Identification and validation of transcriptome-wide association study-derived genes as potential druggable targets for osteoarthritis

From The Affiliated Changzhou Second People's Hospital of Nanjing Medical University, Changzhou, China

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Correspondence should be sent to Luming Nong
nongluming1977@hotmail.com

X. Zhou,[1,2,3] X. Ye,[4,5] J. Yao,[1,2] X. Lin,[4,5] Y. Weng,[1,2] Y. Huang,[1,2] Y. Lu,[1,2] J. Shang,[6] L. Nong[1,2]

[1]Department of Orthopedics, The Affiliated Changzhou Second People's Hospital of Nanjing Medical University, Changzhou, China
[2]Changzhou Medical Center, Nanjing Medical University, Changzhou, China
[3]Department of Orthopedics, Gonghe County Hospital of Traditional Chinese Medicine, Hainan Tibetan Autonomous Prefecture, Qinghai, China
[4]Stomatology Hospital, School of Stomatology, Zhejiang University School of Medicine, Hangzhou, China
[5]Zhejiang Provincial Clinical Research Center for Oral Diseases, Key Laboratory of Oral Biomedical Research of Zhejiang Province, Cancer Center of Zhejiang University, Engineering Research Center of Oral Biomaterials and Devices of Zhejiang Province, Hangzhou, China
[6]Department of Pharmacy, The Affiliated Changzhou Second People's Hospital of Nanjing Medical University, Changzhou, China

Aims

Osteoarthritis (OA) is a widespread chronic degenerative joint disease with an increasing global impact. The pathogenesis of OA involves complex interactions between genetic and environmental factors. Despite this, the specific genetic mechanisms underlying OA remain only partially understood, hindering the development of targeted therapeutic strategies.

Methods

A transcriptome-wide association study (TWAS) was conducted for site-specific OA phenotypes using functional summary-based imputation (FUSION). High-confidence candidate genes were identified through rigorous quality control measures, including joint/conditional analysis, permutation tests, best model evaluation, and colocalization analysis. Co-expression network analysis was performed to elucidate the functional biology of these candidate genes. Druggable gene targets and their structural models were retrieved from the DrugBank and SWISS-MODEL databases. Finally, the enrichment of mitogen-activated protein kinase 3 (*MAPK3*) and *SMAD3* in OA was validated biochemically using in vitro and in vivo OA models, as well as human histological sections.

Results

Utilizing the FUSION algorithm, TWAS identified 794 candidate genes for OA. After quality control, 14 genes were classified as high-confidence genes, with seven recognized as potential drug targets including *GCAT, MAPK3, MST1R, PFKM, RAD9A, SMAD3,* and *USAP8*. Co-expression analysis revealed a strong biological association between *SMAD3* and *MAPK3*. Both in vitro and in vivo experiments demonstrated high activity and enriched expression of these two genes in OA.

Conclusion

The present study identified tissue-specific candidate genes and validated high-confidence druggable targets for OA, providing new insights into the genetic landscape and biological processes involved in OA. Further functional studies are warranted to confirm these findings.

Bone & Joint Research

Article focus

- In this study, we conducted a transcriptome-wide association study (TWAS) to identify tissue-specific candidate genes associated with risk of osteoarthritis (OA). Various quality control methods were employed to confirm high-confidence druggable genes.
- Additionally, we performed both in vitro and in vivo functional experiments on two TWAS-derived genes – mitogen-activated protein kinase 3 (*MAPK3*) and *SMAD3* – to validate our in silico findings.

Key messages

- Our study identified 794 candidate genes for OA through TWAS. After quality control, 14 genes were classified as high-confidence, with seven identified as potential drug targets.
- Co-expression analysis revealed a strong link between *SMAD3* and *MAPK3* genes, supported by in vitro and in vivo experiments demonstrating their high activity and enriched expression in OA.

Strengths and limitations

- The present study identified seven high-confidence, druggable gene targets for OA, the expression of which are correlated with the risk of developing OA.
- Biological experiments confirmed significant enrichment of co-expressed genes *MAPK3* and *SMAD3* in OA. Our research offers new insights into the genetic architecture of and targeted therapies for OA.
- However, the applicability of these findings across diverse populations and disease stages is unclear, necessitating further functional studies.

Introduction

Osteoarthritis (OA) is a prevalent chronic degenerative joint disease that primarily affects the cartilage and surrounding joint tissues. With an ageing population and rising obesity rates, the incidence of OA is increasing, making it a leading cause of disability and reduced quality of life globally.[1] The aetiology of OA is multifaceted and complex, influenced by gene-environment interactions, and the specific molecular and genetic mechanisms remain unclear. Our previous studies focused on exploring the impact of drugs on molecular mechanisms in OA, aiming to lay a scientific foundation for the development of effective targeted therapies.[2-4]

The advent of high-throughput sequencing technologies has greatly enhanced genome-wide association studies (GWAS). These studies have become indispensable in elucidating the genetic foundations of various diseases, providing fresh insights into the molecular mechanisms underlying OA and identifying promising drug targets.[5] A GWAS meta-analysis involving 826,690 participants identified over 100 independent risk variants across 11 OA phenotypes.[6] Bittner et al[7] constructed a genetic map of primary OA chondrocytes, uncovering 77 candidate effector genes through the integration of GWAS and epigenomic data. A recent study demonstrated a dysregulated gene set in osteoarthritic cartilage with high diagnostic accuracy for OA, effectively characterizing cartilage-specific alterations to inform clinical decision-making.[8]

The biological interpretation of GWAS remains challenging, as most GWAS-identified variants are located in non-coding or intergenic regions, limiting their potential as direct therapeutic targets.[9] Furthermore, while distinct molecular OA subtypes – including bone remodelling, immune metabolism, and cartilage degradation – have been identified,[10] GWAS associations frequently fail to provide insights into the tissue specificity, complicating targeted therapeutic approaches.[11] Transcriptome-wide association studies (TWAS) have proven effective in systematically identifying disease susceptibility genes in specific tissues by imputing predicted gene expression into GWAS datasets.[12] TWAS-derived genes provide a clearer understanding of tissue-specific gene targets, thereby enhancing the interpretation of biological processes and druggable targets from a genetic perspective.[13] Within a TWAS framework, our previous research identified immune-regulatory gene targets for periodontitis.[14]

Here, we performed a TWAS to comprehensively identify tissue-specific candidate genes contributing to OA risk. Various quality control methods were employed to confirm high-confidence druggable genes. We also conducted both in vitro and in vivo functional experiments on two TWAS-derived genes, *MAPK3* and *SMAD3*, to validate these in silico findings.

