Investigating the association between uterine fibroids and weight-bearing joint osteoarthritis based on a bidirectional Mendelian randomization study

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Abstract. Uterine fibroids (UFs) are benign smooth-muscle tumors of the uterus that commonly affect women of reproductive age and can influence systemic hormonal and inflammatory states. Osteoarthritis (OA) is a chronic degenerative disease of weight-bearing joints (e.g., hip and knee) and a leading cause of pain and disability. Shared hormonal and immune pathways plausibly link UFs and OA; however, whether a genetic or biological association exists remains unclear. The present study utilized Mendelian randomization (MR) analysis combined with experimental validation to investigate a potential genetic and biological association between UF and OA in weight-bearing joints. Bidirectional MR analyses were carried out using a genome-wide association study to evaluate the potential genetic association between UFs, hip OA (hOA) and knee OA (kOA). The primary method used in the present study was inverse variance weighting, which was supported by complementary approaches including weighted median and MR-Egger. Heterogeneity and pleiotropy were assessed using the Cochran's Q test, MR-Egger intercept and MR-pleiotropy residual sum and outlier. Additionally, sensitivity analyses were conducted using leave-one-out analysis. Furthermore, experimental validation was carried out using a Transwell co-culture system to investigate the effects of UF cells (UFCs) on OA chondrocytes. MR analysis revealed a significant inverse genetic association between UFs and the risk of hOA. However, no genetic association was observed between UFs and kOA. Reverse MR analyses did not support a genetic association between hOA or kOA with UF. Furthermore, experimental results demonstrated that UFCs significantly mitigated OA cartilage degeneration by inhibiting the degradation of Collagen II and Aggrecan at the mRNA level. The present study indicated a potential novel genetic association between UF and hOA, suggesting a potential biological association likely mediated by the tumor microenvironment (such as hormonal or immune alterations), which warrants further mechanistic investigation.

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Abbreviations: UF, uterine fibroid; OA, osteoarthritis; MR, Mendelian randomization; GWAS, genome-wide association study; hOA, hip OA; kOA, knee OA; UFCs, uterine fibroid cells; E2, estradiol; ECM, extracellular matrix; IVs, instrumental variables; SNPs, single-nucleotide polymorphisms; LD, linkage disequilibrium; IVW, inverse variance weighted; SD, standard deviation

Key words: h/kOA, weight-bearing joints, UF, MR study, cell co-culture

Introduction

Osteoarthritis (OA) is a prevalent degenerative joint disorder that affects the quality of life of >344 million individuals worldwide, especially postmenopausal women. This places a substantial burden on healthcare systems as well as a socioeconomic stress (1-3). The pathogenesis of OA involves multifactorial factors, including mechanical stress, inflammation, hormonal imbalance and metabolic dysregulation. However, a complete understanding of its etiology and pathological progression is yet to be elucidated. This has led to a

lack of effective clinical interventions among patients with early-stage OA (4,5).

Among the multifactorial factors involved, estrogen serves a crucial role in female patients (6). A number of studies demonstrate that estrogen, particularly estradiol (E2), is a protective factor against OA as it reduces the release of inflammatory mediators (such as IL-1β, TNF-α and IL-6) and promotes cartilage matrix synthesis, which can mitigate the onset and progression of OA (3,7-9). Clinical evidence also indicates that estrogen replacement therapy can delay cartilage degeneration and reduce the risk of OA among menopausal women, which highlights its anti-inflammatory and cartilage-protective properties (10,11).

However, estrogen-dependent uterine fibroids (UFs) are the most common pelvic tumors in women of childbearing age, affecting >70% of the global population of women (9,12,13). UFs cause symptoms including excessive menstrual bleeding, anemia, pelvic pressure and pain. In addition, UFs elevate the risk of endometrial cancer and compromises the quality of life of women (9,14-16). As typical estrogen-dependent tumors, estrogen binds to estrogen receptors (ERα/ERβ) on myometrial cells, activating proliferation pathways in fibroid stem cells, such as the β-catenin pathway. This promotes the proliferation of smooth muscle cells and inhibits apoptosis (17). Furthermore, estrogen also enhances the expression of various cytokines, such as TGF-β3 and activin A, which leads to an increase in the deposition of extracellular matrix (ECM) in the fibroid. This serves a critical role in the proliferation and enlargement of fibroids (17,18). These dual, yet opposing, roles of estrogen in OA and UFs indicate the complexity of the hormone.

Inflammation is a common risk factor for UFs and OA. In OA, inflammatory cytokines, such as IL-1β, induce cartilage matrix degradation through the upregulation of matrix metalloproteinases and the suppression of cartilage-specific proteins, such as collagen II, aggrecan and cartilage oligomeric matrix protein (19). Furthermore, TGF-β, which is normally protective, is often dysregulated, exacerbating cartilage degradation under inflammatory conditions (20). Additionally, UF tissues have increased levels of inflammatory cytokines (such as MMPs, TNFs and ILs) that promote chronic inflammation and the excessive deposition of ECM (21). Therefore, inflammation-driven ECM remodeling represents a shared, yet divergent, pathway in OA and UF pathogenesis.

