Observational Study

Medicine®

OPEN

Effects of receptor activator nuclear factor κB gene polymorphisms on the susceptibility to knee osteoarthritis

A case–control study

Changcheng Wang, PhD[a], Li Luo, PhD[b], Fengde Tian, PhD[a], Ning An, PhD[a], Yao Zhang, PhD[a], Ruihu Hao, PhD[a], Dongyi Li, PhD[a], Zihao Zhou, PhD[a], Peng Xiao, PhD[a], Lin Guo, PhD[a,*]

Abstract

The present study aimed to explore genetic association of receptor activator nuclear factor κB (RANK) polymorphisms with individual susceptibility to knee osteoarthritis (OA) in different Kellgren–Lawrence (KL) grades.

This case–control study included 138 knee OA patients and 145 healthy individuals. *RANK* rs1805034 and rs8086340 polymorphisms were genotyped through polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The effects of *RANK* polymorphisms on knee OA risk were analyzed via χ^2 test or Fisher exact test, and the results were expressed using odds ratios (ORs) with corresponding 95% confidence intervals (CIs).

The C allele of rs1805034 polymorphism had significantly higher frequency in knee OA patients than in controls ($P = .044$), indicating that this allele could increase the risk of knee OA (OR = 1.424, 95% CI = 1.010–2.008). Besides, the CC genotype and C allele of the rs1805034 polymorphism were significantly associated with elevated risk of knee OA in moderate grade (CC vs TT: $P = .018$, OR = 3.071, 95% CI = 1.187–7.941; C vs T: $P = .012$, OR = 1.787, 95% CI = 1.131–2.823). However, rs8086340 polymorphism had no significant association with knee OA risk

The C allele of *RANK* rs1805034 polymorphism is closely correlated with increased risk of knee OA, especially for moderate grade.

Abbreviations: BMI = body mass index, CI = confidence interval, HWE = Hardy–Weinberg equilibrium, IRB = institutional review board, KL = Kellgren–Lawrence, MRI = magnetic resonance imaging, OA = osteoarthritis, OPG = osteoprotegerin, OR = odds ratio, RA = rheumatoid arthritis, RANK = receptor activator nuclear factor κB, RANKL = RANK-ligand, SNP = single nucleotide polymorphism.

Keywords: classification, knee osteoarthritis, polymorphisms, *RANK*

1. Introduction

Osteoarthritis (OA) is one of common chronic degenerative joint diseases, prevalent in middle, and older people.[1] Joint pain and stiffness are main symptoms of this disease.[2,3] In early stage, OA symptoms may present only during exercise; with its progression, the disease will show constant symptoms. OA reduces the life quality of the patients, representing a main cause of disability in the elderly.[4] In recent years, OA sees an increasing morbidity rate worldwide.[5] Thus, it is necessary to explore the etiology for this disorder. A variety of plausible factors have been confirmed to be involved in this disease.[6–9] The interactions between these factors play a crucial role in the initiation and development of OA.[10,11] However, not all individuals exposing to similar risk factors will eventually suffer from OA, and genetic factors would be responsible for tremendous differences in individual susceptibilities. It is well known that bone lesions are main pathological change in OA.[12] Thus, we hypothesized that genes associated with bone metabolism might affect OA risk.

Receptor activator nuclear factor κB (RANK), also known as TNF receptor superfamily member 11a (TNFRSF11A), is involved in RANK—RANK-ligand (RANKL) —osteoprotegerin (OPG) signaling pathway which participants in the regulation of bone metabolism, including the development and maturity of osteoclasts.[13,14] RANK is a type I transmembrane protein, usually abounding in osteoclasts and cartilage cells.[15,16] It has been reported that RANK was highly expressed in OA patients at advanced stages, especially in cartilage cells.[17] Human *RANK* gene is located at chromosome 18q21.33, and its genetic mutations may alter the protein functions.

