

Preplanned Studies

Putative Adverse Outcome Pathway in Kashin-Beck Disease Suggested by Lipidomics and Network Toxicology — Xi'an City, Shaanxi Province, China, 2023–2025

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Summary

What is already known about this topic?

Lipids are key mediators in cartilage degeneration. While T-2 toxin exposure drives Kashin-Beck disease (KBD), systematic metabolic comparisons between KBD and osteoarthritis (OA) are lacking.

What is added by this report?

KBD and OA share cartilage lipid alterations linked to ferroptosis, but KBD exhibits unique changes indicating severe oxidative stress. Furthermore, prostaglandin-endoperoxide synthase 2 (PTGS2) was identified as the candidate molecular target potentially involved in T-2 toxin-induced cartilage damage.

What are the implications for public health practice?

Uncovering the adverse outcome pathway of T-2 toxin exposure clarifies KBD pathogenesis. Targeting the PTGS2-mediated signaling pathway may provide a potential strategy for clinical intervention and disease management.

and OA when compared with FNF controls. These included reductions in sphingomyelins, ceramides, and 5-hydroxyeicosatetraenoic acid and increases in lysophospholipids and vitamin D3 sulfate. These lipids are predominantly involved in arachidonic acid and linoleic acid metabolism and ferroptosis. Plasma lipidomics revealed alterations in triglyceride and diglyceride levels. KBD was associated with elevated levels of cartilage ether triglycerides and plasma polyunsaturated fatty acids. Within the proposed AOP network, prostaglandin-endoperoxide synthase 2 (PTGS2) was identified as a potential molecular initiating event (MIE).

Conclusion: This study proposes a hypothetical AOP framework that links exposure to T-2 toxin with cartilage injury in KBD and identifies PTGS2 as a potential target for further biochemical and functional validation.

ABSTRACT

Introduction: This study compared the metabolic characteristics of Kashin-Beck disease (KBD) and osteoarthritis (OA) to establish an adverse outcome pathway (AOP) framework linking T-2 toxin exposure with cartilage damage.

Methods: Knee cartilage and plasma samples were collected from 17 patients with KBD, 22 patients with OA, and 5 controls with femoral neck fracture (FNF). Targeted lipidomics, partial least squares discriminant analysis (PLS-DA), pathway analysis, network toxicology, and molecular docking analyses were performed.

Results: Lipidomic analysis of the cartilage identified 158 differential lipids (DLs) common to both KBD

Kashin-Beck disease (KBD) is an endemic, chronic osteoarthropathy characterized by multifocal hyaline cartilage necrosis and significant disabilities. Despite the availability of preventive measures, the burden remains substantial (1). Osteoarthritis (OA) is a degenerative joint disease caused by aging and mechanical stress (2). In contrast to OA, KBD lesions manifest during childhood and are closely associated with environmental exposure, indicating a distinct pathogenic mechanism (3). Although lipids mediate cartilage degeneration (4), comprehensive lipidomic comparisons are lacking.

Environmental T-2 toxin exposure is a primary risk factor for KBD. Besides inducing chondrocyte apoptosis, the T-2 toxin disrupts lipid homeostasis, causing systemic lipid alterations, oxidative stress, and ferroptosis (5). Nevertheless, how the T-2 toxin

coordinates multitissue lipidomic alterations across the cartilage and plasma remains unclear.

To address this gap, this study integrated multitissue lipidomics with an adverse outcome pathway (AOP). Instead of healthy controls, patients with femoral neck fractures (FNF) served as the orthopedic controls. Differential lipids (DLs) were correlated with metabolic enzymes, integrated with KBD-related genes, and T-2 toxin targets were predicted to construct a hypothetical AOP network.

Patients were recruited at Xi'an Honghui Hospital between April 2023 and March 2025. Cartilage and plasma samples were obtained from 17 patients with KBD, 22 patients with OA, and five with FNF (Supplementary Table S1, available at <https://weekly.chinacdc.cn>). Patient selection was based on the WS/T207–2010 criteria for KBD, the Outerbridge grading system for OA, and clinical assessments for FNF. All patients with KBD were Grade II or III and underwent knee arthroplasty, during which cartilage samples were collected. Detailed patient inclusion and exclusion criteria, sample collection, and information processing are provided in the Supplementary Material (available at <https://weekly.chinacdc.cn>).

Targeted lipidomic analysis utilizing liquid chromatography-mass spectrometry (LC-MS) was conducted with a Vanquish Flex ultra-high-performance liquid chromatography (UPLC) system interfaced with a Q-Exactive Plus high-resolution tandem mass spectrometer (Thermo Fisher Scientific, USA). Raw data were processed using the XCMS software (version 4.2.3, Bioconductor, CA, USA), and lipid annotation was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Human Metabolome Database (HMDB). Partial least squares discriminant analysis (PLS-DA) models were evaluated using permutation tests. DLs were defined by t -test $P < 0.05$, fold change (FC) > 1.20 , and variable importance in projection (VIP) ≥ 1.00 . Pearson's correlation assessed the cross-tissue associations. KEGG pathway analysis used hypergeometric testing ($P < 0.05$). Metabolite gene set enrichment analysis identified altered pathways using the absolute value of normalized enrichment score (NES) > 1.00 , $P < 0.05$, and false discovery rate (FDR) < 0.25 .

The KEGGREST R package was used to map the DLs to human genes encoding the corresponding metabolic enzymes. KBD-related genes were sourced from GeneCards and OMIM databases, whereas T-2 toxin targets were obtained from the Comparative Toxicogenomics Database (CTD). The intersection of

the mapped genes, KBD-related genes, and T-2 toxin targets was analyzed using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) and Cytoscape software (version 3.10.2, ISB, WA, USA). Additionally, AutoDock Vina (version 1.1.2, TSRI, CA, USA) was used to predict binding between T-2 toxin and human prostaglandin-endoperoxide synthase 2 (PTGS2) using the Protein Data Bank (PDB ID: 5KIR). The docking poses were prioritized by binding energy. Here, 117 intersecting genes were considered potential molecular initiating events (MIEs) for enrichment analysis. After excluding terms unrelated to cartilage biology, eight gene ontology (GO) phenotypes and seven KEGG pathways were selected as candidate key events (KEs) within the proposed AOP network (Supplementary Table S2, available at <https://weekly.chinacdc.cn>).

Data analysis used R (version 4.5.1, R Foundation, Vienna, Austria). Statistical tests, including Student's t -test, ANOVA, Mann–Whitney U test, and Kruskal–Wallis test, were applied as appropriate, with $P < 0.05$ indicating significance.

