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Causal Role of Oxidative Stress-Related Genes in Osteoarthritis

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Abstract: Background: Osteoarthritis (OA) is a common joint disease, primarily affecting the elderly. Oxidative stress (OS), resulting from an imbalance between oxidants and antioxidants, plays a significant role in OA progression. This study aimed to explore the causal relationship between OS-related genes and OA using summary-based Mendelian randomization (SMR). **Methods:** OS-related genes were selected from the GeneCards database with a relevance score ≥ 7. GWAS data for OA, including 24,955 cases and 378,169 controls, were retrieved from the IEU database. eQTL data were obtained from the eQTLGen Consortium. SMR analysis was conducted to assess the causal effects of gene expression on OA, with the HEIDI test used to confirm the absence of heterogeneity. Colocalization and enrichment analyses were performed, and regulatory networks for key genes were predicted. **Results:** SMR analysis identified 26 OS-related genes with causal links to OA. Seven genes were associated with an increased risk of OA, while 19 were protective. Enrichment analysis revealed pathways such as "response to oxidative stress" and "cellular oxidant detoxification." Colocalization analysis indicated LYRM4 and MAPK3 likely share causal variants with OA, and regulatory network predictions highlighted genes like NFS1 and ELK1 as potential regulators of LYRM4 and MAPK3. **Conclusion:** This study identified several OS-related genes potentially contributing to OA, with LYRM4 and MAPK3 as key candidates. These findings offer insights into OA pathogenesis, suggesting that mitochondrial dysfunction and inflammatory signaling play crucial roles. Further research is needed to validate these results and explore potential therapeutic strategies targeting these genes.

Keywords: Osteoarthritis; Oxidative Stress; Mendelian Randomization; Colocalization Analysis.

1. Introduction

Osteoarthritis (OA) is the most prevalent joint disease worldwide, significantly impairing the quality of life in middle-aged and elderly populations. It has become one of the leading causes of disability. Every year, millions of people are affected by OA, and its incidence continues to rise, particularly in aging societies[1]. The etiology of OA is multifactorial, including joint cartilage degeneration, inflammatory processes, and oxidative stress (OS)[2]. Oxidative stress occurs when the balance between oxidants and antioxidants is disrupted, leading to redox signaling dysregulation and cellular damage[3]. Impaired antioxidant capacity has been associated with the development of OA and may play a critical role in its progression[4].

Genes related to oxidative stress play a pivotal role in the initiation and progression of OA. For instance, the Nrf2 pathway is recognized as a key regulator of redox homeostasis, and studies suggest that excessive activation of Nrf2 in the pathological process of OA may worsen cartilage damage and inflammatory responses[5]. Additionally, GRX1 can inhibit oxidative stress and apoptosis in chondrocytes by regulating the CREB/HO-1 pathway, thereby alleviating osteoarthritis[6].

Investigating the mechanisms of oxidative stress-related genes is crucial for understanding the pathophysiology of OA and identifying potential therapeutic targets. Mendelian randomization (MR) has gained attention as a method for exploring causal relationships between exposures and diseases using genetic variants. The random allocation of genes in MR allows it to minimize confounding factors like environmental and lifestyle influences, reducing bias in causal inference, which is common in traditional epidemiological studies[7]. In two-sample MR analysis, instrumental variables from different populations can be used to assess the association between exposures and outcomes, further enhancing the reliability of causal inference[8].

Summary data-based Mendelian randomization (SMR), an extension of MR, integrates genome-wide association studies (GWAS) with gene expression data, offering a powerful tool for identifying causal effects of gene expression in diseases[9]. Heterogeneity testing using the HEIDI test aids in distinguishing genuine causal relationships from widespread linkage disequilibrium (LD) effects. The aim of this study is to employ SMR analysis to explore the potential causal relationship between oxidative stress-related genes and OA.

2. Methods

2.1. Data Source

OS-related genes were identified by querying the GeneCards database (https://www.genecards.org) using the keyword "oxidative stress" and selecting genes with a relevance score of ≥ 7[10,11]. The GWAS data for OA were retrieved from the IEU database (https://gwas.mrcieu.ac.uk), specifically dataset ebi-a-GCST007090, which comprises 24,955 OA cases and 378,169 controls. Approximately 30 million single nucleotide polymorphisms (SNPs) were included in the final GWAS analysis[12]. eQTL summary statistics used in this study were sourced from the eQTLGen Consortium, a large-scale resource of blood eQTL data generated from 31,648 samples across 37 datasets[13]. We focused on whole blood data from the simplified GTEx V8 eQTL dataset (filtered to include only SNPs with P < 1e-5).