The complex association between OA and UF is yet to be fully elucidated. A number of studies suggest a potential inverse association between UFs and the risk of OA progression (22-24). However, a cross-sectional study by Kovari *et al* (25) reports a higher incidence of UFs in individuals with advanced OA. This raises the possibility that OA may influence the development of UFs through shared hormonal or inflammatory pathways. For instance, both conditions have been linked to hormonal imbalances, such as increased estrogen activity, which can influence the growth and inflammation of tissues. Inflammation-related pathways, including those involving cytokines such as IL-1β, TNF-α and TGF-β, are commonly seen in both OA and UF. These cytokines contribute to cartilage degradation in OA and to the proliferation of smooth muscle cells in uterine fibroids. Additionally, the activation of angiogenic factors, such as VEGF, in both conditions may further promote tissue growth and fibrosis. However, an investigation by Yan *et al* (26) demonstrates that there is no causal link between the concentration of E2 in serum from female patients and the risk of developing OA. The apparent contradictions in the evidence may be attributed to the vulnerability of conventional epidemiological approaches to confounders and reverse causality, rendering the causal link between UF and OA ambiguous (27). However, conducting extensive randomized controlled trials to investigate the potential genetic association between UF and OA is impractical and unethical (28).

Therefore, Mendelian Randomization (MR) was used in the present study to reduce the biases introduced by confounding factors and reverse causation (29). The association between the selected exposures inferred from genetic polymorphisms and their effects were investigated in the present MR analysis. The unaltered characteristics of the genetic polymorphisms after conception suggested that their use as instrumental variables (IVs) strengthened the validity of the conclusions in the present study (30). Additionally, the widespread availability of genetic information in public databases facilitated the strategy used in the present study (29).

Materials and methods

Study summary. Fig. 1 presents the design used in the present study of the bidirectional MR analysis.

Data source and selection of single-nucleotide polymorphisms (SNPs). Publicly available data on hip OA (hOA) and knee OA (kOA) were acquired from a genome-wide study that included >400,000 European patients (31). The genome-wide association study (GWAS) database of UF (https://opengwas.io/datasets/ebi-a-GCST90018934) was obtained from an independent GWAS that included 258,718 European individuals (32). The detailed sample characteristics are presented in Table SI.

To satisfy the MR study criteria (33,34), SNPs with strong associations with UF were selected from the GWAS database ($P<5x10^{-8}$). Linkage disequilibrium (LD) analysis was conducted on these nucleotide polymorphisms to cluster SNPs ($r^2<0.001$), and the genetic variants for pairs in LD were pruned. To minimize bias from sample overlap, instruments (such as those with an F-statistic >10 for the instrument-exposure association) were used. Additionally, SNPs were annotated using the SNiPA SNP annotator online platform (https://snipa.helmholtz-muenchen.de/snipa3/) to find available proxies for unmatchable SNPs in the GWAS database. The Phenoscanner database (http://www.phenoscanner.medschl.cam.ac.uk/) was used to identify SNPs associated with exposure and links to confounding factors were investigated ($P<1x10^{-5}$). Table SII was produced by systematically selecting SNPs from the GWAS database based on the aforementioned criteria. The selected SNPs were then summarized in Table SII, where their chromosomal positions, associated genes, effect alleles, non-effect alleles, effect allele frequencies, β values, P-value, F values and confounders are shown. These tables directly correspond to the instrumental variables selected for the bidirectional MR analysis, which aimed to evaluate causal relationships between UF and OA. Finally, the filtered SNPs were used as IVs for