Cartilage lipidomics identified 214, 204, and 47 DLs for KBD versus FNF, OA versus FNF, and KBD versus OA, respectively (Figure 1A–B). The corresponding plasma analyses revealed 212, 124, and 223 DLs, respectively (Figure 1C–D). PLS-DA permutation tests confirmed model stability without significant overfitting (Supplementary Figure S1, available at <https://weekly.chinacdc.cn>). In the cartilage, KBD versus FNF and OA versus FNF showed reduced 5-hydroxyeicosatetraenoic acid (5-HETE) and increased vitamin D3 sulfate levels, with methyl stearate and elaidic acid additionally elevated in KBD versus FNF (Figure 1E–H). Plasma changes were more heterogeneous and driven by triglyceride and diglyceride shifts versus FNF, along with polyunsaturated fatty acid (PUFA) and membrane lipid variations between KBD and OA. Bredemolonic acid, isoacitretin, cinnacsiol E, and 3-palmitoyl-sn-glycerol were elevated in KBD plasma, whereas isotretinoin levels were higher in OA (Figure 1I–L). KBD versus FNF and OA versus FNF shared 158 cartilage DLs (phospholipids, lysophospholipids, free fatty acids, and sphingolipids) and 80 downregulated plasma DLs. A comparison of KBD and OA revealed 33 cross-tissue DLs with higher levels of fatty acids, ether triglycerides, and glycerolipids in KBD (Supplementary Figure S2, available at <https://weekly.chinacdc.cn>).

Cartilage DLs in KBD versus FNF were enriched in

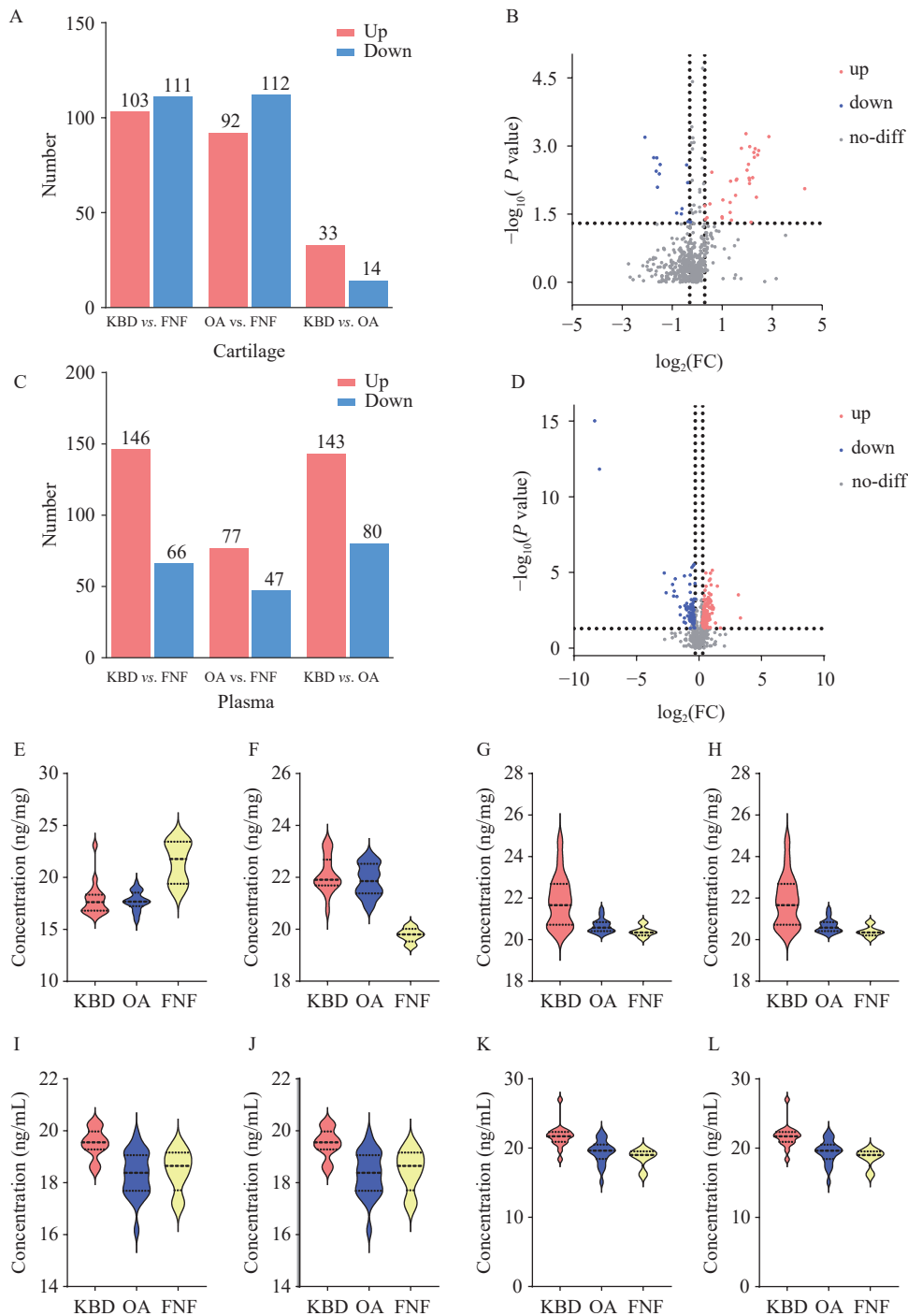


FIGURE 1. DLs in cartilage and plasma. Samples were obtained from patients with KBD ($n=17$), OA ($n=22$), and FNF ($n=5$) at Xi'an Honghui Hospital, Shaanxi Province, China, April 2023–March 2025. (A) Numbers of upregulated and downregulated DLs in cartilage across the three pairwise comparisons. (B) Volcano plot of cartilage DLs in KBD versus OA. (C) Numbers of upregulated and downregulated DLs in plasma across the three pairwise comparisons. (D) Volcano plot of plasma DLs in KBD versus OA. (E) 5-HETE levels in cartilage. (F) Vitamin D3 sulfate levels in cartilage. (G) Methyl stearate levels in cartilage. (H) Elaidic acid levels in cartilage. (I) Bredemolic acid levels in plasma. (J) Isoacitretn levels in plasma. (K) Cinnassiol E levels in plasma. (L) 3-palmitoyl-sn-glycerol levels in plasma.

Note: In panels A and B, red, blue and gray indicate upregulated, downregulated, and non-differential lipids, respectively. In panels C and D, red, blue, and gray indicate upregulated, downregulated, and non-differential lipids, respectively. In panels E–L, red, blue, and yellow represent KBD, OA, and FNF, respectively.

Abbreviation: DLs=differential lipids; KBD=Kashin-Beck disease; OA=osteoarthritis; FNF=femoral neck fracture; FC=fold change; 5-HETE=5-hydroxyeicosatetraenoic acid.

