have shown that the rs632478 variant can alter promoter activity, potentially affecting MMP-3 expression levels. Specifically, the presence of the minor allele was associated with decreased promoter activity in osteoblast-like cells, which could influence extracellular matrix remodeling and contribute to OA pathogenesis (Jehan et al, 2022).

Collectively, these SNPs may modulate MMP-3 expression and activity, thereby influencing the structural integrity of cartilage and bone. Understanding the functional implications of these genetic variations could provide valuable insights into the molecular mechanisms underlying OA and inform the development of targeted therapeutic strategies.

3.2. Statistical Modelling of BMD Risk and Disease Association

The logistic regression analysis evaluated the association between BMD risk scores and disease susceptibility (Table 3). The coefficient for the BMD Risk Score was 0.27, indicating a positive association between BMD risk scores and disease susceptibility. The corresponding odds ratio (OR) was 1.31, suggesting that for each unit increase in the BMD risk score, the odds of developing the disease increased by approximately 31%. This positive association was statistically significant, as evidenced by a p-value less than 0.001. It is important to note that an odds ratio greater than 1 signified an increased likelihood of the event occurring with higher predictor values. In this context, a higher BMD risk score was associated with increased disease susceptibility. This finding implied that genetic variations influencing BMD might have also modified disease risk. The intercept value of -3.72 reflected the log-odds of disease susceptibility when the BMD risk score was zero. However, the primary focus was on the BMD risk score's coefficient and odds ratio, which provided insight into how changes in BMD risk scores related to changes in disease susceptibility. In brief, the analysis indicated that higher BMD risk scores were associated with increased disease susceptibility, highlighting the potential role of genetic factors influencing BMD in modifying disease risk (Chan et al, 2014).

3.3. Comparative Analysis of BMD Risk Scores in Healthy and Diseased Populations

The analysis of BMD risk scores in healthy and diseased individuals revealed a significant disparity in their distributions. Healthy individuals exhibited higher median BMD risk scores, clustering around 80, with a relatively narrow interquartile range (IQR), indicating less variability. In contrast, diseased individuals demonstrated lower median BMD risk scores, approximately 60, with a broader IQR, suggesting greater variability in scores. The presence of outliers, particularly in the diseased group, further highlighted this variation. The box plot visually confirmed this distinction, with the whiskers indicating a wider distribution of BMD risk scores in diseased individuals compared to the more concentrated distribution in healthy individuals (Figure 1). These findings reinforce the association between reduced bone density and increased disease susceptibility, supporting the hypothesis that higher BMD risk scores confer a protective effect against disease. Additionally, the results align with existing literature, suggesting that genetic predisposition plays a crucial role in determining BMD levels and subsequent disease risk (Ralston and de Crombrugghe, 2006; Stewart and Ralston, 2000). Overall, the comprehensive analysis underscores the critical link between BMD and disease susceptibility, emphasizing the potential role of genetic factors in maintaining bone health.

3.4. Risk Stratification Through Quintile-Based Analysis

The analysis stratified individuals into quintiles based on their BMD risk scores to assess the impact on disease susceptibility (Table 4). The ORs calculated for each group revealed a clear inverse relationship between BMD risk scores and disease risk. The lowest quintile (Q1) served as the reference group, while progressively higher quintiles demonstrated a stepwise reduction in disease risk. Individuals in Q2 exhibited a 22% lower risk of disease (OR = 0.78, 95% CI: 0.66–0.91), while those in Q3 had a 38% lower risk (OR = 0.62, 95% CI: 0.49–0.78). The trend continued, with Q4 showing a 55% reduction in disease susceptibility (OR = 0.45, 95% CI: 0.31–0.60), and Q5 (highest BMD scores) presenting a 71% lower risk of disease compared to Q1 (OR = 0.29, 95% CI: 0.18–0.44, $p < 0.001$). These findings strongly reinforce the protective role of higher BMD scores against disease development. The progressive decline in disease risk across quintiles suggests a dose-dependent effect, emphasizing the potential of genetic and environmental factors influencing BMD in mitigating disease susceptibility (Hunter et al, 2002).