2.2. SMR Analysis

We employed the SMR method to investigate potential causal relationships between OS-related gene expression and OA. The SMR method leverages GWAS and eQTL datasets, using SNPs as instrumental variables to estimate the causal effect of gene expression on disease outcomes[14]. This method assumes that all SNPs influence the outcome through the same pathway, such as gene expression. If this assumption holds, SNP effect sizes are expected to be consistent. The HEIDI test, used in SMR to evaluate heterogeneity, checks for consistent SNP effects. A P_HEIDI > 0.05 indicates no significant heterogeneity in SNP effects. Genes with P_SMR < 0.05 and P_HEIDI > 0.05 were considered causally related to OA, and those overlapping with the OS-related gene set were identified as OS-related genes with causal links to OA.

2.3. Enrichment Analysis

To explore the functional and pathway enrichment of OS-related genes with causal links to OA, we uploaded these genes to the Metascape platform for analysis, selecting Homo sapiens as the species of interest[15].

2.4. Colocalization Analysis

We performed Bayesian colocalization analysis using the coloc R package to evaluate whether a single SNP influences two distinct phenotypes within the same gene region. The results are categorized into five posterior probabilities (PPH0-PPH4). Specifically, PPH4 > 0.8 suggests a high likelihood of shared genetic mechanisms underlying the two phenotypes[16].

2.5. Regulatory Network Prediction

We used the GeneMANIA platform (https://genemania.org) to predict potential regulators of LYRM4 and MAPK3 and constructed a regulatory network[17]. Homo sapiens was selected as the species for analysis. Format and save your graphic images using a suitable graphics processing program that will allow you to create the images as PostScript (PS), Encapsulated PostScript (EPS), or Tagged Image File Format (TIFF), sizes them, and adjusts the resolution settings. If you created your source files in one of the following you will be able to submit the graphics without converting to a PS, EPS, or TIFF file: Microsoft Word, Microsoft PowerPoint, Microsoft Excel, or Portable Document Format (PDF).

3. Results

3.1. Identification of OS-Related Genes Causally Associated with OA

We initially identified 1,225 OS-related genes from the GeneCards database. Through SMR analysis, we identified 427 genes causally associated with OA, of which 26 overlapped with the OS-related gene set, confirming their causal links to OA. Causal effects were measured using odds ratios (OR). Among the 26 genes, increased expression of 7 was associated with an elevated risk of OA, while increased expression of 19 was associated with reduced risk (Figure 1).

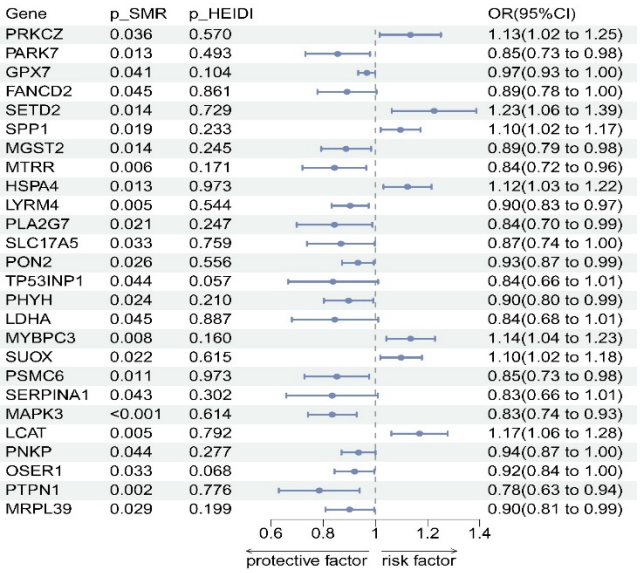

Figure 1. Forest plot of OS-related genes with causal relationships to OA.

3.2. Enrichment Analysis

We performed enrichment analysis on the 26 OS-related genes using Metascape, revealing significant enrichment in pathways such as "response to oxidative stress," "cellular oxidant detoxification," and the "PID MET PATHWAY" (Figure 2A). A network of relationships between the enriched terms was also generated (Figure 2B).

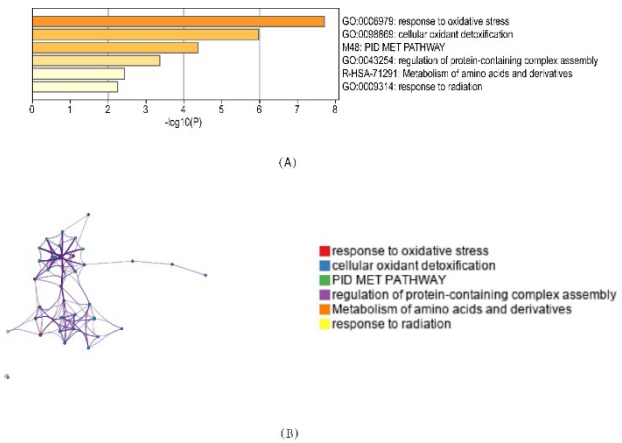

Figure 2. (A) Enrichment analysis results for OS-related genes associated with OA. (B) Network of relationships between enriched terms.

