Heliyon 10 (2024) e28035

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

The potential impact of polymorphisms in *METTL3* gene on knee osteoarthritis susceptibility

Houlin Mi [a], Mingzhi Wang [b,**,1], Yongmei Chang [c,*,1]

[a] *Department of Orthopedics, South China Hospital Affiliated to Shenzhen University, 1# Fuxin Road, Longgang District, Shenzhen City, Guangdong Province, 518111, China*
[b] *Department of Thoracic Surgery, Guangdong Second Provincial General Hospital, 466# Xingang Middle Road, Haizhu District, Guangzhou City, Guangdong Province, 510006, China*
[c] *Department of Respiratory Medicine, Guangdong Second Provincial General Hospital, 466# Xingang Middle Road, Haizhu District, Guangzhou City, Guangdong Province, 510006, China*

ARTICLE INFO

Keywords:
Knee osteoarthritis
METTL3
Susceptibility
Polymorphism

ABSTRACT

Objective: This study was aimed to explore the correlation between *METTL3* polymorphisms and susceptibility to knee osteoarthritis (KOA).
Methods: The relationship of five single nucleotide polymorphisms (SNPs) in the *METTL3* gene with the susceptibility of KOA was analyzed through multinomial logistic regression analysis in this a case-control study. Genotyping was performed on 228 KOA patients and 252 unaffected individuals from South China based on the TaqMan method. The MDR software (version 3.0.2) was utilized for the analysis of SNP interactions.
Results: Out of the five SNPs examined, the T > G change in the *METTL3* gene at the rs1061026 locus increased the risk of KOA, while rs1139130 A > G and rs1263802 C > T variants were found to be linked with a reduced risk of developing KOA with statistical significance. The rs1061027 A > C and rs1263801 C > G variants did not show significant association ($p > 0.05$). The rs1061026 TG/GG genotype showed a significant correlation with an increased risk of KOA in the following subgroups: the males, individuals with a BMI ranging from 24 to 28, smokers, those who were not engaged in physical exercise (PE), patients who had experienced KOA symptoms for eight years or longer, and those without a family history of the disease or reported swelling. On the other hand, the rs1139130 AG/GG genotype demonstrated a protective effect against KOA among the females, individuals with a BMI greater than or equal to 24, a unilateral KOA, or a KOA duration of 8 years or less, non-smokers, non-alcohol drinkers, those who were not engaged in PE, and those who had no injury or family history, or no experience of knee swelling. Additionally, it was observed that the rs1263802 CT/TT genotypes showed a protective effect among patients without a history of injury. Furthermore, individuals with the haplotypes GAT, GGC, TAT, and TGC were found to have a significantly lower susceptibility to KOA compared to the reference haplotype TAC.
Conclusions: The *METTL3* gene variant rs1061026 could increase the risk of KOA, whereas the variants of rs1139130 as well as rs1263802 might exert a protective effect against KOA. These

* Corresponding author.
** Corresponding author.
E-mail addresses: wmz891@163.com (M. Wang), cym891@163.com (Y. Chang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e28035
Received 24 August 2023; Received in revised form 29 February 2024; Accepted 11 March 2024
Available online 19 March 2024

variants could potentially function as susceptibility markers for KOA among the population from South China.

1. Introduction

As a chronic condition that affects the joints along with their associated tissues, Osteoarthritis (OA) can cause progressive damage to the articular cartilage, subchondral bone, as well as adjacent synovial structures [1]. It stands as a widespread orthopedic disorder, exerting a significant impact on both affected patients and healthcare systems [2]. The majority of the discomfort and disability caused by OA is linked to the hips and knees. Knee osteoarthritis (KOA) is a prevalent degenerative disease that leads to disability in elderly patients [3]. It is characterized by pathological changes in a variety of joint components, contributing to the dysfunction, pain, stiffness, and limited functionality [4]. Currently, no specific medications are available for treating KOA. Non-steroidal anti-inflammatory drugs, along with physical therapy, are acknowledged as key treatments for managing symptoms, while joint replacement surgery may be considered for more advanced cases.

RNA N6-methyladenosine (m6A) modification is prevalent in mRNA and is considered a crucial component of the epiregulome. RNA methyltransferases (*METTL3*, METTL14, and WTAP), referred to as 'writers,' are responsible for introducing m6A modifications, while demethylases (FTO alongside ALKBH5), named 'erasers,' are involved in their removal. m6A-binding proteins (YTHDF1/2/3 and IGF2BP1), referred to as 'readers,' recognize these modifications [5–7]. The installation of the m6A modification involves the 'writers' complex, which includes methyltransferase-like 3 (*METTL3*), METTL14, and WTAP [8]. *METTL3* are the catalytic core in this complex. METTL14 together with WTAP serve as its regulatory subunits [9]. As the pioneering methyltransferase as well as the essential subunit, *METTL3* is instrumental in facilitating the addition of m6A. Extensive studies have demonstrated that *METTL3* catalyzes the methylation of certain target transcripts and takes part in diverse physiological processes, such as the development of the embryo [10], the brain growth [11], sperm production [12], cell reprogramming [10], as well as T cell homeostasis [13]. Recently, accumulating evidence suggests that *METTL3* is crucial for musculoskeletal disorders, depending on the presence or absence of m6A modifications. *METTL3* has been linked to the inflammatory processes in OA as well. *METTL3* expression was reduced among pre-osteoblast *MC3T3-E1* cells treated with lipopolysaccharide (LPS). Knockdown of *METTL3* resulted in decreased levels of markers associated with bone formation, while it modulated the levels of several cytokines involved in inflammatory processes (including IL6,

Table 1
Demographic characteristics of KOA cases and control individuals.

Clinical characteristics	KOA	HC	P value
	n = 228	n = 252	
Sex			0.836
Females	163	178	
Males	65	74	
Age, years (mean ± SD)	65.3 ± 7.6	63.2 ± 7.9	
Smoking			0.333
Yes	35	31	
No	193	221	
Drinking			0.405
Yes	59	57	
No	169	195	
Pysical exercise			0.038
Yes	58	86	
No	170	166	
BMI (kg/m^2)	25.7 ± 3.2	23.5 ± 2.2	<0.001
Height (cm)	159.9 ± 65.9	158.7 ± 66.8	0.068
Location			
Unilateral	183		
Bilateral	45		
Cause, years			
≥8	83		
<8	145		
History of injury			
Yes	12		
No	216		
Family history			
Yes	22		
No	206		
Swelling			
Yes	70		
No	158		

SD, standard deviation.
[a] Two-sided $\chi 2$ test for distributions between KOA cases and cancer-free controls.