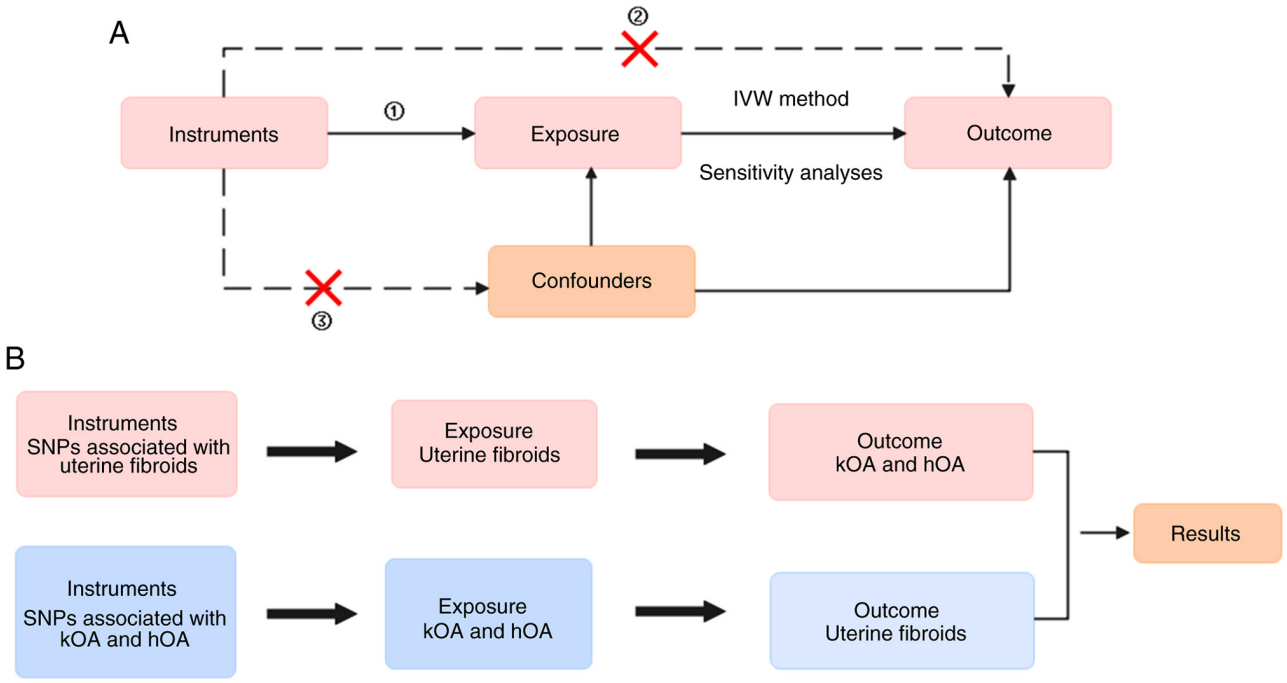

Figure 1. Procedure for the bidirectional MR analysis. (A) Underlying principle of MR analysis. A-1) Correlation hypothesis: IVs exhibited a correlation with the exposure. A-2) Independence hypothesis: IVs were not associated with confounders (such as the body mass index and age). A-3) Exclusive hypothesis: Selected IVs were not directly engaged in the outcome except via the exposure route. (B) The process of bidirectional MR in the present study. MR, Mendelian Randomization; IVs, instrumental variables; SNP, single nucleotide polymorphism; hOA, hip osteoarthritis; kOA, knee osteoarthritis; IVW, inverse variance weighted.

MR analysis. The F-statistic was calculated as: $F=R^2$ x (sample size-2)/(1-R^2), where R^2 is the explained variance in the exposure by each IV (35,36).

MR analysis. MR analyses were conducted using the version 4.3.1 of TwoSampleMR package (MRC Biostatistics Unit; https://github.com/MRCIEU/TwoSampleMR) (36), including inverse variance weighted (IVW), simple median, MR-Egger, weighted mode and weighted median. If the P-value of the Egger intercept was >0.05, no potential pleiotropic effects were suggested (37). The MR-pleiotropy residual sum and outlier method was used to assess the presence of horizontal pleiotropic outliers. Subsequently, the degree of heterogeneity was quantified using Cochran's Q statistic (38). 'Leave-one-out' analysis, the sequential exclusion of each SNP, was used to support the sensitivity and reliability of the outcomes (29). As the results were binary variables, the β effect estimates were converted to odds ratios (OR), which were interpreted as the odds of an outcome per unit increase in exposure (29). The P-values were adjusted using the Benjamini-Hochberg method (39).

Cell culture conditions. For the chondrocytes isolated from rats (cat. no. CP-R087; Procell Life Science & Technology Co., Ltd.), the culture medium used was rat Articular Chondrocyte Complete Medium (cat. no. CM-R092; Procell Life Science & Technology Co., Ltd.), which primarily contains DMEM/F12, 10% fetal bovine serum (FBS), 1% penicillin-streptomycin (P-S) solution (cat. no. C0222; Beyotime Institute of Biotechnology), transferrin and selenium. For the ELT3 cells (cat. no. 4616; BioVector NTCC Inc.), the complete culture medium mainly contained DMEM/F12 (cat. no. PM150312; Procell Life Science & Technology Co., Ltd.), 10% FBS (cat. no. 164250; Procell Life Science & Technology Co., Ltd.), 1% P-S solution (cat. no. C0222; Beyotime Institute of Biotechnology), ferrous sulfate and sodium selenite. All the cells were incubated at 37˚C in a humidified atmosphere containing 5% CO_2.

Cell viability analysis. Using the Cell Counting Kit-8 (CCK-8) assay (cat. no. C0037; Beyotime Institute of Biotechnology) and a Calcein AM/propidium iodide (PI) kit (cat. no. CA1630; Beijing Solarbio Science & Technology Co., Ltd.), the viability of chondrocytes was evaluated. Chondrocytes were seeded in 6-well plates at a density of 1-1.5x10^4 cells/well and pretreated with 10 ng/ml IL-1β for 2 h at 37˚C (OA group). Afterward, they were incubated at 37˚C for 24 h either alone or with ELT3 cells (0.4-0.8x10^4 cells/well) in the OA + UF cell (UFC) group using a 6-well Transwell co-culture system with a 0.4-μm pore size (cat. no. 3460; Corning, Inc.). Subsequently, 10 μl of CCK-8 reagent was added to each well, followed by a 2 h incubation at 37˚C. The absorbance at 450 nm was recorded using a Rayto RT-6100 microplate reader.

For live/dead staining, chondrocytes were cultured under the aforementioned conditions and then treated with Calcein AM and a PI kit, according to the manufacturer's protocol. Live cells were stained green with Calcein AM for 20 min at room temperature and protected from light, while dead cells were stained red with PI for 5 min under the same conditions. Stained cells were visualized under a fluorescence microscope and quantified using Image-Pro (version 6.0; Media Cybernetics, Inc.). All experiments were performed in

Table I. Primer sequences used in reverse transcription-quantitative PCR.

Target genes	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
Collagen II	GAGTGGAAGAGCGGAGACTACTG	GTCTCCATGTTGCAGAAGACTTTCA
Aggrecan	CTAGCTGCTTAGCAGGGATAACG	GATGACCCGCAGAGTCACAAAG
GAPDH	GAAGGTCGGTGTGAACGGATTTG	CATGTAGACCATGTAGTTGAGGTCA

triplicate. Data are presented as the mean ± standard deviation (SD). To avoid phenotypic loss, only passages 1-3 of primary cells were used.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was isolated from the chondrocytes using the RNA Easy Fast Tissue/Cell kit (Tiangen Biotech Co., Ltd.). The extracted RNA was reverse-transcribed into cDNA using the HiScript III RT SuperMix for qPCR (+gDNA wiper) kit (cat. no. R323-01; Vazyme Biotech Co., Ltd.) following the manufacturer's protocol.. The cDNA served as a template for qPCR analysis using TB Green PreMix Ex Taq (cat. no. RR420A; Takara Bio, Inc.), with the following thermocycling conditions: 95˚C for 30 sec, followed by 40 cycles of 95˚C for 5 sec and 60˚C for 30 sec. Gene expression levels were determined by calculating the cycle threshold values, normalized to the internal control GAPDH, and analyzed using the $2^{-\Delta\Delta Cq}$ method (40). The primer sequences used for the target genes are listed in Table I.