Methods

Data source

The GWAS summary-level datasets for OA were obtained from the Genetics of Osteoarthritis Consortium. This database includes data from 13 cohorts spanning nine populations, with up to 826,690 individuals, of whom 177,517 are OA patients. We selected OA data in four different sites: hand OA (20,901 patients), hip OA (34,445 patients), knee OA (36,445 patients), and spine OA (28,372 patients).[6] Tissue-specific transcriptomic data were obtained from the Genotype-Tissue Expression (GTEx) project (V8), which includes 15,201 RNA-sequencing samples from 49 tissues of 838 donors.[15] We selected data from muscle skeletal tissue (n = 706) and whole blood tissue (n = 670), both of which are potentially associated with OA (Figure 1).[16]

Transcriptome-wide association study

We used functional summary-based imputation (FUSION) to conduct a summary-based TWAS employing the GRCh38 genome from European populations in the 1000 Genomes project.[17] FUSION constructs predictive models of the genetic components of functional or molecular phenotypes and tests these components for disease association. Gene expression weights were calculated using multiple predictive models within FUSION, including best linear unbiased prediction (BLUP), Bayesian sparse linear mixed model (BSLMM), least absolute shrinkage and selection operator (LASSO), Elastic Net, and Top1. The model with the highest predictive accuracy was then selected for subsequent TWAS analysis.[18] Additionally, we performed a permutation test to shuffle the quantitative trait locus (QTL) weights and prioritize the empirical associations based on the GWAS effects at the locus. The multiple correction set the lenient significance threshold at 0.01 and the genome-wide significance threshold at 1×10^{-5}, balancing the detection of relevant genetic associations with

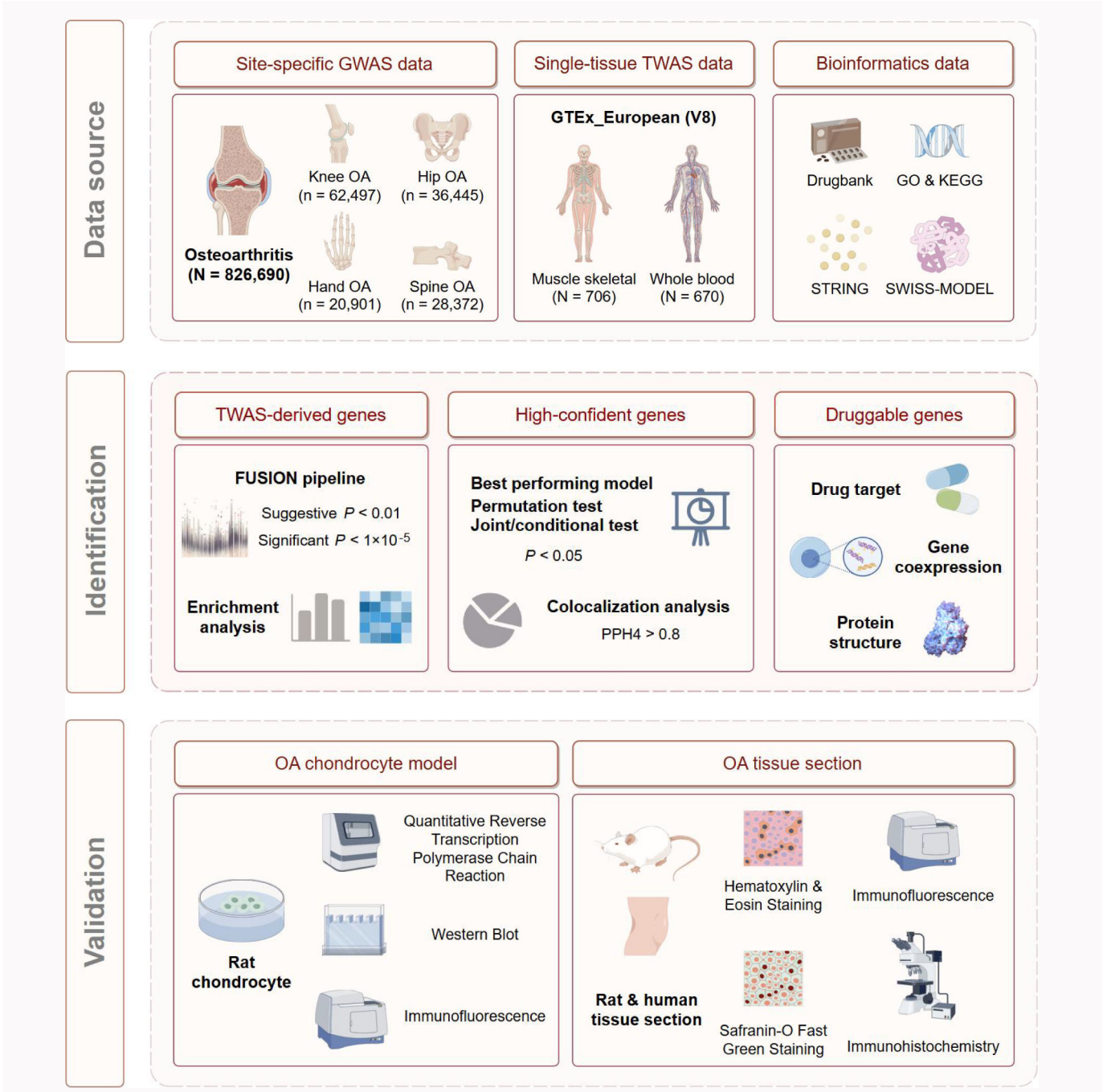

Fig. 1
Study design. FUSION, functional summary-based imputation; GO, Gene Ontology; GTEx, genotype-tissue expression; GWAS, genome-wide association study; KEGG, Kyoto Encyclopedia of Genes and Genomes; OA, osteoarthritis; PP, posterior probability; TWAS, transcriptome-wide association study.

statistical rigour to minimize Type I errors.[19] To investigate the potential biological functions and pathways of significant genes derived from the TWAS, we used Enrichr, a web-based functional annotation analysis tool.[20] The enrichment analysis incorporated data from the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases.

Conditional and joint analysis

Multiple associated features may arise within a locus or the same feature across different tissues, which can result in an overestimation of gene significance. To assess the conditional independence of genes, we used conditional and joint analysis through the "FUSION.post_process.R" script in FUSION software.[17] Genes that retain significance after conditioning are classified as jointly significant, whereas those that exhibit reduced significance due to coregulation are categorized as marginally significant.

