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Genetic variants in mTOR-pathway-related genes contribute to osteoarthritis susceptibility

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ABSTRACT

The mTOR signaling pathway has been demonstrated to be related to the development of osteoarthritis (OA) by regulating expression of autophagy regulators. Few studies have shed light on the association of mTOR-pathway-related gene variants with OA risk. Totally 441 OA patients and 533 controls were recruited and their genotypes for mTOR-pathway-related gene variants were determined based on the matrix-assisted laser desorption/ionization time of flight mass spectrometry. Genetic risk scores (GRS) were calculated to evaluate the combined effect of these polymorphisms on OA risk. No significant differences were observed in genotypic and allelic frequencies of *AKT* rs1130233/*REDD1* rs1053639 polymorphisms. However, the *mTOR* rs1034528 polymorphism was demonstrated to be related to an increased risk of OA, especially among smokers and individuals aged ≥60 years. This single nucleotide polymorphism (SNP) also showed significantly correlation with the Lequesne's index. Similarly, the *IRS1* rs1801278 polymorphism increased the risk of OA among smokers, drinkers and individuals aged ≥60 years. A strong positive correlation was found between *PTEN* rs3830675 polymorphism and OA risk, and this SNP was more frequent in the smokers and drinkers groups. No association was found between *IRS1* rs1801278/*PTEN* rs3830675 polymorphism and OA characteristics. Additionally, there was a strong interaction between genetic factors and lifestyles under the combined models (*IRS1* rs1801278/*PTEN* rs3830675 polymorphism and smoking/drinking). A high GRS was positively related to an increased risk of OA. In summary, three gene polymorphisms (*mTOR* [rs1034528], *IRS1* [rs1801278] and *PTEN* [rs3830675]) were found to affect the risk of OA development by regulating the mTOR pathway.

1. Introduction

Osteoarthritis (OA) is a degenerative joint disease with joint pain and dysfunction [1,2]. Moreover, aging and obesity increase the incidence, which is becoming an escalating worldwide health burden [3]. There is evidence of a complex interaction between environmental and genetic factors in the risk of OA, including sex, age, obesity, daily diet and genetics [4]. Several studies have confirmed that genetic components and heritability are related to the etiology of OA [5,6].

Autophagy is an essential cellular homeostasis mechanism that plays an antiaging role in the removal of dysfunctional cellular organelles and macromolecules [7]. The up-regulation of autophagy was observed in OA chondrocytes and cartilage in the early phase of degeneration to protect chondrocytes from environmental changes by regulating apoptosis and reactive oxygen species [8]. However, autophagy decreases as the cartilage gradually degenerates, eventually leading to cell death [8]. Mammalian target of rapamycin (mTOR) associates with raptor and rictor to form the mTOR complex 1 (mTORC1) and the mTOR complex 2 (mTORC2). mTORC1 is a critical negative regulator of general autophagy [9]. Additionally, mTOR is highly expressed in human OA cartilage, while cartilage-specific deletion of mTOR enhances autophagy and prevents chondrocyte death [10]. Thus, we hypothesized that mTOR plays important roles in the development of OA by regulating autophagy.

Functional polymorphisms in several genes (*AKT*, *mTOR*, *IRS1*, *PTEN* and *REDD1*) in the mTOR signaling pathway for analysis have

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been identified as susceptibility variants in various human cancers [11–13]. However, an association of these polymorphisms with OA susceptibility has not yet been reported. This hospital-based case-control study was conducted to investigate the role of mTOR pathway-related genetic variants in the risk of OA in a Chinese population.

2. Materials and methods

2.1. Study population

The present study consisted of 441 patients with knee OA and 533 sex and age-matched controls; all participants were recruited from the Affiliated Changzhou No.2 People's Hospital of Nanjing Medical University (Changzhou, China), the Second Affiliated Hospital, Zhejiang University School of Medicine (Hangzhou, China) and the Second Affiliated Hospital of Jiaxing University (Jiaxing. China). The knee OA diagnosis was in accordance with the clinical and radiographic criteria of the American College of Rheumatology (1987). Inclusion criteria were: (1) any symptom and/or sign of knee OA, (2) no evidence for any other form of arthritis, and (3) informed consent obtained. Exclusion criteria were as follows: (1) autoimmune disorders, malignant tumor, or liver and kidney dysfunction; (2) history of arthritis; (3) history of drug abuse exceeding 3 months. The control group were selected from the subjects receiving medical examination at the above-mentioned hospitals. Symptoms of knee OA were confirmed by X-ray analysis. Demographic and clinical information (C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), visual analogue score (VAS) and Kellgren–Lawrence (K–L) grade) was collected from written questionnaires and medical records. We defined individuals who smoked at least one cigarette daily more than 1 year as smokers. Individuals were defined as alcohol consumers if they drank three alcoholic drinks every week.

Ethical approval was obtained by the Ethics Committees of the three participating hospitals and confidentiality was performed in accordance with the Declaration of Helsinki. Each individual was first surveyed by means of a short questionnaire to obtain the written informed consent.