Statistical analysis. Experimental data are presented as the mean ± SD and were visualized using GraphPad Prism (version 6.02; Dotmatics). Statistical comparisons between groups were carried out using the unpaired Student's t-test. All experiments were conducted in triplicate. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Genetic associations between UFs and hOA. In total, 49 SNPs were initially extracted from the GWAS database for UF. However, one SNP that was unmatchable in the GWAS for UF lacked an available proxy on the SNipA SNP annotator online platform and was removed from the analysis. Using the Phenoscanner database, two SNPs associated with confounding factors, such as age, body mass index and ankylosing spondylitis, were removed. Therefore, 46 genetically independent variants were selected as IVs for UFs for use in the subsequent MR analyses (Table SII). As shown in Table II, no heterogeneity was observed in the associations between the selected IVs of UF and hOA using the Cochran's Q test (Q=55.24; P=0.14); therefore, IVW was used as the primary random-effects model. The IVW results indicated a significant inverse genetic association between UFs and hOA (OR, 0.941; 95% CI, 0.895-0.990; adjusted P=0.042). Additionally, the weighted median (OR, 0.914; 95% CI, 0.854-0.979; adjusted P=0.045) and weighted mode (OR, 0.906; 95% CI, 0.833-0.985; adjusted P=0.042) were in line with the IVW method (Fig. 2A and B; Table II). However, MR-Egger analysis did not indicate a genetic association (OR, 0.928; 95% CI, 0.843-1.021; adjusted P=0.161) nor the presence of horizontal pleiotropy in the IVs (intercept P=0.73) (Fig. 2A and B; Table II), which indicated uncertainty regarding the robustness of the association estimates.

A 'leave-one-out' analysis was conducted to assess the influence of each UF-associated SNP on the overall association with hOA, which provided a sensitivity evaluation of the results (Fig. 2C). The analysis revealed that the removal of rs11,7245,733 had a significant impact on the association, suggesting that this SNP plays a critical role in the observed relationship between UF and hOA.. The funnel plots are presented in Fig. S1A.

In the reverse MR analysis, 27 SNPs were extracted from the hOA GWAS database. However, two SNPs associated with confounding factors were removed, and 25 genetically independent variants were selected as the IVs of hOA (Table SII). As shown in Table II, the IVW method did not find a genetic association between hOA and UFs (OR, 1.030; 95% CI, 0.974-1.089; adjusted P=0.891), and Cochran's Q test did not reveal any significant heterogeneity (Q=35.71; P=0.06). Additionally, the MR-Egger analysis corroborated the IVW findings (OR, 0.985; 95% CI, 0.795-1.220; adjusted P=0.891) (Fig. 2D and E; Table II). The MR-Egger analysis also revealed no evidence of horizontal pleiotropy in the IVs (intercept P=0.68) (Fig. 2D and E; Table II).

A 'leave-one-out' analysis was carried out to assess the influence of each hOA-associated SNP on UF, which provided a sensitivity evaluation and consistency of the findings. The findings indicated that none of the SNPs exhibited a discernible effect on the pooled results (Fig. 2F). The funnel plots are presented in Fig. S1B.

Genetic associations between UFs and kOA. As aforementioned, 46 genetically independent variants were identified and selected as IVs for the UF analysis (Table SII). In the primary analysis using the IVW method, no genetic association was found between UFs and kOA (OR, 0.985; 95% CI, 0.945-1.025; adjusted P=0.569). Cochran's Q test indicated the absence of significant heterogeneity in the results (Q=57.20; P=0.10) (Fig. 3A and B; Table III). The results of the MR-Egger analysis were consistent with those of the IVW analysis (OR, 0.983; 95% CI, 0.910-1.062; adjusted P=0.662), which indicated the absence of horizontal pleiotropy in the IVs (intercept P=0.956) (Fig. 3A and B; Table III). Furthermore, the result of the 'leave-one-out' analysis indicated that the genetic association between UF and kOA was not associated to a single SNP (Fig. 3C). The funnel plots are presented in Fig. S1C.

In the reverse MR analysis, the genetic associations between kOA and UFs were analyzed using the same aforementioned methodology. In total, 10 SNPs were extracted

Table II. MR results of UF and hOA genetic association.

A, 46 SNPs (Exp, UF; Out, hOA)

				Heterogeneity test		Pleiotropy test	
Methods	OR (95% CI)	P-value	Adjusted P-value	Cochran's Q	P-value	Intercept	P-value
IVW	0.941 (0.895, 0.990)	0.018	0.042	55.24	0.14		
Weighted median	0.914 (0.854, 0.979)	0.011	0.045				
MR-Egger	0.928 (0.843, 1.021)	0.131	0.161			0.002	0.73
Simple mode	0.909 (0.797, 1.037)	0.161	0.161				
Weighted mode	0.906 (0.833, 0.985)	0.025	0.042				

B, 25 SNPs (Exp, hOA; Out, UF)

				Heterogeneity test		Pleiotropy test	
Methods	OR (95% CI)	P-value	Adjusted P-value	Cochran's Q	P-value	Intercept	P-value
IVW	1.030 (0.974, 1.089)	0.300	0.891	35.71	0.06		
Weighted median	1.017 (0.953, 1.086)	0.612	0.891				
MR-Egger	0.985 (0.795, 1.220)	0.891	0.891			0.004	0.68
Simple mode	1.029 (0.909, 1.163)	0.657	0.891				
Weighted mode	1.010 (0.907, 1.125)	0.859	0.891				

Exp, exposure; Out, outcome; SNP, single nucleotide polymorphism; UF, uterine fibroids; hOA, hip osteoarthritis; OR, odds ratio; IVW, inverse variance weighted; MR, Mendelian Randomization.

