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Association between transforming growth factor-beta 1 gene single nucleotide polymorphisms and knee osteoarthritis susceptibility in a Chinese Han population

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Abstract

Objective: To investigate associations between single polymorphisms (SNPs) rs1800469 and rs1982073 in the transforming growth factor-β1 gene (*TGF-β1*) and knee osteoarthritis (OA) susceptibility in a Chinese Han population.

Methods: *TGF-β1* rs1800469 and rs1982073 were genotyped in patients with knee OA and age- and sex-matched OA-free controls from a Chinese Han population. The association was further analyzed according to gender and age.

Results: A total of 765 patients with knee OA and 780 controls were included. CT and CT + CC genotypes of rs1982073, and variant C, were associated with a significantly increased risk of knee OA. Stratification analysis showed that the association between the OA risk and rs1982073 CT heterozygotes compared with TT homozygotes was stronger in females and those aged >65 years. In contrast, CT, TT, and CT + TT genotypes of rs1800469 were not significantly associated with the risk of knee OA, even after further stratification analysis for gender and age.

Conclusions: The *TGF-β1* rs1982073 T to C change and the variant C genotype may contribute to knee OA risk in the Chinese Han population.

Keywords

Knee osteoarthritis, polymorphism, transforming growth factor-beta 1

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Introduction

Osteoarthritis (OA) is a multifactorial disease characterized by degeneration of the articular cartilage,[1] which affects almost 27 million people in the United States.[2] The etiology of OA is complex, but it has been shown to be linked with environmental factors including aging, obesity, and previous injury.[3–5] Genetic studies have also shown that a number of candidate genes, such as those encoding collagens, vitamin D, bone, and cartilage growth factors, are associated with OA.[6,7]

TGF-β1 is one of the candidate genes contributing to the risk of knee OA development. It is composed of six large introns and seven exons, and is localized on chromosome 19q13.1–q13.3.[10] The TGF-β family consists of the three isoforms TGF-β1, TGF-β2, and TGF-β3,[8] which encode protein precursors of 20–30 amino acids with an N-terminal signal peptide[9] Following proteolytic cleavage, the protein precursors become mature TGF-β molecules.[9] TGF-β1 plays an important role in osteoblast differentiation and growth, acting on bone resorption and recovery,[11] while TGF-β/Smad3 signals repress chondrocyte hypertrophic differentiation and are required for maintaining articular cartilage.[12] Recently, both *in vitro* and *in vivo* studies suggested that the TGF-β/SMAD pathway plays a critical role during OA development[14,15] and aneurysms-osteoarthritis syndrome.[16,17] Moreover, the TGF-β type II receptor was shown to promote terminal chondrocyte differentiation and osteoarthritis in mouse skeletal tissue.[13]

A recent study showed that *Mmp13* and *Adamts5* are critical downstream target genes involved in the TGF-β signaling pathway during the development of OA.[18] Previous studies showed that the *TGF-β1* single nucleotide polymorphism (SNPs) rs1982073,[19] in exon 1, is associated with being overweight and may contribute to the risk of osteoporosis (OP) and OA. Additionally, SNP rs1800469, in the promoter of *TGF-β1*, is linked with OP in Japanese women.[20]

In this study, we examined whether SNPs and genetic variants of *TGF-β1* are associated with the pathogenesis of knee OA and determined their impact on the parameters of OA.

Materials and methods

Patients

In this case–control study, Han Chinese patients diagnosed with knee arthritis between March 2009 and May 2016 at the Department of Orthopaedics, Tongde Hospital of Zhejiang Province, Hangzhou, China were evaluated for inclusion. Patients were included if their chief complaint was usage-related pain that often worsened at the end of the day and was relieved by rest. Anteroposterior extended-view weight-bearing radiographs of the involved knees were obtained. Two examiners who were blinded to the grouped information assessed the radiographic data to provide a Kellgren–Lawrence (KL) score ranging from 0 to 4,[21] where 0 = none; 1 = possible osteophytes only; 2 = definite osteophytes and possible joint space narrowing; 3 = moderate osteophytes and/or definite joint space narrowing; and 4 = large osteophytes, severe joint space narrowing, and/or bony sclerosis. Patients with a KL score of $\geq$2 were defined as radiographic OA and included in the study. Other etiologies of knee joint disease such as inflammatory arthritis (rheumatoid, polyarthritic, or autoimmune disease), post-traumatic or postseptic arthritis, skeletal dysplasia, or developmental dysplasia were excluded.