IL12, as well as TNF-α) by enhancing the phosphorylation of components within the MAPK signaling pathway. *Liu* et al. [14] illustrated the crucial effect of *METTL3* on the progression as well as the fundamental mechanisms of OA. *METTL3*'s expression varies across different cells and tissues depending on the experimental conditions. However, it influences the progression of OA by regulating NF-κB signaling alongside the synthesis of the extracellular matrix in chondrocytes. Moreover, *Sang* et al. [15] demonstrated *METTL3* overexpression to activate the NF-κB pathway and also to downregulate the levels of inflammatory cytokines. Although the importance of m6A modifications by *METTL3* in numerous musculoskeletal conditions have been widely well acknowledged, its essential function in specific disorders like KOA, is not systematically revealed.

Genome-wide association studies have effectively pinpointed thousands of common genetic variations in relation to complex diseases and characteristics; yet, these variations account for only a modest portion of the genetic inheritance [16–18]. Variations in single nucleotide polymorphisms (SNPs) within key regions of genes regulating m6A methylation have been found to influence m6A methylation and impact disease development. Notably, SNPs in genes like *METTL3* as well as METTL14 have been linked to neuroblastoma [19], acute lymphoblastic leukemia [20], as well as autoimmune thyroid disease [21]. Additionally, SNPs exhibit functions on osteoarthritis. A study revealed that the rs1871054 polymorphism of *ADAM12* was linked to osteoarthritis in dominant, recessive, allelic, and homozygote genetic models [22]. However, the impact of SNPs in m6A modifier genes on the risk of KOA has not yet been investigated.

In light of the evidence suggesting that the *METTL3* gene contributes to the development of osteoarthritis, a case-control study was carried out to explore the possible link of *METTL3* gene mutations and the susceptibility to KOA among individuals in South China.

2. Materials and methods

2.1. Patients and healthy controls

KOA patients (n = 228) as well as healthy controls (n = 252), with ages ranging from 49 to 89 years old, were enrolled from Guangdong Second Provincial General Hospital between 2018 and 2022. Two chief physicians independently confirmed the diagnosis. Written informed consent was obtained from all participants, and the research was approved by the Ethics Committee of Guangdong Second Provincial General Hospital, conducted by the Declaration of Helsinki. Table 1 lists the participants' demographic details. SNPs within the *METTL3* genes, specifically rs1061026 T > G, rs1139130 A > G, alongside rs1263802 C > T, were categorized according to a variety of factors like age, sex, BMI, smoking, drinking, physical exercise (PE), geographical location, duration of symptoms, previous injuries, family medical history, and the presence of joint swelling.

2.2. SNP selection and genotyping

Five single nucleotide polymorphisms (SNPs) (rs1061027 A > C, rs1263801 C > G, rs1061026 T > G, rs1139130 A > G, as well as rs1263802 C > T) were chosen from the NCBI dbSNP database [20]. As brief, SNPs of *METTL3* with 1000 Genomes Minimum Alternate Allele Frequency (MAF)≥0.05 from NCBI dbSNP (https://www.ncbi.nlm.nih.gov/snp/) were selected by SNPviewer. A total of 50 SNPs were screened. Then the function assessment of these 50 SNPs was carried out by the SNPinfo Web server (https://manticore.niehs.nih.gov/snpinfo/guide.html). In "SNP function prediction", these 50 SNPs' names were filled into the box, CHB Genotype Data from HapMap and Asian Genotype Data from HapMap was chosen. The potential function according to SNPs' location at or near transcriptional factor binding site (TFBS), splicing site, miRNA binding site, stop codon were predicted. Eight SNPs were identified to be with potential function, three of them were at TFBS, one was at splicing site, the other four SNPs were at miRNA binding sites. Next step, the Linkage Disequilibrium (LD) SNPs of these 8 SNPs were analyzed by SNPinfo Web Server. LDSNP >0.8 were screened. More, Asian MAF and CHB <0.95 or >0.05 were selected. In result, 5 SNPs were selected.

Reagents and methods for DNA extraction, as well as the instruments used for genotyping, were as described by Liu et al. [23]. The purity and concentration of the DNA were determined using a UV absorption spectrophotometer (NanoDrop). As a quality control measure, a random sample comprising 5% of the total ones was adopted as positive control and negative control.

2.3. The miRNA binding sites and TFBS prediction

The miRNA binding sites were predicted by ENCORI (https://rnasysu.com/encori/rbpClipRNA.php?source=mRNA&flag=none&clade=mammal&genome=human&assembly=hg38&RBP=all&clipNum=1&regionType=None&pval=0.05&clipType=None&panNum=0&target=*METTL3*#modal). Upstream of *METTL3* gene was searched by UCSC (https://genome-asia.ucsc.edu/index.html), and the transcriptional factors and their binding sites were predicted by Jaspar which is connected to UCSC.

2.4. SNP-SNP interaction analysis

Based on the MDR software (version 3.0.2, Computational Genetics Laboratory, University of Pennsylvania, USA, available at https://www.epistasis.org), the method of multifactor dimensionality reduction (MDR) was used to examine and define epistasis between the SNPs. The top interaction models were determined based on cross-validation consistency (CVC) as well as test accuracy [24].

Table 2
Prediction and selection of *METTL3* SNPs.

No.	SNP	Position	Allele	TFBS	Splicing(ESE or ESS)	miRNA(miRanda)	1000G	Site
1	rs1061026	21498965	G/T	–	–	Y	0.893	intron8
2	rs1061027	21498989	A/C	–	–	Y	0.767	intron8
3	rs1139130	21499772	A/G	–	Y	–	0.388	exon5
4	rs1263801	21510742	C/G	Y	–	–	0.316	intron1
5	rs1263802	21512847	C/T	Y	–	–	0.316	upstream

2.5. Statistical analyses

The differences in genotypes and ages between groups was examined by a chi-square test. Besides, the relationship between SNP and the risk of KOA was evaluated based on a generalized linear regression model. Besides, crude/adjusted odds ratios (cORs/ORs) as well as 95% confidence intervals (CIs) were calculated. In addition, a chi-square test was employed to examine deviation from Hardy-Weinberg equilibrium (HWE) within the control group. Data analyses were carried out using SAS software (Version 9.4; SAS Institute, Cary, NC, USA). Statistical significance was determined by a two-sided P vale of less than 0.05 [25].

3. Results

3.1. Functional prediction of METTL3 polymorphisms

The *METTL3* gene is located on chromosome 14q11.2. The detail information of the selected 5 SNPs was listed in Table 6. Rs1061026 T > G and rs1061027 A > C located in intron 8 maybe miRNA binding sites, rs1139130 A > G located in exon 5 might affect splicing, rs1263801 C > G located in intron 1 might be TFBS, and rs1263802 C > T located in the upstream of *METTL3* also may be TFBS (Table 2). Rs1061026 is located 336 bp upstream and 351 bp upstream, while rs1061027 is positioned at 312 bp and 321 bp upstream of hsa-miR-875-5p and hsa-miR-23a-3p binding sites, respectively (Fig. 1A). Rs1263801 was identified to be positioned within the binding site of MYF6 (Fig. 1B). Rs1263802 was found to be suited within the binding sites of CTCF, ZNF460, ZNF135, ELF3, IKZF3 and ELF1 (Fig. 1C).