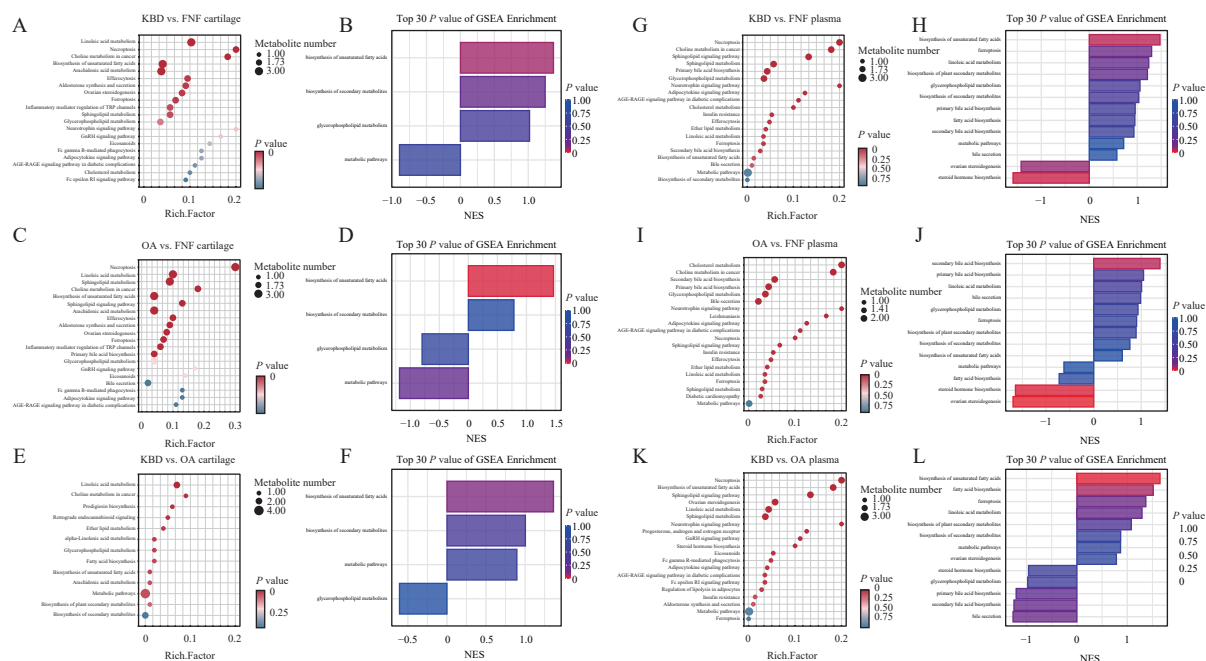


FIGURE 2. KEGG and GSEA of cartilage and plasma DLs. (A) KEGG enrichment of cartilage DLs in KBD versus FNF. (B) GSEA of cartilage DLs in KBD versus FNF. (C) KEGG enrichment of cartilage DLs in OA versus FNF. (D) GSEA of cartilage DLs in OA versus FNF. (E) KEGG enrichment of cartilage DLs in KBD versus OA. (F) GSEA of cartilage DLs in KBD versus OA. (G) KEGG enrichment of plasma DLs in KBD versus FNF. (H) GSEA of plasma DLs in KBD versus FNF. (I) KEGG enrichment of plasma DLs in OA versus FNF. (J) GSEA of plasma DLs in OA versus FNF. (K) KEGG enrichment of plasma DLs in KBD versus OA. (L) GSEA of plasma DLs in KBD versus OA.

Note: In panels A, C, E, G, I and K, bubble size represents the number of metabolites and color represents the P value. In panels B, D, F, H, J and L, the x-axis represents the NES, and bar color represents the P value.

Abbreviation: DLs=differential lipids; KBD=Kashin-Beck disease; OA=osteoarthritis; FNF=femoral neck fracture; KEGG=Kyoto Encyclopedia of Genes and Genomes; GSEA=gene set enrichment analysis; NES=normalized enrichment score.

linoleic acid, arachidonic acid, unsaturated fatty acid biosynthesis, ferroptosis, necroptosis, and apoptosis pathways. OA versus FNF showed similar enrichment but lacked apoptosis. KBD versus OA showed enrichment in linoleic acid, arachidonic acid, and glycerophospholipid metabolism without cellular process enrichment (Figure 2A–F). Plasma enrichment paralleled cartilage, highlighting the sphingolipid, glycerophospholipid, and ferroptosis pathways, with metabolite gene set enrichment suggesting the involvement of fatty acid and sterol pathways (Figure 2G–L).

Systematic mapping identified 165 common genes between KBD and OA DLs (Figure 3A). The protein-protein interaction (PPI) network analysis revealed that core hub genes, notably PTGS2, arachidonate 5-lipoxygenase (ALOX5), and cytochrome P450 family 4 subfamily F (CYP4F), were involved in lipid metabolism (Figure 3B–C). Intersecting the mapped genes with KBD disease genes and T-2 toxin targets identified 117 genes (Figure 3D). Further intersection

with the core network genes highlighted PTGS2 as the singular critical overlapping gene associated with the tumor necrosis factor (TNF) and interleukin-17 (IL-17) signaling pathways (Figure 3E–F). Molecular docking predicted a favorable interaction between T-2 toxin and the active site of PTGS2, which was mediated through hydrogen bonds and hydrophobic interactions (Figure 3G).

A putative AOP network was constructed from 117 candidate MIEs (Figure 3H). These genes were computationally associated with eight GO phenotypes and seven KEGG pathways potentially involved in cartilage injury, including the TNF signaling pathway (Supplementary Figure S1, available at <https://weekly.chinacdc.cn/>). Frequency analysis of these pathways identified PTGS2 as the only consistently overlapping candidate MIE (Figure 3I).