Age- ($\pm$ 5 years) and sex-matched healthy volunteers were recruited from the same hospital during the same period. Inclusive

criteria for the controls included no history of OA and a KL score <2 on radiographic examination. The baseline of all study participants including age, sex, weight, height, and body mass index (BMI) was recorded.

Written informed consent was provided from all participants and the study protocol was approved by the Ethics Committee of the Tongde Hospital of Zhejiang Province, Hangzhou, China.

Genotyping

Venous blood samples from all participants were obtained using 20 g/L ethylene diamine tetra-acetic acid anticoagulant. Genomic DNA was extracted from 250 µl of venous blood using a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) and stored at –80°C until analysis according to the manufacturer's instructions. The *TGF-β1* SNPs rs1800469 and rs1982073 were genotyped using the TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA). We performed PCR amplification[22] with 100 ng of genomic DNA, 0.1 µM of each probe, 0.2 µM of each primer, 200 µM of each deoxyribonucleotide, 3 mM $MgCl_2$, and 1 U Platinum Taq DNA polymerase. PCR amplification was carried with an initial denaturation at 95°C for 2 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing at 65°C for 30 s using the 7900HT Fast Real-Time PCR System (Applied Biosystems).[23] ABI PRISM 7900 Sequence Detection System software version 2.3 (Applied Biosystems) was used to perform data analyses. For confirmation, approximately 5% of the samples were selected randomly for repeated genotyping.

Reverse-transcription PCR analysis

Total RNA was extracted from venous blood using TRIzol reagent (Invitrogen, Waltham, MA, USA) according to the manufacturer's instructions. A total of 2 µg mRNA was then converted into cDNA using the PrimeScript RT reagent kit (Takara Biotechnology Co., Ltd., Dalian, China). The following primers were used: matrix metalloproteinases-13 (MMP-13) forward 5'-TTCGGCTTAGAGGTGACA GG-3' and reverse 5'-ACTCTTGCCGGT GTAGGTGT-3'; and type II collagen (Col II) forward 5'-GCACCCATGGACATT GGAGG-3' and reverse 5'-AGCCCCGCA CGGTCTTGCTTGCTT-3'. The housekeeping gene 18 S rRNA was amplified as an internal reference using the primers: forward 5'-GACGGACCAGAGCGAAA GC-3' and reverse 5'-CGCCAGTCGGCA TCGTTTATG-3'.

Statistical analyses

We tested the Hardy–Weinberg equilibrium (HWE) using a goodness-of-fit χ^2-test to compare observed and expected genotype frequencies in controls. We also evaluated differences in the distributions of demographic characteristics and rs1800469 and rs1982073 genotypes between patients with knee OA and controls using the Wilcoxon's test for age and the χ^2-test for all other variances. Associations between *TGF-β1* variants and OA risk were estimated by calculating odds ratios and 95% confidence intervals using both univariate and multivariate logistic regression analysis with adjustments for age, sex, and BMI. We performed two-sided tests for statistical analyses and a P-value < 0.05 was considered to be statistically significant. All statistical analyses were performed using SPSS® version 16.0 (SPSS Inc., Chicago, IL, USA). The statistical power of the study as calculated using Power and Sample Size Calculation software version 3.0 (Department of Biostatistics, Vanderbilt University, Nashville, TN, USA) was

0.90, according to the methods described previously.[24]

Results

A total of 515 Chinese Han participants were included. Of these, 765 were diagnosed with radiographic knee OA and were included in the study. A total of 780 age- and sex-matched healthy controls were recruited. There were no significant differences in demographic characteristics between the two groups (Table 1). Clinical symptoms of OA patients included knee pain which was worse at the end of the day and was relieved by rest. Radiographic findings included KL = 4 defined as large osteophytes and severe joint space narrowing.

Table 1. Demographic characteristics of Chinese Han patients with osteoarthritis (OA) of the knee and controls.