Bayesian colocalization

A Bayesian-based statistical method was performed to investigate the presence of shared causal variants between the functional feature and the trait.[21] The posterior probabilities (PP) for hypotheses 0–4 were calculated as follows: H0, no association; H1, functional association only; H2, GWAS

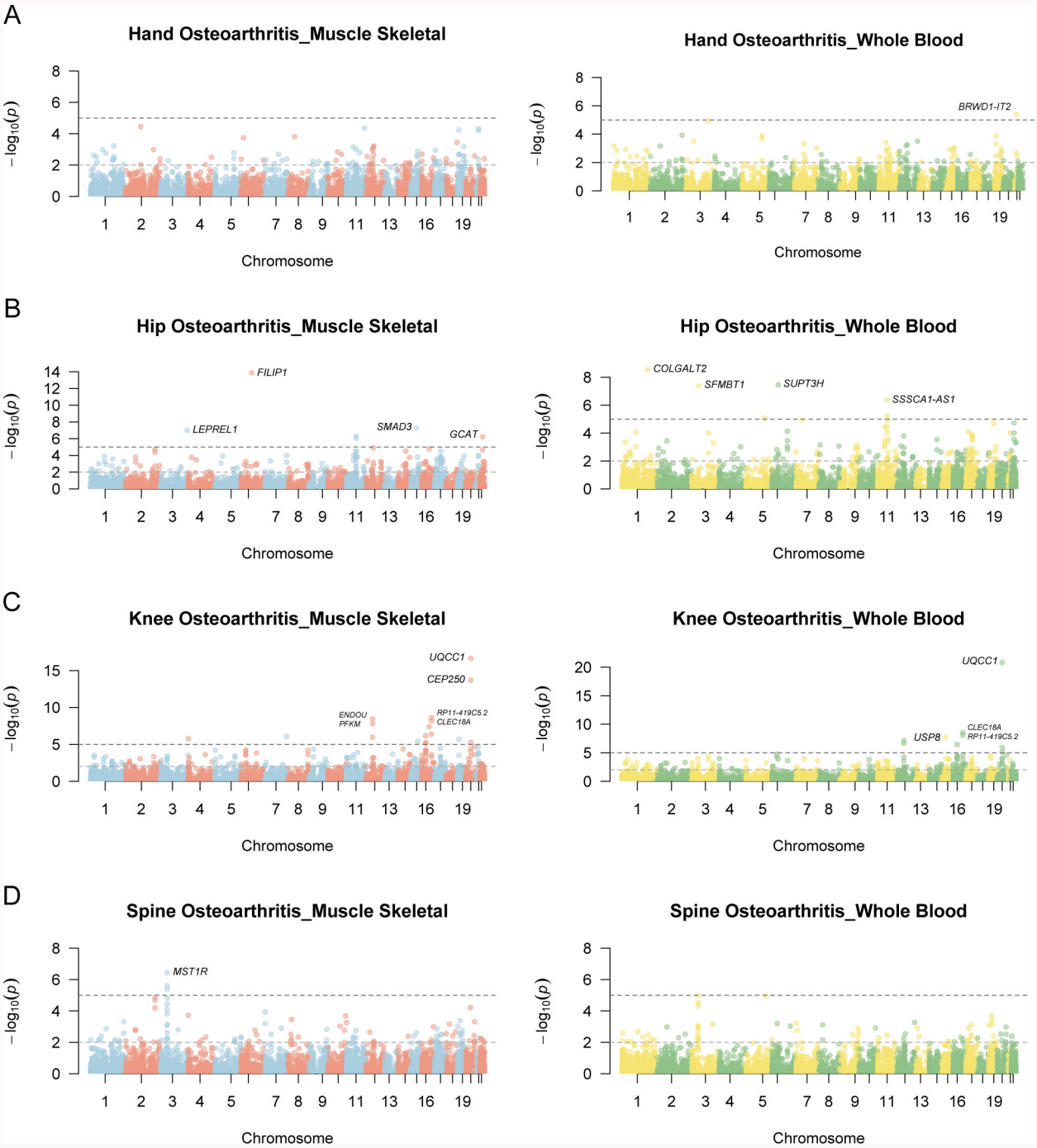

Fig. 2
Manhattan plots of the transcriptome-wide association study (TWAS) results. The x-axis represents chromosomal positions, while the y-axis denotes the p-values on a $-\log_{10}$ scale. The suggestive and genome-wide significance thresholds are set at $p < 0.01$ and $p < 1 \times 10^{-5}$, respectively. a) Regional Manhattan plots in chromosome (muscle skeletal of hand osteoarthritis) and chromosome (whole blood of hand osteoarthritis). b) Regional Manhattan plots in chromosome (muscle skeletal of hip osteoarthritis) and chromosome (whole blood of hip osteoarthritis). c) Regional Manhattan plots in chromosome (muscle skeletal of knee osteoarthritis) and chromosome (whole blood of knee osteoarthritis). d) Regional Manhattan plots in chromosome (muscle skeletal of spine osteoarthritis) and chromosome (whole blood of spine osteoarthritis).

association only; H3, independent associations; H4, colocalized associations. A value of PP.H4 > 0.8 was considered indicative of colocalization.[22]

High-confidence druggable targets

We conducted a comprehensive analysis to identify high-confidence genes, defined by several criteria: FUSION methodology ($p < 1\times10^{-5}$), joint/conditional analysis ($p < 0.05$), permutation test ($p < 0.05$), best model evaluation ($p < 0.05$), and colocalization analysis (PP.H4 > 0.8). Using the DrugBank dataset, a web-based pharmaceutical database, we explored druggable targets for these genes. Additionally, we obtained co-expression and interaction profiles from the STRING database and retrieved model structures of the related proteins from SWISS-MODEL.[23]