DISCUSSION

This study combined multitissue lipidomics and

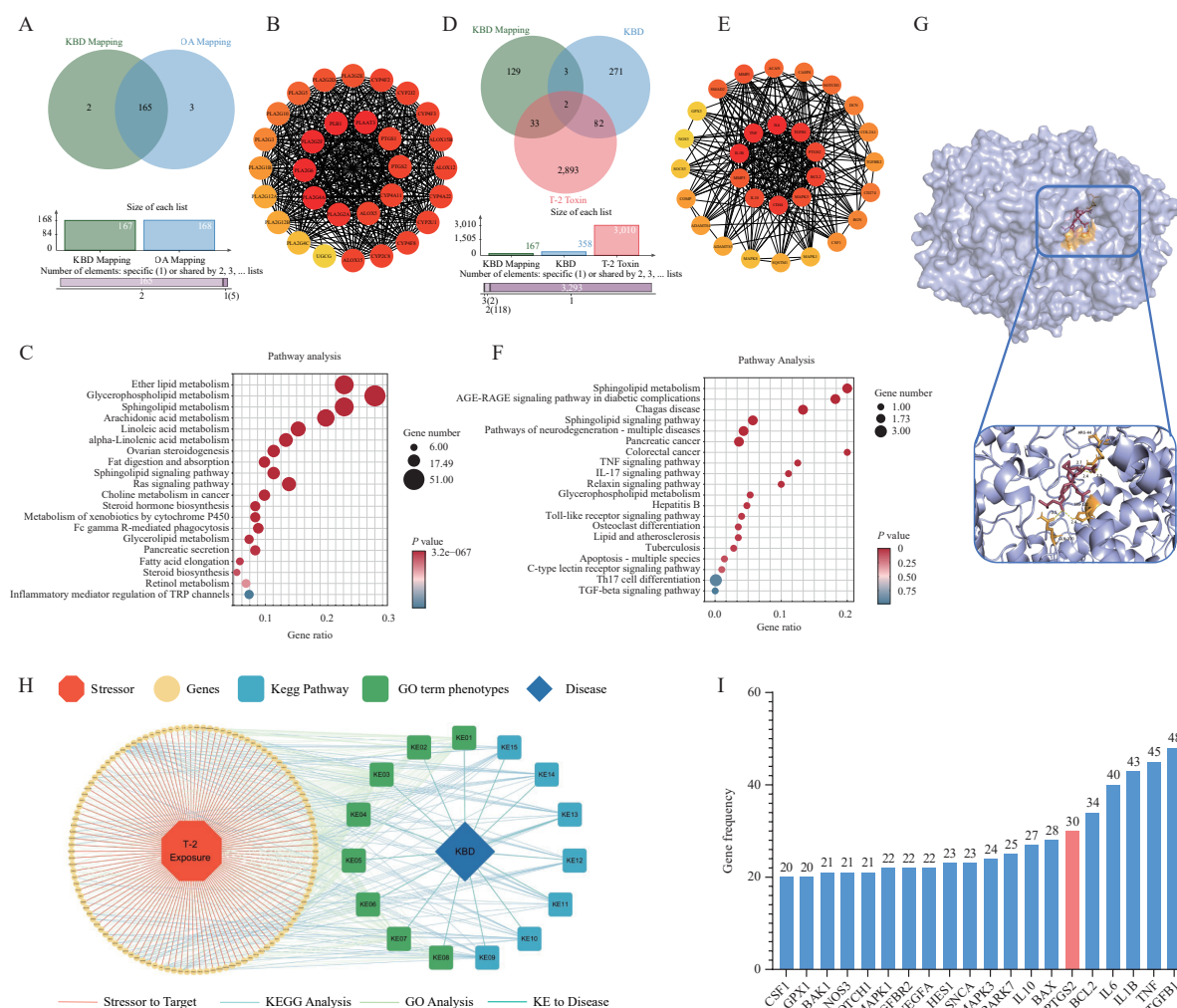


FIGURE 3. Network toxicology analysis of DL mapping genes. (A) Venn diagram of common mapped genes between KBD and OA. (B) PPI network of shared mapped genes between KBD and OA. (C) KEGG enrichment of shared mapped genes. (D) Venn diagram of KBD mapped genes, KBD disease genes, and T-2 toxin targets. (E) PPI network of genes intersecting between KBD and T-2 toxin targets. (F) KEGG enrichment of the intersecting genes. (G) Molecular docking of PTGS2 and T-2 toxin. (H) Integrated network linking T-2 toxin exposure to candidate genes, Gene Ontology (GO) phenotypes, KEGG pathways, and KBD. (I) Frequency of genes across the selected phenotypes and pathways; PTGS2 is highlighted in red. Abbreviation: DLs=differential lipids; KBD=Kashin-Beck disease; OA=osteoarthritis; PPI=protein-protein interaction; KEGG=Kyoto Encyclopedia of Genes and Genomes; GO=Gene Ontology; PTGS2=prostaglandin-endoperoxide synthase 2.

network toxicology to compare KBD and OA and proposed a hypothetical AOP linking T-2 toxin with cartilage damage. Both diseases share alterations in lysophospholipids (LPLs), 5-HETE, vitamin D3 sulfate, arachidonic and linoleic acid metabolism, and ferroptosis, which are consistent with joint lipid remodeling (2,6–7). PTGS2, ALOX5, and CYP4F participate in arachidonic acid metabolism and inflammatory lipid mediator production (8). Conversely, KBD-specific shifts in glycerides, ether lipids, and plasma PUFAs suggest distinct metabolic responses (5). Reduced 5-HETE levels may indicate altered ALOX5-dependent arachidonic acid flux through substrate redistribution toward the

cyclooxygenase (COX)/PTGS2 or alternative lipoxygenase (LOX) pathways (9). Elevated vitamin D3 sulfate suggests altered vitamin D metabolism, although its role remains unclear.

The 117 intersecting genes were associated with signaling pathways related to TNF, IL-17, mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase/protein kinase B (PI3K-Akt), and sphingolipids. The TNF and IL-17 signaling may activate nuclear factor kappa B (NF- κ B) and MAPK pathways, potentially converging on PTGS2 regulation and prostanoid metabolism, linking inflammatory signaling to lipid dysregulation (10). Nonetheless, this model is derived from network relationships rather than

empirical validation. Consequently, PTGS2 should be considered a candidate rather than a confirmed causal MIE.

This study has two limitations that merit attention. First, the small FNF group reduced the statistical power and limited the generalizability. This was due to stringent inclusion criteria and practical challenges in obtaining matched human cartilage specimens. Owing to the ethical constraints associated with the collection of completely healthy articular cartilage, FNF cartilage is often used as a relatively normal orthopedic control. However, it does not entirely represent healthy tissue, and the acute inflammatory and metabolic responses may complicate plasma comparisons. Second, key lipids and PTGS2 lacked orthogonal validation, and the study did not include PTGS2 knockdown or inhibition experiments, or ferroptosis assays. Larger cohorts, targeted biochemical validation, and functional experiments are required to test the proposed AOP.

In summary, KBD and OA share substantial lipid remodeling, whereas KBD shows distinct glyceride and PUFA features. The proposed T-2 toxin-PTGS2 AOP provides a testable framework for mechanistic validation.

Conflicts of interest: No conflicts of interest.

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Ethical statement: This study was conducted in accordance with the Declaration of Helsinki. This study was approved by the Ethics Committee of Xi'an Jiaotong University (Approval Date: March 1, 2022; Reference Number: 2022SLCJ0421).

