CARTILAGE
2021, Vol. 13(Suppl 1) 797S–808S

DOI: 10.1177/1947603520988165
journals.sagepub.com/home/CAR
SAGE

Patients with Osteoarthritis and Kashin-Beck Disease Display Distinct CpG Methylation Profiles in the *DIO2, GPX3*, and *TXRND1* Promoter Regions

Rongqiang Zhang[1,2], Hao Guo[2], Xiaoli Yang[2], Dandan Zhang[2], Di Zhang[2], Qiang Li[2], Chen Wang[2], Xuena Yang[2], and Yongmin Xiong[2]

Abstract

Objective. We aimed to analyze deoxycytidine-deoxyguanosine dinucleotide (CpGs) methylation profiles in *DIO2, GPX3*, and *TXNRD1* promoter regions in osteoarthritis (OA) and Kashin-Beck disease (KBD) patients. *Methods.* Blood samples were collected from 16 primary OA patients and corresponding 16 healthy individuals and analyzed for methylations in the CpGs of *DIO2, GPX3*, and *TXNRD1* promoter regions using MALDI-TOF-MS. The methylation profiles of these regions were then compared between OA and KBD patients. *Results. DIO2*-1_CpG_2 and *DIO2*-1_CpG_3 methylations were significantly lower in OA than KBD patients ($P < 0.05$). A similar trend was observed for *GPX3*-1_CpG_4, *GPX3*-1_CpG_7, *GPX3*-1_CpG_8.9.10, *GPX3*-1_CpG_13.14.15 and *GPX3*-1_CpG_16 ($P < 0.05$) as well as *TXNRD1*-1_CpG_1 and *TXNRD1*-1_CpG_2 methylation between OA and KBD patients ($P < 0.05$). However, there was no difference in methylation levels of other CpGs between the 2 groups ($P > 0.05$). *Conclusion.* OA and KBD patients display distinct methylation profiles in the CpG sites of *DIO2, GPX3*, and *TXNRD1* promoter regions. These findings provide a strong background and new perspective for future studies on mechanisms underlying epigenetic regulation of selenoprotein genes associated with OA and KBD diseases.

Keywords

osteoarthritis, Kashin-Beck disease, DNA methylation, CpG, CpG-SNP

Osteoarthritis (OA), a degenerative joint disorder, is one of the leading causes of disability around the globe. The disease affects almost all joints, but more than 80% of OA occurs in the knees.[1,2] Apart from disability, OA increase in the risk of developing cardiovascular diseases.[3] In addition, OA negatively affects mental well-being. In particular, the disease condition is associated with depression, suicidal ideation, memory loss, insomnia, and bipolar disorder.[4-7] Current studies are focused on understanding the pathogenesis of OA with a view of rationalizing treatment approaches. However, the mechanism underlying OA pathogenesis remains to be validated.

Kashin-Beck disease (KBD) is an endemic OA, mainly prevalent in China, North Korea, and Russia.[8,9] Generally, KBD manifests with pain, swelling, and stiffness of the joints as well as flexion of finger joints. Patients with this conditions find it very difficult to work or to take care of themselves.[10] By the end of 2017, there were 535,878 first-degree KBD patients in China. Among these, 12,730 were children ≤13 years old.[11] Exploring the etiology, pathogenesis, as well as biomarkers for early diagnosis of KBD is critical in guiding management strategies of the disease.

Primary OA and KBD share not only similar clinical characteristics but also the associated pathologic articular cartilage disorders, including chondrocyte apoptosis, inflammation, and cartilage degeneration manifest in both disease conditions.[4,12,13] Nonetheless, OA and KBD exhibit distinct epidemiological characteristics. Whereas OA is globally distributed, KBD is only endemic in some regions. As search, discerning marked differences and similarities

[1]School of Public Health, Shaanxi University of Chinese Medicine, Xianyang, China
[2]Institute of Key Laboratory of Trace Elements and Endemic Diseases, National Health Commission of the People's Republic of China, School of Public Health, Xi'an Jiaotong University Health Science Center, Xi'an, Shaanxi, China

Corresponding Author:
Yongmin Xiong, Institute of Key Laboratory of Trace Elements and Endemic Diseases, National Health Commission of the People's Republic of China, School of Public Health, Xi'an Jiaotong University Health Science Center, No.76 Yanta West Road, Xi'an, Shaanxi, 710061, China.
Emails: xiongym@mail.xjtu.edu.cn, 754274301@qq.com

Table 1. The Basic Characteristics of the Subjects for MALDI-TOF MS Experiment.

Characteristics	OA Patients ($n = 16$)	KBD Patients ($n = 16$)	t/x^2	P
Age, y (mean ± SD)	55.23 ± 6.27	52.35 ± 8.79	1.067	0.295
Gender (female/male)	7/9	8/8	0.125	0.723

OA = osteoarthritis; KBD = Kashin-Beck disease; MALDI-TOF MS = matrix-assisted laser desorption/ionization time of flight mass spectrometry.

between OA and KBD will promote early diagnosis and effective treatment of the 2 diseases.

In recent years, increasing evidence shows that both OA and KBD are caused by epigenetic DNA modification. For instance, methylation of selenoprotein genes has been implicated in the development of OA and KBD.[14,15] As such, selenoproteins are important targets in understanding arthritis diseases. Meanwhile, iodothyronine deiodinase (IDs), GPXs, and TrxRs family of proteins are key members of selenoproteins. These proteins participate in regeneration of skeletal muscles. Indeed, research shows that DIO2 is central to KBD progression in children.[16] On the other hand, GPX3 protects against oxidative-related damage of human chondrocytes[14]. Similar to GPX3, TXRND1 protects against oxidative-related cell damages.[17-19] Increasing evidence shows that the cross-talk between inflammation and epigenetic regulators contribute to the development and/or progression of OA.[20] Nevertheless, methylation profiles of selenoprotein genes in OA and KBD patients remain unknown. Therefore, we evaluated the epigenetic changes in *DIO2, GPX3*, and *TXRND1* genes in OA and KBD patients.

CpGs methylations in *DIO2, GPX3*, and *TXRND1* promoter regions of OA and KBD patients were assessed using the high-throughput, cheap, and highly accurate matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) technique,[21] DNA methylation differences between KBD and OA will deepen our understanding of the epigenetic mechanism underlying modification of selenoprotein genes in OA and KBD patients.

Materials and Methods

Participant Selection

We collected 32 blood samples from 16 primary OA patients and 16 healthy subjects. All study participants were Han Chinese, drawn from all ages and either gender (**Table 1**). Clinical examinations and radiographic test were performed on each participant. Primary OA diosmosis was based on the proposed guidelines by the Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association (ACR). KBD was diagnosed based on the clinical diagnosis criteria of China (WS/T 207-2010). Patients with underlying genetic bone-, cartilage-, and arthritis-related diseases as well as other skeletal disorders were not included in the study.