3.2. Association of METTL3 genes SNPs and the risk of KOA

We analyzed the genetic variations of 5 SNPs in the *METTL3* gene, namely rs1061027 A > C, rs1263801 C > G, rs1061026 T > G, rs1139130 A > G, as well as rs1263802 C > T, in a total of 228 samples from patients with KOA and 252 healthy controls matched for age. Our findings indicated that all five SNPs (rs1061027 A > C, rs1263801 C > G, rs1061026 T > G, rs1139130 A > G, and rs1263802 C > T) were in HWE ($p > 0.05$). But the rs1061027 A > C as well as rs1263801 C > G variants were not in relation to the risk of KOA ($p > 0.05$). To explore the association between rs1061026 T > G, rs1139130 A > G, rs1263802 C > T, and the risk of KOA, we performed a detailed analysis focusing on each individual genetic locus. Our results indicated a significant increase in the vulnerability to KOA when comparing individuals with rs1061026 TG/GG variants to those with TT variants. (OR = 1.451, 95% CI = 1.008–2.088, p = 0.0453). Conversely, the variants of rs1139130 GG (OR = 0.573, 95% CI = 0.404–0.812, p = 0.0018) together with rs1263802 TT (OR = 0.286, 95% CI = 0.141–0.582, p = 0.0005) were linked to a reduced risk of KOA (Table 3).

3.3. Stratification analysis of rs1061026, rs1139130, and rs1263802 with the susceptibility to KOA

The results of stratification analysis were shown in Table 4. The genotype rs1061026 TG/GG had a adverse effect on specific subgroups, including males (OR = 3.133, 95% CI = 1.51–6.329, p = 0.0015), individuals with a BMI<24 (OR = 1.927, 95% CI = 1.070–3.469, p = 0.0288) or ≥ 28 (OR = 2.266, 95% CI = 1.206–4.256, p = 0.0110), smokers (OR = 3.993, 95% CI = 1.741–9.159, p = 0.0011), non-drinkers (OR = 1.504, 95% CI = 1.005–2.251, p = 0.0474), those suffering from KOA ≥8 years (OR = 1.868, 95% CI = 1.110–3.139, p = 0.0186), individuals without a family history (OR = 1.520, 95% CI = 1.044–2.218, p = 0.029), as well as those experiencing swelling (OR = 1.830, 95% CI = 1.046–3.200, p = 0.0341).

The rs1139130 AG/GG genotype may protect against KOA risk in individuals aged 65 years or younger (OR = 0.479, 95% CI = 0.296–0.775, p = 0.0027). This protective effect was observed in the females (OR = 0.564, 95% CI = 0.360–0.882, p = 0.012), individuals with a BMI <24 or ≥ 24 (OR = 0.432, 95% CI = 0.266–0.703, p = 0.0007) or BMI≥28 (OR = 0.486, 95% CI = 0.257–0.920, p = 0.0266), non-smokers (OR = 0.527, 95% CI = 0.354–0.785, p < 0.0016), alcohol drinkers (OR = 0.520, 95% CI = 0.279–0.968, p < 0.039), non-alcohol drinkers (OR = 0.527, 95% CI = 0.348–0.798, p = 0.0025), those who were not engaged in PE (OR = 0.473, 95% CI = 0.312–0.717, p = 0.0004), those with a unilateral KOA (OR = 0.471, 95% CI = 0.314–0.705, p = 0.0003), KOA duration of 8 years or more (OR = 0.439, 95% CI = 0.255–0.756, p = 0.003), or KOA duration of less than 8 years (OR = 0.577, 95% CI = 0.376–0.886, p = 0.0119), those who had no history of knee injury (OR = 0.527, 95% CI = 0.359–0.772, p = 0.001), no family history of KOA (OR = 0.520, 95% CI = 0.353–0.765, p = 0.0009), or no experience of knee swelling (OR = 0.465, 95% CI = 0.304–0.713, p = 0.0004).

The rs1263802 CT/TT was also identified to decrease the risk of KOA in the individuals of age >65 years (OR = 0.114, 95% CI =

A

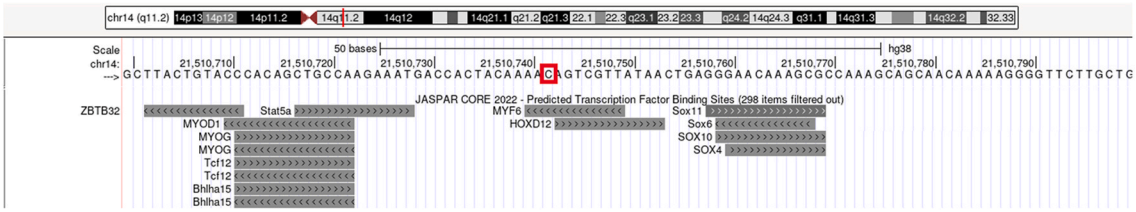
Binding Site of hsa-miR-875-5p on METTL3:

TargetRegion	Type	Alignment	TDMDScore	AgoExpNum	CleaveExpNum	phyloP
chr14:21499301-21499329[+]	7mer-m8	Target: 5' GAUGUGAUCGUAGCUGAGGUAUG 3' |: |||| : |:|||||||| miRNA : 3' GUGGACUA-UUUUGACUCCAUAU 5'	1.5133	2	0	4.727

Binding Site of hsa-miR-23a-3p on METTL3:

TargetRegion	Type	Alignment	TDMDScore	AgoExpNum	CleaveExpNum	phyloP
chr14:21499316-21499344[-]	6mer	Target: 5' GGGUCUGGAUUGUGAUGUGAU 3' ||: | | |::|||||| miRNA : 3' CCUUUAGGGACCGUUACACUA 5'	0.9175	2	0	4.974

B



C

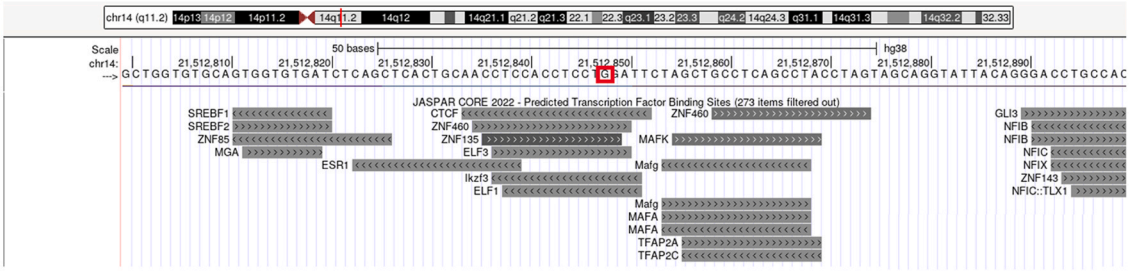


Fig. 1. Functional prediction for the polymorphisms of *METTL3*. (A) Prediction of miRNA binding sites for rs1061026 and rs1061027; (B) Prediction of protein binding sites for rs1263801; (C) Prediction of protein binding sites for rs1263802.