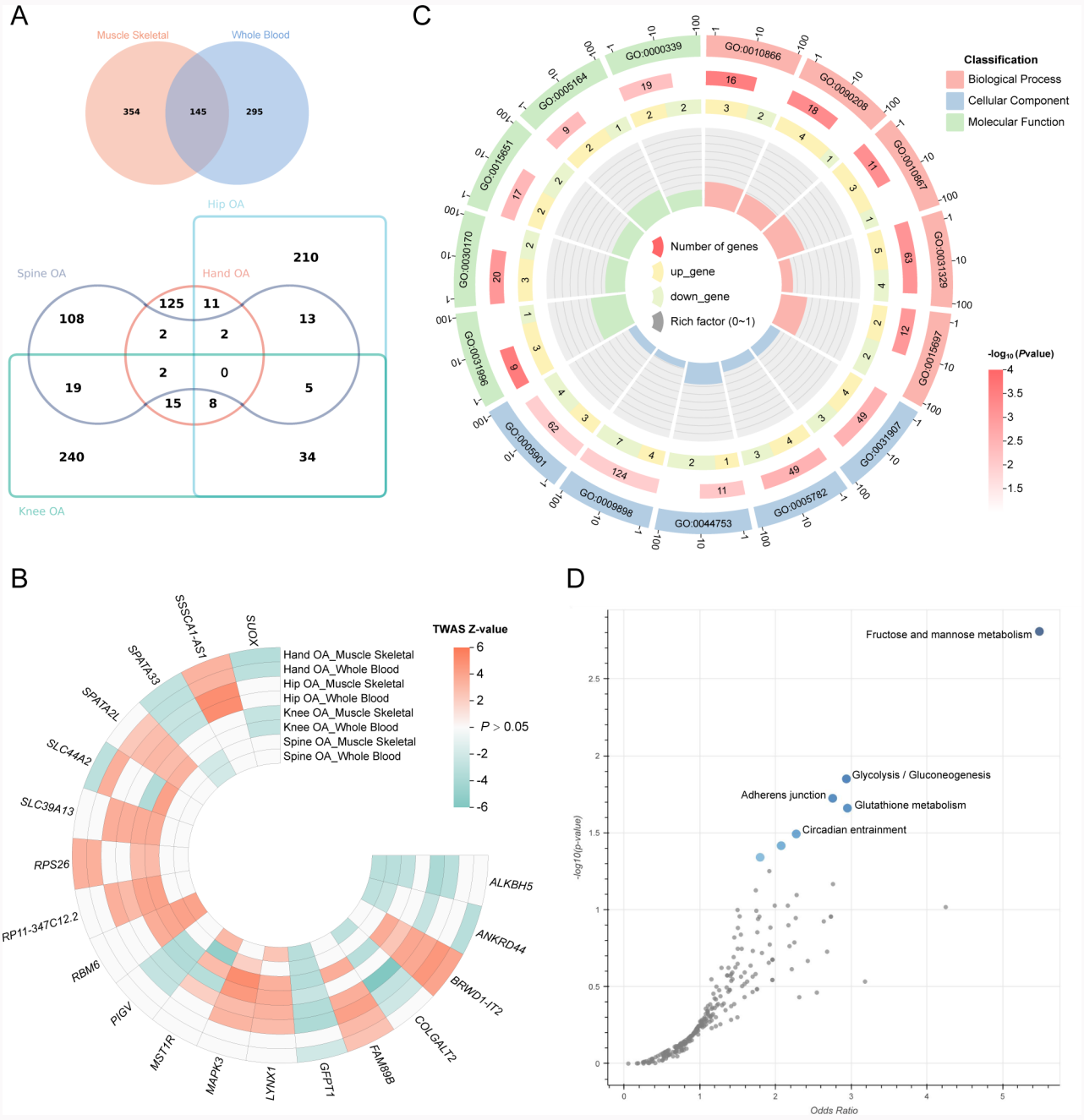

Fig. 3
Identification and enrichment analysis of cross-site genes in osteoarthritis (OA). a) Venn diagrams displaying the intersecting genes shared across different tissues and sites. b) Circular heatmap of cross-site genes (present in at least four panels). c) Circular diagram of enriched Gene Ontology (GO) pathways. The concentric layers represent enrichment tags, total gene count, upregulated and downregulated gene numbers, and the enrichment factor, from outermost to innermost, respectively. d) Volcano plot of enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

Chondrocyte OA model

Primary chondrocytes were extracted from the knee joints of 12 six-week-old Sprague Dawley (SD) male rats. The detailed isolation and culture methods have been described in our previous studies.[3] Chondrocytes from the third and fourth passages were used for subsequent experiments (Supplementary Figure a). All procedures were conducted in accordance with approved animal protocols and ethical guidelines. To simulate the in vitro inflammatory environment of OA, chondrocytes in the experimental group were preincubated with interleukin (IL)-1β (10 ng/ml; MedChemExpress, USA) for 24 hours.[3] The control group was treated with an equivalent amount of phosphate-buffered saline (PBS).

Quantitative reverse-transcription PCR

In accordance with the MIQE guidelines,[24] total RNA was isolated from chondrocytes using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, USA). RNA quantities were evaluated through complementary DNA (cDNA) synthesis using a reverse transcription system (Applied Biosystems; Thermo Fisher Scientific), followed by quantitative real-time polymerase chain reaction (qRT-PCR) analysis with SYBR Green PCR Master Mix (Applied Biosystems), using β-actin as the reference gene.