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REFERENCES

1. Cui SL, Liu H, Pei JR, Li JX, Jiao Z, Deng Q, et al. Summary analysis of national surveillance on Kashin-beck disease from 1990 to 2023. *Biomed Environ Sci* 2024;37(9):1056 – 66. <https://doi.org/10.3967/bes2024.109>.
2. Mei ZX, Yilamu K, Ni WY, Shen PY, Pan N, Chen HS, et al. Chondrocyte fatty acid oxidation drives osteoarthritis via SOX9 degradation and epigenetic regulation. *Nat Commun* 2025;16(1):4892. <https://doi.org/10.1038/s41467-025-60037-4>.
3. Han LX, Cheng BL, Wei WM, Liu L, Cheng SQ, Liu H, et al. Whole-transcriptome sequencing of knee joint cartilage from Kashin-beck disease and osteoarthritis patients. *Int J Mol Sci* 2024;25(8):4348. <https://doi.org/10.3390/ijms25084348>.
4. Chang H, Liu L, Zhang QP, Xu GY, Wang JP, Chen P, et al. A comparative metabolomic analysis reveals the metabolic variations among cartilage of Kashin-Beck disease and osteoarthritis. *Bone Joint Res* 2024;13(7):362 – 71. <https://doi.org/10.1302/2046-3758.137.Bjr-2023-0403.R1>.
5. An KY, Shi BZ, Lv XZ, Liu YH, Xia ZF. T-2 toxin triggers lipid metabolism disorder and oxidative stress in liver of ducks. *Ecotoxicol Environ Saf* 2024;286:117169. <https://doi.org/10.1016/j.ecoenv.2024.117169>.
6. Wang GY, Qin SL, Zheng YN, Xia C, Zhang P, Zhang LX, et al. T-2 toxin induces ferroptosis by increasing lipid reactive oxygen species (ROS) and downregulating solute carrier family 7 member 11 (SLC7A11). *J Agric Food Chem* 2021;69(51):15716 – 27. <https://doi.org/10.1021/acs.jafc.1c05393>.
7. Miao Y, Chen YW, Xue F, Liu KX, Zhu B, Gao JJ, et al. Contribution of ferroptosis and GPX4's dual functions to osteoarthritis progression. *EBioMedicine* 2022;76:103847. <https://doi.org/10.1016/j.ebiom.2022.103847>.
8. Wu XX, Sun AR, Crawford R, Xiao Y, Wang YP, Prasadam I, et al. Inhibition of leukotriene A₄ hydrolase suppressed cartilage degradation and synovial inflammation in a mouse model of experimental osteoarthritis. *Cartilage* 2024;15(2):184 – 94. <https://doi.org/10.1177/19476035231169940>.
9. Jónasdóttir HS, Brouwers H, Kwekkeboom JC, van der Linden HMJ, Huizinga T, Kloppenburg M, et al. Targeted lipidomics reveals activation of resolution pathways in knee osteoarthritis in humans. *Osteoarthritis Cartilage* 2017;25(7):1150 – 60. <https://doi.org/10.1016/j.joca.2017.01.018>.
10. Mimpfen JY, Baldwin MJ, Cribbs AP, Philpott M, Carr AJ, Dakin SG, et al. Interleukin-17A causes osteoarthritis-like transcriptional changes in human osteoarthritis-derived chondrocytes and synovial fibroblasts *in vitro*. *Front Immunol* 2021;12:676173. <https://doi.org/10.3389/fimmu.2021.676173>.

SUPPLEMENTARY MATERIAL

Section 1: Patient Sources and Inclusion and Exclusion Criteria

Patient Sources

A total of 44 patients were recruited at Xi'an Honghui Hospital between April 10, 2023, and March 25, 2025, and all patients signed informed consent forms. The 17 patients with Kashin-Beck disease (KBD) were primarily from KBD-endemic areas in Shaanxi Province, such as Xianyang, Yan'an, and Baoji. The 22 patients with osteoarthritis (OA) were mainly from Xi'an and Weinan in Shaanxi Province, and the 5 patients with femoral neck fracture (FNF) were all from Shaanxi Province.

Criteria for Identification of Patients with KBD

According to the National Diagnostic Criteria for KBD (WS/T207-2010), the diagnosis of KBD in patients and controls is made by combining clinical signs and right-hand orthopantomograms, and the diagnostic basis is as follows.

1) Epidemiological investigation: Patients with KBD have a history of contact with the endemic area of the disease.

2) Clinical signs and symptoms: Patients with KBD often have multiple deformed symmetrical epiphyseal plates, and articular cartilage damage, which is mainly manifested as thickening of finger joints, pain and limitation of flexion and extension, and other clinical signs. According to the severity of clinical symptoms and signs, patients are classified into I, II and III degrees.

3) X-ray manifestations: Before the epiphyseal-plate isometric stage, the metacarpophalangeal epiphyses are first indented and sclerotic with calcified bands, and some of them are accompanied by deformational changes of the bone ends or epiphyses and carpal bones. KBD can be classified as mild, moderate, or severe according to the degree of lesions on the X-ray film, or as active, inactive, and obsolete according to the changes on the X-ray film, which can be used to evaluate the degree of activity of KBD.

4) Differential diagnosis: KBD is distinguished from bone and cartilage-related diseases such as osteoarthritis, rheumatoid arthritis, hereditary osteoarthropathy, chondrodysplasia, and epiphyseal dysplasia based on its spatial, temporal, and population distribution.

Criteria for Identification of Patients with OA

OA was diagnosed based on a combination of clinical symptoms, physical examinations, and imaging and arthroscopic findings according to the Outerbridge classification system for cartilage damage.

1) Clinical history: Patients with OA usually had a long-term history of chronic joint pain, most commonly involving weight-bearing joints such as the knee or hip.

2) Clinical signs and symptoms: Patients with OA often showed localized joint tenderness, bony enlargement, crepitus during joint movement, restricted flexion and extension, joint deformity, or varus/valgus malalignment in advanced cases.

3) Imaging and cartilage manifestations: Radiographic examination, computed tomography (CT), magnetic resonance imaging (MRI), or intraoperative observation was used to evaluate degenerative changes of the affected

SUPPLEMENTARY TABLE S1. Baseline information of patients with KBD, OA and FNF recruited at Xi'an Honghui Hospital, Shaanxi Province, China, April 2023–March 2025.

Primary diagnosis	Subjects (n)	Sex (male/female)	Age (years)	P	BMI (kg/m ²)	P
KBD	17	2/15	61.59 (5.78)	0.100	24.52 (4.56)	0.327
OA	22	3/19	63.50 (4.83)		25.43 (3.41)	
FNF	5	2/3	68.80 (13.01)		21.85 (1.63)	

Note: Age and BMI values are mean standard deviation.

Abbreviations: KBD=Kashin-Beck disease; OA=osteoarthritis; FNF=femoral neck fracture; BMI=body mass index.

SUPPLEMENTARY TABLE S2. Classification of phenotypes and pathways.

Classification	Ancestor terms	Phenotypes	Gene count
GO terms	Homeostatic process	Amide metabolic process	19
		Olefinic compound metabolic process	12
		Homeostasis of number of cells	15
		Tissue homeostasis	13
		Anatomical structure homeostasis	13
		Regulation of cellular catabolic process	16
		Maintenance of location	13
		Membrane organization	14
		Carboxylic acid biosynthetic process	12
		Organic acid biosynthetic process	12
		Regulation of membrane potential	14
		Regulation of metal ion transport	13
		Regulation of monoatomic ion transport	14
		Regulation of protein-containing complex assembly	12
		Calcium ion transport	12
		Negative regulation of catalytic activity	12
		Regulation of transmembrane transport	12
		Positive regulation of protein localization	12
	Immune system process	Response to lipopolysaccharide	18
		Leukocyte proliferation	16
		Regulation of leukocyte proliferation	14
		Mononuclear cell proliferation	14
		T cell proliferation	12
		Lymphocyte proliferation	13
		Regulation of mononuclear cell proliferation	12
		Cellular response to biotic stimulus	12
		Regulation of leukocyte cell-cell adhesion	13
		Leukocyte cell-cell adhesion	13
		Regulation of inflammatory response	13
		Chemotaxis	13
		Taxis	13
		Lymphocyte differentiation	12
	Anatomical structure development	Mesenchyme development	18
		Ossification	20
		Connective tissue development	15
		Glial cell differentiation	14
		Mesenchymal cell differentiation	14
		Positive regulation of cell development	17
		Gliogenesis	15
		Cartilage development	12
		Skeletal system morphogenesis	12
		Epithelial cell proliferation	16
		Morphogenesis of an epithelium	13