2.2. SNP selection

For our analysis, we selected five single nucleotide polymorphisms (SNPs): *AKT* (rs1130233 C > T), *mTOR* (rs1034528 G > C), *IRS1* (rs1801278 C > G), *PTEN* (rs3830675 C > T) and *REDD1* (rs1053639 T > A) in the mTOR signaling pathway based on the following criteria: (1) minor allele frequency > 0.05; (2) location: promoter, exon or 3′-UTR; (3) genetic variant on the mTOR pathway; (4) these SNPs have been studied in other diseases, but not in OA.

2.3. Genotyping

Genomic DNA was extracted from peripheral blood (2 mL) using the TIANamp Blood DNA kit (Tiangen Biotech, Beijing, China) following the manufacturer's instructions. The purity and concentration of DNA samples for genotyping analysis were tested with NanoDrop 2000 UV–VIS spectrophotometer (Thermo Scientific, USA). Genotyping was conducted by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF–MS) using the MassARRAY system (Sequenom, San Diego, CA, USA). The primers used in this article could be found in supplemental Table 1. Genotype calling was performed in real time with MassARRAY RT software (version 3.1; Sequenom), and analyzed using MassARRAY Typer software (version 4.0; Sequenom). A fraction (10%) of the samples was randomly selected for repeated analysis to ensure the accuracy of genotyping.

2.4. Genotype based mRNA expression analysis

We downloaded the genotype and mRNA expression data from the GTEx portal database (http://www.gtexportal.org/home/) to evaluate the potential effects of these positive polymorphisms on target gene expression.

2.5. Statistical analysis

Categorical variables (frequency and percentage) were compared by chi-squared test and Student's *t*-test or one-way ANOVA was utilized to compare different levels of clinical parameters. The departure from Hardy–Weinberg equilibrium (HWE) among controls was assessed through a goodness-of-fit chi-squared test. The SNP-associated OA risk was evaluated by calculating odds ratios (ORs) and 95% confidence intervals (CIs). Stratification analysis was conducted according to sex, age, smoking, alcohol consumption and body mass index (BMI). In the mTOR signaling pathway, five SNPs were genotyped to investigate their associations with OA risk. We assumed that these SNPs associated independently with RA risk, and the genetic risk scores (GRS) were equivalent to the number of risk alleles among the five SNPs carried by an individual. Crossover analyses were used to evaluate the interaction gene-environmental interaction. False-positive report probability (FPRP) was used to evaluate the positive findings [14]. FPRP < 0.2 at a prior probability of 0.1 implied a significant relationship. All statistical analyses were performed on SAS 9.1.3 (SAS Institute, Cary, NC, USA). $P < 0.05$ was the cutoff value for statistical significance.

3. Results

3.1. Subjects

The average ages of OA cases and controls were 57.75 and 57.45 years, respectively (Table 1). The female-to-male ratios in the cases and controls were 1.23:1 and 1.17:1 respectively. The average BMIs didn't differ significantly between cases and controls ($P = 0.610$). The prevalence of smoking and drinking was much higher in cases than in controls (55.6% vs. 45.8%, $P = 0.002$ for smoking; 59.2% vs. 45.4%, $P < 0.001$ for drinking). Approximately 86% of OA patients were assigned K–L grade III + IV. Clinical characteristics (e.g. ESR, CRP, VAS and Lequesne's index) of patients were also collected to explore the association of these parameters with polymorphisms in the mTOR signaling pathway.

Table 1
Patient demographics and risk factors in osteoarthritis.

Variable	Cases (n = 441)	Controls (n = 533)	*P*
Age (years)	57.75 ± 8.90	57.45 ± 9.13	0.603
BMI	25.07 ± 3.96	24.94 ± 3.75	0.610
Sex			0.695
Male	198(44.9%)	246(46.2%)	
Female	243(55.1%)	287(53.8%)	
Smoking			0.002
Yes	245(55.6%)	244(45.8%)	
No	196(44.4%)	289(54.2%)	
Alcohol			< 0.001
Yes	261(59.2%)	242(45.4%)	
No	180(40.8%)	291(54.6%)	
ESR, mm/h	11.40 ± 13.09		
CRP, mg/L	7.05 ± 15.84		
VAS	7.92 ± 1.27		
Lequesnes' index	15.35 ± 1.85		
Left/Right knee OA			
Left	235(53.3%)		
Right	206(46.7%)		
K-L grade			
II	61(13.8%)		
III	186(42.2%)		
IV	194(44.0%)		

BMI, body mass index; ESR, erythrocyte sedimentation rate; CRP, C-reaction protein; VAS, Visual Analogue Scale; K-L grade, Kellgren-Lawrence grade.

Table 2
Logistic regression analysis of associations between polymorphisms on mTOR signal pathway and risk of osteoarthritis.