3.3. Colocalization Analysis

Colocalization analysis was performed on the 26 genes identified through SMR, assessing the likelihood of shared causal genetic variants between OA and OS-related gene expression. The results showed that LYRM4 (PPH4 = 0.873) and MAPK3 (PPH4 = 0.829) likely share causal variants with OA(Table1).

3.4. Regulatory Network Prediction

Using the GeneMANIA platform, we predicted genes potentially involved in regulating LYRM4 and MAPK3 and constructed a protein-protein interaction (PPI) network. The analysis revealed close associations between LYRM4 and genes such as NFS1, SLC25A37, and SLC25A28, and between MAPK3 and genes such as ELK1, MAP2K1, and RPS6KA2, suggesting these genes may play roles in LYRM4 and MAPK3 regulation.

Table 1. The results

ProbeChr	Gene	H0	H1	H2	H3	H4
1	PRKCZ	<0.001	0.674	<0.001	0.315	0.012
1	PARK7	<0.001	0.659	<0.001	0.25	0.091
1	GPX7	<0.001	0.757	<0.001	0.214	0.028
3	FANCD2	<0.001	0.308	<0.001	0.683	0.009
3	SETD2	<0.001	0.594	<0.001	0.324	0.083
4	SPP1	<0.001	0.725	<0.001	0.25	0.025
4	MGST2	<0.001	0.822	<0.001	0.172	0.006
5	MTRR	<0.001	0.67	<0.001	0.326	0.004
5	HSPA4	<0.001	0.666	<0.001	0.189	0.145
6	LYRM4	<0.001	0.087	<0.001	0.04	0.873
6	PLA2G7	<0.001	0.596	<0.001	0.395	0.008
6	SLC17A5	<0.001	0.727	<0.001	0.209	0.064
7	PON2	<0.001	0.074	<0.001	0.92	0.006
8	TP53INP1	<0.001	0.608	<0.001	0.37	0.022
10	PHYH	<0.001	0.435	<0.001	0.556	0.01
11	LDHA	<0.001	0.749	<0.001	0.222	0.029
11	MYBPC3	<0.001	0.683	<0.001	0.188	0.13
12	SUOX	<0.001	0.644	<0.001	0.31	0.046
14	PSMC6	<0.001	0.486	<0.001	0.502	0.012
14	SERPINA1	<0.001	0.668	<0.001	0.328	0.004
16	MAPK3	<0.001	0.08	<0.001	0.091	0.829
16	LCAT	<0.001	0.4	<0.001	0.31	0.29
19	PNKP	<0.001	0.72	<0.001	0.254	0.026
20	OSER1	<0.001	0.763	<0.001	0.201	0.036
20	PTPN1	<0.001	0.46	<0.001	0.527	0.013
21	MRPL39	<0.001	0.565	<0.001	0.396	0.04

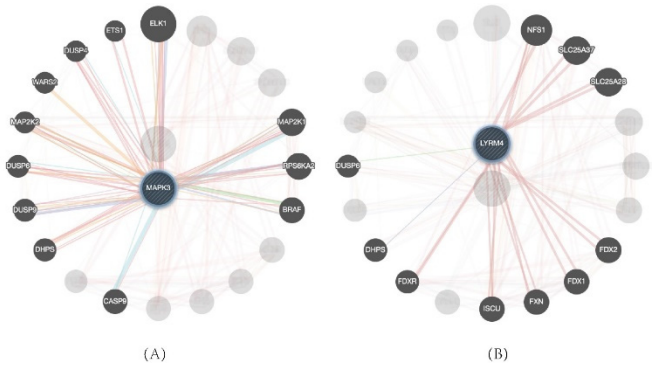

Figure 3. (A) Regulatory network for MAPK3. (B) Regulatory network for LYRM4

4. Discussion

This study aims to explore the potential causal relationship between OS-related genes and OA using the SMR method. SMR analysis identified 26 OS-related genes causally associated with OA, with the expression of 7 genes linked to an increased risk of OA, while the expression of 19 genes was associated with a reduced risk. Notably, LYRM4 and MAPK3 were found to share causal genetic variants with OA in colocalization analysis, further highlighting their potential roles in OA pathogenesis.