	Patients with OA	Controls
Characteristic	$n = 765$	$n = 780$
Age, years	63.9 ± 7.6	63.6 ± 8.8
Weight, kg	67.6 ± 8.5	66.2 ± 7.6
Height, cm	161.2 ± 7.2	160.6 ± 7.6
Body mass index, kg/cm^2	26.0 ± 3.8	25.7 ± 4.2

Data presented as mean ± SD. No significant between-group differences observed ($P \geq 0.05$, using Wilcoxon's test for age and χ^2-test for other parameters).

Genotype frequencies for SNPs rs1800469 and rs1982073 in the controls were consistent with HWE (data not shown). The repeated genotyping results of randomly selected samples were 100% concordant.

Genotype and allele distributions of SNPs rs1800469 and rs1982073 in patients with knee OA and healthy controls are shown in Table 2. The genotype distribution of rs1982073 was significantly different between the two groups ($P < 0.001$). After adjustment for age, sex, and BMI,

Table 2. Associations between *TGF-β1* polymorphisms rs1982073 and rs1800469 and risk of osteoarthritis (OA) in Chinese Han patients with OA of the knee and controls.

SNP	Genotype/ allele	Patients with knee OA $n = 765$	Controls $n = 780$	Statistical significance[a]	Adjusted odds ratio[b]	95% confidence intervals[b]
rs1982073	TT	309 (40.3)	453 (58.1)	$P = 0.001$	0.49	0.34, 0.69
	CT	450 (58.8)	318 (40.8)	$P = 0.001$	2.08	1.46, 2.95
	CC	6 (0.9)	9 (1.1)	$P = 0.67$	0.68	0.11, 4.09
	CT + CC	456 (59.7)	327 (41.9)	$P = 0.001$	2.04	1.44, 2.90
	C	462 (30.2)	336 (21.5)	$P = 0.001$	1.58	1.19, 2.09
	T	1068 (69.8)	1494 (78.5)	$P = 0.001$	0.10	0.06, 0.16
rs1800469	CC	420 (55.0)	435 (55.8)	$P = 0.84$	0.97	0.68, 1.37
	CT	288 (37.6)	285 (36.5)	$P = 0.79$	1.05	0.73, 1.50
	TT	57 (7.4)	60 (7.7)	$P = 0.92$	0.97	0.50, 1.86
	CT + TT	354 (45.0)	345 (44.2)	$P = 0.64$	1.09	0.77, 1.54
	T	411 (26.9)	405 (26.0)	$P = 0.74$	1.05	0.79, 1.38
	C	1137 (73.1)	1125 (74.0)	$P = 0.43$	1.12	0.85, 1.47

Data presented as *n* (%) of patients. [a]Patients with knee OA versus controls; χ^2-test. [b]Versus TT genotype or T allele; estimated using multiple logistic regression analyses and adjusted for age, sex, and body mass index. No statistically significant differences ($P \geq 0.05$).

a significantly increased risk of knee OA was associated with the CT genotype of rs1982073 compared with the TT genotype. Additionally, those carrying at least one C allele (CT + CC) had a significantly increased risk of knee OA compared with those with no C allele (TT). On the other hand, the CT and TT genotypes of rs1800469 were not significantly associated with the risk of knee OA.

We also performed stratification analysis to evaluate the potential association of genetic variants of *TGF-β1* rs1982073 with knee OA risk in subgroups based on age and gender. When stratified by age, both younger (≤65 years) and older (>65 years) patients showed significant differences in genotype frequencies compared with healthy controls ($P < 0.001$) (Table 3). Evaluation of the association between C (CT + CC) allele carriers and the risk of OA using logistic regression analysis showed that CT heterozygotes had a 1.84-fold increased risk of OA compared with TT homozygotes in the younger patients group (Table 3). Similarly, CT heterozygotes carried a 2.33-fold increased risk of OA compared with TT homozygotes in the older patients group (Table 3). When stratified by gender, both male and female patients showed significant differences in genotype frequencies between patients with OA and healthy controls ($P < 0.01$) (Table 3). Additionally, when compared with TT homozygotes, CT heterozygotes showed a 1.76- and 2.40-fold increased risk of knee OA in males and females, respectively (Table 3).

In contrast, logistic regression analysis of *TGF-β1* rs1800469 genetic variants showed that the CT and TT genotypes were not associated with knee OA susceptibility when stratified by age or gender (Table 4).