Genotype	Cases*(n = 441)		Controls*(n = 533)		OR (95% CI)	*P*	*OR (95% CI)	**P*
	n	%	n	%				
AKT (rs1130233 C/T)								
CC	217	49.3%	273	51.4%	1.00	–	1.00	–
CT	184	41.8%	215	40.5%	1.08(0.83–1.41)	0.563	1.08(0.83–1.41)	0.558
TT	39	8.9%	43	8.1%	1.14(0.71–1.82)	0.581	1.14(0.71–1.82)	0.589
CT + TT	223	50.7%	258	48.6%	1.09(0.85–1.41)	0.497	1.09(0.85–1.41)	0.495
CC + CT	401	91.1%	488	91.9%	1.00	–	1.00	–
TT	39	8.9%	43	8.1%	1.10(0.70–1.73)	0.675	1.10(0.70–1.73)	0.686
C allele	618	70.2%	761	71.7%	1.00	–	–	–
T allele	262	29.8%	301	28.3%	1.07(0.88–1.31)	0.489	–	–
REDD1 (rs1053639T/A)								
TT	176	39.9%	205	38.5%	1.00	–	1.00	–
TA	205	46.5%	250	47.0%	0.96(0.78–1.26)	0.764	0.96(0.73–1.26)	0.770
AA	60	13.6%	77	14.5%	0.91(0.61–1.35)	0.629	0.91(0.61–1.34)	0.623
TA + AA	265	60.1%	327	61.5%	0.95(0.73–1.23)	0.679	0.95(0.73–1.23)	0.681
TT + TA	381	86.4%	455	85.5%	1.00	–	1.00	–
AA	60	13.6%	77	14.5%	0.93(0.65–1.34)	0.690	0.93(0.64–1.33)	0.680
T allele	557	63.2%	660	62.0%	1.00	–	–	–
A allele	325	36.8%	404	38.0%	0.95(0.79–1.15)	0.611	–	–
MTOR (rs1034528 G/C)								
GG	216	49.1%	299	56.3%	1.00	–	1.00	–
GC	184	41.8%	199	37.5%	1.29(0.99–1.68)	0.064	1.29(0.99–1.68)	0.063
CC	40	9.1%	33	6.2%	**1.68(1.03–2.75)**	**0.040**	**1.68(1.02–2.74)**	**0.041**
GC + CC	224	50.9%	232	43.7%	**1.34(1.04–1.73)**	**0.023**	**1.34(1.04–1.73)**	**0.023**
GG + GC	400	90.9%	498	93.8%	1.00	–	1.00	–
CC	40	9.1%	33	6.2%	1.51(0.93–2.43)	0.094	1.50(0.93–2.43)	0.096
G allele	616	70.0%	797	75.0%	1.00	–	–	–
C allele	264	30.0%	265	25.0%	**1.29(1.05–1.58)**	**0.013**	–	–
IRS1 (rs1801278 C/G)								
CC	238	54.1%	327	61.5%	1.00	–	1.00	–
CG	171	38.9%	182	34.2%	1.29(0.99–1.68)	0.064	1.29(0.99–1.69)	0.062
GG	31	7.0%	23	4.3%	**1.85(1.05–3.25)**	**0.033**	**1.85(1.05–3.26)**	**0.033**
CG + GG	202	45.9%	205	38.5%	**1.35(1.05–1.74)**	**0.022**	**1.35(1.05–1.75)**	**0.021**
CC + CG	409	93.0%	509	95.7%	1.00	–	1.00	–
GG	31	7.0%	23	4.3%	1.67(0.96–2.92)	0.069	1.68(0.96–2.92)	0.068
C allele	647	73.5%	836	78.6%	1.00	–	–	–
G allele	233	26.5%	228	21.4%	**1.32(1.07–1.63)**	**0.009**	–	–
PTEN (rs3830675 C/T)								
CC	199	45.1%	278	52.2%	1.00	–	1.00	–
CT	194	44.0%	214	40.2%	1.27(0.98–1.66)	0.076	1.27(0.97–1.66)	0.080
TT	48	10.9%	41	7.7%	**1.64(1.04–2.58)**	**0.034**	**1.64(1.04–2.59)**	**0.033**
CT + TT	242	54.9%	255	47.8%	**1.33(1.03–1.72)**	**0.027**	**1.33(1.03–1.71)**	**0.028**
CC + CT	393	89.1%	492	92.3%	1.00	–	1.00	–
TT	48	10.9%	41	7.7%	1.46(0.94–2.27)	0.088	1.47(0.95–2.28)	0.084
C allele	592	67.1%	770	72.2%	1.00	–	–	–
T allele	290	32.9%	296	27.8%	**1.27(1.05–1.55)**	**0.014**	–	–

The genotyping was successful in 440 cases and 531 controls for rs1130233; 441 cases and 532 controls for rs1053639; 440 cases and 531 controls for rs1034528; 440 cases and 532 controls for rs1801278; 441 cases and 533 controls for rs3830675
Bold values are statistically significant ($P < 0.05$).
* Adjust for age and sex.

3.2. mTOR-pathway-related genes variant analyses

As is shown in Table 2, there was no significant deviation from HWE for the five SNPs among the controls. The genotypic and allelic frequencies did not differ significantly for the AKT rs1130233 or REDD1 rs1053639 polymorphism.