LYRM4, also known as ISD11, plays a crucial role in mitochondrial and cytosolic iron homeostasis. The protein it encodes is essential for the assembly of mitochondrial complex I [18], a key component of the electron transport chain[19]. Mitochondrial dysfunction, often resulting in excessive reactive oxygen species (ROS) production[20], triggers oxidative stress responses in cells. Studies suggest that mitochondrial ROS accumulation is closely linked to the development of various degenerative diseases, including OA[21]. In OA pathogenesis, mitochondrial dysfunction disrupts energy metabolism and induces chondrocyte apoptosis, contributing to cartilage degeneration[22]. Therefore, abnormal LYRM4 expression may promote OA progression by impairing mitochondrial function and elevating ROS levels.

The MAPK3 gene encodes the ERK1 protein, a critical component of the MAPK signaling pathway, which regulates cell proliferation, differentiation, inflammation, and stress responses. Oxidative stress activates the MAPK pathway, inducing the expression of inflammatory mediators such as NF-κB and AP-1, thereby intensifying inflammation[23,24]. In cartilage, excessive MAPK3 activation may upregulate matrix metalloproteinases (MMPs)[25], leading to cartilage matrix degradation and accelerating OA progression. Furthermore, MAPK3 influences chondrocyte survival and apoptosis, contributing to cartilage destruction. Therefore, MAPK3 likely plays a pivotal role in OA pathogenesis by regulating inflammation and oxidative stress.

This study's strength lies in the use of Mendelian randomization, which mitigates the influence of unmeasured confounding factors common in observational studies, thereby enhancing causal inference. By integrating GWAS and eQTL data, SMR analyses assessed the association between gene expression and OA, identifying causal variants.

The addition of colocalization analysis, specifically Bayesian colocalization, reinforces the causal implications of LYRM4 and MAPK3 in OA. This method revealed that LYRM4 and MAPK3 likely share genetic variants with OA, suggesting that both genes may operate through overlapping genetic mechanisms in contributing to OA risk. This comprehensive approach enhances the credibility of the findings and provides new insights into the role of OS-related genes in OA.

Despite the significant findings, this study has several limitations. First, the reliance on publicly available GWAS and eQTL data may lead to population-specific biases and limited coverage of relevant gene expression profiles. Additionally, the analysis used whole blood eQTL data, which may not fully capture the expression patterns and regulatory mechanisms in articular cartilage, the primary tissue affected by OA. Future research should focus on validating these findings in tissue-specific samples, particularly cartilage, to better understand the role of OS-related genes in the local microenvironment of OA-affected joints.

Future studies could also benefit from integrating single-cell RNA sequencing data to uncover cell-type-specific expression profiles for LYRM4, MAPK3, and other OS-related genes identified. Such approaches would clarify how these genes operate within specific cell populations, such as chondrocytes or synovial cells, and could reveal new insights into the cellular and molecular pathways that drive OA progression.

In summary, this study identifies LYRM4 and MAPK3 as key OS-related genes causally linked to OA through both SMR and colocalization analyses. The overlapping genetic pathways associated with these genes point to a potentially shared mechanism by which OS influences OA risk, providing promising avenues for therapeutic intervention. Further experimental validation and exploration of targeted therapies for LYRM4 and MAPK3 could offer new, dual-action strategies for mitigating both oxidative and inflammatory components in OA, ultimately advancing the prevention and treatment of this debilitating condition.

5. Conclusion

This study employed SMR to explore the potential causal relationship between OS-related genes and OA. A total of 26 OS-related genes with causal links to OA were identified,

with LYRM4 and MAPK3 emerging as key candidates. Colocalization analysis further supported that these two genes likely share causal genetic variants with OA, suggesting a strong genetic overlap between OS-related pathways and OA pathogenesis. LYRM4 is crucial for mitochondrial function, where its dysregulation may lead to increased reactive oxygen species (ROS) production and contribute to cartilage degeneration. MAPK3, on the other hand, regulates key inflammatory and oxidative stress pathways, potentially accelerating cartilage matrix degradation and OA progression.

The strength of this study lies in the use of SMR analysis and Colocalization analysis, which minimizes confounding factors and improves causal inference between gene expression and OA. However, some limitations remain, including the reliance on publicly available data from whole blood samples, which may not fully capture the gene expression patterns in affected joint tissues.

In conclusion, our findings provide novel insights into the genetic underpinnings of OA and highlight LYRM4 and MAPK3 as potential therapeutic targets. Future experimental studies are required to validate the functional roles of these genes in OA and explore targeted therapeutic interventions that may mitigate oxidative stress and inflammation, thereby preventing or slowing OA progression.

Acknowledgments

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