Gene polymorphisms refer to the existence of 2 or more discontinuous variants, genotypes or alleles in biological genomes. Single nucleotide polymorphism (SNP) is a common type of gene polymorphisms and is abundant in human genome. Some SNPs have the capability to influence the gene functions, thus resulting in changes in biological traits, even diseases. Genetic association of SNPs with individual susceptibility to diseases has become a research hot in recent years. According to the 1000 genomes projects, minor allele frequencies of *RANK*

Editor: Yan Li.

The authors have no conflicts of interest to disclose.

[a] Department II of Orthopedics, [b] Department of Respiratory Medicine, Affiliated Zhongshan Hospital of Dalian University, Dalian, Liaoning, China.

[*] Correspondence: Lin Guo, Department II of Orthopedics, Affiliated Zhongshan Hospital of Dalian University, Dalian 116001, Liaoning, China (e-mail: owij3dh@163.com).

Medicine (2019) 98:13(e14933)

Received: 24 August 2017 / Received in final form: 16 January 2019 / Accepted: 28 February 2019

http://dx.doi.org/10.1097/MD.0000000000014933

Table 1

Primer sequences of *RANK* SNPs.

SNP	Position	Primer sequences	Annealing temperature, °C	Fragments length, bp	Restriction enzyme
rs1805034	Exon6	F: 5′-TGCTGTTCTCCTCCCTGTGG-3′ R: 5′-AGGGATCTAAACCAGCTGAC-3′	61	323	SsiI
rs8086340	Intron1	F: 5′-AGTGAAGATGCCTTTGACAGTGTGATG-3′ R: 5′-ACTGTTCCCCGCAAAGAGGCT-3′	61	210	TaqI

RANK = receptor activator nuclear factor κB, SNP = single nucleotide polymorphism.

gene polymorphisms rs1805034 and rs8086340 were more than 0.1 in Chinese Han in Beijing (CHB) population. Besides, rs1805034 and rs8086340 SNPs have already been explored in rheumatoid arthritis (RA).[18,19] The aim of the present study was to explore the associations of *RANK* gene rs1805034 and rs8086340 SNPs with OA risk in a Chinese Han population.

2. Materials and methods

2.1. Participants

This case-control study was approved by the institutional review board (IRB) of Affiliated Zhongshan Hospital of Dalian University (IRB number: DLU124-201411). Written informed consent was signed by every participant. Study process conformed to the Declaration of Helsinki.

The participants were recruited from Affiliated Zhongshan Hospital of Dalian University between January 2015 and December 2016. Knee OA patients were diagnosed via physical examinations and magnetic resonance imaging (MRI), according to corresponding criteria.[20] The patients with Kellgren–Lawrence (KL) score ≥2 were included in the case group.[21] KL classification was performed by experienced radiologist through MRI examinations. Healthy individuals had no signs of knee OA or the histories of other arthritis or joint diseases. Age and gender distributions in the controls were matched with those in the cases. Besides, individuals with inflammatory arthritis, severe trauma of knee joint or skeletal dysplasia were excluded from this study. Participants lacking essential data were also excluded from this study. During sample collection period, only 138 knee OA patients and 145 healthy controls met the included criteria. The statistical power based on these included subjects was 0.49, less than 0.7, and this condition might reduce the accountability of our results.

2.2. Sample collection and genotyping method

After 12 hours of fasting, peripheral blood was collected from the elbow vein of each participant and put into EDTA tub. Genomic DNA was extracted from the blood samples using TIANamp Blood DNA Kit (TIANGEN, Beijing), and the procedures were carried out according to the introduction of the manufacturer.