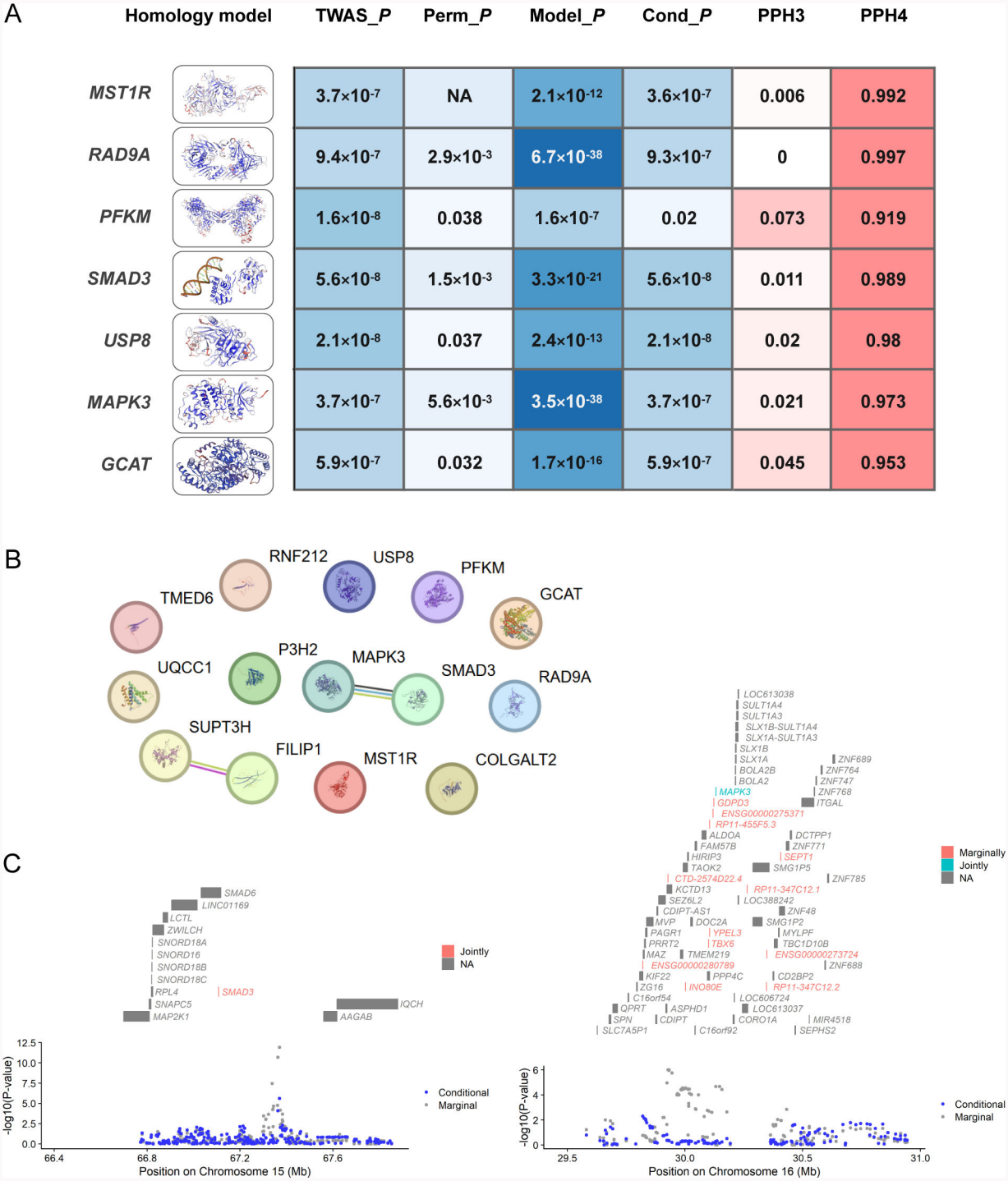

Fig. 4

Identification of high-confidence genes in osteoarthritis (OA). a) Transcriptome-wide association study (TWAS) results and structural model of high-confidence druggable genes. b) Coexpression results of high-confidence genes in STRING. c) Regional Manhattan plots in chromosome 15 (muscle skeletal of hip osteoarthritis) and chromosome 16 (whole blood of knee osteoarthritis). Cond, conditional analysis; FILIP1, filamin A interacting protein 1; GCAT, glycine C-acetyltransferase gene; MAPK3, mitogen-activated protein kinase 3; MST1R, macrophage stimulating 1 receptor; NA, not applicable; P3H2, prolyl 3-hydroxylase 2; Perm, permutation test; PP, poster probability; TMED6, transmembrane P24 Trafficking Protein 6; UQCC1, ubiquinol-cytochrome c reductase complex assembly factor 1; USP8, ubiquitin-specific protease 8.

Primers were optimized at an annealing temperature of 60°C, with efficiencies between 90% and 110%, and were designed to span intron-exon boundaries to ensure cDNA specificity. Relative mRNA levels for each gene were calculated using the $2^{-\Delta\Delta Ct}$ method. Detailed primer sequences are listed in Supplementary Table i.

Western blot

Total protein was isolated from chondrocytes using a complete cell lysis buffer, and quantified with the BCA protein assay kit (Beyotime, China). The protein samples were separated by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto 0.2 μm PVDF membranes (Sigma-Aldrich, USA). The membranes were