Collection of Blood Specimens and DNA Extraction

Protocol for this study was approved by the Human Ethics Committee of Xi'an Jiaotong University, People's Republic of China. All participants consented to the study in writing. Briefly, 3 mL of blood from all subjects was first collected in biochemical anticoagulation test tubes. Genomic DNA was then extracted from the blood samples based on the QIAamp DNA Blood Mini Kit (QIAGEN, Germany), following the manufacture's protocol. Efficiency of extraction was validated by agarose gel electrophoresis.

Primer Design and Synthesis

Primers for the methylated CpG sites in the *DIO2, GPX3*, and *TXNRD1* promoter regions were designed using the Agena software (http://www.epidesigner.com/index.html). Methylation patterns in CpG sites were determined based on the sequence of the three target genes. The size of the target fragments ranged between 200 and 600 bp for all the genes. *DIO2, GPX3*, and *TXNRD1* fragments contain 4, 25, and 13 CpGs, respectively. Each forward primer was conjugated with a 10-mer tag. Also, reverse primers were conjugated with a T7 promoter tag to adjust for the differences in melting temperature. The properties of primers used in this experiment including sequences of the target fragments and CpGs are highlighted in **Tables 2** and **3**, respectively. The primers were synthesized by Liuhe Huada Gene Technology Co., Ltd. (Beijing, China).

Methylation Assessment

Briefly, 200 ng of genomic DNA of each participant was treated with bisulfite based on the EZ-96 DNA methylation kit (Zymo Research, Irvine, CA, USA), according to the manufacturer's instructions. Methylation of *DIO2, GPX3*, and *TXNRD1* was then quantitatively analyzed using the Agena MassARRAY platform (CapitalBio Corporation, Beijing, China). The platform composes of a MALDI-TOF mass spectrometer and an RNA base-specific cleavage (Mass CLEAVE) module. The methylations were detected using the Spectro CHIP (Agena Bioscience, California, USA) and Mass ARRAY Compact System (Agena Bioscience, California, USA) on the Agena MassARRAY platform. The data imported and analyzed using the

Table 2. The Information of Methylation Primers of *DIO2, GPX3, TXNRD1* Used in This Study.

Amplicon Name			Primer	Target Length	Target CpG	CpG Analyzed in Target Sequence
*DIO2*_1	Before bisulfite modification	F	TTTATAAGGATATTGAGTAGTTTGAGAAGA	442	4	4
		R	CCCAACACCTAAAACCATACTTAAC			
	After bisulfite modification	F	AGGAAGAGAGTTTATAAGGATATTGAGTAGTTTGAGAAGA			
		R	CAGTAATACGACTCACTATAGGGAGAAGGCTCCCAACACCTAAAACCATACTTAAC			
*GPX3*_8	Before bisulfite modification	F	GGAATAAGAAATGTTTTTTAGAATGGA	456	25	20
		R	ACCAAAAAACAAAAAAAAACAAACAAA			
	After bisulfite modification	F	AGGAAGAGAGGGAATAAGAAATGTTTTTTAGAATGGA			
		R	CAGTAATACGACTCACTATAGGGAGAAGGCTACCAAAAACAAAAAAAAACAAACAAA			
*TXNRD1*_38	Before bisulfite modification	F	TTTGAGAATGATGAAGATATTAGGTT	440	13	12
		R	AAACACCAAAATTCTAAAAACCCTC			
	After bisulfite modification	F	AGGAAGAGAGTTTGAGAATGATGAAGATATTAGGTT			
		R	CAGTAATACGACTCACTATAGGGAGAAGGCTAAACACCAAAATTCTAAAAACCCTC			

F = forward primers; R = reverse primers.

Table 3. The CpGs in the Target Sequences.

Amplicon Name	Target Sequence
*DIO2*_1	CCCATAAGGACATTGAGTAGTTTGAGAAGATACATGCAGTAAATGTACATTGCATATATTAGTAA TATTATTTTGCTAACATTGTATGCTAGTCATAGGGAAGGTGTAATTTTTCTATGTGGTTTATATTATTTT TCTAAGCTTTGAAGTCTTTTAACAATTT**CG**[1]TCCATGAGATGCAAATTCAAAACTCATTAGGAGCCAAAC TAATGAAAAAAATGAAAGAATTTGTAGGGAATGCTGAGGGTTGTGTCACAAGAGAGGGCATTGCACAA GGCCACCA**CG**[2]TTTTACAATTCCAGGGCACCCCCATTCACATCATATTGTATGTGAATGGTGCCCCCTA GTGTAGAGCAATGCAGAGGCCCTCTACATGCCCTGTTCATTCATTCATTCAGGA**CG**[3]TCCTCAATGAGC CAACTGTG**CG**[4]CCAAGCATGGTTCTAGGTGTTGGG
*GPX3*_8	GGAATAAGAAATGCTTCCCAGAATGGAGACTTCCATCAGTTCTAGGGAGCTATTAGCCCCCTTGCCC TGGCTGTAATGGAGAC**CG**[1]CTGTGTCTGCCTCCTTT**CG**[2]CACTTTGGAGCCAAAAGAGGAAGGGAC**C G**[3]CCTCCCA**CG**[4]TCCACAGGGACCTGACTTCCACCTCTCTGCCCAGATTTGCTTATGTCACTGT**CG**[5]C CC**CG**[6]GGA**CG**[7]GGGAGGTGGGGAGCTGAGGGCAAGT**CG**[8]**CG**[9]CC**CG**[10]CCCCTGAAATCCCAGC**CG**[11] CCTAG**CG**[12]ATTGGCTGCAAGGGTCT**CG**[13]GCTTGGC**CG**[14]**CG**[15]GATTGGTCACACC**CG**[16]AGGGCTTGA AAGGTGGCTGGGAG**CG**[17]C**CG**[18]GACACCTCAGA**CG**[19]GA**CG**[20]GTGGCCAGGGATCAGGCAG**CG**[21]GCTC AGG**CG**[22]ACCCTGAGTGTGCCCCCACCC**CG**[23]CCATGGCC**CG**[24]GCTGCTGCAGG**CG**[25]TCCTGCCTGCTT TCCCTGCTCCTGGC
*TXNRD1*_38	TTTGAGAATGATGAAGACATCAGGCCCTCCTTCAGGACT**CG**[1]ACCACCTTT**CG**[2]CTCTGCTGCAAAT GC**CG**[3]GAGTGAAGAAAACTGAGGTGAAAGGGTTGCTGTTTCCTAATGGCTA**CG**[4]AGAAGCCA**CG**[5]CC CTG**CG**[6]C**CG**[7]AG**CG**[8]GG**CG**[9]GGCTAACTGCCAGAGTCAGAATGACAAAGCAGAAATCCACTCCT**CG**[10] TGCAGCCCTGGCAACCTATGCTAATTGCCT**CG**[11]TAAGCTGAGAGCTCCTAATTGGGTGACACCACATC CTCTGCCTGCTCATAGC**CG**[12]CCCCCAACCTAAGAACAGAGCAAAAACAATTTAAGGCAATTCTGGAA CCAAATTTGGAATAAGTGTGTTTTTACTGAAGAAATTAGACTATGCCCTAAGGCAGTAGCTACAGAAC TTTGCTAGCTGTTGTCATCA**CG**[13]TGAGGGTCTTTAGAATCCTGGTGCCT

EpiTYPER software version 1.0 (Agena Bioscience, San Diego, CA, USA).