RANK gene rs1805034 SNP was amplified via polymerase chain reaction (PCR) and digested by restriction enzyme, according to the steps in previous study.[22] Primer sequences for *RANK* gene rs8086340 SNP was designed by Primer Premier 5.0 and synthesized by Sangon Biotech (Shanghai) Co., Ltd. PCR reaction was performed in a system of 25 μL, including 2 μL template DNA, 2.5 μL 10×buffer, 0.3 μL DNA Taq polymerase, 0.5 μL dNTP, each 0.7 μL of forward and reverse primers, and ddH_2O. PCR reaction was carried out abiding by the following procedures: 95°C initial denaturation for 10 minutes; 40 cycles of 94°C denaturation for 50 seconds, 61°C annealing for 40 seconds and 72°C extension for 1 minute; and 72°C final extension for 10 minutes. Then these PCR products were digested using restriction enzymes at 37°C overnight (Table 1).

2.3. Statistical analysis

PASW18.0 was used for data calculations. Significant level was set at 0.05 (2 side). Continuous variables were compared between OA patients and controls adopting *t* test or Mann–Whitney *U* test. Genotype distributions of our studied polymorphisms both in case and control groups were examined for their conformity to Hardy–Weinberg equilibrium (HWE). Genotype and allele frequencies were obtained via direct counting. Association of *RANK* gene SNPs with knee OA susceptibility was assessed through χ^2 test or Fisher exact test. The effects of *RANK* SNPs on the susceptibility to knee OA in different KL grades were also detected employing χ^2 test or Fisher exact test. Association strength was appraised applying odds ratios (ORs) with 95% confidence intervals (CIs). Furthermore, the results were adjusted for age, gender, body mass index (BMI), smoking, and drinking using logistic regression analysis.

3. Results

3.1. Characteristics of the participants

Knee OA patients included 57 males and 81 females with a mean age of 55.80 ± 11.46 years. While the control group was constituted by 60 males and 85 females, and their mean age was 56.58 ± 11.56 years. No significant difference existed in age

Table 2

Basic characteristics of participants.

Characteristics	Knee OA n = 138, %	Control n = 145, %	*P*
Age	55.80 ± 11.46	56.58 ± 11.56	.667
Gender			
male	57 (41.30)	60 (41.38)	.990
female	81 (58.70)	85 (58.62)	
BMI, Kg/m^2	24.93 ± 4.28	23.67 ± 4.55	.072
Smoking			
no	97 (70.29)	105 (72.41)	.693
yes	41 (29.71)	40 (27.59)	
Drinking			
no	98 (71.01)	108 (74.48)	.512
yes	40 (28.99)	37 (25.52)	
KL classification			
Mild (K-L score = 2)	55 (39.86)	–	
Moderate (K-L score = 3)	52 (37.68)	–	
Severe (K-L score = 4)	31 (22.46)	–	

BMI = body mass index, KL = Kellgren–Lawrence, OA = osteoarthritis,

Table 3

Association of *RANK* SNPs with knee OA susceptibility.

Genotype/allele	OA n = 138, %	Control n = 145, %	*P*	OR (95% CI)	*P**	OR (95% CI)*
rs1805034						
TT	49 (35.51)	67 (46.21)	–	–	–	–
TC	66 (47.83)	62 (42.76)	.145	1.456 (0.878–2.414)	.875	1.056 (0.538–2.072)
CC	23 (16.67)	16 (11.03)	.070	1.966 (0.941–4.106)	.223	1.821 (0.694–4.781)
T	164 (59.42)	196 (67.59)	–	–		
C	112 (40.58)	94 (32.41)	.044	1.424 (1.010–2.008)		
P_{HWE}	.923	.772				
rs8086340						
GG	47 (34.06)	57 (39.31)	–	–	–	–
GC	70 (50.72)	68 (46.90)	.394	1.248 (0.749–2.080)	.119	1.728 (0.868–3.438)
CC	21 (15.22)	20 (13.79)	.512	1.273 (0.617–2.627)	.685	1.217 (0.471–3.146)
G	164 (59.42)	182 (62.76)	–	–		
C	112 (40.58)	108 (37.24)	.415	1.151 (0.821–1.614)		
P_{HWE}	.543	.969				