For mTOR rs1034528 polymorphism, subjects with the CC genotype had a significantly higher risk of knee OA in comparison with those carrying GG genotype (CC vs. GG: OR 1.68; 95%CI: 1.03–2.75; $P = 0.040$). Moreover, the elevated risk of OA was observed in the dominant (CC + GC vs. GG: OR 1.34; 95%CI: 1.04–1.73; $P = 0.023$) and allelic models (C vs. G: OR 1.29; 95%CI: 1.05–1.58; $P = 0.013$). Similarly, IRS1 rs1801278 or PTEN rs3830675 polymorphism was related to an increased risk of OA under the homozygous, dominant and allelic models. Notably, these associations were still significant after adjusting for sex and age.

Furthermore, we carried out stratified analyses according to sex, age, smoking, drinking and BMI to evaluate the effect of these positive polymorphisms on OA risk (Table 3). We noted a significant association of mTOR rs1034528 polymorphism among smokers and the elderly. When stratified according to smoking, drinking or age, significant associations between the IRS1 rs1801278 polymorphism and OA risk were limited to smokers, drinkers and the elderly (age > 60 years). For PTEN rs3830675 polymorphism, the increased effect was strong in the subgroups of smokers and drinkers.

Subsequently, we investigated the effects of three polymorphisms on clinical features (CRP, ESR, VAS, Lequesne's index and K–L grade) of OA patients (Table 4). For mTOR rs1034528 polymorphism, the CC genotype was more frequent in the group of Lequesne's index ≥ 6 versus the group with Lequesne's index < 6. The CC or GC + CC genotype carriers of the rs1034528 polymorphism had lower K–L grade in comparison with the GG genotype. No significant association was observed between IRS1 rs1801278 or PTEN rs3830675 polymorphisms and other clinical features such as ESR, CRP, K-L grade, VAS and Lequesne's index.

We calculated the ORs and 95% CI for the combined exposure

Table 3
Stratified analyses between mTOR rs1034528/ IRS1 rs1801278/ PTEN rs3830675 polymorphisms on the mTOR signaling pathway and the risk of osteoarthritis.