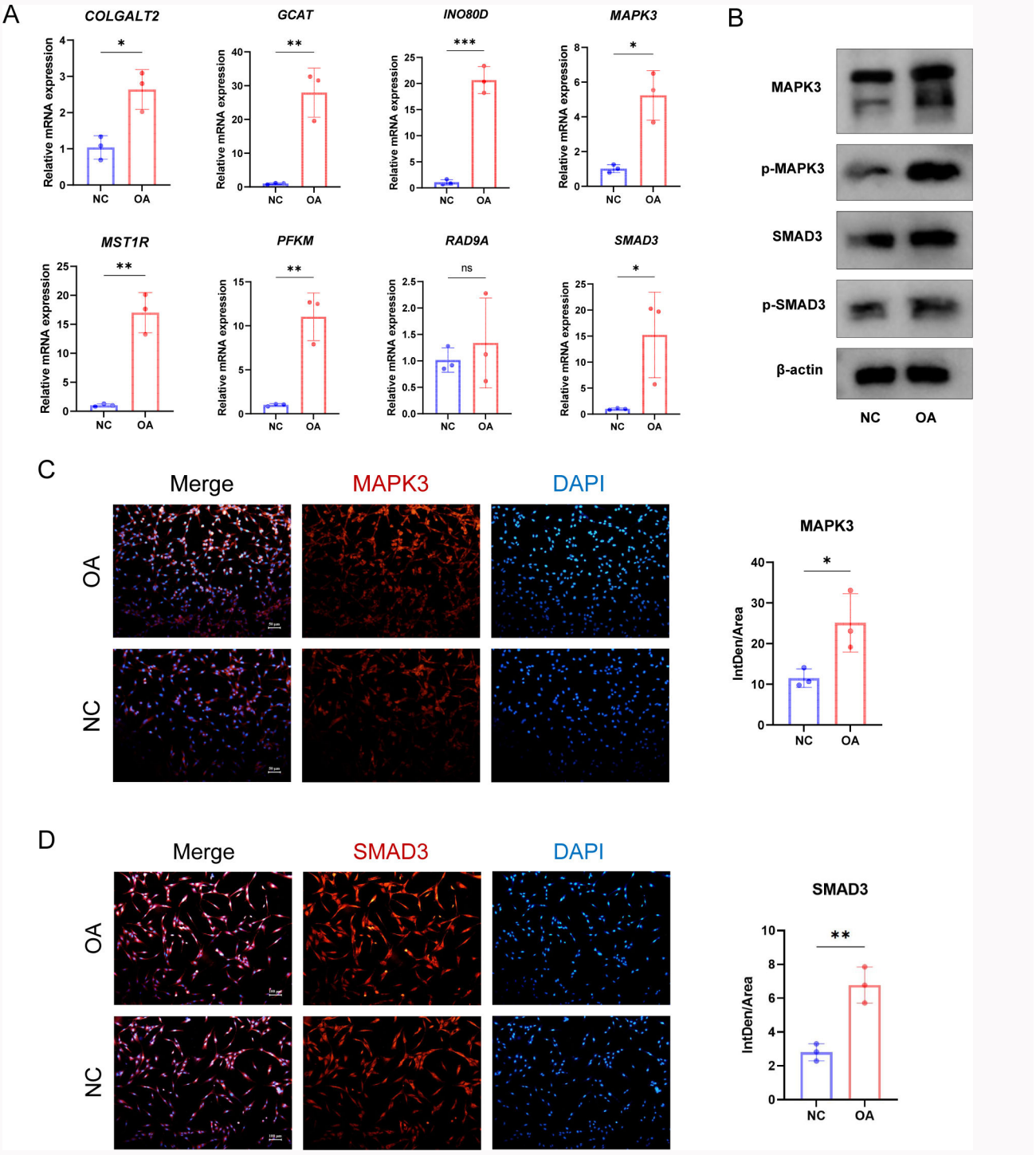

Fig. 5

Validation of risk genes in interleukin (IL)1β-induced chondrocyte osteoarthritis (OA) model. a) Quantitative reverse transcription polymerase chain reaction (RT-PCR) was employed to measure the expression levels of high-confidence genes. b) Western blot analysis was performed to evaluate the expression levels of mitogen-activated protein kinase 3 (MAPK3), SMAD3, phosphorylated MAPK3 (p-MAPK3), and p-SMAD3. c) Immunofluorescence staining for mitochondria, MAPK3 was stained for cellular actin, and 4',6-diamidino-2-phenylindole (DAPI) was stained for nuclear. d) Immunofluorescence staining for mitochondria, SMAD3 was stained for cellular actin, and DAPI was stained for nuclear. The experiment was repeated three times. Data represent the mean (SD) . *p < 0.05, **p < 0.01, ***p < 0.001.

blocked with a 5% skim milk solution at room temperature, then incubated overnight at 4°C with primary antibodies specific to MAPK3, p-MAPK3, SMAD3, p-SMAD3, and β-actin (Abcam, UK). After three washes with Tris-buffered saline with Tween 20 (TBST), the membranes were treated with secondary antibodies (Abcam) at room temperature. Finally, protein bands were detected using an enhanced chemiluminescence reagent (Thermo Fisher Scientific, USA), and their gray intensity was quantified by densitometric analysis using ImageJ software (National Institutes of Health, USA).

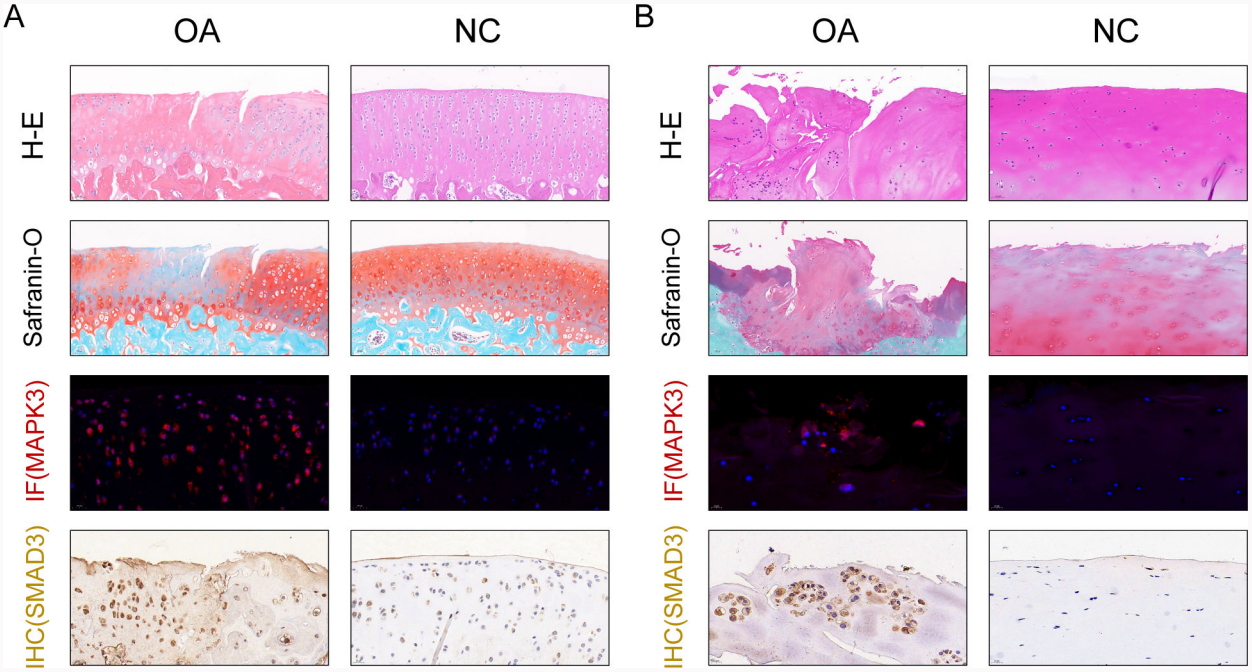

Fig. 6
Validation of risk genes in osteoarthritis (OA) histological sections. Haematoxylin and eosin (H-E) staining, Safranin-O staining, immunofluorescence, and immunohistochemical staining for mitogen-activated protein kinase 3 (MAPK3) and SMAD3 were performed on a) rat and b) human histological sections. The tissues were obtained from the lateral femoral condyle region. IF, immunofluorescence; IHC, immunohistochemical; NC, non-OA control.

Immunofluorescence analysis

Primary chondrocytes were thoroughly washed with PBS, fixed with 4% paraformaldehyde, and permeabilized using 0.1% Triton X-100. The cells were then incubated overnight at 4°C with primary antibodies against MAPK3 and SMAD3 (Abcam, dilution 1:100) in 3% bovine serum albumin (BSA). Following three PBS washes, the cells were incubated with Alexa Fluor 594-conjugated secondary antibody (Abcam, dilution 1:200), Alexa Fluor 488 phalloidin (Sigma-Aldrich), and DAPI (Sigma-Aldrich). Finally, the cells were imaged using a fluorescence microscope, and the mean immunofluorescence (IF) intensity was quantitatively measured with ImageJ software (National Institutes of Health, USA).

Animal OA models

A total of 12 six-month-old male SD rats from Joinn Laboratories (China) were randomly divided into OA and control groups. The OA model was established by surgically resecting the medial monoconidial ligament to induce destabilization of the medial meniscus (DMM), following previously described methods.[2] After four weeks, the rats were euthanized, and their joints from the lateral femoral condyle region were extracted and fixed in 4% paraformaldehyde. The experimental protocol was approved by the Animal Care and Use Committee of the School of Medicine, Zhejiang University in accordance with the ARRIVE guidelines, and an ARRIVE checklist is included in the Supplementary Material to show that the ARRIVE guidelines were adhered to in this study.