CI = confidence interval, HWE = Hardy–Weinberg equilibrium, OA = osteoarthritis, OR = odds ratio, RANK = receptor activator nuclear factor κB, SNP = single nucleotide polymorphism
* adjusted by age, gender, BMI, smoking, and drinking.

or gender distribution between case and control groups (Table 2, $P > .05$), suggesting that the cases and controls were well matched. Smokers and drinkers were more common in case group than in control group, though the difference had no significant difference. According to disease severity, the patients were divided into mild (KL score = 2), moderate (KL score = 3), and severe (KL score = 4) subgroups and the number of the patients in these 3 subgroups were 55 (39.86%), 52 (37.68%), and 31 (22.46%), respectively.

3.2. Association of RANK SNPs with knee OA susceptibility

Genotype and allele distributions of *RANK* rs1805034 and rs8086340 SNPs accorded with HWE in both case and control groups (Table 3, $P > .05$), indicating that the participants could represent general population.

The frequencies of the TT, TC, and CC genotypes of the polymorphism rs1805034 were 35.51%, 47.83%, 16.67% in case group, and 46.21%, 42.76%, 11.03% in control group. Although the TC and CC genotypes had higher frequencies in OA patients than in healthy individuals, the differences had no statistical significance (Table 3, $P > .05$). After adjustment for age, gender, BMI, smoking, and drinking, rs1805034 polymorphism showed no significant association with knee OA susceptibility either. Meanwhile, the C allele of the polymorphism rs1805034 was more frequent in OA patients than in controls ($P = .044$), indicating that the C allele was associated with increased susceptibility to knee OA (OR = 1.424, 95% CI = 1.010–2.008).

The GC and CC genotypes of the rs8086340 SNP had higher frequencies in OA patients. Besides the C allele of this polymorphism was more frequent in cases than in controls. However, we failed to find any significant association between rs8086340 and OA susceptibility ($P > .05$).

3.3. Association of RANK SNPs with knee OA susceptibility in different KL grades

Genotype distributions of *RANK* rs1805034 and rs8086340 SNPs in OA cases of different grades are shown in Table 4. We evaluated the association of *RANK* SNPs with OA risk in different grades. *RANK* rs1805034 SNP was slightly correlated with increased risk of knee OA at both mild and severe grades. The CC genotype and C allele of the rs1805034 SNP were significantly correlated with increased susceptibility to moderate OA (CC vs TT: $P = .018$, OR = 3.071, 95% CI = 1.187–7.941; C vs T: $P = .012$, OR = 1.787, 95% CI = 1.131–2.823). Additionally, the polymorphism rs8086340 had no distinct association with knee OA risk at any grades (Table 5).

4. Discussion

Growing evidence have demonstrated that RANK/RANKL/OPG signaling pathway might be involved in OA development.[2,3–2,5] Polymorphisms in *RANK* gene might alter the signaling way, thus contributing to disorders related to bone metabolism. In this case-control study, we investigated the genetic association of *RANK* rs1805034 and rs8086340 polymorphisms with individual susceptibility to knee OA. We found that the C allele of rs1805034 polymorphism might increase the disease risk, especially for moderate grade.

In the present study, the frequency of the C allele of the polymorphism rs1805034 was significantly higher in knee OA patients than in controls, increasing the disease risk by 1.424

Table 4

Genotype distributions of *RANK* SNPs in different KL grades of OA.