0.057–0.225, $p < 0.001$), ≤65 years (OR = 0.514, 95% CI = 0.312–0.847, $p = 0.008$), males (OR = 0.144, 95% CI = 0.066–0.310, $p < 0.0001$), females (OR = 0.381, 95% CI = 0.239–0.609, $p < 0.0001$), those with BMI <24 (OR = 0.282, 95% CI = 0.148–0.538, $p = 0.0001$), BMI ≥24 but <28 (OR = 0.286, 95% CI = 0.171–0.479, $p < 0.0001$), BMI ≥28 (OR = 0.244, 95% CI = 0.119–0.503, $p = 0.0001$), smoking (OR = 0.309, 95% CI = 0.137–0.700, $p < 0.0049$), non-smokers (OR = 0.264, 95% CI = 0.172–0.407, $p < 0.0001$), alcohol drinkers (OR = 0.158, 95% CI = 0.074–0.339, $p < 0.0001$), non-alcohol drinkers (OR = 0.332, 95% CI = 0.215–0.514, p=<0.0001), those engaged in PE (OR = 0.302, 95% CI = 0.156–0.584, $p = 0.0004$), not engaging in PE (OR = 0.272, 95% CI = 0.174–0.424, $p < 0.0001$), those with a unilateral KOA (OR = 0.321, 95% CI = 0.204–0.476, $p < 0.0001$), bilateral KOA (OR = 0.164, 95% CI = 0.070–0.388, $p < 0.0001$), those suffering from KOA≥8 years (OR = 0.321, 95% CI = 0.183–0.562, $p < 0.0001$), suffering from KOA<8years (OR = 0.256, 95% CI = 0.159–0.413, $p < 0.0001$), those who had no history of injury (OR = 0.283, 95% CI = 0.188–0.425, $p < 0.0001$), had family history (OR = 0.040, 95% CI = 0.005–0.310, $p = 0.002$), no family history (OR = 0.317, 95% CI = 0.211–0.476, $p < 0.0001$), had the experience of swelling (OR = 0.352, 95% CI = 0.193–0.642, $p = 0.0007$) and had no experience of swelling (OR = 0.251, 95% CI = 0.158–0.399, $p < 0.0001$).

3.4. Haplotype analysis of METTL3 gene SNPs related with the susceptibility to KOA

We conducted an investigation into the relationship between the SNP haplotypes of *METTL3* gene and the susceptibility to KOA. The reference group was the haplotype containing the wild-type alleles (TAC). We found that the haplotypes GAT (OR = 0.496, 95% CI = 0.276–0.893, $p = 0.0193$), TAT (OR = 0.408, 95% CI = 0.252–0.661, $p = 0.0003$), and TGC (OR = 0.090, 95% CI = 0.035–0.232, $p < 0.0001$) significantly linked to the reduced risk of KOA (Table 5). However, the haplotype GGC (OR = 1.755, 95% CI = 1.095–2.812, $p = 0.0194$) elevated the susceptibility to KOA with statistical significance.

Table 3
Logistic regression analysis of association of *METTL3* Gene Polymorphisms with KOA susceptibility.

Genotype	Cases	Controls	P[a]	Crude OR	P	Adjusted OR	P[b]
	(N = 228)	(N = 252)		(95% CI)		(95% CI) b	
rs1061026 T > G (HWE = 0.625)							
TT	99 (43.42)	133 (52.78)		1.0000		1.0000	
TG	103 (45.18)	98 (38.89)		1.412 (0.966–2.064)	0.0750	1.405 (0.958–2.063)	0.0821
GG	26 (11.40)	21 (8.33)		1.663 (0.885–3.127)	0.1141	1.662 (0.882–3.132)	0.1164
Additive			0.1081	1.334 (1.014–1.756)	0.0394	1.331 (1.009–1.756)	0.0430
Dominant	129 (56.58)	119 (47.22)	0.0405	1.456 (1.016–2.088)	0.0408	1.451 (1.008–2.088)	0.0453
Recessive	202 (88.60)	231 (91.67)	0.2584	1.416 (0.773–2.593)	0.2603	1.414 (0.771–2.593)	0.2635
rs1061027 A > C (HWE = 0.137)							
AA	103 (45.18)	132 (52.38)		1.0000		1.0000	
AC	107 (46.93)	94 (37.30)		1.459 (0.999–2.130)	0.0505	1.441 (0.981–2.119)	0.0629
CC	18 (7.89)	26 (10.32)		0.887 (0.461–1.706)	0.7199	0.887 (0.460–1.707)	0.7186
Additive			0.0960	1.120 (0.850–1.475)	0.4212	1.109 (0.840–1.465)	0.4658
Dominant	125 (54.82)	120 (47.62)	0.1148	1.335 (0.932–1.912)	0.1151	1.318 (0.915–1.898)	0.1386
Recessive	210 (92.11)	226 (89.68)	0.3583	0.745 (0.397–1.398)	0.3596	0.749 (0.399–1.408)	0.3697
rs1139130 A > G (HWE = 0.821)							
AA	153 (67.11)	130 (51.39)		1.0000		1.0000	
AG	67 (29.39)	103 (40.87)		0.553 (0.376–0.816)	0.0026	0.561 (0.379–0.830)	0.0039
GG	8 (3.51)	19 (7.54)		0.358 (0.152–0.844)	0.0190	0.357 (0.151–0.843)	0.0188
Additive			0.0017	0.572 (0.419–0.781)	0.0004	0.577 (0.421–0.789)	0.0006
Dominant	75 (32.89)	122 (48.41)	0.0006	0.522 (0.361–0.757)	0.0006	0.527 (0.362–0.768)	0.0008
Recessive	220 (96.49)	233 (92.46)	0.0556	0.446 (0.191–1.040)	0.0616	0.440 (0.188–1.027)	0.0576
rs1263801 C > G (HWE = 0.793)							
TT	133 (58.33)	133 (52.78)		1.0000		1.0000	
TC	85 (37.28)	99 (39.29)		0.859 (0.589–1.251)	0.4274	0.836 (0.571–1.224)	0.3565
CC	10 (4.39)	20 (7.94)		0.500 (0.226–1.109)	0.0880	0.501 (0.225–1.113)	0.0895
Additive			0.2012	0.783 (0.583–1.052)	0.1046	0.772 (0.573–1.039)	0.0876
Dominant	95 (58.33)	119 (47.22)	0.2214	0.798 (0.556–1.146)	0.2216	0.778 (0.540–1.120)	0.1775
Recessive	218 (95.61)	232 (92.06)	0.1085	0.532 (0.244–1.162)	0.1135	0.537 (0.245–1.177)	0.1202
rs1263802 C > T (HWE = 0.084)							
CC	177 (77.63)	126 (50.00)		1.0000		1.0000	
CT	39 (17.11)	96 (38.10)		0.289 (0.187–0.448)	<0.0001	0.285 (0.184–0.442)	<0.0001
TT	12 (5.26)	30 (11.90)		0.285 (0.140–0.578)	0.0005	0.286 (0.141–0.582)	0.0005
Additive			<0.0001	0.411 (0.301–0.563)	<0.0001	0.410 (0.299–0.562)	<0.0001
Dominant	51 (22.37)	126 (50.00)	<0.0001	0.288 (0.194–0.429)	<0.0001	0.285 (0.191–0.426)	<0.0001
Recessive	216 (94.74)	222 (88.10)	0.0101	0.411 (0.205–0.824)	0.0122	0.417 (0.208–0.836)	0.0137