Human OA samples

Joint tissue samples from the lateral femoral condyle region were collected from ten patients undergoing joint arthroplasty surgery at the Affiliated Changzhou Second People's Hospital of Nanjing Medical University. Specifically, five OA samples were obtained from patients with severe, irreparable OA, while five control samples were sourced from patients with femoral neck fractures. Patient information is provided in Supplementary Table ii. Informed consent was obtained from all patients, and the study was approved by the Ethics Committee of the Affiliated Changzhou Second People's Hospital of Nanjing Medical University.

Histological evaluation

The specimens were decalcified in 10% EDTA for 15 days, dehydrated in a series of ethanol solutions, and embedded in paraffin. Subsequently, 4 μm-thick sections were prepared and stained with haematoxylin and eosin (HE) and Safranin O-Fast Green. The detailed staining procedures have been described in our previous study.[3] For immunohistochemical analysis, the bone samples were fixed in 4% paraformaldehyde for 20 minutes, followed by antigen retrieval through heating for 30 minutes. They were then treated with 3% hydrogen peroxide for 20 minutes and blocked with 5% BSA for one hour at room temperature. Primary antibodies against MAPK3 (Abcam, 1:200) and SMAD3 (Abcam, 1:200) were incubated in 5% BSA at 4°C overnight. Standard 3,3'-diaminobenzidine (DAB) staining was used for chromogenic detection. Finally, the bone tissues were imaged using a Canon microscopic imaging system (Canon, Japan).

Statistical analysis

Data are presented as the mean with SD. Statistical comparisons between two groups were conducted using the independent-samples *t*-test, while differences among three or more groups were assessed using one-way analysis of variance (ANOVA). All analyses were performed using GraphPad Prism

9.0 (GraphPad Software, USA). For population data, the measurement data were measured by median and IQR, and tested by non-parameters. The counting data were expressed as a quantity, and the comparison between the two groups was performed by Fisher's exact test. Statistical significance was indicated at the following intervals: $p < 0.05$, $p < 0.01$, and $p < 0.001$.

Results

TWAS revealed tissue-specific risk genes for OA

Using the FUSION methodology, we identified 794 genes that met the significance threshold ($p < 0.01$) and 39 genes that met the genome-wide significance threshold ($p < 1 \times 10^{-5}$) after removing probes without specific gene names (Figure 2). Knee and hip OA exhibited the highest number of tissue-specific genes with significant associations, likely due to larger sample sizes. Specifically, 12 genes, such as *FILIP1, COLGALT2, SMAD3, SSSCA1-AS1,* and *GCAT*, demonstrated high significance in hip OA, while 23 genes, such as *UQCC1, CEP250, RP11-419C5.2, CLEC18A,* and *PFKM*, were significant in knee OA. By analyzing the intersection of these genes, we identified 145 genes that are involved in both the muscle-skeleton system and the whole blood panel (Figure 3a). Additionally, 17 genes were shared among at least three OA-affected sites (Figure 3b). In the GO database, these genes are predominantly enriched in the regulation of triglyceride biosynthetic processes, microbody lumen, and thioester binding (Figure 3c, Supplementary Table iii). Furthermore, in the KEGG database, these genes are primarily enriched in fructose and mannose metabolism, glycolysis/gluconeogenesis, and adherens junctions (Figure 3d, Supplementary Table iv).

Identification of high-confidence druggable genes for OA

After a rigorous selection process employing the FUSION methodology ($p < 1 \times 10^{-5}$), joint/conditional analysis ($p < 0.05$), permutation test ($p < 0.05$), best model evaluation ($p < 0.05$), and colocalization analysis (PP.H4 > 0.8), we identified 14 high-confidence risk genes for OA (Supplementary Table iv). Among these, seven genes (*GCAT, MAPK3, MST1R, PFKM, RAD9A, SMAD3,* and *USAP8*) were established as druggable targets in DrugBank (Figure 4a). We further explored the co-expression patterns and structural models of these genes using STRING and SWISS-MODEL (Figure 4b). Of particular interest, a strong correlation was observed between *SMAD3* and *MAPK3*. Furthermore, the regional Manhattan plot revealed a concomitant driving effect of these genes within the regions of chromosomes 15 and 16, respectively (Figures 4c and 4d).

Validation experiments for candidate genes in OA

For initial validation, we assessed the mRNA expression levels of the aforementioned genes in rat chondrocytes. The results revealed high expression of most genes under in vitro OA conditions induced by IL-1β stimulation. Specifically, *COLGALT2, GCAT, MAPK3, MST1R, PFKM,* and *SMAD3* exhibited statistically significant changes, while the expression levels of *RAD9A* and *USAP8* were below the detection limit (Figure 5a). Subsequently, we prioritized *MAPK3* and *SMAD3*, which demonstrated significant associations, for further validation. Western blot analysis demonstrated activation of pathways associated with *SMAD3* and *MAPK3* under OA conditions, as evidenced by elevated levels of phosphorylated *SMAD3* and *MAPK3* in chondrocytes (Figure 5b). Immunofluorescence analysis revealed that *MAPK3* and *SMAD3* were localized in both the cytoplasm and nucleus, with notably higher fluorescence intensity observed in OA samples (Figures 5c and 5d). Finally, we corroborated these findings in histological sections of rat and human knee joints. Immunofluorescence results indicated heightened expression of *MAPK3* in OA joint tissues, while immunohistochemistry results revealed an enrichment of *SMAD3* in inflamed joint tissues (Figure 6).

Discussion

OA is a chronic degenerative disease that affects the articular cartilage and exhibits significant familial aggregation. However, its genetic mechanisms remain poorly understood.[25] Recent advancements in bioinformatics and the exploration of GWAS databases have provided efficient and comprehensive methods for identifying disease risk genes through multiomics analysis.[19] For instance, a recent investigation used proteomic QTL GWAS data to explore protein-mediated mechanisms and therapeutic targets for OA.[26] Additionally, the TWAS method integrates tissue-specific genetic information and analyzes gene expression patterns across various tissues, offering new strategies for identifying genetic and molecular targets for the disease. Mei et al[27] identified candidate genes and chemicals associated with OA by utilizing TWAS and chemical-gene interaction analysis. The present research parallels this approach, focusing on the exploration of druggable genes for OA, with preliminary validation.