We regarded individuals with the rs1982073 CC genotype as SNP-positive and those with the TT genotype as SNP-negative. We found that *MMP-13* mRNA expression in SNP-positive patients was

Table 3. Logistic regression analysis of rs1982073 genotype frequencies and risk of osteoarthritis (OA) in Chinese Han patients with OA of the knee and controls.

	Patients with knee OA ($n = 765$)				Controls ($n = 780$)				CC versus TT		CT versus TT	
Parameter	*n*	TT	CT	CC	*n*	TT	CT	CC	Adjusted OR[a]	95% CI[a]	Adjusted OR[a]	95% CI[a]
Age group												
≤65 years	375	162 (43.2)	210 (56.0)	3 (0.8)	330	192 (58.2)	135 (40.1)	3 (1.7)	0.88	0.05, 14.22	1.84	1.09, 3.09
>65 years	390	147 (37.7)	240 (61.5)	3 (0.8)	450	261 (58.0)	183 (40.7)	6 (1.3)	0.57	0.05, 6.40	2.33	1.44, 3.77
Sex												
Male	366	153 (41.8)	210 (57.4)	3 (0.8)	360	201 (55.8)	156 (43.3)	3 (0.9)	0.98	0.06, 15.91	1.76	1.06, 2.93
Female	399	156 (39.1)	240 (60.2)	3 (0.7)	420	252 (60.0)	162 (38.6)	6 (1.4)	0.52	0.05, 5.83	2.40	1.48, 3.91

Data presented as *n* (%) of patients.
OR, odds ratio.
CI, confidence interval.
[a]Adjusted for the other covariate presented in this table and for body mass index using a logistic regression model for each stratum.

Table 4. Logistic regression analysis of rs1800469 genotype frequencies and the risk of osteoarthritis (OA) in Chinese Han patients with OA of the knee and controls.

	Patients with knee OA ($n = 765$)				Controls ($n = 780$)				TT versus CC		CT versus CC	
Parameter	*n*	CC	CT	TT	*n*	CC	CT	TT	Adjusted OR[a]	95% CI[a]	Adjusted OR[a]	95% CI[a]
Age group												
≤65 years	375	204 (54.4)	144 (38.4)	27 (7.2)	330	180 (54.5)	126 (38.2)	24 (7.3)	0.99	0.37, 2.66	1.01	0.60, 1.71
> 65 years	390	216 (55.4)	144 (36.9)	30 (7.7)	450	255 (56.7)	159 (35.3)	36 (8.0)	1.07	0.66, 1.75	1.51	0.91, 2.49
Sex												
Male	366	204 (55.7)	138 (37.7)	24 (6.6)	360	189 (52.5)	150 (41.7)	21 (5.8)	1.13	0.40, 3.23	0.85	0.51, 1.42
Female	399	216 (54.1)	150 (37.6)	33 (8.3)	420	246 (58.6)	135 (32.1)	39 (9.3)	0.88	0.38, 2.04	1.27	0.77, 2.09

Data presented as number of patients (%).
OR, odds ratio.
CI, confidence interval.
[a]Adjusted for the other covariate presented in this table and for body mass index using a logistic regression model for each stratum.

higher than that in SNP-negative patients (Figure 1), while type II collagen mRNA expression was lower in SNP-positive patients than in SNP-negative patients (Figure 2).

Discussion

In this case–control hospital-based study, associations between the *TGF-β1* SNPs rs1800469 and rs1982073 and the risk of knee OA were investigated in a Chinese Han population. We found that the CT genotype of SNP rs1982073 and variant C may contribute to the risk of knee OA, and that this risk was increased in older (i.e., >65 years of age) and female patients. To our knowledge, this is the first report linking *TGF-β1* SNP rs1982073 with knee OA in a Chinese Han population.

TGF-β1 is an inflammatory cytokine involved in the pathogenesis of OA[25] that participants in bone remodeling[26] and the regulation of cartilage development.[27] *TGF-β1* has nine polymorphic sites, among which rs1982073 causes a Leu to Pro substitution at position 10 of the TGF-β1 molecule which affects the peptide export efficiency caused by the T to C transition in the signal sequence. Previous studies reported that carriers of the variant C allele (CT or CC genotype) had higher serous TGF-β1 levels than those with other genotypes.[26,28]