Abbreviations: KOA, knee osteoarthritis; HWE, Hardy-Weinberg equilibrium; OR, odds ratios; CI, confidence interval.
[a] $\chi 2$ test for genotype distributions between KOA cases and cancer-free controls.
[b] Adjusted for age and gender.

3.5. SNP-SNP interaction analysis

MDR analysis showed the third-order interplay involving polymorphisms rs1061026, rs1139130, and rs1263802 to be the most significant model for the prediction of a possible risk of KOA, with the highest CVC (10/10) as well as test accuracy (0.6896) (OR: 5.5527, 95% CI 1.5456–19.9484, p = 0.0065) (Table 6). Fig. 2 shows the interaction map depicting the interaction of rs1061026 with rs1139130, with high positive entropy/synergism (4.56%, marked in red). Specifically, low values of entropy indicated the presence of redundancy or suggested independence.

4. Discussion

The present study offered the initial population-based evidence demonstrating the impact of *METTL3* gene polymorphisms on the susceptibility to KOA among individuals from southern China. Based on a case-control study, we have identified a plausible link between the polymorphisms of *METTL3* gene and the risk to KOA among the individuals from southern China. Within the 5 chosen genetic variations, it was observed that rs1061026 showed a higher risk of KOA, whereas rs1139130 and rs1263802 were linked to a decreased risk. Our research reported the connection of the polymorphisms of *METTL3* gene and KOA for the first time.

Epigenetic changes, encompassing DNA methylation, post-translational histone modifications, as well as noncoding RNAs, have been identified as influencing factors for the advancement of OA [26]. One prevalent posttranscriptional modification of RNA is m6A, which is crucial in regulating mRNA stability, splicing, transport, localization, and translation efficiency. Growing evidence suggests that m6A is deeply involved in a variety of cellular processes, like DNA damage, autophagy, as well as cellular senescence [27]. The m6A modification process is dynamic and reversible, with methyltransferases increasing their levels and methyltransferases decreasing them [28]. Shi et al. found a significant elevation of *METTL3* in the synovium of human rheumatoid arthritis (RA). *METTL3* has been shown to inhibit the apoptosis alongside the autophagy of chondrocytes during inflammation by regulating the stability of Bcl2 through m6A modification mediated by Ythdf1 [29]. However, the specific mechanism by which *METTL3* alleviates KOA has not been clearly elucidated.

Table 4
Stratification analysis of *METTL3* polymorphisms with KOA susceptibility.

Variables	rs1061026 (cases/controls)		Adjusted OR[a] (95% CI)	*P*[a]	rs1139130 (cases/controls)		Adjusted OR[a] (95% CI)	*P*[a]	rs1263802 (cases/controls)		Adjusted OR[a] (95% CI)	*P*[a]
	TT	TG/GG			AA	AG/GG			CC	CT/TT		
Age, years												
>65	42/133	58/119	1.596 (0.909–2.802)	0.1037	69/130	31/122	0.614 (0.342–1.101)	0.1016	85/126	15/126	0.114 (0.057–0.225)	<0.001
≤65	57/133	71/119	1.363 (0.852–2.181)	0.1960	84/130	44/122	0.479 (0.296–0.775)	0.0027	92/126	36/126	0.514 (0.312–0.847)	0.0080
Sex												
Males	29/133	36/119	3.133 (1.51–6.329)	0.0015	39/130	26/122	0.430 (0.217–0.849)	0.0151	52/126	13/126	0.144 (0.066–0.310)	<0.0001
Females	70/133	93/119	1.085 (0.707–1.664)	0.7104	114/130	49/122	0.564 (0.360–0.882)	0.0120	125/126	38/126	0.381 (0.239–0.609)	<0.0001
BMI (kg/m^2)												
<24	24/133	39/119	1.927 (1.070–3.469)	0.0288	37/130	26/122	0.772 (0.434–1.374)	0.3794	48/126	15/126	0.282 (0.148–0.538)	0.0001
≥24, <28	57/133	56/119	1.030 (0.654–1.624)	0.8971	80/130	32/122	0.432 (0.266–0.703)	0.0007	87/126	25/126	0.286 (0.171–0.479)	<0.0001
≥28	18/133	35/119	2.266 (1.206–4.256)	0.0110	36/130	17/122	0.486 (0.257–0.920)	0.0266	42/126	11/126	0.244 (0.119–0.503)	0.0001
Smoking												
Yes	12/133	23/119	3.993 (1.741–9.159)	0.0011	21/130	14/122	0.521 (0.239–1.132)	0.0994	24/126	11/126	0.309 (0.137–0.700)	0.0049
No	87/133	106/119	1.266 (0.863–1.857)	0.2281	132/130	51/122	0.527 (0.354–0.785)	0.0016	153/126	40/126	0.264 (0.172–0.407)	<0.0001
Drinking												
Yes	31/133	28/119	1.284 (0.703–2.343)	0.4161	37/130	22/122	0.520 (0.279–0.968)	0.0390	49/126	10/126	0.158 (0.074–0.339)	<0.0001
No	68/133	101/119	1.504 (1.005–2.251)	0.0474	116/130	53/122	0.527 (0.348–0.798)	0.0025	128/126	41/126	0.332 (0.215–0.514)	<0.0001
Pysical exercise												
Yes	24/133	34/119	1.605 (0.889–2.900)	0.1168	35/130	23/122	0.711 (0.394–1.284)	0.2582	44/126	14/126	0.302 (0.156–0.584)	0.0004
No	75/133	95/119	1.411 (0.949–2.097)	0.0888	118/130	52/122	0.473 (0.312–0.717)	0.0004	133/126	37/126	0.272 (0.174–0.424)	<0.0001
Location												
Unilateral	79/133	104/119	1.474 (0.999–2.174)	0.0507	127/130	56/122	0.471 (0.314–0.705)	0.0003	139/126	44/126	0.312 (0.204–0.476)	<0.0001
Bilateral	20/133	25/119	1.388 (0.723–2.664)	0.3240	26/130	19/122	0.829 (0.428–1.607)	0.5789	38/126	7/126	0.164 (0.070–0.388)	<0.0001
Cause, years												
≥8	32/133	51/119	1.868 (1.110–3.139)	0.0186	58/130	25/122	0.439 (0.255–0.756)	0.0030	52/126	21/126	0.321 (0.183–0.562)	<0.0001
<8	67/133	78/119	1.269 (0.837–1.925)	0.2617	95/130	50/122	0.577 (0.376–0.886)	0.0119	115/126	30/126	0.256 (0.159–0.413)	<0.0001
History of injury												
Yes	5/133	7/119	1.714 (0.502–5.858)	0.3901	8/130	4/122	0.517 (0.149–1.795)	0.2991	9/126	3/126	0.300 (0.077–1.170)	0.0829
No	94/133	122/119	1.442 (0.997–2.085)	0.9516	145/130	71/122	0.527 (0.359–0.772)	0.0010	168/126	48/126	0.283 (0.188–0.425)	<0.0001
Family history												
Yes	12/133	10/119	0.952 (0.389–2.333)	0.9143	14/130	8/122	0.603 (0.240–1.519)	0.2835	21/126	1/126	0.040 (0.005–0.310)	0.0020
No	87/133	119/119	1.520 (1.044–2.218)	0.0290	139/130	67/122	0.520 (0.353–0.765)	0.0009	156/126	50/126	0.317 (0.211–0.476)	<0.0001
Swelling												
Yes	25/133	45/119	1.830 (1.046–3.200)	0.0341	44/130	26/122	0.698 (0.400–1.215)	0.2037	52/126	18/126	0.352 (0.193–0.642)	0.0007
No	74/133	84/119	1.308 (0.873–1.960)	0.1936	109/130	49/122	0.465 (0.304–0.713)	0.0004	125/126	33/126	0.251 (0.158–0.399)	<0.0001