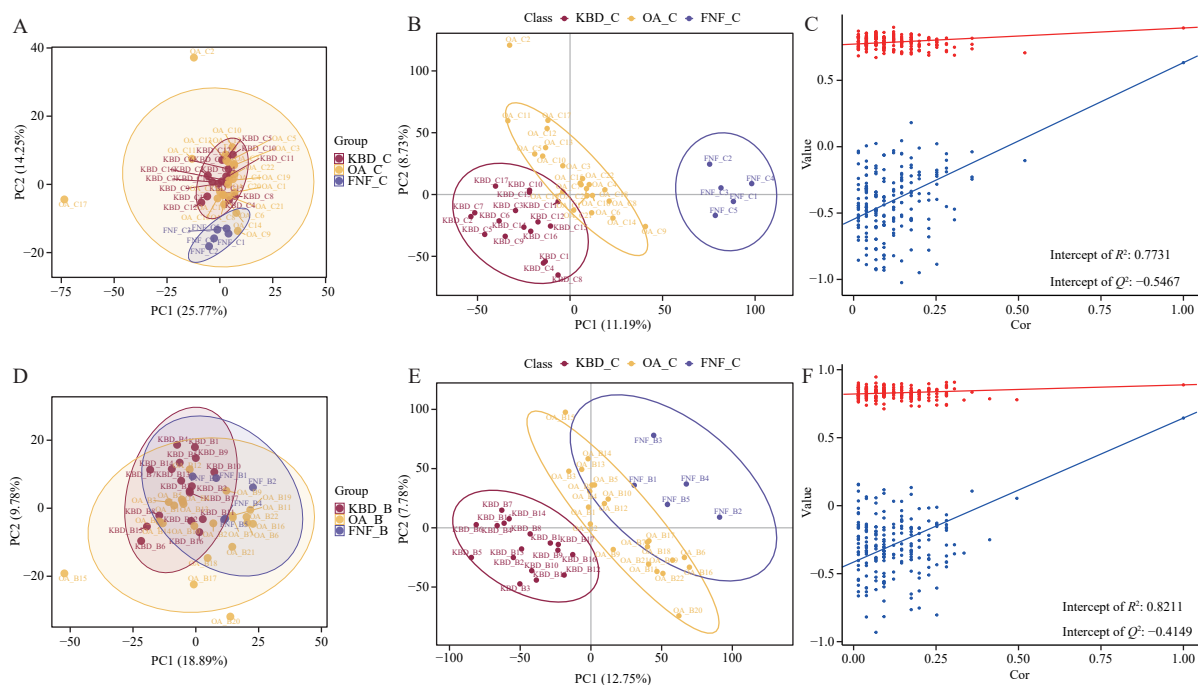
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Classification	Ancestor terms	Phenotypes	Gene count
GO terms	Response to stress	Response to xenobiotic stimulus	25
		Reactive oxygen species metabolic process	16
		Response to oxidative stress	18
		Response to toxic substance	15
		Response to reactive oxygen species	13
		Response to mechanical stimulus	13
		Cellular response to chemical stress	14
		Cellular response to abiotic stimulus	14
		Cellular response to environmental stimulus	14
		Cellular response to oxidative stress	12
		Response to steroid hormone	12
	Lipid metabolic process	Sphingolipid metabolic process	15
		Ceramide metabolic process	13
		Membrane lipid metabolic process	16
		Lipid catabolic process	17
		Fatty acid metabolic process	17
		Steroid metabolic process	14
	Extracellular matrix organization	Phospholipid metabolic process	13
		Extracellular matrix organization	15
		Extracellular structure organization	15
		External encapsulating structure organization	15
		Positive regulation of cell-cell adhesion	12
		Positive regulation of cell adhesion	14
	Programmed cell death	Negative regulation of apoptotic signaling pathway	17
		Regulation of apoptotic signaling pathway	20
		Regulation of intrinsic apoptotic signaling pathway	13
		Extrinsic apoptotic signaling pathway	14
		Intrinsic apoptotic signaling pathway	15
	Signaling	Positive regulation of phosphorylation	14
		Positive regulation of protein phosphorylation	13
		Positive regulation of MAPK cascade	13
KEGG pathways	Not further categorized	Sphingolipid metabolism	14
		Sphingolipid signaling pathway	13
		TNF signaling pathway	11
		Osteoclast differentiation	11
		MAPK signaling pathway	13
		Ras signaling pathway	11
		PI3K-Akt signaling pathway	13

Note: GO terms were grouped into eight functional categories for clarity, whereas KEGG pathways were not further categorized due to the limited number of enriched pathways.

Abbreviations: DLs=differential lipids; KBD=Kashin-Beck disease; OA=osteoarthritis; FNF=femoral neck fracture.

joint. Typical related changes included joint space narrowing, osteophyte formation, subchondral sclerosis, subchondral cysts, cartilage thinning, cartilage defects, and deformity of the articular surface. The severity of



SUPPLEMENTARY FIGURE S1. Quality control of cartilage and plasma lipidomics. (A) PCA of cartilage lipids. (B) PLS-DA of cartilage lipids. (C) Permutation test of the PLS-DA model for cartilage lipids. (D) PCA of plasma lipids. (E) PLS-DA of plasma lipids. (F) Permutation test of the PLS-DA model for plasma lipids.

Note: In the PCA and PLS-DA plots, red, yellow, and blue represent KBD, OA, and FNF samples, respectively. In the permutation tests, red and blue represent R^2 and Q^2 , respectively.

Abbreviation: KBD=Kashin-Beck disease; OA=osteoarthritis; FNF=femoral neck fracture; PCA=principal component analysis; PLS-DA=partial least squares-discriminant analysis; R^2 =coefficient of determination; Q^2 =cross-validated predictive ability.

cartilage degeneration was assessed according to the Outerbridge classification:

- Grade 0: normal articular cartilage;
- Grade I: cartilage softening and swelling;
- Grade II: superficial fragmentation or fissuring with partial-thickness cartilage damage;
- Grade III: deep fissuring or fragmentation extending toward the subchondral bone;
- Grade IV: full-thickness cartilage erosion with exposed subchondral bone.