A preliminary study revealed an association between SNP rs1982073 and severe adult hip OA secondary to developmental dysplasia of the hip in a Croatian population.[29] Other studies demonstrated that SNP rs1982073 may contribute to an increased risk of OP in Japanese and Chinese women.[20,30] However, no studies have previously examined the association between SNP rs1982073 and the risk of knee OA in a Chinese Han population. Combined with previous findings, the present study supports the suggestion that SNP rs1982073 might be

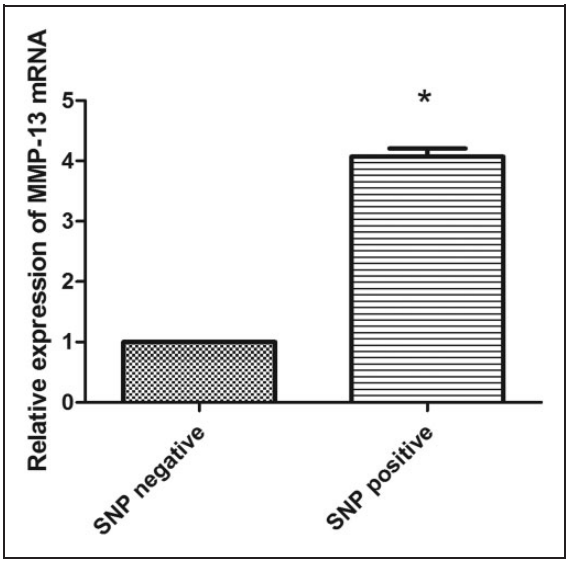

Figure 1. Relative *MMP-13* mRNA expression levels in SNP-negative and SNP-positive patients were analyzed by RT-PCR. The bar represents the mean ± SEM of two independent experiments. *$P < 0.05$ compared with SNP-negative patients.

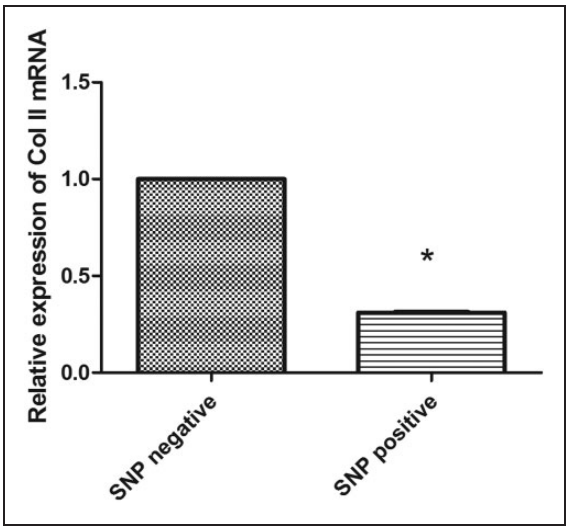

Figure 2. Relative Col II mRNA expression levels in SNP-negative and SNP-positive patients were analyzed by RT-PCR. The bar represents the mean ± SEM of two independent experiments. *$P < 0.05$ compared with SNP-negative patients.

a risk factor for OA. Individuals carrying *TGF-β1* rs1982073 CT + CC or CT genotypes had a higher risk of developing knee OA than those carrying the TT genotype, suggesting that the rs1982073 C allele might be associated with the development of knee OA.

According to the stratification analysis performed in this study, the association between the risk of knee OA and rs1982073 CT heterozygotes compared with TT homozygotes was stronger in female patients and older patients (>65 years). This is consistent with the increased incidence of knee OA reported in those aged >50 years and in females.[31] Because OA is a multifactorial disease, both gene–environment and gene–gene interactions may occur, and a single genetic variant is likely to be insufficient to predict overall risk. Further research is therefore needed to reveal the role of other SNPs in *TGF-β1* and related genes involved in similar biological pathways that may affect the etiology of OA.

Previous studies have also identified a significant association between the CT genotype of SNP rs1800469 and the risk of OP in Thai women.[32] However, no studies elucidated the association between SNP rs1800469 and the risk of knee OA. Thus, the present study investigated the associations between CT and TT genotypes of rs1800469 and the risk of knee OA, but the results were negative. Further stratification analysis by gender and age also did not reveal any significant association between knee OA susceptibility and rs1800469 genotypes.