Statistical Analysis

Continuous variables were expressed as means ± standard deviations (SD). Differences between OA and KBD patients were analyzed using chi-square (χ^2) test and Student *t* test for 2 independent samples. All statistical analyses were performed using SPSS V. 23.0 (IBM Corp Armonk, NY, USA). Statistical significance was set at $P < 0.05$.

Results

Baseline Characteristics of the Study Population

There was no significant difference in demographic characteristics of the 16 OA and 16 KBD patients evaluated in this study between the 2 groups with regard to age and gender ($P > 0.05$) (Table 1).

DIO2 *Methylation*

Methylation profile of the 4 CpGs in *DIO2* promoter region is shown in **Figure 1**. We found methylation levels of *DIO2*-1_CpG_2 and *DIO2*-1_CpG_3 were significantly lower in OA than KBD patients ($P < 0.05$). However, there was no significant difference in the methylation level of *DIO2*-1_CpG_1 and *DIO2*-1_CpG_4 between the 2 groups of patients ($P > 0.05$) (**Fig. 2**).

GPX3 *Methylation*

Methylation profile of 14 CpGs (CpG Units) in *GPX3* promoter region is summarized in **Figure 3**. Methylated *GPX3*-1_CpG_4, *GPX3*-1_CpG_7, *GPX3*-1_CpG_8.9.10, *GPX3*-1_CpG_13.14.15, and *GPX3*-1_CpG_16 were significantly lower in OA than KBD patients ($P < 0.05$). Conversely, there was no difference in the levels of methylated *GPX3*-1_CpG_3, *GPX3*-1_CpG_5.6, *GPX3*-1_CpG_11, *GPX3*-1_CpG_17.18, *GPX3*-1_CpG_20, *GPX3*-1_CpG_21, *GPX3*-1_CpG_22, *GPX3*-1_CpG_23, *GPX3*-1_CpG_24 between the groups ($P > 0.05$) (**Fig. 4**). However, the methylation patterns of *GPX3*-1_CpG_1, *GPX3*-1_CpG_2, *GPX3*-1_CpG_12, *GPX3*-1_CpG_19, and *GPX3*-1_CpG_25 were not assessed because they were either large or small, beyond the detection range of the Mass ARRAY platform.

TXNRD1 *Methylation*

Methylation profiles for 9 CpGs (CpG Units) in the *TXNRD1* promoter region are shown in **Figure 5**. Methylated *TXNRD1*-38_CpG_1 and *TXNRD1*-38_CpG_2 were significantly lower in OA than KBD patients

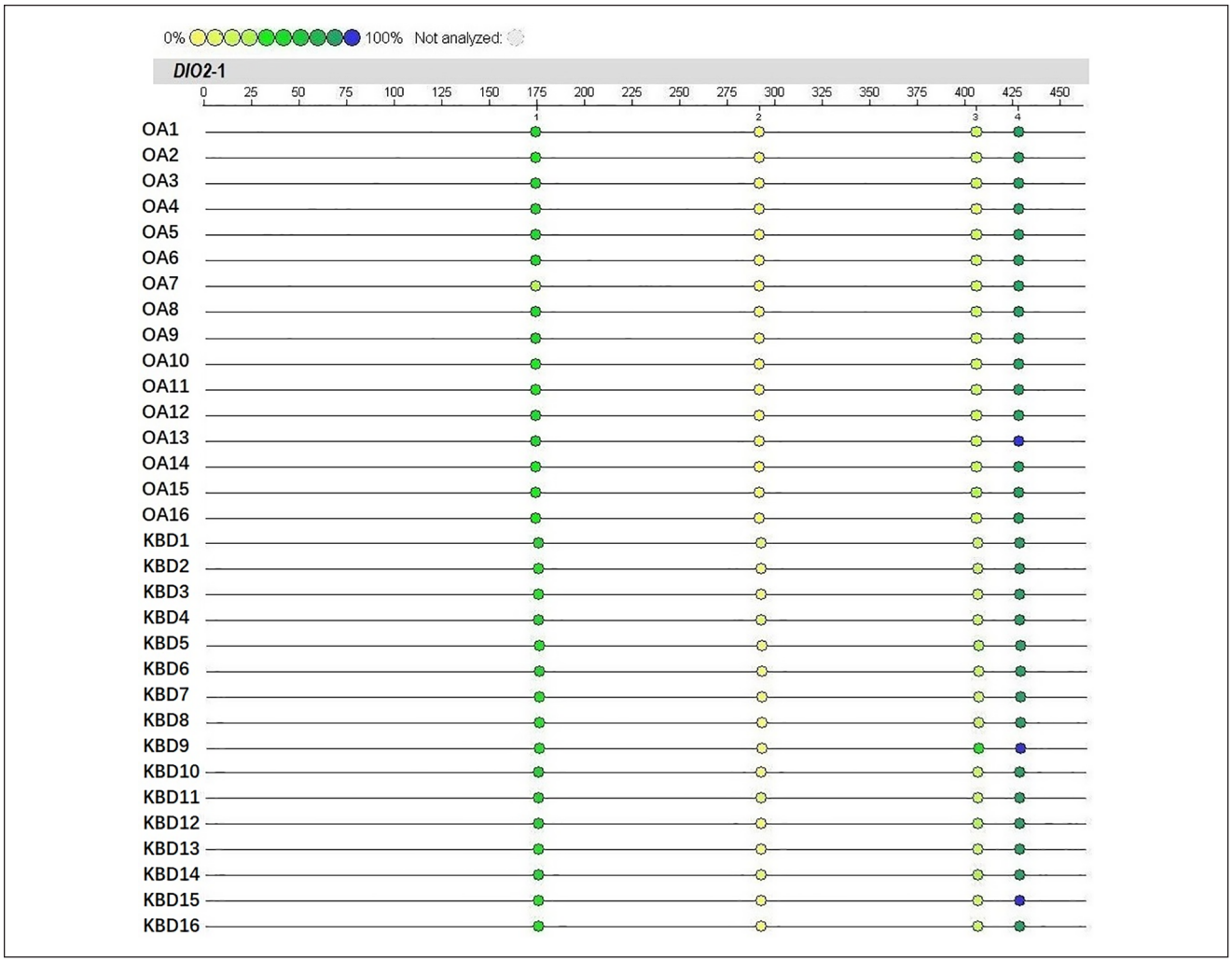

Figure 1. Methylation levels of CpGs in the promoter region of *DIO2* gene in patients with osteoarthritis (OA) and Kashin-Beck disease (KBD). The color intensity is directly proportional to the methylation level. The numbers 1, 2, 3, and 4 represent CpGs *DIO2*-1_CpG_1, *DIO2*-1_CpG_2, *DIO2*-1_CpG_3, and *DIO2*-1_CpG_4, respectively.