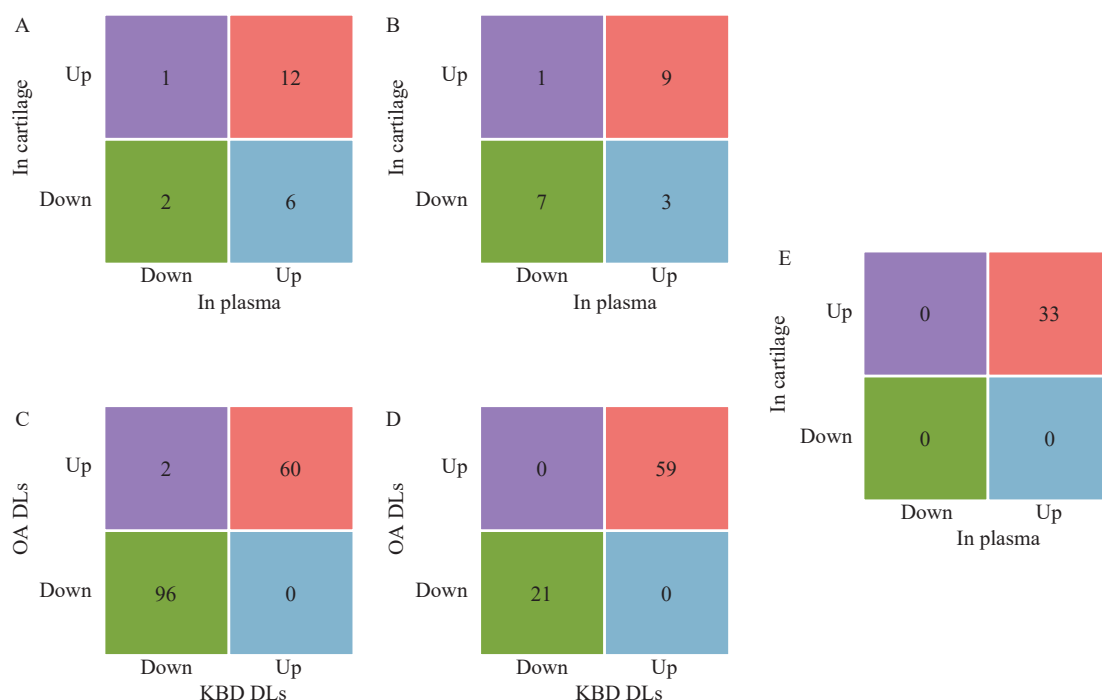
In this study, patients with OA were identified when degenerative cartilage lesions consistent with OA were observed, particularly moderate-to-severe cartilage degeneration corresponding to advanced Outerbridge grades.

4) Patients were excluded if they had evidence of KBD, rheumatoid arthritis, gouty arthritis, septic arthritis, ankylosing spondylitis, psoriatic arthritis, systemic lupus erythematosus-related arthritis, osteonecrosis of the femoral head, congenital or hereditary skeletal dysplasia, traumatic cartilage injury, tumor-related joint destruction, or other secondary causes of joint degeneration. Patients with a clear history of residence in or exposure to KBD-endemic areas combined with typical symmetrical hand joint deformities or characteristic hand X-ray changes were not classified as OA cases.

Criteria for Identification of Patients with FNF

The diagnosis of FNF was based on clinical assessment and imaging confirmation. The clinical evaluation included medical history, trauma history, physical examination, and radiological examination of the hip joint. The diagnostic criteria were as follows.

1) Clinical history: Patients with FNF usually had a definite history of trauma, such as a fall from standing height in elderly patients or high-energy trauma in younger patients. Some patients, especially elderly individuals with osteoporosis, could develop FNF after minor trauma.



SUPPLEMENTARY FIGURE S2. Common DLs across tissues and groups. (A) Common DLs in KBD cartilage and plasma (Consistency rate-66.7%). (B) Common DLs in OA cartilage and plasma (Consistency rate-80.0%). (C) Common DLs in KBD versus OA cartilage (Consistency rate-98.7%). (D) Common DLs in KBD versus OA plasma (Consistency rate-100.0%). (E) Common DLs in cartilage and plasma of KBD and OA (Consistency rate-100.0%).

Note: Red and green indicate concordant upregulation and downregulation, respectively. Purple indicates upregulation in the dataset represented on the y-axis and downregulation in the dataset represented on the x-axis, whereas blue indicates the opposite directional pattern. The consistency rate represents the proportion of common DLs showing concordant directional changes in the two compared datasets.

Abbreviation: DLs=differential lipids; KBD=Kashin-Beck disease; OA=osteoarthritis; FNF=femoral neck fracture.

2) Clinical signs and symptoms: Patients with FNF often presented with pain around the hip or groin, reduced range of motion of the hip joint, tenderness over the anterior hip or groin region, pain during passive hip rotation, and inability to perform straight leg raising. Displaced FNF could be accompanied by shortening, abduction, and external rotation of the affected lower limb.

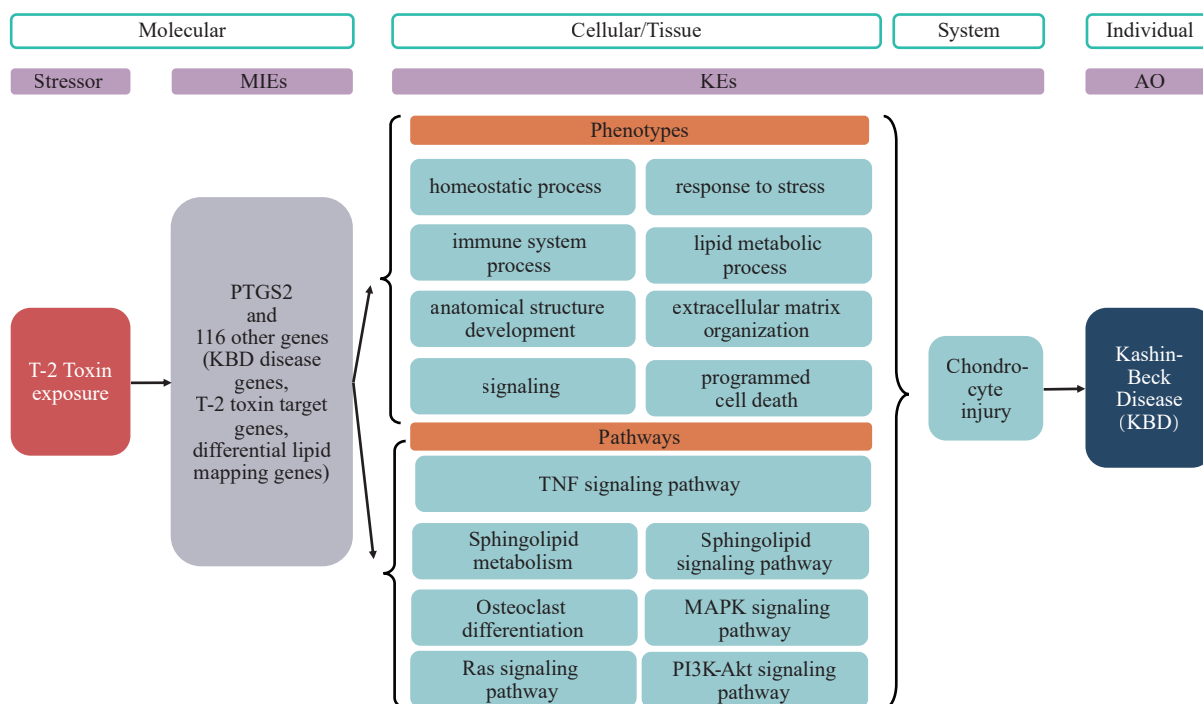
3) Imaging manifestations: The diagnosis of FNF was confirmed by imaging examination. FNF was identified by the presence of a fracture line through the femoral neck, cortical interruption, trabecular discontinuity, impaction, angulation, or displacement of the femoral head-neck fragment. When plain radiographs were negative or equivocal but clinical suspicion remained high, CT or MRI was used to detect occult, non-displaced, impacted, or stress-related FNF.