[a] Adjusted for age and gender.

Table 5
Association between inferred haplotypes of the *METTL3* genes and KOA risk.

Haplotypes[a]	Cases(n = 456)	Controls(n = 504)	Crude OR (95% CI)	P	Adjusted OR (95% CI)	P[b]
	No.%	No.%				
TAC	267 (58.55)	219 (43.45)				
GAC	57 (12.50)	53 (10.52)	0.882 (0.583–1.335)	0.5531	0.875 (0.577–1.327)	0.5311
GAT	20 (4.39)	33 (6.55)	0.497 (0.277–0.891)	0.0189	0.496 (0.276–0.893)	0.0193
GGC	63 (13.82)	30 (5.95)	1.722 (1.076–2.756)	0.0234	1.755 (1.095–2.812)	0.0194
GGT	15 (3.29)	24 (4.76)	0.513 (0.262–1.001)	0.0504	0.515 (0.263–1.007)	0.0524
TAT	29 (6.36)	58 (11.51)	0.410 (0.254–0.663)	0.0003	0.408 (0.252–0.661)	0.0003
TGC	5 (1.10)	45 (8.93)	0.091 (0.036–0.234)	<0.0001	0.090 (0.035–0.232)	<0.0001
TGT	0	42 (8.33)	/	0.9743	/	0.9743

[a] The haplotypes order was rs1061027, rs1263801, rs1061026, rs1139130, and rs1263802.
[b] Obtained in logistic regression models with adjustment for age and gender.

Table 6
Best multifactor dimensionality reduction (MDR) interaction models.

Locus number	Testing Accuracy	CVC	OR	95% CI	P
rs1061026, rs1139130, rs1263802	0.6896	10/10	5.5527	(1.5456,19.9484)	0.0065

The model was considered as the best model.

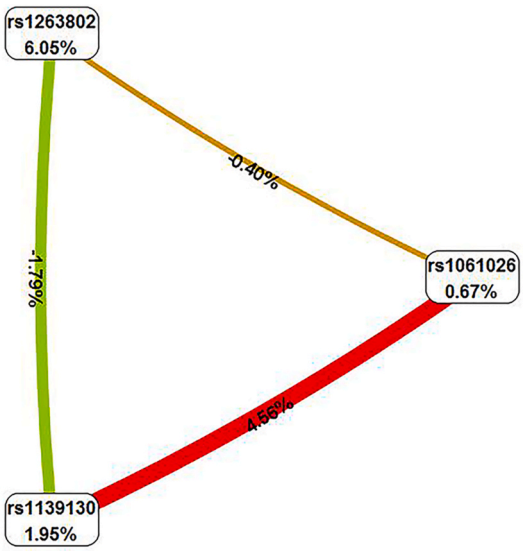


Fig. 2. Interaction map for the risk of knee osteoarthritis. The interaction model describes the percentage of the entropy (information gain) explained by each factor or 2-way interaction. Positive entropy (plotted in red) indicates the interaction, which can be interpreted as a synergistic or nonadditive relationship. In contrast, negative entropy (plotted in yellow-green or green) indicates the independence or additivity (redundancy). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Even though there is no report on the expression and function of miR-875-5p within KOA, evidence has shown the effect of miR-875-5p on the inflammation among gestational diabetes rats [30]. MiR-23a-3p was certified as a prognostic biomarker for KOA [31]. Chondrocyte miR-23a-3p facilitates the initiation of osteoarthritis by inhibiting SMAD3 [32]. Among the predicted transcription factors of *METTL3*, most of them have not been verified to be associated with KOA. However, some of them are involved in arthritis or inflammation. Myogenic regulatory factor 6 (MYF6) binds to the E-box sequence CANNTG and induces myogenic conversion upon transfection into fibroblasts [33]. CCCTC-binding factor (CTCF) is pivotal for the regulatory network of RA and plays a role in regulating differentially methylated genes [34]. ZNF460 is a member of the Zinc finger protein family [35]. CTCF SUMOylation could inhibit human lung inflammation injury [36] Among the differentially methylated genes selected, ZNF135 shows down-regulated expression in esophageal adenocarcinoma (NOS) [37].) However, no empirical evidence has been found to establish its correlation