TGF-β signaling in OA is associated with catabolic events that induce the production of MMPs. Col II is the major component of extracellular matrix that is degraded by MMP-13, and we showed that knee OA patients with the rs1982073 CC genotype had higher expression of *MMP-13* and lower expression of Col II than those with the TT genotype, which is consistent with previous findings.[33]

The present study has several limitations. First, as a hospital-based study, it may be subject to inherent bias; however, the C allele frequency in the control subjects was similar to that in the haplotype map database, and the genotype distributions of SNPs rs1800469 and rs1982073 in the controls were in HWE, indicating that the results did not suffer from selection bias. Second, the sample size was modest, so the results should be further confirmed in studies of a larger scale. Third, only two *TGF-β1* SNPs were investigated, but it is possible that SNPs at other loci may also be associated with susceptibility to OA.

In conclusion, this is the first study to demonstrate that the genotype distribution of the *TGF-β1* rs1982073 SNP differs significantly between patients with knee OA and healthy controls in the Chinese Han population. Larger-scale and more in-depth molecular studies are now required to confirm these findings and to elucidate the detailed roles of the rs1982073 SNP in the pathology of OA.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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References

1. Felson DT and Zhang Y. An update on the epidemiology of knee and hip osteoarthritis with a view to prevention. *Arthritis Rheum* 1998; 41: 1343–1355.
2. Lawrence RC, Helmick CG, Arnett FC, et al. Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. *Arthritis Rheum* 1998; 41: 778–799.
3. Jensen CH and Rofail S. Knee injury and obesity in patients undergoing total knee replacement: a retrospective study in 115 patients. *J Orthop Sci* 1999; 4: 5–7.
4. Blagojevic M, Jinks C, Jeffery A, et al. Risk factors for onset of osteoarthritis of the knee in older adults: a systematic review and meta-analysis. *Osteoarthritis Cartilage* 2010; 18: 24–33.
5. Grotle M, Hagen KB, Natvig B, et al. Obesity and osteoarthritis in knee, hip and/or hand: an epidemiological study in the general population with 10 years follow-up. *BMC Musculoskelet Disord* 2008; 9: 132.
6. Loughlin J. The genetic epidemiology of human primary osteoarthritis: current status. *Expert Rev Mol Med* 2005; 7: 1–12.
7. Valdes AM, Loughlin J, Oene MV, et al. Sex and ethnic differences in the association of ASPN, CALM1, COL2A1, COMP, and FRZB with genetic susceptibility to osteoarthritis of the knee. *Arthritis Rheum* 2007; 56: 137–146.
8. Patil AS, Sable RB and Kothari RM. An update on transforming growth factor-beta (TGF-beta): sources, types, functions and clinical applicability for cartilage/bone healing. *J Cell Physiol* 2011; 226: 3094–3103.
9. Brophy TM, Coller BS and Ahamed J. Identification of the thiol isomerase-binding peptide, mastoparan, as a novel inhibitor of shear-induced transforming growth factor beta1 (TGF-beta1) activation. *J Biol Chem* 2013; 288: 10628–10639.
10. Campos-Xavier B, Saraiva JM, Savarirayan R, et al. Phenotypic variability at the TGF-beta1 locus in Camurati-Engelmann disease. *Hum Genet* 2001; 109: 653–658.
11. Ochiai H, Okada S, Saito A, et al. Inhibition of insulin-like growth factor-1 (IGF-1) expression by prolonged transforming growth factor-beta1 (TGF-beta1) administration suppresses osteoblast differentiation. *J Biol Chem* 2012; 287: 22654–22661.
12. Yang X, Chen L, Xu X, et al. TGF-beta/Smad3 signals repress chondrocyte hypertrophic differentiation and are required for maintaining articular cartilage. *J Cell Biol* 2001; 153: 35–46.
13. Serra R, Johnson M, Filvaroff EH, et al. Expression of a truncated, kinase-defective TGF-beta type II receptor in mouse skeletal tissue promotes terminal chondrocyte differentiation and osteoarthritis. *J Cell Biol* 1997; 139: 541–552.
14. Shen, J, Li, S and Chen, D. TGF-β signaling and the development of osteoarthritis. *Bone Res* 2014; 2.
15. Valdes AM, Spector TD, Tamm A, et al. Genetic variation in the SMAD3 gene is associated with hip and knee osteoarthritis. *Arthritis Rheum* 2010; 62: 2347–2352.
16. van de Laar IM, van der Linde D, Oei EH, et al. Phenotypic spectrum of the SMAD3-related aneurysms-osteoarthritis syndrome. *J Med Genet* 2012; 49: 47–57.
17. van de Laar IM, Oldenburg RA, Pals G, et al. Mutations in SMAD3 cause a syndromic form of aortic aneurysms and dissections with early-onset osteoarthritis. *Nat Genet* 2011; 43: 121–126.
18. Shen J, Li J, Wang B, et al. Deletion of the transforming growth factor beta receptor type II gene in articular chondrocytes leads to a progressive osteoarthritis-like phenotype in mice. *Arthritis Rheum* 2013; 65: 3107–3119.
19. Utennam D, Tungtrongchitr A, Phonrat B, et al. Association of T869C gene polymorphism of transforming growth factor-beta1 with low protein levels and anthropometric indices in osteopenia/osteoporosis postmenopausal Thai women. *Genet Mol Res* 2012; 11: 87–99.
20. Yamada Y, Miyauchi A, Takagi Y, et al. Association of the C-509–>T