3.5. Medical Significance and Applications

The findings of this study provide compelling evidence that SNPs within the MMP-3 gene influence BMD and disease susceptibility in OA, underscoring their potential clinical significance. From a genetic screening perspective, SNP profiling could serve as a valuable tool for identifying individuals predisposed to OA due to reduced BMD (Honsawek et al, 2013). Early detection of high-risk genotypes may facilitate timely implementation of preventative strategies, potentially mitigating disease onset or progression. In terms of risk stratification and preventative measures, individuals exhibiting lower BMD risk scores may benefit from targeted lifestyle interventions, including calcium and vitamin D supplementation, structured physical therapy, and bone-strengthening exercises (Shahid et al, 2022). Conversely, those with higher BMD risk scores may have a lower predisposition to disease progression, highlighting the heterogeneity in genetic susceptibility. Furthermore, the study highlights the therapeutic potential of elucidating the molecular mechanisms by which SNPs influence BMD regulation. A deeper understanding of these pathways could drive the advancement of precision medicine, enabling the development of gene-based therapies or pharmacological interventions that modulate disease-associated molecular targets. These insights pave the way for a more personalized approach to OA management, integrating genetic risk assessment with tailored therapeutic strategies.

CONCLUSION

In conclusion, this study provides compelling evidence that SNP variations within the MMP-3 gene significantly influence BMD and disease susceptibility in OA. The observed inverse correlation between BMD risk scores and disease risk underscores the protective effect of higher BMD, with individuals in the highest risk quintile exhibiting markedly reduced susceptibility to disease progression. These findings highlight the potential of SNP profiling as a predictive tool for identifying high-risk individuals and implementing early intervention strategies. Furthermore, the molecular insights gained from this research pave the way for precision medicine approaches, including targeted therapies aimed at modulating SNP-

associated pathways. To establish the causal mechanisms underlying these associations, future research should incorporate functional validation studies, large-scale GWAS, and longitudinal cohort analyses. Such advancements will enhance the accuracy of risk stratification models and contribute to the development of personalized therapeutic interventions for OA prevention and management.

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Conflict of Interest

The authors declare that they have no conflicts of interest related to the publication of this study.

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S/no.	Primer Name	Oligo-Sequence	Tm(°C)	GC-Content
1.	Forward Primer	5′ GGTCACTGTCTCATTGTGTGT 3′	62	47.62%
2.	Reverse Primer	5′ TCAGGTAGAGGTGACAAGTG 3′	60	50%

Table 1: Primers used during PCR amplification of MMP-3 gene. Here, Tm is Melting temperature

S/No.	Nucleotide Position	Healthy Allele	Diseased Allele
1.	72	G	A
2.	150	C	T
3.	302	T	C
4.	478	A	G
5.	682	G	T

Table 2. Single Nucleotide Polymorphisms (SNPs) detected at distinct nucleotide positions

Variable	Coefficient	Standard Error	P-Valuer	Odds-Ratio
Intercept	-3.72	1.12	<0.001	--
BMD Risk Score	0.27	0.06	<0.001	1.31

Table 3. Significant association between BMD risk scores and OA susceptibility.