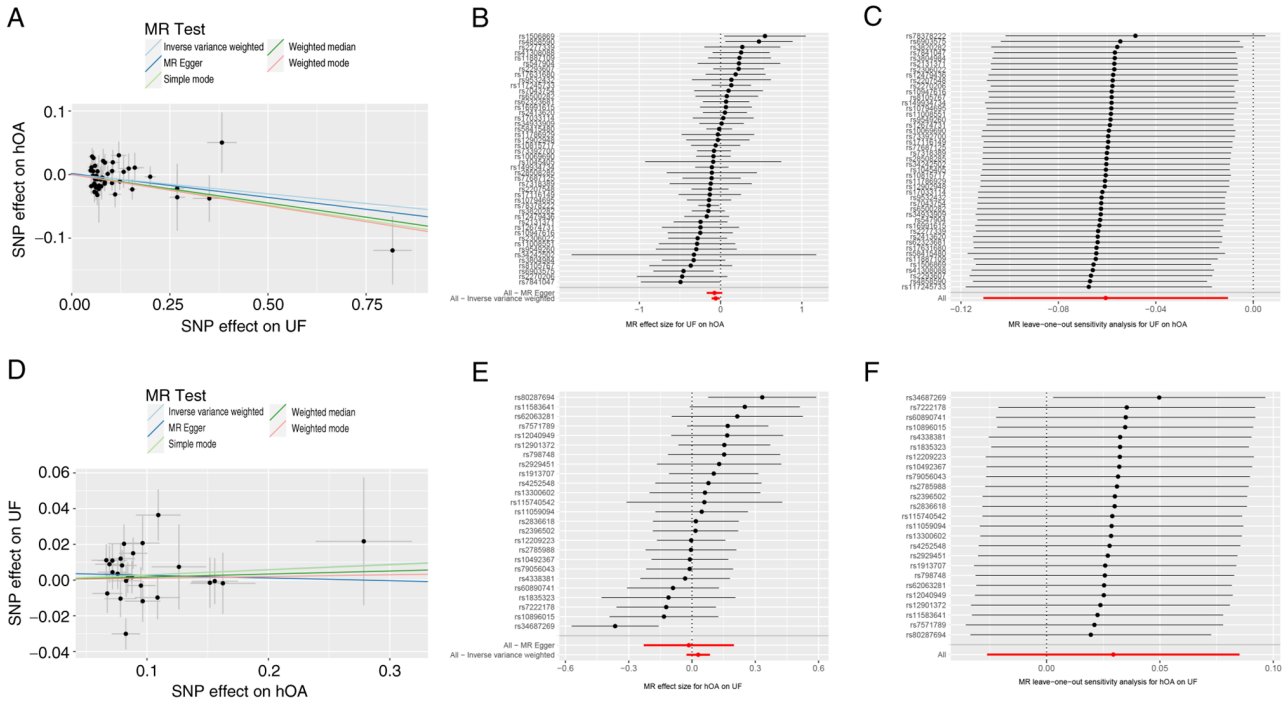

Figure 2. Genetic associations between UF and hOA. (A) Scatter plots, (B) forest plots and (C) leave-one-out sensitivity analysis for the estimated genetic association of UF on hOA. (D) Scatter plots, (E) forest plots and (F) leave-one-out sensitivity analysis for estimated genetic association of hOA on UF. UF, uterine fibroids; hOA, hip osteoarthritis; SNP, single nucleotide polymorphism; MR, Mendelian Randomization.

from the GWAS for UFs, and eight variants were identified as IVs (Table SII). The subsequent analysis revealed the absence of a genetic association of kOA on UFs using the IVW method (OR, 0.961; 95% CI, 0.820-1.126; adjusted P=0.975),

Table III. MR results of UF and kOA genetic association.

A, 46 SNPs (Exp, UF; Out, kOA)

				Heterogeneity test		Pleiotropy test	
Methods	OR (95% CI)	P-value	Adjusted P-value	Cochran's Q	P-value	Intercept	P-value
IVW	0.985 (0.945, 1.025)	0.455	0.569	57.20	0.10		
Weighted median	0.976 (0.921, 1.034)	0.409	0.569				
MR-Egger	0.983 (0.910, 1.062)	0.662	0.662			0.0002	0.956
Simple mode	0.930 (0.835, 1.035)	0.191	0.569				
Weighted mode	0.967 (0.886, 1.054)	0.454	0.569				

B, 8 SNPs (Exp, kOA; Out, UF)

				Heterogeneity test		Pleiotropy test	
Methods	OR (95% CI)	P-value	Adjusted P-value	Cochran's Q	P-value	Intercept	P-value
IVW	0.961 (0.820, 1.126)	0.624	0.975	13.18	0.07		
Weighted median	1.016 (0.872, 1.182)	0.841	0.975				
MR-Egger	0.960 (0.075, 12.247)	0.975	0.975			7.68×10^{-5}	0.999
Simple mode	1.033 (0.833, 1.283)	0.774	0.975				
Weighted mode	1.033 (0.855, 1.249)	0.744	0.975				

Exp, exposure; Out, outcome; SNP, single nucleotide polymorphism; UF, uterine fibroids; kOA, knee osteoarthritis; OR, odds ratio; IVW, inverse variance weighted; MR, Mendelian randomization.