($P < 0.05$). On the other hand, there no difference in the levels of methylated *TXNRD1*-38_CpG_3, *TXNRD1*-38_CpG_5.6.7, *TXNRD1*-38_CpG_8.9, *TXNRD1*-38_CpG_10, *TXNRD1*-38_CpG_11, *TXNRD1*-38_CpG_12, and *TXNRD1*-38_CpG_13 between the 2 groups ($P > 0.05$) (**Fig. 6**). Methylation of *TXNRD1*-38_CpG_4 was not detectable because it is too small, beyond the detection range of the Mass ARRAY platform.

Discussion

Herein, we explored the methylation patterns of selenoprotein genes in patients with OA and KBD using the MALDI-TOF MS technique. Overall, 42 CpG sites in the promoter region of three selenoprotein genes (*DIO2, GPX3, TXRND1*) were analyzed. We found significant pathogenetic differences between OA and KBD patients. Most previous OA research focused on the pathogenesis, management, and treatment modules of the disease.[2,15,22-24] Clinical evidence shows that symptom management does not sufficiently ameliorate OA. Research on mechanism underlying OA development is particularly important in guiding the development of better treatment therapeutic strategies, which will improve the quality of life of affected patients. On the other hand, KBD is characterized by joint deformation. In extreme cases, the effected individual becomes disabled. Though critical, early diagnosis and prevention of KBD remain a persistent challenge. Numerous research studies have shown that OA and KBD patients exhibit oxidative damage and apoptosis

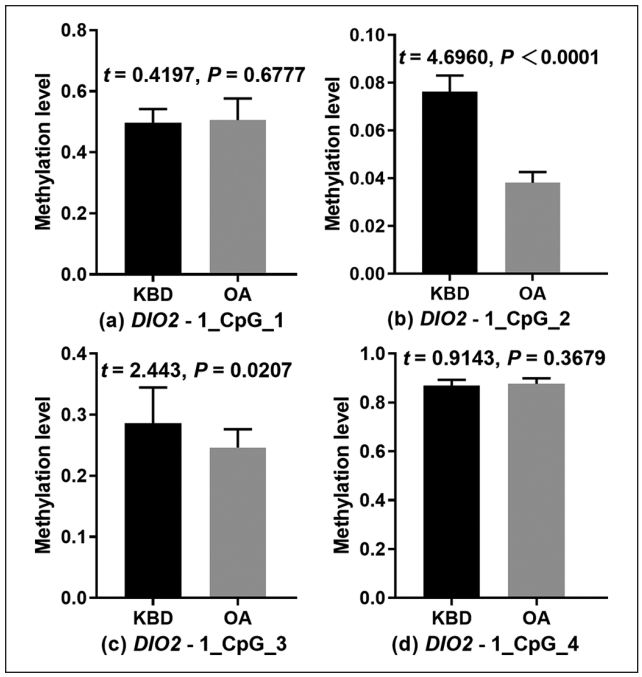

Figure 2. Methylation levels of CpG sites in *DIO2* promoter region in osteoarthritis (OA) and Kashin-Beck disease (KBD) patients.

of cartilage chondrocytes.[25-30] Accordingly, reducing the levels of reactive oxygen species (ROS) in chondrocytes has been reported to effectively delay and prevent oxidative related damage to the cells.[31,32]

Selenium (Se) is a natural mineral widely distributed in the environment.[33] It is an essential trace element in human and animals, easily obtained from food.[34] In the body, selenium is distributed in various tissues, organs, and body fluids, with the highest concentration found in the kidneys. Besides the known toxic effects, there has been a keen interest in selenium owing to its nutritional value.[35] In one study, it was found that selenium deficiency is closely associated with Keshan disease (KSD) and KBD.[36,37] Given that several inorganic selenium antioxidant compounds have been implicated in the oxidation of the selenium, it is particularly necessary to explore the biological functions of the mineral. Meanwhile, selenium compounds prevent oxidative DNA damage.[31,36] Oxidative stress also causes degeneration of the extracellular matrix, which exacerbate apoptosis and necrosis of KBD and OA articular chondrocytes.[31,38,39] These findings demonstrate the protective antioxidant properties of selenium on apoptosis and necrosis of chondrocytes.

To date, 25 selenoproteins have been identified and isolated in humans. Functionally, selenoprotein (selenocysteine) mainly regulates various biological functions such as oxidation, inflammation, and apoptosis.[17,40,41] The iodothyronine deiodinase (ID) family participates in maintaining metabolic balance of thyroid hormones in the body, as well as regulates regeneration of skeletal tissues.[17,40,41] Several studies show that *DIO2* mRNA is overexpressed in the articular cartilage of OA patients.[22,42] Meanwhile, *DIO2* has been implicated in KBD progression in children.[16]

Recent studies show that, GPXs protects against oxidative damage by degrades ROS. For instance, *GPX3* exerts an anti-inflammatory biological effect against activated H_2O_2-mediated lipoxygenase. Selenium up-regulates the level expression of *GPX3* mRNA following oxidative-related damage of human chondrocytes.[14]

On the other hand, most research on the biological function of TrxRs mainly focuses on removing excess free radicals in the cytoplasm and mitochondria to protect cells against oxidative stress.[29,43] In related studies, it has been found that low Se level in the body increases production of ROS and activates the Nrf2 signaling pathway. Together, they enhance the antioxidant capacity of the body.[17-19] This underlines the role of selenoproteins in genetic regulation of OA and KBD diseases.

Recent studies have revealed significant difference in the methylation patterns of *DIO2, GPX3*, and *TXNRD1* promoter regions between OA and KBD patients. In this study, we found there were significantly fewer methylated CpGs sites in the *DIO2, GPX3*, and *TXNRD1* regions in OA than KBD patients. Although the 2 diseases exhibit several similarities across many characteristics, the above methylation differences suggest of distinct epigenetic modification of selenoprotein genes toward OA and KBD disease development. Even so, the exact mechanism underlying epigenetic modification of selenoprotein genes in the development of OA and KBD remain to be elucidated. Nevertheless, findings of this study provide a strong background that will guide future research perspectives on OA and KBD.

Overall, compared with individual with KBD, OA patients exhibit higher methylations in the *DIO2, GPX3*, and *TXNRD1* promoter regions. This implies that KBD and OA are caused by distinct epigenetic modifications. These changes disrupt the expression of selenoproteins, modulating anti-oxidation and anti-chondrocyte apoptosis. In general, findings of this study provide new insights into epigenetic mechanism underlying repressed expression of selenoprotein in OA and KBD disease conditions.