4) Patients were excluded if the lesion was not located in the femoral neck or if imaging suggested intertrochanteric fracture, subtrochanteric fracture, femoral shaft fracture, acetabular fracture, hip dislocation, isolated greater trochanteric fracture, pelvic fracture, osteonecrosis of the femoral head, hip osteoarthritis, inflammatory arthritis, bone tumor, metastatic bone disease, osteomyelitis, or pathological fracture unrelated to ordinary trauma or osteoporosis. Patients with chronic degenerative joint disease without an acute FNF line were not classified as FNF cases.

Section 2: Cartilage and Plasma Sample Collection and Preprocessing Procedures

Cartilage and Plasma Sample Collection Methods

Peripheral venous blood was collected from all patients and centrifuged within 2 h of collection. After centrifugation at 3,000 rpm for 15 min at 4 °C, the supernatant plasma was carefully aspirated, aliquoted into



SUPPLEMENTARY FIGURE S3. Putative adverse outcome pathway (AOP) framework linking T-2 toxin exposure to Kashin-Beck disease.

Note: T-2 toxin exposure is shown as the stressor; PTGS2 and 116 other intersecting genes are presented as candidate molecular initiating events (MIEs). Candidate key events (KEs) include eight Gene Ontology phenotype categories and seven pathways. These KEs converge on chondrocyte injury at the system level and KBD as the adverse outcome (AO).

nuclease-free cryovials, and immediately stored at -80°C for subsequent lipidomic analysis. Samples from the femoral condyles were collected within 1 h following joint replacement surgery, ensuring the inclusion of an intact cartilage layer and a 0.5–1 cm layer of subchondral bone, in preparation for subsequent lipidomic analysis.

Cartilage and Plasma Sample Preprocessing Methods

A plasma sample (200 μL) was extracted using methyl tert-butyl ether (MTBE, 400 μL) and ice-cold methanol (80 μL). Cartilage samples (50 mg) were homogenized in 50% cold methanol (200 μL) and extracted with cold MTBE (600 μL). Both mixtures were centrifuged and the supernatants were dried and reconstituted with dichloromethane-methanol (v/v, 1:1) for instrumental analysis.

Section 3: Lipidomics Data Acquisition Process and Chromatographic Conditions

Lipidomics Data Acquisition Process

Lipidomics data were acquired using a Thermo Fisher Scientific Q-Exactive Plus high-resolution tandem mass spectrometer coupled to a Thermo Vanquish Flex UPLC system. Data acquisition employed the full mass spectrometry (MS) and data-dependent acquisition (DDA) modes. In each acquisition cycle, the full scan range was 100–1,500 Da, with a resolution of 70,000, an automatic gain control (AGC) target of $3\text{E}6$, and a maximum ion injection time (IT) of 100 ms. The top six ions with response intensities exceeding 100,000 were selected from the full scan for MS/MS analysis, with an MS/MS resolution of 17,500 and a maximum IT of 50 ms. The dynamic exclusion time was set to 6 s.

Chromatographic and Mass Spectrometric Conditions

Lipidomic separation was performed using an ACQUITY UPLC CSH C18 column (100 mm $\times$ 2.1 mm, 1.7 μm ; Waters). The mobile phase consisted of solvent A (acetonitrile:water, 6:4 v/v, containing 10 mM ammonium

formate and 0.1% formic acid) and solvent B (isopropanol:acetonitrile, 9:1, v/v, containing 10 mM ammonium formate and 0.1% formic acid). The gradient elution program was as follows: 0–0.5 min, 30% B; 0.5–2.0 min, 30%–75% B; 2.0–5.2 min, 75%–100% B; 5.2–6.8 min, 100% B; 6.8–6.81 min, 100%–30% B; and 6.81–10.0 min, 30% B. The flow rate was maintained at 0.35 mL/min, the injection volume was 4 μ L, and the column temperature was kept at 40 °C. Mass spectrometry (MS) was performed using a Q-Exactive Plus high-resolution tandem mass spectrometer (Thermo Fisher Scientific) equipped with an electrospray ionization (ESI) source. Each sample was analyzed in both the positive and negative ion modes. The ESI source temperature was set to 350 °C. The spray voltage was +3,800 V in the positive ion mode and –3,500 V in the negative ion mode. The pressures of sheath, auxiliary, and sweep gases were maintained at 45, 12, and 0 arbitrary units (Arb), respectively. Data acquisition was performed using the full scan-data-dependent acquisition (full scan-DDA) mode. The mass-to-charge ratio (m/z) range for the full scan was 100–1,500 Da, with a resolution of 70,000, an automatic gain control (AGC) target of 3E6, and a maximum ion injection time (IT) of 100 ms. Subsequently, the six most intense precursor ions with signal intensities exceeding 100,000 were selected from the full-scan results for DDA MS/MS fragmentation analysis. The DDA scan parameters included a resolution of 17,500, a maximum ion injection time of 50 ms, and dynamic exclusion time of 6 s.

Section 4: Data Processing and Analysis Process

Data Preprocessing Process

The acquired MS data were preprocessed using XCMS software, involving steps such as peak picking, peak grouping, retention time correction, secondary peak grouping, and annotation of isotopes and adducts. Raw LC-MS data files were converted to mzXML format and subsequently processed using R-based toolkits, including XCMS, CAMERA, and metaX. Individual ions were identified based on the retention time (RT) and mass-to-charge ratio (m/z). Peak intensities were recorded to generate a three-dimensional matrix containing specific peak indices (RT- m/z combinations), sample names (observations), and ion intensity information (variables).

Information Analysis Process

Metabolites were annotated using online KEGG and HMDB databases by matching the samples' accurate molecular mass data (m/z) against database entries. Annotations were assigned when the mass deviation between the observed and database values was less than 10 ppm; molecular formulas were further determined and verified through isotopic distribution analysis. Additionally, metabolite identification results were validated using an in-house metabolite fragmentation spectral library. Metabolite data underwent three key processing steps: data filtering (removing samples with >80% missing values or quality control [QC] samples with >50% missing data), data imputation using the K-nearest neighbors (KNN) method, and data normalization via probabilistic quotient normalization (PQN). Principal component analysis (PCA) and differential metabolite analysis were performed using the 'metaX' package. Partial least squares-discriminant analysis (PLS-DA) was conducted using the 'ropls' package, and variable importance in projection (VIP) values were calculated. Finally, significantly differential metabolites were selected based on three criteria: a t -test $P < 0.05$, $FC > 1.20$, and a VIP value ≥ 1.00 in the PLS-DA analysis. Hypergeometric testing was employed for KEGG pathway enrichment analysis, with a $P < 0.05$ indicating significant enrichment. GSEA (v4.1.0) was used for metabolite set enrichment analysis; KEGG pathways meeting the criteria of $|NES| > 1.00$, nominal $P < 0.05$, and FDR < 0.25 were considered significantly different between the two groups.