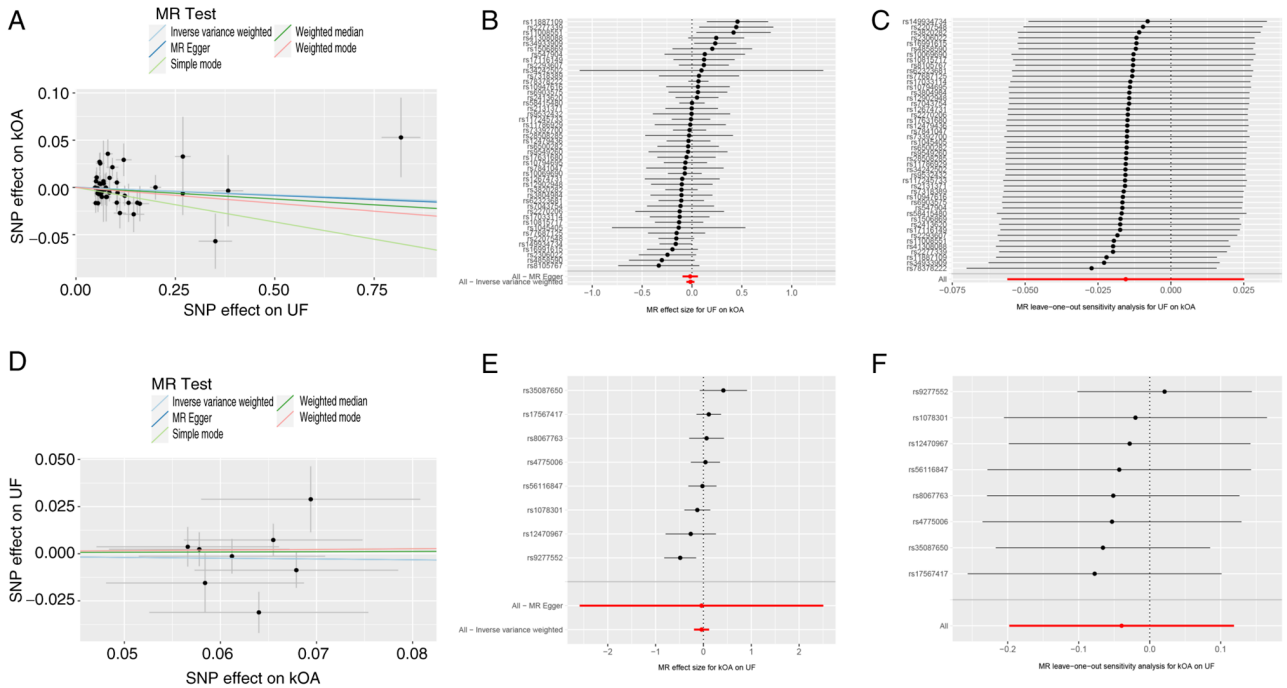

Figure 3. Genetic associations between UF and kOA. (A) Scatter plots, (B) forest plots and (C) leave-one-out sensitivity analysis for the estimated genetic association of UF on kOA. (D) Scatter plots, (E) forest plots and (F) leave-one-out sensitivity analysis for estimated genetic association of kOA on UF. UF, uterine fibroids; kOA, knee osteoarthritis; SNP, single nucleotide polymorphism; MR, Mendelian Randomization.

which aligned with the result obtained from the MR-Egger method (OR, 0.960; 95% CI, 0.075-12.247; adjusted P=0.975) (Fig. 3D and E; Table III). The MR-Egger analysis showed no evidence of horizontal pleiotropy among the IVs (intercept