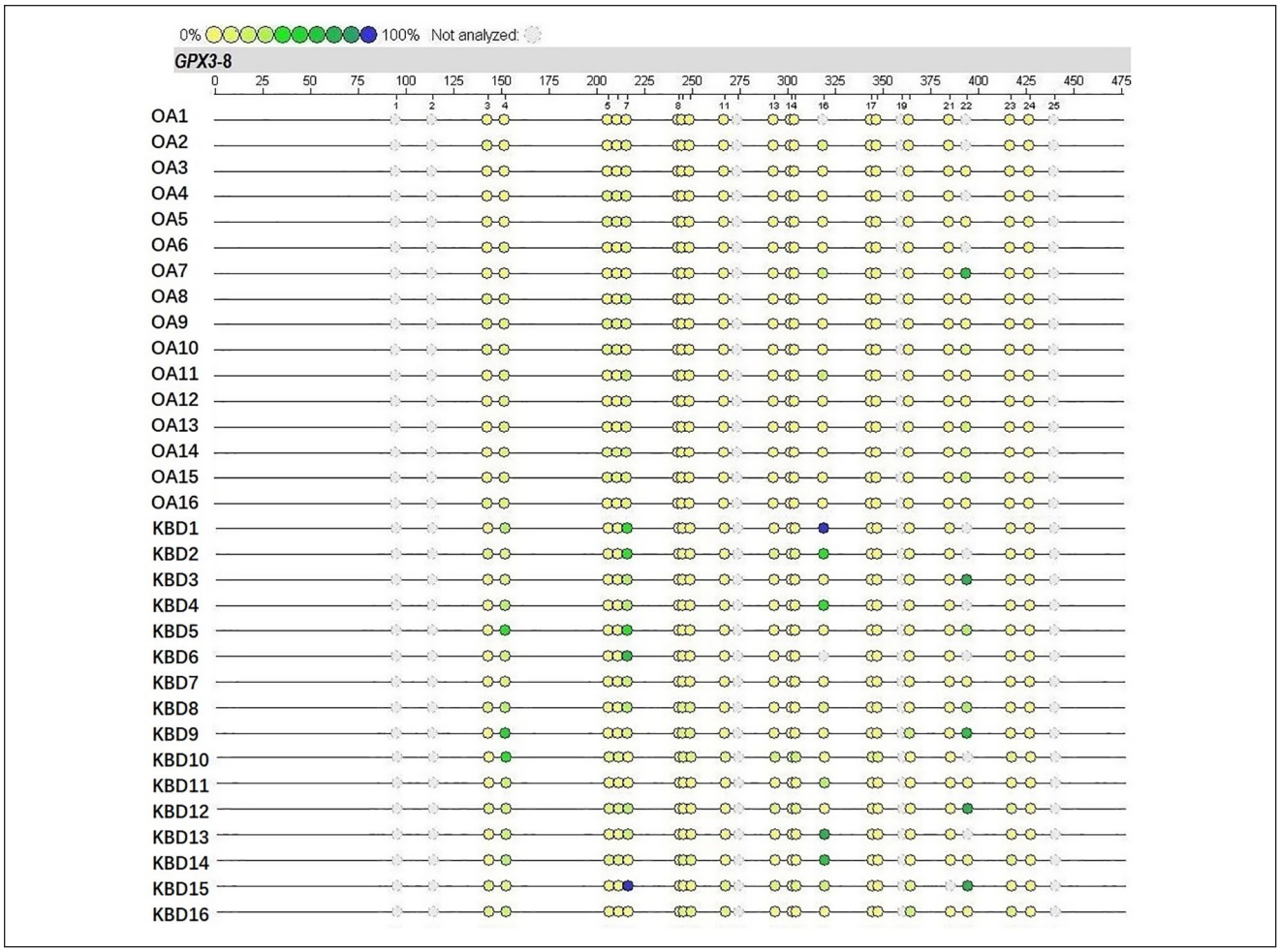

Figure 3. Methylation levels of CpGs in the promoter region of *GPX3* gene in osteoarthritis (OA) and Kashin-Beck disease (KBD) patients. The color intensity of the dots is directly proportional to the methylation level. The numbers 1 to 25 in the figure represent CpGs *GPX3*-8_CpG_1 - *GPX3*-8_CpG_25.