Variable	case/control			Heterozygous model	Homozygous model	Recessive model	Dominant model
MTOR (rs1034528)	**GG**	**GC**	**CC**	**GA vs. GG**	**AA vs.GG**	**AA vs. GA + GG**	**AA + GA vs.GG**
Sex							
Male	97/135	85/99	16/12	1.21(0.82–1.78); 0.345	1.86(0.84–4.10); 0.126	1.71(0.79–3.70); 0.175	1.28(0.88–1.86); 0.201
Female	119/164	99/101	24/21	1.36(0.95–1.97); 0.095	1.58(0.84–2.96); 0.159	1.38(0.75–2.55); 0.298	1.40(0.99–1.98); 0.055
Smoking							
Yes	126/148	99/118	20/14	1.44(0.98–2.10); 0.061	1.68(0.81–3.46); 0.161	1.61(0.84–3.11); 0.154	**1.47(1.03–2.11); 0.035**
No	90/151	85/81	20/19	1.22(0.83–1.79); 0.310	1.77(0.90–3.49); 0.101	1.45(0.72–3.95); 0.300	1.30(0.90–1.87); 0.164
Alcohol							
Yes	124/129	115/98	21/15	1.22(0.85–1.76); 0.284	1.46(0.72–2.95); 0.297	1.33(0.67–2.64); 0.416	1.25(0.88–1.78); 0.209
No	92/170	69/101	19/18	1.28(0.86–1.90); 0.232	1.95(0.98–3.90); 0.059	1.77(0.90–3.47); 0.097	1.38(0.95–2.01); 0.094
Age (years)							
< 60	129/172	112/118	19/20	1.27(0.90–1.79); 0.181	1.27(0.65–2.47); 0.488	1.14(0.60–2.19); 0.686	1.27(0.91–1.76); 0.162
≥60	87/127	72/81	21/13	1.31(0.86–2.00); 0.202	**2.36(1.12–4.96); 0.024**	**2.01(1.02–4.33); 0.044**	1.46(0.98–2.17); 0.061
BMI							
< 25	106/155	92/96	19/18	1.40(0.96–2.05); 0.080	1.54(0.77–3.08); 0.218	1.34(0.68–2.62); 0.395	1.42(0.99–2.04); 0.054
≥25	110/144	92/103	21/15	1.18(0.81–1.72); 0.386	1.83(0.90–3.72); 0.093	1.70(0.86–3.39); 0.129	1.26(0.88–1.81); 0.200
IRS1 (rs1801278)	**CC**	**CG**	**GG**	**CG vs. CC**	**GG vs.CC**	**GG vs. CG + CC**	**GG + CG vs.CC**
Sex							
Male	106/150	76/86	15/9	1.25(0.84–1.86); 0.269	2.36(0.99–5.59); 0.051	2.16(0.93–5.05); 0.075	1.36(0.93–1.98); 0.117
Female	132/177	95/96	16/14	1.32(0.92–1.90); 0.134	1.52(0.72–3.23); 0.272	1.37(0.65–2.87); 0.404	1.35(0.95–1.90); 0.094
Smoking							
Yes	104/137	118/99	22/7	**1.57(1.09–2.27); 0.017**	**4.14(1.70–10.06); 0.002**	**3.34(1.40–7.98); 0.007**	**1.74(1.22–2.49); 0.003**
No	134/190	53/83	9/16	0.90(0.60–1.36); 0.617	0.79(0.34–1.85); 0.592	0.82(0.35–1.89); 0.639	0.88(0.60–1.30); 0.530
Alcohol							
Yes	120/143	117/87	23/11	**1.60(1.11–2.32); 0.012**	**2.49(1.17–5.32); 0.018**	**2.03(0.97–4.26); 0.061**	**1.70(1.20–2.43); 0.003**
No	118/184	54/95	8/12	0.88(0.59–1.32); 0.543	1.03(0.41–2.61); 0.944	1.08(0.43–2.69); 0.872	0.90(0.61–1.33); 0.590
Age (years)							
< 60	151/192	95/103	14/15	1.17(0.83–1.67); 0.374	1.19(0.56–2.54); 0.658	1.12(0.53–2.37); 0.767	1.18(0.84–1.64); 0.349
≥60	87/135	76/79	17/8	1.48(0.98–2.24); 0.063	**3.28(1.35–7.91); 0.009**	**2.78(1.17–6.59); 0.021**	**1.65(1.11–2.45); 0.014**
BMI							
< 25	119/167	83/90	16/12	1.29(0.89–1.89); 0.183	1.87(0.85–4.10); 0.118	1.70(0.79–3.67); 0.179	1.36(0.95–1.96); 0.095
≥25	119/160	88/92	15/11	1.28(0.88–1.86); 0.202	1.82(0.81–4.11); 0.148	1.65(0.74–3.68); 0.217	1.34(0.93–1.92); 0.117
PTEN (rs3830675)	**CC**	**CT**	**TT**	**CT vs. CC**	**TT vs.CC**	**TT vs. CT + CC**	**TT + CT vs.CC**
Sex							
Male	89/131	86/93	23/22	1.36(0.91–2.03); 0.129	1.54(0.81–2.93); 0.189	1.34(0.72–2.48); 0.355	1.40(0.96–2.03); 0.082
Female	110/147	108/121	25/19	1.20(0.84–1.72); 0.313	1.76(0.92–3.35); 0.087	1.61(0.87–3.00); 0.133	1.28(0.91–1.80); 0.160
Smoking							
Yes	92/129	125/97	28/18	**1.80(1.24–2.63); 0.002**	**2.18(1.14–4.18); 0.019**	1.62(0.87–3.01); 0.128	**1.87(1.30–2.68); 0.001**
No	107/149	69/117	20/23	0.83(0.56–1.22); 0.341	1.21(0.63–2.32); 0.563	1.31(0.70–2.46); 0.401	0.89(0.62–1.29); 0.537
Alcohol							
Yes	95/121	135/108	31/13	**1.59(1.10–2.30); 0.014**	**3.04(1.51–6.12); 0.002**	**2.37(1.21–4.65); 0.012**	**1.75(1.22–2.50); 0.002**
No	104/157	59/106	17/28	0.85(0.57–1.27); 0.425	0.92(0.48–1.76); 0.793	0.98(0.52–1.84); 0.940	0.86(0.59–1.26); 0.440
Age (years)							
< 60	118/156	118/134	25/21	1.16(0.83–1.64); 0.387	1.57(0.84–2.95); 0.157	1.46(0.80–2.68); 0.218	1.22(0.88–1.70); 0.238
≥60	81/122	76/80	23/20	1.45(0.95–2.21); 0.085	1.73(0.89–3.36); 0.104	1.47(0.78–2.78); 0.232	**1.51(1.01–2.24); 0.043**
BMI							
< 25	100/146	93/101	25/23	1.34(0.92–1.97); 0.127	1.59(0.85–2.95); 0.145	1.39(0.77–2.53); 0.278	1.39(0.97–1.99); 0.072
≥25	99/132	101/113	23/18	1.20(0.83–1.75); 0.335	1.70(0.87–3.33); 0.119	1.56(0.82–2.97); 0.177	1.27(0.89–1.82); 0.189

BMI, body mass index.
Bold values are statistically significant ($P < 0.05$).

models (gene variants in the mTOR signaling pathway and smoking; gene variants in the mTOR signaling pathway and drinking) (Table 5). The IRS1 rs1801278 polymorphism or drinking/smoking was not correlated with the risk of OA. Interestingly, the combined effect of the IRS1 rs1801278 variant and drinking/smoking conferred susceptibility to OA, which confirmed the gene-environment interaction. This result also held true for PTEN rs3830675 polymorphism.

A strong significant interaction was obtained between GRS and OA risk in patients and controls. Individuals with 4–6 risk alleles having a higher risk (2.42-fold) of developing OA compared to individuals without the risk alleles (Table 6).

The FPRP values for mTOR rs11034528, IRS1 rs1801278 or PTEN rs3830675 polymorphism at the different P levels are summarized in Table 7. At the level of 0.1, some FPRPs were ≤0.20, indicating that the significant associations between mTOR rs11034528 or IRS1 rs1801278 polymorphism and OA risk were noteworthy under the allelic model (Table 7).