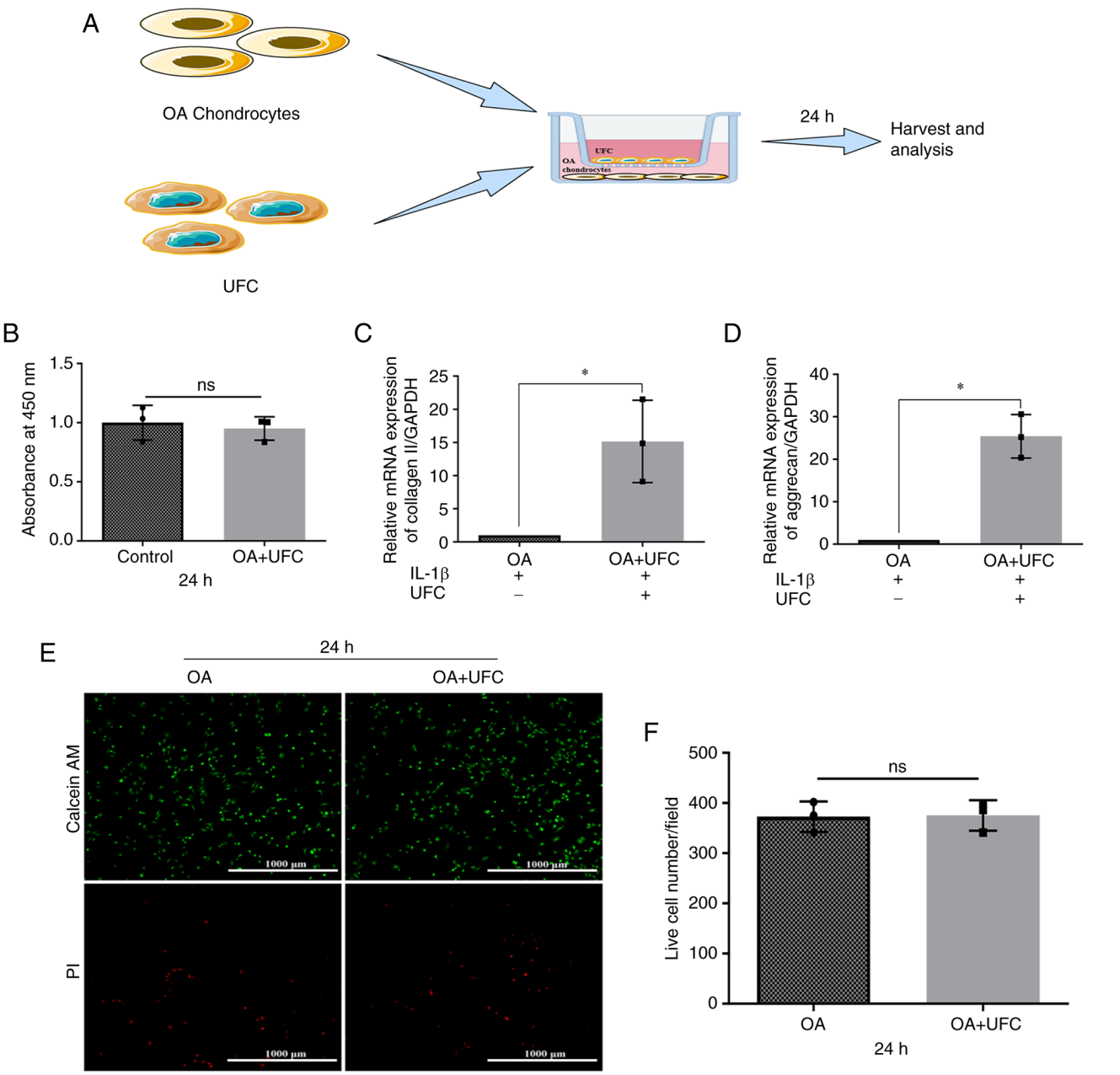

Figure 4. Potential role of UFCs in OA chondrocyte degeneration. (A) A schematic diagram of the experimental procedure. (B) Cell viabilities of OA chondrocytes and OA chondrocytes co-cultured with UFCs were assessed using Cell Counting Kit-8 assays after 24 h with absorbance measured at 450 nm. (C) mRNA levels of Collagen II were measured using reverse transcription-quantitative PCR. (D) mRNA levels of Aggrecan were measured using reverse transcription-quantitative PCR. (E) Live (green)/dead (red) staining of OA chondrocytes or OA chondrocytes co-cultured with UFCs after 24 h using a Calcein AM/PI kit. Scale bar, 1,000 μm. (F) The number of live/dead IL-1β-induced chondrocytes or IL-1β-induced chondrocytes co-cultured with UFCs were measured using a Calcein AM/PI kit, analyzed with Image-Pro 6.0 and quantified. In all experiments, n=3. *P<0.05. UFC, uterine fibroid cells; OA, osteoarthritis; ns, not significant.

P=0.999) (Fig. 3D and E; Table III). Furthermore, Cochran's Q test revealed no heterogeneity (Q=13.18; P=0.07) (Table III). Additionally, the results of the 'leave-one-out' analysis indicated that the genetic association of kOA on UF was not attributable to a single SNP (Fig. 3F). The funnel plots are presented in Fig. S1D.

Investigating the role of UFCs in OA chondrocyte degeneration. A Transwell co-culture system was used to investigate the effects of UFCs on chondrocytes of OA. Rat chondrocytes were cultured in the lower chamber and pre-treated with IL-1β for 2 h to mimic an inflammatory cartilage environment. UFCs were then cultured in the upper chamber for another 24 h (Fig. 4A). The CCK-8 assay indicated that there was no significant difference in the viability of chondrocytes after exposure to UFCs compared with those in the control group not exposed to UFCs (Fig. 4B). This was further confirmed by live/dead cell staining (Fig. 4E and F). Furthermore, it was demonstrated that the mRNA levels of Collagen II and Aggrecan were increased in the chondrocytes treated with UFCs compared with the chondrocytes that were not co-cultured with UFCs (Fig. 4C and D). These

results suggested that UFCs reduced the levels of markers of chondrocyte degeneration *in vitro*, which indicated a possible biological interaction that warrants further basic mechanistic investigation.

Discussion

In the present study, a bidirectional MR analysis was carried out using population-based genetic datasets to investigate the potential genetic association between OA of weight-bearing joints and the risk of UF. Analysis using the IVW method indicated an inverse genetic association between genetic liability to UF and hOA, whereas this association was not observed in the MR-Egger analysis. This discrepancy highlights the need for cautious interpretation of the findings of the present study. The IVW method estimates the association between genetically predicted exposure and outcome through a weighted regression of SNP-specific Wald ratios ($\beta_{outcome}/\beta_{exposure}$), which may be more sensitive to undetected pleiotropy or confounding factors (29). The MR-Egger method tests for directional pleiotropy and provides an assessment of potential associations, but the strength of evidence is reduced when accounting for pleiotropy or other unmeasured confounders (35,41). Therefore, the inconsistency between these two analyses introduced uncertainty regarding the robustness and interpretation of these associations. Given the potential limitations in the robustness of these association inferences from MR analysis, *in vitro* co-culture experiments were carried out. These *in vitro* experiments used OA chondrocytes and UFCs to provide additional biological insights. In the present study, IL-1β-stimulated rat chondrocytes were co-cultured with UFCs to mimic an inflammatory cartilage environment. The experiments demonstrated that the presence of UFCs in the co-culture system significantly inhibited the IL-1β-induced reduction in Aggrecan and Collagen II mRNA expression in chondrocytes, compared with the OA group. These results suggest a potential interaction between UFCs and IL-1β-stimulated chondrocytes, but further mechanistic studies are needed to validate these findings.