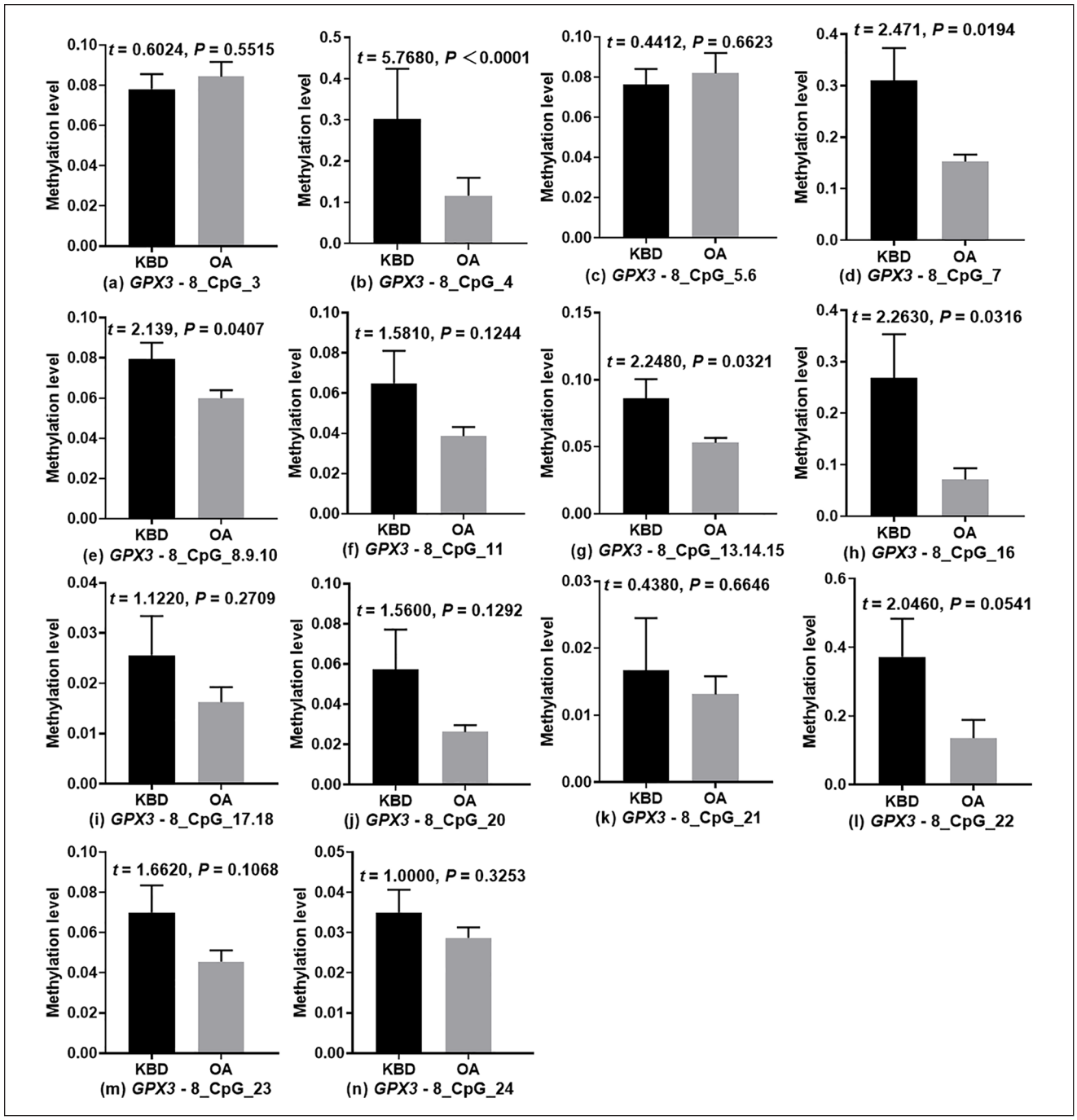

Figure 4. Methylation levels of CpGs in the promoter region of *GPX3* in osteoarthritis (OA) and Kashin-Beck disease (KBD) patients.

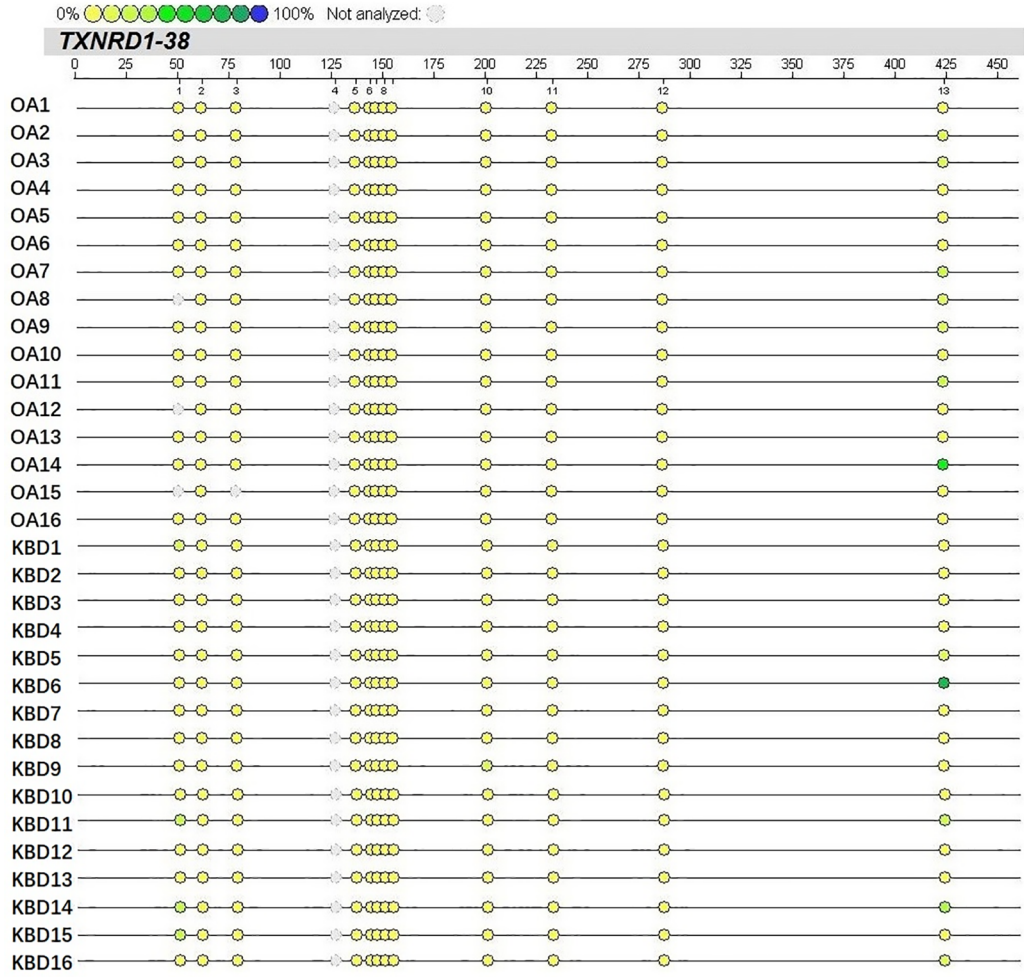

Figure 5. Methylation levels of CpGs in the promoter region of the *TXNRD1* gene in osteoarthritis (OA) and Kashin-Beck disease (KBD) patients. The color intensity of the dots is directly proportional to the methylation level. The numbers 1 to 13 represent CpGs *TXNRD1*-38_CpG_1- to *TXNRD1*-38_CpG_13.