3.3. Genotype and mRNA expression correlation analysis

Three polymorphisms (mTOR [rs1034528], IRS1 [rs1801278] and PTEN [rs3830675]) were significantly associated with an increased risk of OA, while the GTEx portal database had data for only genotype and mRNA expression for mTOR rs1034528 polymorphism. Up-regulated mTOR expression was observed in the whole blood of individuals carrying the CC genotype of the mTOR rs1034528 polymorphism compared with those carrying the GG genotype (Fig. 1, $P = 5.5 \times 10^{-12}$).

4. Discussion

In this study, mTOR-pathway-related gene polymorphism analyses showed that AKT rs1130233 or REDD1 rs1053639 polymorphism was negatively associated with OA risk. However, mTOR rs1034528, IRS1 rs1801278 or PTEN rs3830675 polymorphism was demonstrated to be related to an increased risk of OA. A strong interaction was found between genetic and environmental factors under the combined models (IRS1 rs1801278/PTEN rs3830675 polymorphism and smoking/

Table 4
The associations between rs1034528/ rs1801278/ rs3830675 polymorphism on the mTOR signaling pathway and clinical characteristics of osteoarthritis.

Characteristics	Genotype distributions			
rs1034528	**GG**	**GC**	**CC**	**GC + CC**
ESR				
≥10/ < 10	90/126	70/114	22/18	92/132
OR (95%CI); *P*-value	1.0 (reference)	1.16(0.78–1.74); 0.461	0.58(0.30–1.15); 0.121	1.03(0.70–1.50); 0.899
CRP				
≥25/ < 25	14/202	9/125	2/38	11/163
OR (95%CI); *P*-value	1.0 (reference)	0.96(0.41–2.29); 0.931	1.32(0.29–6.03); 0.723	1.03(0.45–2.32); 0.949
K-L grade				
III + IV/II	189/27	156/28	35/5	191/33
OR (95%CI); *P*-value	1.0 (reference)	1.26(0.71–2.22); 0.432	1.00(0.36–2.77); 1.000	1.21(0.70–2.09); 0.496
VAS				
≥6/ < 6	203/13	171/13	38/2	209/15
OR (95%CI); *P*-value	1.0 (reference)	1.19(0.54–2.63); 0.672	0.82(0.18–3.79); 0.801	1.12(0.52–2.41); 0.771
Lequesne's index				
≥12/ < 12	207/9	177/7	40/0	217/0
OR (95%CI); *P*-value	1.0 (reference)	0.91(0.33–2.49); 0.854	**1.04(1.02–1.07); 0.189**	**1.04(1.02–1.07); 0.002**
rs1801278	**CC**	**CG**	**GG**	**CG + GG**
ESR				
≥10/ < 10	101/137	69/102	11/20	80/122
OR (95%CI); *P*-value	1.0 (reference)	0.92(0.62–1.37); 0.673	0.75(0.34–1.63); 0.461	0.89(0.61–1.30); 0.547
CRP				
≥25/ < 25	13/225	10/161	2/29	12/190
OR (95%CI); *P*-value	1.0 (reference)	1.08(0.46–2.51); 0.867	1.19(0.26–5.56); 0.822	1.09(0.49–2.45); 0.829
K-L grade				
III + IV/II	207/31	145/26	27/4	172/30
OR (95%CI); *P*-value	1.0 (reference)	0.84(0.48–1.47); 0.531	1.01(0.33–3.09); 0.985	0.86(0.50–1.48); 0.581
VAS				
≥6/ < 6	224/14	159/12	29/2	188/14
OR (95%CI); *P*-value	1.0 (reference)	0.83(0.37–1.84); 0.643	0.91(0.20–4.19); 0.900	0.84(0.39–1.81); 0.654
Lequesne's index				
≥12/ < 12	230/8	164/7	30/1	194/8
OR (95%CI); *P*-value	1.0 (reference)	0.82(0.29–2.29); 0.698	1.04(0.13–8.64); 0.969	0.84(0.31–2.29); 0.738
rs3830675	**CC**	**CT**	**TT**	**CT + TT**
ESR				
≥10/ < 10	81/118	78/116	23/25	101/141
OR (95%CI); *P*-value	1.0 (reference)	0.98(0.66–1.47); 0.920	1.34(0.71–2.52); 0.365	1.08(0.74–1.57); 0.711
CRP				
≥25/ < 25	14/185	8/186	3/45	11/231
OR (95%CI); *P*-value	1.0 (reference)	0.57(0.23–1.39); 0.215	0.88(0.24–3.20); 0.847	0.63(0.28–1.42); 0.264
K-L grade				
III + IV/II	172/27	167/27	41/7	208/34
OR (95%CI); *P*-value	1.0 (reference)	0.97(0.55–1.72); 0.920	0.92(0.37–2.26); 0.855	0.96(0.56–1.66); 0.884
VAS				
≥6/ < 6	183/16	187/7	43/5	230/12
OR (95%CI); *P*-value	1.0 (reference)	2.34(0.94–5.81); 0.068	0.75(0.26–2.17); 0.597	1.68(0.77–3.63); 0.191
Lequesne's index				
≥12/ < 12	192/7	187/7	46/2	133/9
OR (95%CI); *P*-value	1.0 (reference)	0.97(0.34–2.83); 0.961	0.84(0.17–4.17); 0.830	0.54(0.20–1.48); 0.231

ESR, erythrocyte sedimentation rate; CRP, C-reaction protein; VAS, Visual Analogue Scale; K-L grade, Kellgren-Lawrence grade
Bold values are statistically significant ($P < 0.05$).

drinking). Furthermore, a high GRS significantly increased the risk of OA.