Numerous studies have investigated the potential associations of various exposures on OA, but the results have been unfavorable (26,28,42). For example, a previous MR analysis demonstrates that elevated serum testosterone and dihydrotestosterone levels may increase the risk of total hip arthroplasty (26). IVs linked to genetic predispositions for osteoporosis are associated with an increased risk of OA, and increasing bone mineral density serves as an effective strategy to prevent OA (42). Furthermore, MR analysis reveals an association between OA and bladder cancer (28). However, the association between UFs and OA is yet to be elucidated. UFs, the most common benign tumor among women of reproductive age, have an etiology that is still not completely understood (9). An observational study by Kovari *et al* (25) reports a notably higher prevalence of UFs in patients diagnosed with hOA, which contradict the MR findings of the present study. One possible explanation for this discrepancy may involve estrogen (specifically, E2). Previous studies reveal that elevated serum E2 levels are associated with an increased risk of UFs, while emerging evidence suggests that E2 may exert anti-degenerative effects on cartilage, potentially influencing OA onset or progression (7-9).

Systemic E2 levels in patients with UFs may contribute to protection against OA via estrogen receptor signaling (43). However, this mechanism alone cannot explain the results of the present study, which demonstrated *in vitro* that UFCs restrained the IL-1β-induced reduction of Aggrecan and Collegen II in chondrocytes at the mRNA level. Given that UFCs do not secrete E2, the observed reduction in IL-1β-induced reduction of Aggrecan and Collagen II in chondrocytes may involve alternative mechanisms, such as paracrine anti-inflammatory signaling or modulation of receptor sensitivity in chondrocytes (44). Integrating these findings with the findings of the MR analysis in the present study, we hypothesize that the inverse association between UF and hOA likely reflects indirect biological interactions mediated by alterations in the tumor microenvironment instead of direct effects of the tumor itself. For example, changes in hormonal levels, immune cell modulation and inflammatory cytokine profiles within the tumor microenvironment may influence cartilage metabolism or immune homeostasis in anatomically adjacent tissues, such as the hip joint (7,44). This localized paracrine mechanism may partially account for the anatomical specificity observed in the MR findings of the present study, in which UF was inversely associated with hOA, an anatomically adjacent site, but not with kOA. This specificity may be attributed to the proximity of the uterus to the hip, which potentially facilitates local crosstalk or signaling gradients that may not extend to more distant joints.

The findings of the present study indicated novel evidence of a potential genetic link between UF and hOA, suggesting that the association between UF pathophysiology and joint degeneration may be more complex than previously considered (45,46). However, as the simplified *in vitro* model used in the present study cannot fully recapitulate the complex biological environment of OA, the proposed hypothesis is speculative. Further comprehensive mechanistic experiments involving *in vivo* models and clinical samples are required to validate these interactions.

However, there were a number of limitations in the present study that should be considered. Firstly, the present study was limited by the relatively small number of SNPs. Therefore, to enhance the validity of the MR analyses, larger GWAS datasets should be used and a broader array of SNPs should be incorporated as IVs in future investigations. Secondly, the background of the patients included in the present study were restricted to those with European ancestry, and the instruments identified within European populations may not be applicable to non-European groups. Therefore, additional MR analyses that include a broader range of ethnic groups are required. Furthermore, the weak instruments used in MR analyses can lead to biased and inconsistent association estimates. Therefore, to assess the strength of the IVs used in the present study, F-statistics for each SNP was calculated. The results of the present study showed that the F-statistics for the instruments exceeded the commonly accepted threshold of 10, indicating that the instruments were strong and the risk of weak instrument bias was minimized. However, despite the apparent strength of the instruments, the possibility of a weak instrument

bias affecting the association estimates cannot be excluded. Weak instrument bias could lead to attenuated or distorted estimates, undermining the validity of the conclusions of the present study. Although, precautions to mitigate this risk were taken in the present study, further research using alternative IVs or additional methodological approaches may strengthen the robustness of the findings of the present study. Finally, the MR analysis combined with the cellular experiments used in the present study assumed a unidirectional association between exposure and outcome. However, biological systems may include complex feedback loops, potentially affecting the interpretation of results (47).

In conclusion, the novelty of the present study was in the integration of the bidirectional MR with *in vitro* co-culture experiments, an approach that, to the best of our knowledge, has not previously been used to investigate the association between UF and hOA. Using this combined strategy the present study proposed a novel microenvironment-mediated mechanism that may explain the possible inverse association and its anatomical specificity. Furthermore, a possible inverse genetic association between UF and the risk of hOA was demonstrated in the present study. This was further suggested by the findings of the *in vitro* experiments, which demonstrated that Aggrecan and Collagen II mRNA levels were increased in IL-1β-induced chondrocytes in the presence of UFCs, compared with that in the OA group. Furthermore, we hypothesize that this association potentially reflects indirect effects mediated by the tumor microenvironment instead of the direct effects of fibroids themselves. However, due to the discrepancies across the MR analytical approaches, further detailed mechanistic studies are warranted to validate the hypothesis of the present study, which may potentially provide a framework for integrating genetic epidemiology with experimental biology when investigating complex disease interactions.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

XM, SM, TW and YZ conceived and designed the present study. XM, JM, XJ, HZ and SM collected, analyzed and interpreted the data. XM and SM drafted the manuscript. XM, JM, XJ, TW and YZ revised the manuscript. All authors read and approved the final version of the manuscript. XM and YZ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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