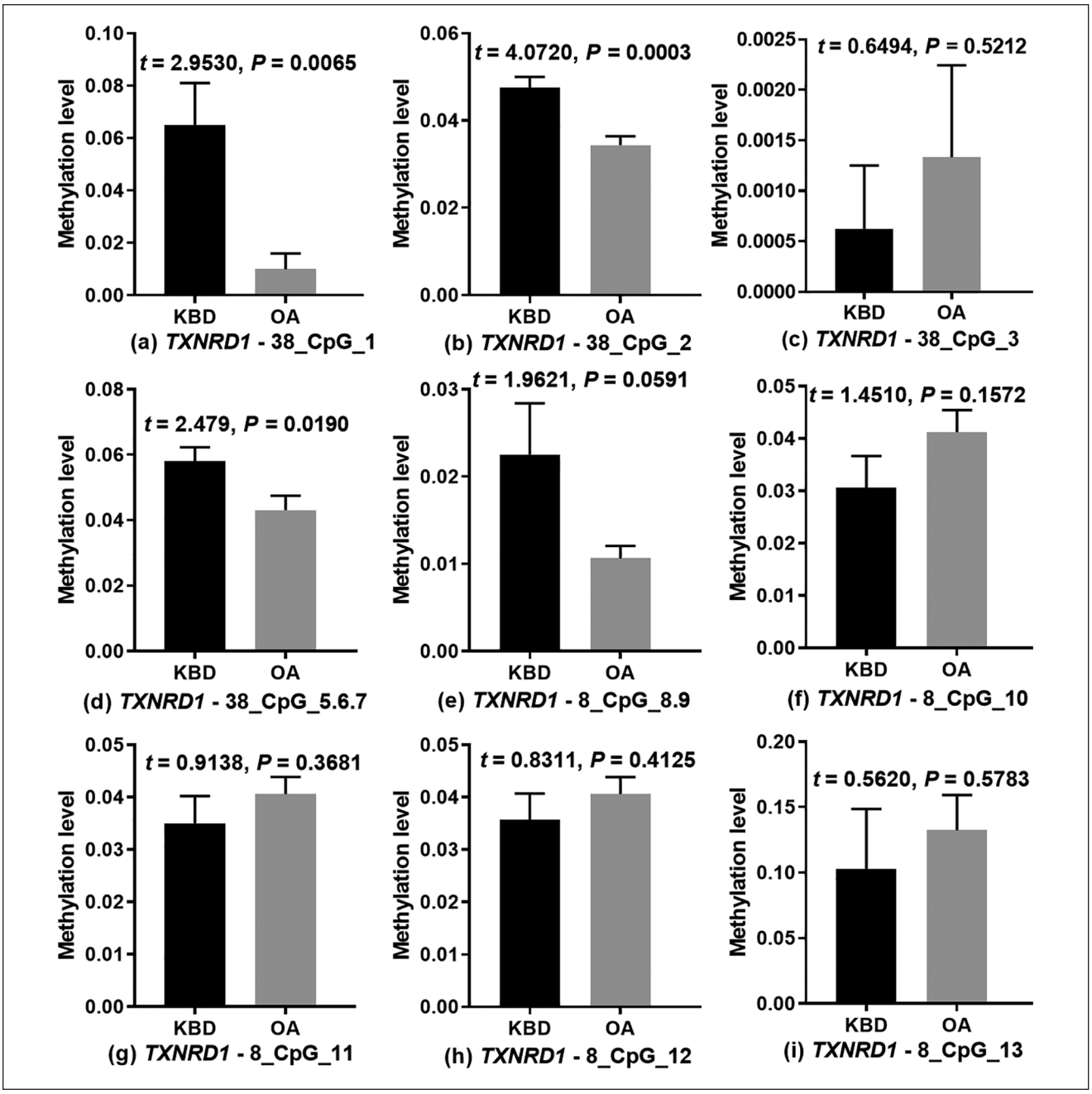

Figure 6. Methylation levels of CpGs in the promoter region of *TXNRD1* in osteoarthritis (OA) and Kashin-Beck disease (KBD) patients.

Author Contributions

Study design and conception: Yongmin Xiong, Rongqiang Zhang. Data collection: Hao Guo, Rongqiang Zhang, Xiaoli Yang, Di Zhang, Dandan Zhang, Qiang Li, Chen Wang, Xuena Yang. Data analysis and interpretation: Hao Guo, Rongqiang Zhang, Yongmin Xiong. All authors read, revised and approved submission of the final draft.

Acknowledgments and Funding

We appreciate Chao Lu (Honghui Hospital Affiliated to Xi'an Jiaotong University) for assisting in collecting clinical materials. The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported financially by the National Natural Science Foundation of China (81773372, 81573104), Key Research and

Development Program in Shaanxi Province (2020SF-076), Research Project from Health Commission of Shaanxi Provincial Government (2018A017) and the Education Department of Shaanxi Provincial Government (19JS015).

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

Protocol for this study was approved by the Human Ethics Committee of Xi'an Jiaotong University, People's Republic of China.

Informed Consent

All participants consented to the study in writing.

Trial Registration

Not applicable.

ORCID iD

Yongmin Xiong https://orcid.org/0000-0002-8263-2970

References

1. Ebell MH. Osteoarthritis: rapid evidence review. Am Fam Physician. 2018;97(8):523-6.
2. Kloppenburg M, Berenbaum F. Osteoarthritis year in review 2019: epidemiology and therapy. Osteoarthritis Cartilage. 2020;28(3):242-8.
3. Vina ER, Kwoh CK. Epidemiology of osteoarthritis: literature update. Curr Opin Rheumatol. 2018;30(2):160-7.
4. Hubertsson J, Turkiewicz A, Petersson IF, Englund M. Understanding occupation, sick leave, and disability pension due to knee and hip osteoarthritis from a sex perspective. Arthritis Care Res (Hoboken). 2017;69(2):226-33.
5. Innes KE, Sambamoorthi U. The association of perceived memory loss with osteoarthritis and related joint pain in a large Appalachian population. Pain Med. 2018;19(7):1340-56.
6. Veronese N, Stubbs B, Solmi M, Smith TO, Noale M, Cooper C, *et al*. Association between lower limb osteoarthritis and incidence of depressive symptoms: data from the osteoarthritis initiative. Age Ageing. 2017;46(3):470-6.
7. Kye SY, Park K. Suicidal ideation and suicidal attempts among adults with chronic diseases: a cross-sectional study. Compr Psychiatry. 2017;73:160-7.
8. Fu Q, Cao J, Renner JB, Jordan JM, Caterson B, Duance V, *et al*. Radiographic features of hand osteoarthritis in adult Kashin-Beck Disease (KBD): the Yongshou KBD study. Osteoarthritis Cartilage. 2015;23(6):868-73.
9. Guo Y, Li H, Yang L, Li Y, Wei B, Wang W, *et al*. Trace element levels in scalp hair of school children in Shigatse, Tibet, an endemic area for Kaschin-Beck disease (KBD). Biol Trace Elem Res. 2017;180(1):15-22.
10. Sun LY, Meng FG, Li Q, Zhao ZJ, He CZ, Wang SP, *et al*. Effects of the consumption of rice from non-KBD areas and selenium supplementation on the prevention and treatment of paediatric Kaschin-Beck disease: an epidemiological intervention trial in the Qinghai Province. Osteoarthritis Cartilage. 2014;22(12):2033-40.
11. National Health and Family Planning Commission. China health and family planning statistical yearbook. Peking Union Medical College Press; 2018.
12. Lei J, Amhare AF, Wang LY, Lv YZ, Deng H, Gao H, *et al*. Proteomic analysis of knee cartilage reveals potential signaling pathways in pathological mechanism of Kashin-Beck disease compared with osteoarthritis. Sci Rep. 22 2020;10(1):6824.
13. Liu HM, Wang YF, Wu JM, Li BY, Dong F, Lu DF, *et al*. A comparative study of clinical effect of total knee arthroplasty in the treatment of primary osteoarthritis and osteoarthritis of Kashin-Beck disease. Int Orthop. 2020;44(9):1719-26.
14. Han LX, Yang XL, Sun WY, Li ZF, Ren H, Li BR, *et al*. The study of GPX3 methylation in patients with Kashin-Beck Disease and its mechanism in chondrocyte apoptosis. Bone. 2018;117:15-22.
15. Jeffries MA. Osteoarthritis year in review 2018: genetics and epigenetics. Osteoarthritis Cartilage. 2019;27(3):371-7.
16. Wen Y, Zhang F, Li CY, He SL, Tan WH, Lei YX, *et al*. Gene expression analysis suggests bone development-related genes GDF5 and DIO2 are involved in the development of Kashin-Beck disease in children rather than adults. PLoS One. 2014;9(7):e103618.
17. Li YY, Mo XY, Xiong YM. The study on polymorphism of TrxR and Nrf2/HO-1 signaling pathway in Kaschin-Beck disease. Biol Trace Elem Res. 2019;190(2):303-8.
18. Ekström L, Johansson M, Monostory K, Rundlöf AK, Arnér E SJ, Björkhem-Bergman L. Simvastatin inhibits the core promoter of the TXNRD1 gene and lowers cellular TrxR activity in HepG2 cells. Biochem Biophys Res Commun. 2013;430(1):90-4.
19. Dafre AL, Goldberg J, Wang T, Spiegel DA, Maher P. Methylglyoxal, the foe and friend of glyoxalase and Trx/TrxR systems in HT22 nerve cells. Free Radic Biol Med. 2015;89:8-19.
20. Shen J, Abu-Amer Y, O'Keefe RJ, McAlinden A. Inflammation and epigenetic regulation in osteoarthritis. Connect Tissue Res. 2017;58(1):49-63.
21. Hou TY, Chiang-Ni C, Teng SH. Current status of MALDI-TOF mass spectrometry in clinical microbiology. J Food Drug Anal. 2019;27(2):404-14.
22. Bomer N, den Hollander W, Ramos YF, Bos SD, van der Breggen R, Lakenberg N, *et al*. Underlying molecular mechanisms of DIO2 susceptibility in symptomatic osteoarthritis. Ann Rheum Dis. 2015;74(8):1571-9.
23. Bos SD, Bovee JV, Duijnisveld BJ, Raine E VA, van Dalen WJ, Ramos Y FM, *et al*. Increased type II deiodinase protein in OA-affected cartilage and allelic imbalance of OA risk polymorphism rs225014 at DIO2 in human OA joint tissues. Ann Rheum Dis. 2012;71(7):1254-8.
24. Du XA, Wang HM, Dai XX, Kou Y, Wu RP, Chen Q, *et al*. Role of selenoprotein S (SEPS1) -105G>A polymorphisms and PI3K/Akt signaling pathway in Kashin-Beck disease. Osteoarthritis Cartilage. 2015;23(2):210-6.