polymorphism, alone of in combination with the T869–>C polymorphism, of the transforming growth factor-beta1 gene with bone mineral density and genetic susceptibility to osteoporosis in Japanese women. *J Mol Med (Berl)* 2001; 79: 149–156.
21. Qiu XM, Jin CT and Wang W. Association between single nucleotide polymorphisms of sterol regulatory element binding protein-2 gene and risk of knee osteoarthritis in a Chinese Han population. *J Int Med Res* 2014; 42: 320–328.
22. Jia S, Dong W, Zhou X, et al. Association between TNFSF10 polymorphism and migraine susceptibility in a Chinese population. *J Int Med Res* 2015; 43: 326–331.
23. Qiu XM, Jin CT and Wang W. Association between single nucleotide polymorphisms of sterol regulatory element binding protein-2 gene and risk of knee osteoarthritis in a Chinese Han population. *J Int Med Res* 2014; 42: 320–328.
24. Dupont WD and Plummer WJ. Power and sample size calculations for studies involving linear regression. *Control Clin Trials* 1998; 19: 589–601.
25. Malemud CJ. Anticytokine therapy for osteoarthritis: evidence to date. *Drugs Aging* 2010; 27: 95–115.
26. Hinke V, Seck T, Clanget C, et al. Association of transforming growth factor-beta1 (TGFbeta1) T29 –> C gene polymorphism with bone mineral density (BMD), changes in BMD, and serum concentrations of TGF-beta1 in a population-based sample of postmenopausal german women. *Calcif Tissue Int* 2001; 69: 315–320.
27. Roelen BA and Dijke P. Controlling mesenchymal stem cell differentiation by TGFBeta family members. *J Orthop Sci* 2003; 8: 740–748.
28. Tzakas P, Wong BY, Logan AG, et al. Transforming growth factor beta-1 (TGFB1) and peak bone mass: association between intragenic polymorphisms and quantitative ultrasound of the heel. *BMC Musculoskelet Disord* 2005; 6: 29.
29. Kolundzic R, Trkulja V, Mikolaucic M, et al. Association of interleukin-6 and transforming growth factor-beta1 gene polymorphisms with developmental hip dysplasia and severe adult hip osteoarthritis: a preliminary study. *Cytokine* 2011; 54: 125–128.
30. Lau EM, Wong SY, Li M, et al. Osteoporosis and transforming growth factor-beta-1 gene polymorphism in Chinese men and women. *J Bone Miner Metab* 2004; 22: 148–152.
31. Zhang W, Doherty M, Peat G, et al. EULAR evidence-based recommendations for the diagnosis of knee osteoarthritis. *Ann Rheum Dis* 2010; 69: 483–489.
32. Utennam D, Tungtrongchitr A, Phonrat B, et al. Association of T869C gene polymorphism of transforming growth factor-beta1 with low protein levels and anthropometric indices in osteopenia/osteoporosis postmenopausal Thai women. *Genet Mol Res* 2012; 11: 87–99.
33. Vilar JM, Rubio M, Spinella G, et al. Serum Collagen Type II Cleavage Epitope and Serum Hyaluronic Acid as Biomarkers for Treatment Monitoring of Dogs with Hip Osteoarthritis. *PLoS One* 2016; 11: e149472.