BMD Risk Score Quintile	Odds Ratio (OR) for OA	95% Confidence Interval (CI)
Q1 (Lowest BMD Score)	Reference Group	--
Q2	0.78	(0.66 – 0.91)
Q3	0.62	(0.49 – 0.78)
Q4	0.45	(0.31 – 0.60)
Q5 (Highest BMD Score)	0.29	(0.18 – 0.44)

Table 4. BMD risk score on OA after individuals were divided into quintiles

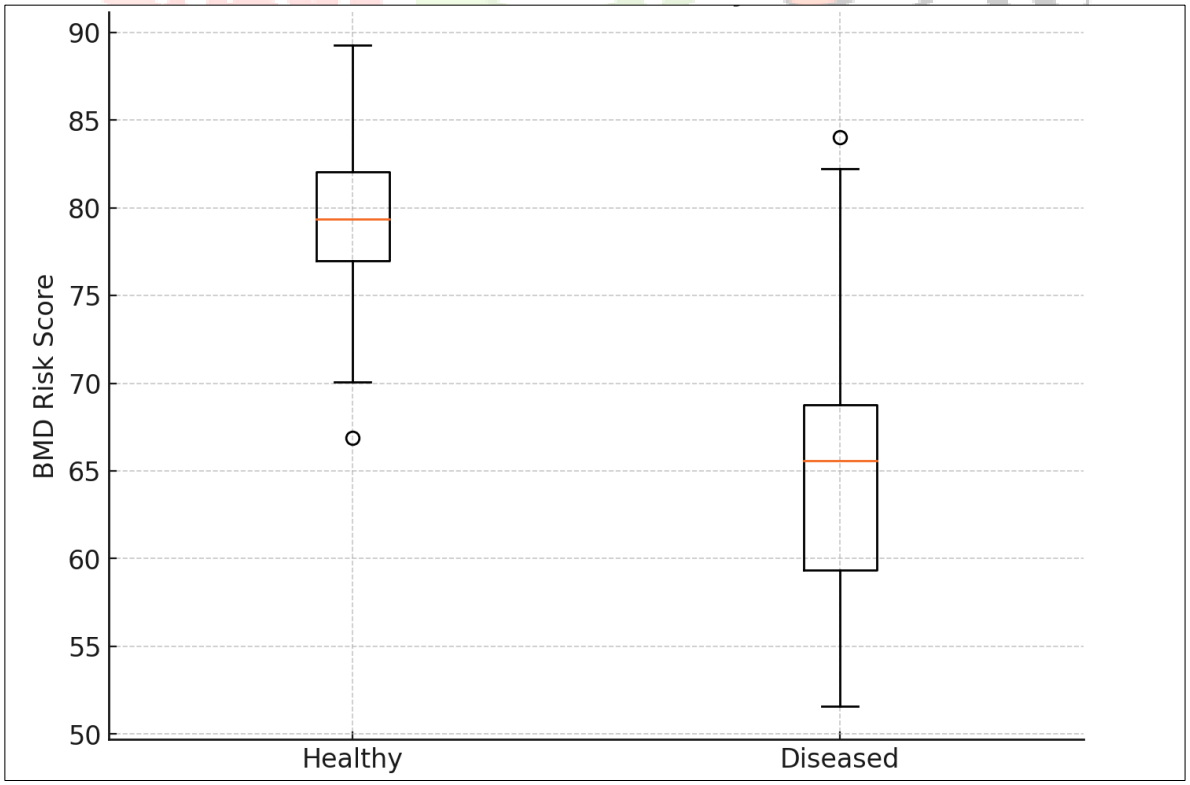

Figure 1. Distribution of BMD risk scores in healthy vs. osteoarthritis (OA) individuals

ABBREVIATIONS

OA	: Osteoarthritis
SNPs	: Single Nucleotide Polymorphisms
MMP	: Matrix Metalloproteinase
BMD	: Bone Mineral Density
GWAS	: Genome-wide association study
PCR	: Polymerase Chain Reaction
DNA	: Deoxyribonucleic acid
BMI	: Body Mass Index
ECM	: Extra-cellular Matrix
EDTA	: Ethylenediaminetetraacetic Acid
TE	: Tris- ethylenediamine tetra-acetic acid
QC	: Quality Control
IQR	: Interquartile Range
UV-VIS	: Ultraviolet-Visible
dNTPs	: Deoxynucleotide triphosphates
OR	: Odds Ratio
CI	: Confidence Interval