with joint inflammation. Taipale et al. [38] revealed that individuals of Finnish descent possess a susceptibility locus on chromosome 2q21 and exhibit recognition sequences for E74 like ETS transcription factor 3 (ELF3), which plays a pivotal role in maintaining articular cartilage homeostasis. ELF3 also takes part in cartilage degradation in a post-traumatic osteoarthritis mouse model [39]. IKAROS family zinc finger 3 (IKZF3) plays a direct role in the differentiation or function of Th17 cells, representing novel therapeutic targets for enhancing current treatments or leading to the development of new strategies for RA treatment [40]. E74 like ETS transcription factor 1 (ELF1) is associated with autoimmunity, highlighting the dual role of ETS factors as both positive and negative regulators of immune responses [41]. The function of these predicted transcription factors in KOA is still unrevealed. In a publication by Lin et al. in 2020, it was shown that children with four protective genotypes (rs1061026 TG/GG and rs1139130 GG) had a relatively lower risk of Wilms tumor compared to children without these protective genotypes [42]. The *METTL3* gene polymorphism rs1139130 AA has been linked to an eleveated risk of pediatric ALL [43] as well as Graves' disease [21]. While SNPs have been studied to examine the genetic predisposition to OA, there is no previous investigation specifically focusing on the relationship between SNPs in the *METTL3* gene and KOA. Our research indicates that the TG/GG genotype of the *METTL*3 rs1061026 gene is associated with an increased risk of KOA in women experiencing swelling. Conversely, the *METTL3* rs1139130 gene has the opposite effect. Therefore, we believe that the association between METTL3 SNPs and disease risk varies among different diseases.

Numerous studies have investigated the link of smoking and KOA. Some research suggests that smoking less is linked to a relatively higher risk of incident KOA [44]. However, the evidence for the impact of cigarette smoking on OA is contradictory. Besides, reports have linked smoking to an elevated prevalence of incident knee pain [45]. Additionally, significant findings have emerged regarding the interaction of cigarette smoking and gene polymorphisms associated with inflammation, like the genotypes of IL-1 [46] alongside IL-4 receptor [47], impacting the susceptibility to various disorders. Meanwhile, they may also be linked to KOA. Our findings suggest that individuals with the TG/GG genotype of *METTL3* rs1061026 have a higher risk of KOA when they smoke, while those with the AG/GG genotype of *METTL3* rs1139130 show a protective effect of smoking against KOA.

Multiple factors, such as alcohol consumption, PE, obesity, acute joint injury, and swelling, have been extensively studied in relation to OA's development. Pain, joint stiffness, instability, swelling, as well as muscle weakness can contribute to physical and psychological disability, ultimately impacting an individual's quality of life [29]. The present study examined the link of various genotypes of *METTL3* SNPs and different lifestyles in KOA patients. The findings suggest that adopting a healthier lifestyle could potentially reduce the risk of developing the disease for KOA patients. Surprisingly, our study also revealed that smoking might have a risk-reducing effect on certain patient groups, although the underlying mechanism remains unclear. Further research is necessary to gain a better understanding of this unexpected finding. However, additional investigation is required to determine if *METTL3* rs1139130 protects against KOA through anti-inflammatory mechanisms.

Despite our research findings, there were certain limitations in the present study. Firstly, Further studies on functional/molecular biology are necessary to ascertain the importance of the connections observed in the present study. Moreover, the relatively small sample size could have affected the reliability of our results. While our study suggests an elevated risk of KOA among southern Chinese population with genetic variation in *METTL3* genes, it is pivotal to acknowledge the limited sample size as well as the potential for larger-scale studies. Additionally, the extent to which genetic/biological variances contribute to KOA across different ethnic groups is not yet fully understood. More importantly, we didn't consider lifestyle factors associated with KOA risk when performing association analysis between SNPs and KOA since the data obtained were qualitative rather than quantitative. Therefore, further investigation is necessary to gain a comprehensive understanding of the mechanisms that underlie the observed associations of rs1061026, rs1139130, and rs1263802 with KOA. Moreover, the exercise time, drinking volume, and smoking quantity of both patients and healthy controls should be recorded.

Our research findings indicate a significant correlation between genetic variation in *METTL3* and the susceptibility to KOA. Specifically, we identified 3 genetic markers (rs1061026, rs1139130, and rs1263802) associated with KOA in the southern Chinese population. These markers might have the potential to serve as biomarkers for assessing susceptibility to KOA, providing guidance for the improvement of the lifestyles of patients.

Ethics declarations

All participants signed an informed consent approved by the Institutional Review Board and agreed to participate in the study.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Houlin Mi: Writing – original draft. **Mingzhi Wang:** Writing – review & editing. **Yongmei Chang:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