During the identification phase, we analyzed differential gene expression in OA across four distinct joint sites using tissue-specific transcriptomic data. After stringent quality control, 19 genes were deemed high-confidence candidates, with nine pinpointed as potential drug targets. Of these, six genes demonstrated elevated messenger RNA (mRNA) expression levels in an in vitro OA model. We performed detailed validation for two co-expressed genes of interest, *MAPK3* and *SMAD3*, which showed increased protein expression and phosphorylation under OA conditions. Histological analysis confirmed their enrichment in OA tissue compared to healthy controls. Our findings highlight novel genes associated with OA risk, offering new insights into the genetic landscape of OA.

MAPK3, also known as ERK1, is a promising drug target due to its crucial role in the MAPK/ERK signalling pathway, which regulates various cellular processes such as proliferation, differentiation, and survival. Consistent with our findings, Zou and Shao[28] identified MAPK3 as a core therapeutic target for OA using proteome-wide Mendelian randomization and colocalization analysis. Recent studies have further confirmed that certain drugs or herbal medicines can regulate the MAPK/ERK signalling pathway by targeting MAPK3 to control OA.[29–31] These results provide scientific evidence for MAPK3 as a potential druggable target for OA, although further clinical application awaits the development of specific biologics.

SMAD3, activated by transforming growth factor-beta (TGF-β) signalling, transmits signals from TGF-β receptors to the nucleus. Previous studies have shown that genetic variants and polymorphisms in the TGF-β/SMAD3 pathway influence the prevalence of OA.[32] SMAD3 is crucial for maintaining cartilage homeostasis and regulating inflammatory

responses, impacting chondrocyte function and OA progression. This influence occurs through processes such as mitochondrial dynamics, mechanical stress response, and pyroptosis activation.[33,34] Our previous research demonstrated that intra-articular injections of calcipotriol significantly reduce cartilage degradation in OA by inhibiting GPX4-mediated ferroptosis of chondrocytes via suppression of the TGF-β1 pathway.[35] Additionally, other studies have confirmed that the TGF-β/SMAD3 pathway plays a crucial role in pharmacological and physical therapies for OA, underscoring the potential of targeting SMAD3 for OA treatment.[36,37]

The present study has further identified several candidate drug targets for OA, including *COLGALT2, GCAT, KCNH2, MST1R, PFKM, RAD9A,* and *USAP8*. These findings are supported by existing research. Kehayova et al[38] discovered the genetic and epigenetic interplay within a COLGALT2 enhancer, emphasizing its role in collagen post-translational glycosylation, which is crucial for cartilage homeostasis. Mullin et al[39] used quantitative trait loci datasets from osteoclast genetic regulatory data to identify GCAT as colocalized with OA traits, highlighting its regulatory role in OA. Xu et al[40] identified PFKM as a potential drug-binding target in the glycolysis pathway, which could protect and activate chondrocytes while inhibiting inflammation. These insights offer new avenues for OA drug development, necessitating further biological validation.

In explaining the clinical significance of our research, several limitations need to be considered. First, due to data source constraints, we used data from European populations in the exploratory cohort, whereas Asian tissue samples were used in the validation process. Similarly, we broadly analyzed risk genes for OA at four sites during the exploration phase, but validation was conducted only on human knee joint samples. This discrepancy might introduce some bias. Second, although the whole blood and muscle skeleton tissues used in our study are the most relevant to OA in GTEx, the absence of directly affected tissues, such as cartilage, may reduce the power of the analysis. Furthermore, our study only conducted preliminary validation; we did not perform deeper targeted blockade, which requires the development of more efficient inhibitors or blocking techniques. Finally, high-quality clinical drugs should be designed for specificity to minimize side effects; our study focused only on OA-related druggable gene targets without evaluating their potential impacts on other diseases.

In conclusion, our TWAS study identified seven high-confidence, druggable gene targets for OA, the expression of which are correlated with the risk of developing OA. Through biological experiments, we validated that *MAPK3* and *SMAD3*, two co-expressed genes, are highly enriched in OA. These findings contribute fresh insights into the genetic architecture of and targeted therapies for OA. However, further functional studies are warranted to confirm these findings.

Supplementary material

Chondrocyte phenotype verification, primer sequence, and clinical specimen population data. An ARRIVE checklist is also included to show that the ARRIVE guidelines were adhered to in this study.

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Author information

X. Zhou, MD, Associate Professor,
Department of Orthopedics, The Affiliated Changzhou Second People's Hospital of Nanjing Medical University, Changzhou, China;
Changzhou Medical Center, Nanjing Medical University, Changzhou, China;
Department of Orthopedics, Gonghe County Hospital of Traditional Chinese Medicine, Hainan Tibetan Autonomous Prefecture, Qinghai, China.

X. Ye, BDS, Research Fellow, PhD Student
X. Lin, PhD, Associate Professor
Stomatology Hospital, School of Stomatology, Zhejiang University School of Medicine, Hangzhou, China; Zhejiang Provincial Clinical Research Center for Oral Diseases, Key Laboratory of Oral Biomedical Research of Zhejiang Province, Cancer Center of Zhejiang University, Engineering Research Center of Oral Biomaterials and Devices of Zhejiang Province, Hangzhou, China.

J. Yao, PhD, Research Fellow
Y. Weng, PhD, Research Fellow
Y. Huang, PhD, Research Fellow
Y. Lu, PhD, Research Fellow
L. Nong, PhD, Professor, Research Fellow
Department of Orthopedics, The Affiliated Changzhou Second People's Hospital of Nanjing Medical University, Changzhou, China; Changzhou Medical Center, Nanjing Medical University, Changzhou, China.

J. Shang, PhD, MD, Research Fellow, Department of Pharmacy, The Affiliated Changzhou Second People's Hospital of Nanjing Medical University, Changzhou, China.

Author contributions

X. Zhou: Funding acquisition, Investigation, Project administration, Writing – original draft, Writing – review & editing.
X. Ye: Data curation, Formal analysis, Software, Supervision, Writing – original draft.
J. Yao: Investigation, Methodology.
X. Lin: Conceptualization, Data curation.
Y. Weng: Software, Supervision.
Y. Huang: Resources, Validation, Visualization.
Y. Lu: Conceptualization, Data curation.
J. Shang: Formal analysis, Investigation, Supervision, Validation, Visualization.
L. Nong: Project administration, Writing – review & editing, Funding acquisition.

J. Shang and L. Nong contributed equally to this work.

X. Zhou and X. Ye are joint first authors.

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Data sharing

The data that support the findings for this study are available to other researchers from the corresponding author upon reasonable request.

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Ethical review statement

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