Chondrocyte death and degeneration of the extracellular matrix are the central characteristics of OA pathogenesis [15]. OA is associated with decreased autophagy, which is reported to provide cytoprotective effects in articular cartilage [15]. In the rat OA model, inhibition of the phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR signaling pathway enhances autophagy of articular chondrocytes and reduces inflammatory responses [16]. Notably, SNPs may affect OA susceptibility by influencing gene expression and function [17,18]. Many studies have investigated the effect of the mTOR signaling pathway on the development of OA [16,19]; however, mTOR-related gene polymorphisms have not yet been reported in OA.

We selected five polymorphisms that are significantly associated with cancer to explore their associations with OA susceptibility. The AKT rs1130233 polymorphism is correlated with an increased risk of gastric cancer [20] and breast cancer [21]. In a meta-analysis of 23 original articles, Jin et al. demonstrated that the mTOR rs1034528 polymorphism increased the risk of cancer [22]. Similarly, a meta-analysis of four studies involving 3708 patients with colorectal cancer and 4176 controls conducted by Li et al. revealed that the IRS-1 rs1801278 polymorphism exhibited a negative association with susceptibility to colorectal cancer [11]. A small meta-analysis indicated a strong correlation between PTEN rs3830675 polymorphism and cancer risk under five models [23]. In contrast, genotypic and allelic frequencies of the REDD1 rs1053639 polymorphism did not differ significantly among two groups under the allelic model [24].

Our results indicated that the mTOR rs1034528, IRS1 rs1801278 or PTEN rs3830675 polymorphism, but not AKT rs1130233 or REDD1 rs1053639 polymorphisms, correlated with an increased risk of OA in a Chinese population. The significant correlation of mTOR rs1034528 or REDD1 rs1053639 polymorphism with increased risk of OA is similar to the results of two previous studies [22,24], whereas the correlation of mTOR rs1034528, IRS1 rs1801278 or PTEN rs3830675 polymorphism is inconsistent with previous reports [11,20,23]. Three factors may account for these discrepancies. First, there are differences in the pathogenesis of cancer and OA and these gene polymorphisms may have disease-dependent functionality. Second, false-positive or false-negative

Table 5
Interaction between genetic polymorphisms on the mTOR signaling pathway and smoking/drinking.

G[a]	E[b]	Case	Control	OR (95%CI); P value	Reflecting information
AKT (rs1130233)	Smoking				
+	+	127	123	**1.60(1.12,2.27); 0.010**	G, E combined effect
+	–	96	135	1.10(0.76,1.58); 0.611	G alone effect
–	+	118	120	**1.52(1.06,2.18); 0.022**	E alone effect
–	–	99	153	1.00	Common control
	Drinking				
+	+	130	119	**1.91(1.33,2.74); < 0.001**	G, E combined effect
+	–	93	139	1.17(0.80,1.70); 0.417	G alone effect
–	+	131	123	**1.86(1.29,2.67); 0.001**	E alone effect
–	–	86	150	1.00	Common control
REDD1 (rs1053639)	Smoking				
+	+	151	156	1.39(0.97,2.00); 0.071	G, E combined effect
+	–	114	171	0.96(0.66,1.39); 0.825	G alone effect
–	+	94	87	**1.56(1.04,2.33); 0.033**	E alone effect
–	–	82	118	1.00	Common control
	Drinking				
+	+	156	152	**1.66(1.15,2.41); 0.007**	G, E combined effect
+	–	109	175	1.01(0.69,1.48); 0.964	G alone effect
–	+	105	90	**1.89(1.26,2.84); 0.002**	E alone effect
–	–	71	115	1.00	Common control
MTOR (rs1034528)	Smoking				
+	+	119	95	**2.10(1.44,3.06); < 0.001**	G, E combined effect
+	–	105	137	1.29(0.89,1.85); 0.176	G alone effect
–	+	126	148	**1.43(1.00,2.03); 0.047**	E alone effect
–	–	90	151	1.00	Common control
	Drinking				
+	+	136	113	**2.22(1.56,3.17); < 0.001**	G, E combined effect
+	–	88	119	1.37(0.94,1.99); 0.102	G alone effect
–	+	124	129	**1.78(1.25,2.53); 0.001**	E alone effect
–	–	92	170	1.00	Common control
IRS1 (rs1801278)	Smoking				
+	+	140	106	**1.87(1.34,2.62); < 0.001**	G, E combined effect
+	–	62	99	0.89(0.60,1.31); 0.547	G alone effect
–	+	104	137	1.08(0.77,1.51); 0.669	E alone effect
–	–	134	190	1.00	Common control
	Drinking				
+	+	140	98	**2.23(1.58,3.15); < 0.001**	G, E combined effect
+	–	62	107	0.90(0.61,1.33); 0.609	G alone effect
–	+	120	143	1.31(0.94,1.83); 0.116	E alone effect
–	–	118	184	1.00	Common control
PTEN (rs3830675)	Smoking				
+	+	153	115	**1.85(1.31,2.62); < 0.001**	G, E combined effect
+	–	89	140	0.89(0.62,1.27); 0.511	G alone effect
–	+	92	129	0.99(0.69,1.43); 0.970	E alone effect
–	–	107	149	1.00	Common control
	Drinking				
+	+	166	121	**2.07(1.47,2.91); < 0.001**	G, E combined effect
+	–	76	134	0.86(0.59,1.25); 0.417	G alone effect
–	+	95	121	1.19(0.82,1.71); 0.362	E alone effect
–	–	104	157	1.00	Common control