25. Han YC, Li XH, Yan MJ, Yang MJ, Wang SJ, Pan J, *et al*. Oxidative damage induces apoptosis and promotes calcification in disc cartilage endplate cell through ROS/MAPK/NF-kappaB pathway: Implications for disc degeneration. Biochem Biophys Res Commun. 2019;516(3):1026-32.
26. Lepetsos P, Papavassiliou AG. ROS/oxidative stress signaling in osteoarthritis. Biochim Biophys Acta. 2016;1862(4):576-91.
27. Wu CY, Lei RH, Tiainen M, Wu SX, Zhang Q, Pei FX, *et al*. Disordered glycometabolism involved in pathogenesis of Kashin-Beck disease, an endemic osteoarthritis in China. Exp Cell Res. 2014;326(2):240-50.
28. Wang SJ, Guo X, Zuo H, Zhang YG, Xu P, Ping ZG, *et al*. Chondrocyte apoptosis and expression of Bcl-2, Bax, Fas, and iNOS in articular cartilage in patients with Kashin-Beck disease. J Rheumatol. 2006;33(3):615-9.
29. Chang CH, Ho CT, Liao VH. *N*-γ-(L-glutamyl)-L-selenomethionine enhances stress resistance and ameliorates aging indicators via the selenoprotein TRXR-1 in *Caenorhabditis elegans*. Mol Nutr Food Res. 2017;61(8). doi:10.1002/mnfr.201600954
30. Yu FF, Zhang YX, Zhang LH, Li WR, Guo C, Lammi MJ. Identified molecular mechanism of interaction between environmental risk factors and differential expression genes in cartilage of Kashin-Beck disease. Medicine (Baltimore). 2016;95(52):e5669.
31. Wang W, Wei S, Luo M, Yu B, Cao J, Yang Z, *et al*. Oxidative stress and status of antioxidant enzymes in children with Kashin-Beck disease. Osteoarthritis Cartilage. 2013;21(11):1781-9.
32. Wu C, Zheng J, Yao X, Shan H, Li Y, Xu P, *et al*. Defective autophagy in chondrocytes with Kashin-Beck disease but higher than osteoarthritis. Osteoarthritis Cartilage. 2014;22(11):1936-46.
33. Lobanov AV, Hatfield DL, Gladyshev VN. Eukaryotic selenoproteins and selenoproteomes. Biochim Biophys Acta. 2009;1790(11):1424-8.
34. Avery JC, Hoffmann PR. Selenium, selenoproteins, and immunity. Nutrients. 2018;10(9):1203.
35. Majumdar B, Saini N, Agrawal S, Prakash C. Familiar manifestations of unfamiliar selenium toxicity. Indian J Dermatol. 2018;63(5):430-1.
36. Loscalzo J. Keshan disease, selenium deficiency, and the selenoproteome. N Engl J Med. 2014;370(18):1756-60.
37. Vinceti M, Filippini T, Wise LA. Environmental selenium and human health: an update. Curr Environ Health Rep. 2018;5(4):464-85.
38. Li DY, Han J, Guo X, Qu CJ, Yu FF, Wu XF. The effects of T-2 toxin on the prevalence and development of Kashin-Beck disease in China: a meta-analysis and systematic review. Toxicol Res (Camb). 2016;5(3):731-51.
39. Dai XX, Li YY, Zhang RQ, Kou Y, Mo XY, Cao JL, *et al*. Effects of sodium selenite on c-Jun N-terminal kinase signalling pathway induced by oxidative stress in human chondrocytes and c-Jun N-terminal kinase expression in patients with Kashin-Beck disease, an endemic osteoarthritis. Br J Nutr. 2016;115(9):1547-55.
40. Xiong YM, Mo XY, Zou XZ, Song RX, Sun WY, Lu W, *et al*. Association study between polymorphisms in selenoprotein genes and susceptibility to Kashin-Beck disease. Osteoarthritis Cartilage. 2010;18(6):817-24.
41. Wu RP, Zhang RQ, Xiong YM, Sun WY, Li YY, Yang XL, *et al*. The study on polymorphisms of Sep15 and TrxR2 and the expression of AP-1 signaling pathway in Kashin-Beck disease. Bone. 2019;120:239-45.
42. Meulenbelt I, Min JL, Bos S, Riyazi N, Houwing-Duistermaat JJ, van der Wijk HJ, *et al*. Identification of DIO2 as a new susceptibility locus for symptomatic osteoarthritis. Hum Mol Genet. 2008;17(12):1867-75.
43. Cheng Y, Qi Y. Current progresses in metal-based anticancer complexes as mammalian TrxR inhibitors. Anticancer Agents Med Chem. 2017;17(8):1046-69.