References

[1] R. Giorgino, et al., Knee Osteoarthritis: Epidemiology, Pathogenesis, and Mesenchymal Stem Cells: What Else Is New? An Update 24 (7) (2023) 6405.
[2] D.J. Hunter, D. Schofield, E. Callander, The individual and socioeconomic impact of osteoarthritis, Nat. Rev. Rheumatol. 10 (7) (2014) 437–441.
[3] H.S. Kan, et al., Non-surgical treatment of knee osteoarthritis, Hong Kong Med. J. 25 (2) (2019) 127–133.
[4] J.N. Katz, K.R. Arant, R.F. Loeser, Diagnosis and treatment of hip and knee osteoarthritis: a review, JAMA 325 (6) (2021) 568–578.
[5] N. Liu, et al., N(6)-methyladenosine-dependent RNA structural switches regulate RNA-protein interactions, Nature 518 (7540) (2015) 560–564.
[6] G. Zheng, et al., ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility, Mol. Cell 49 (1) (2013) 18–29.
[7] J. Han, et al., METTL3 promote tumor proliferation of bladder cancer by accelerating pri-miR221/222 maturation in m6A-dependent manner, Mol. Cancer 18 (1) (2019) 110.
[8] J. Xiong, et al., Lactylation-driven METTL3-mediated RNA m(6)A modification promotes immunosuppression of tumor-infiltrating myeloid cells, Mol. Cell 82 (9) (2022) 1660–1677. .e10.
[9] J. Huang, et al., Solution structure of the RNA recognition domain of METTL3-METTL14 N6-methyladenosine methyltransferase, Protein & Cell 10 (4) (2018) 272–284.
[10] F. Aguilo, et al., Coordination of m(6)A mRNA methylation and gene transcription by ZFP217 regulates pluripotency and reprogramming, Cell Stem Cell 17 (6) (2015) 689–704.
[11] M.E. Hess, et al., The fat mass and obesity associated gene (Fto) regulates activity of the dopaminergic midbrain circuitry, Nat. Neurosci. 16 (8) (2013) 1042–1048.
[12] K. Xu, et al., Mettl3-mediated m(6)A regulates spermatogonial differentiation and meiosis initiation, Cell Res. 27 (9) (2017) 1100–1114.
[13] H.B. Li, et al., m(6)A mRNA methylation controls T cell homeostasis by targeting the IL-7/STAT5/SOCS pathways, Nature 548 (7667) (2017) 338–342.
[14] Q. Liu, et al., METTL3 promotes experimental osteoarthritis development by regulating inflammatory response and apoptosis in chondrocyte, Biochem. Biophys. Res. Commun. 516 (1) (2019) 22–27.
[15] W. Sang, et al., METTL3 involves the progression of osteoarthritis probably by affecting ECM degradation and regulating the inflammatory response, Life Sci. 278 (2021) 119528.
[16] X. Li, et al., Dynamic incorporation of multiple in silico functional annotations empowers rare variant association analysis of large whole-genome sequencing studies at scale, Nat. Genet. 52 (9) (2020) 969–983.
[17] Z. Li, et al., A framework for detecting noncoding rare-variant associations of large-scale whole-genome sequencing studies, Nat. Methods 19 (12) (2022) 1599–1611.
[18] H. Zhou, et al., FAVOR: functional annotation of variants online resource and annotator for variation across the human genome, Nucleic Acids Res. 51 (D1) (2023) D1300–d1311.
[19] J. Bian, et al., Association between METTL3 gene polymorphisms and neuroblastoma susceptibility: a nine-centre case-control study, J. Cell Mol. Med. 24 (16) (2020) 9280–9286.
[20] A. Luo, et al., Genetic variants in METTL14 are associated with the risk of acute lymphoblastic leukemia in southern Chinese children: a five-center case-control study, Cancer Manag. Res. 13 (2021) 9189–9200.
[21] R.H. Song, et al., METTL3 gene polymorphisms contribute to susceptibility to autoimmune thyroid disease, Endocrine 72 (2) (2021) 495–504.
[22] S. Yang, et al., The association between ADAM12 gene polymorphisms and osteoarthritis: an updated meta-analysis, J. Orthop. Surg. Res. 18 (1) (2023) 149.
[23] S. Liu, et al., Relationship between PIWIL1 gene polymorphisms and epithelial ovarian cancer susceptibility among southern Chinese woman: a three-center case-control study, BMC Cancer 23 (1) (2023) 1149.
[24] J. Fernández-Torres, et al., Multifactor dimensionality reduction reveals a strong gene-gene interaction between STC1 and COL11A1 genes as a possible risk factor of knee osteoarthritis, Mol. Biol. Rep. 47 (4) (2020) 2627–2634.
[25] Y.T. Lu, et al., The interaction between glycemic index, glycemic load, and the genetic variant ADIPOQ T45G (rs2241766) in the risk of colorectal cancer: a case-control study in a Korean population, Eur. J. Nutr. 61 (5) (2022) 2601–2614.
[26] Z. Cai, et al., Epigenetic regulation in knee osteoarthritis, Front. Genet. 13 (2022) 942982.
[27] S. Jin, et al., m(6)A RNA modification controls autophagy through upregulating ULK1 protein abundance, Cell Res. 28 (9) (2018) 955–957.
[28] Y. Niu, et al., N6-methyl-adenosine (m6A) in RNA: an old modification with a novel epigenetic function, Dev. Reprod. Biol. 11 (1) (2013) 8–17.
[29] Y. He, et al., Mettl3 inhibits the apoptosis and autophagy of chondrocytes in inflammation through mediating Bcl2 stability via Ythdf1-mediated m(6)A modification, Bone 154 (2022) 116182.
[30] S. Fu, et al., miR-875-5p regulates IR and inflammation via targeting TXNRD1 in gestational diabetes rats, Mol. Med. Rep. 23 (5) (2021).
[31] Y.H. Kwak, et al., Significant changes in synovial fluid microRNAs after high tibial osteotomy in medial compartmental knee osteoarthritis: identification of potential prognostic biomarkers, PLoS One 15 (1) (2020) e0227596.
[32] L. Kang, et al., MicroRNA-23a-3p promotes the development of osteoarthritis by directly targeting SMAD3 in chondrocytes, Biochem. Biophys. Res. Commun. 478 (1) (2016) 467–473.
[33] N. Moncaut, P.W. Rigby, J.J. Carvajal, Dial M(RF) for myogenesis, FEBS J. 280 (17) (2013) 3980–3990.
[34] Y. Lin, Z. Luo, Aberrant methylation patterns affect the molecular pathogenesis of rheumatoid arthritis, Int. Immunopharm. 46 (2017) 141–145.
[35] C. Zou, et al., Circular RNA mitochondrial translation optimization 1 homologue (CircMTO1) induced by zinc finger protein 460 (ZNF460) promotes oral squamous cell carcinoma progression through the microRNA miR-320a/alpha thalassemia/mental retardation, X-linked (ATRX) axis, Bioengineered 12 (2) (2021) 9585–9597.
[36] L. Kang, et al., SENP1 knockdown-mediated CTCF SUMOylation enhanced its stability and alleviated lipopolysaccharide-evoked inflammatory injury in human lung fibroblasts via regulation of FOXA2 transcription, Biochim. Biophys. Acta Gen. Subj. 1868 (1) (2023) 130500.
[37] T. Xi, G. Zhang, Epigenetic regulation on the gene expression signature in esophagus adenocarcinoma, Pathol. Res. Pract. 213 (2) (2017) 83–88.
[38] M. Taipale, et al., Targeted re-sequencing of linkage region on 2q21 identifies a novel functional variant for hip and knee osteoarthritis, Osteoarthritis Cartilage 24 (4) (2016) 655–663.
[39] E.B. Wondimu, et al., Elf3 contributes to cartilage degradation in vivo in a surgical model of post-traumatic osteoarthritis, Sci. Rep. 8 (1) (2018) 6438.
[40] J.P. van Hamburg, S.W. Tas, Molecular mechanisms underpinning T helper 17 cell heterogeneity and functions in rheumatoid arthritis, J. Autoimmun. 87 (2018) 69–81.
[41] S. Gallant, G. Gilkeson, ETS transcription factors and regulation of immunity, Arch. Immunol. Ther. Exp. 54 (3) (2006) 149–163.
[42] A. Lin, et al., METTL3 polymorphisms and Wilms tumor susceptibility in Chinese children: a five-center case-control study, J. Gene Med. 22 (11) (2020) e3255.
[43] X. Liu, et al., Novel associations between METTL3 gene polymorphisms and pediatric acute lymphoblastic leukemia: a five-center case-control study, Front. Oncol. 11 (2021) 635251.
[44] R. Takiguchi, et al., Modifiable factors associated with symptomatic knee osteoarthritis: the Murakami cohort study, Maturitas 128 (2019) 53–59.
[45] H. Miranda, et al., A prospective study on knee pain and its risk factors, Osteoarthritis Cartilage 10 (8) (2002) 623–630.
[46] P. Jerrard-Dunne, et al., Inflammatory gene load is associated with enhanced inflammation and early carotid atherosclerosis in smokers, Stroke 35 (11) (2004) 2438–2443.
[47] S.A. Rosner, et al., Interaction between inflammation-related gene polymorphisms and cigarette smoking on the risk of myocardial infarction in the Physician's Health Study, Hum. Genet. 118 (2) (2005) 287–294.