[a] G (+): rs1130233/rs1053639/rs11034528/rs1801278/rs3830675 variants (Heterozygous and homozygous); G (−): wild type.
[b] E(+): smoking/non-smoking; E(−): non-smoking/non-drinking.

results may have been obtained in this study due to the moderate sample size. Third, clinical heterogeneity and differences in ethnicity also exert significant effects on the results.

Several limitations of this study warrant careful consideration. First, environmental factors, such as occupation and diet, were not taken into consideration. Second, selection bias of hospital-based study could not be avoided during the collection process. Third, the sample size was not sufficient to obtain significant results. In addition, we could not rule out

Table 6
The association of genetic risk score of mTOR signaling pathway with risk of osteoarthritis.

GRS	Case (n = 438)	Control (n = 527)	OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
	N (%)	N (%)				
0	9(2.1%)	20(3.8%)	1		1	
1–3	261(59.6%)	352(66.8%)	1.65(0.74,3.68)	0.223	1.66(0.74,3.71)	0.215
4–6	159(36.3%)	146(27.7%)	**2.42(1.07,5.49)**	**0.034**	**2.44(1.08,5.53)**	**0.033**
7–8	9(2.1%)	9(1.7%)	2.22(0.66,7.48)	0.197	2.23(0.66,7.52)	0.195

The genotyping was successful in: 438 cases and 527 controls.
Adjusted for sex and age.
*Bold values are statistically significant ($P < 0.05$)

Table 7
False-positive report probability values for associations between mTOR pathway-related gene polymorphisms and risk of osteoarthritis.

Variables	OR (95%CI)	*P* value	Power	Prior Probability				
				0.25	0.1	0.01	0.001	0.0001
MTOR (rs11034528 G/C)								
CC vs. GG	**1.68(1.03–2.75)**	**0.040**	0.504	0.192	0.417	0.887	0.988	0.999
GC + CC vs. GG	**1.34(1.04–1.73)**	**0.023**	0.489	0.124	0.297	0.823	0.979	0.998
C vs. G	**1.44 (1.18–1.75)**	**0.013**	0.881	0.042	0.117	0.594	0.936	0.993
IRS1 (rs1801278 C/G)								
GG vs. CC	**1.85(1.05–3.25)**	**0.033**	0.503	0.164	0.371	0.867	0.985	0.998
CG + GG vs. CC	**1.35(1.05–1.74)**	**0.022**	0.511	0.114	0.279	0.810	0.977	0.998
G vs. C	**1.32(1.07–1.63)**	**0.009**	0.487	0.053	0.143	0.647	0.949	0.995
PTEN (rs3830675 C/T)								
TT vs. CC	**1.64(1.04–2.58)**	**0.034**	0.508	0.167	0.376	0.869	0.985	0.999
CT + TT vs. CC	**1.33(1.03–1.72)**	**0.027**	0.485	0.143	0.334	0.846	0.982	0.998
T vs. C	**1.27(1.05–1.55)**	**0.014**	0.458	0.084	0.216	0.752	0.968	0.997

Bold values are statistically significant ($P < 0.05$).

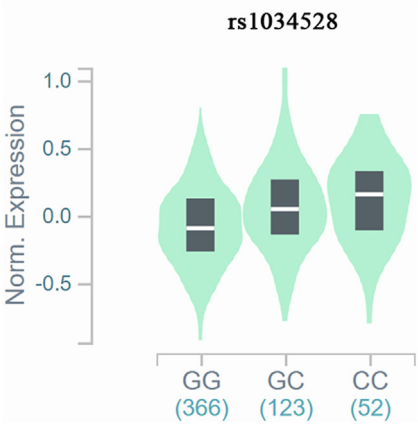

Fig. 1. Functional implication of mTOR rs1034528 polymorphism.

the possibility of false-positive and false-negative results. Fourth, we did not confirm the positive association between three SNPs and OA risk experimentally.

This study confirms that mTOR rs1034528 or IRS1 rs1801278 or PTEN rs3830675 polymorphisms on the mTOR signaling pathway conferred susceptibility to knee OA in a Chinese population. Further population studies are required to identify the genetic profiles of individuals with knee OA.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.105960.

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