Genotypes/alleles	Knee OA patients		
	Mild	Moderate	Severe
rs1805034			
TT	24 (43.64)	15 (28.84)	10 (32.26)
TC	24 (43.64)	26 (50.00)	16 (51.61)
CC	7 (12.72)	11 (21.15)	5 (16.13)
T	72 (65.45)	56 (53.85)	36 (58.06)
C	38 (34.55)	48 (46.15)	26 (41.94)
rs8086340			
GG	22 (40.00)	12 (23.08)	13 (41.94)
GC	26 (47.27)	30 (57.69)	14 (45.16)
CC	7 (12.73)	10 (19.23)	4 (12.90)
G	70 (63.64)	54 (51.92)	40 (64.52)
C	40 (36.36)	50 (48.08)	22 (35.48)

KL = Kellgren–Lawrence, OA = osteoarthritis, RANK = receptor activator nuclear factor κB, SNP = single nucleotide polymorphism.

Table 5

Association between *RANK* SNPs and OA susceptibility in different KL grades of knee OA.

	Mild		Moderate		Severe	
Genotypes/alleles	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**
rs1805034						
TT	–	–	–	–	–	–
TC	.819	1.081 (0.557–2.097)	.087	1.873 (0.909–3.861)	.210	1.729'(0.730–4.095)
CC	.696	1.221 (0.448–3.330)	.018	3.071 (1.187–7.941)	.222	2.094 (0.628–6.980)
T	–	–	–	–	–	–
C	.686	1.100 (0.692–1.749)	.012	1.787 (1.131–2.823)	.151	1.506 (0.859–2.639)
rs8086340						
GG	–	–	–	–	–	–
GC	.313	0.709 (0.364–1.383)	.053	2.096 (0.984–4.465)	.810	0.903 (0.392–2.076)
CC	.847	0.907 (0.336–2.444)	.080	2.375 (0.890–6.339)	1.000	0.877 (0.256–3.003)
G	–	–		–	–	–
C	.871	0.963 (0.611–1.519)	.053	1.560 (0.993–2.453)	.795	0.927 (0.523–1.642)

CI = confidence interval, KL = Kellgren–Lawrence, OA = osteoarthritis, OR = odds ratio, RANK = receptor activator nuclear factor κB, SNP = single nucleotide polymorphism.

times. Gianfrancesco et al found that the C allele was positively correlated with the severity of Paget's disease of bone (PDB).[22] Meanwhile, the CC gentoype of the rs1805034 SNP might act as a risk factor for osteoporosis in women with RA.[18] However, Assmann and co-workers reported that the rs1805034 polymorphism had no significant association with psoriatic arthritis.[26] Besides, they observed no significant correlation between this SNP and RA either.[27] These studies could testify our results indirectly.

The GC and CC genotypes of the rs8086340 SNP exhibited higher frequencies in case group than in control group, but these differences were not significant, so this polymorphism had no significant association with knee OA risk. While previous study demonstrated that the rs8086340 SNP was closely related to the presence of anticitrullinated peptide antibodies (ACPA) but not erosion in RA patients.[19]

Knee OA patients in our research were divided into 3 grades according to their severity, so we further analyzed the association of our interested polymorphisms with the disease in different grade groups. However, no significant association was discovered between the rs1805034 SNP and OA risk in either mild or severe grade. Whereas, the CC genotype of the rs1805034 SNP had significantly higher frequency in moderate OA patients than in controls, indicating a promoting effect of the CC genotype on moderate OA. Meanwhile, the C allele of the polymorphism rs1805034 was also more frequent in moderate OA, demonstrating its enhancing effect on moderate OA. However, we failed to find any significant association between the rs8086340 SNP and knee OA susceptibility in either mild moderate or severe grade group. This was the first study on the relationship of *RANK* polymorphisms with knee OA severity in Chinese Han population.

Limitations in the present study should be noticed. First of all, the sample size was not large enough, so the statistical power was restricted, particularly in stratified analysis. However, our study adopted strict sample collection, experimental procedures, and scientific statistical analyses, so our results had certain representativeness. Second, study participants from only 1 region could not represent general Chinese Han population. Third, OA is a complex disease affected by the interactions between genetic and environmental factors. However, such interactions were not considered in this study. Besides, confounding factors were not adjusted either, which might influence the accuracy of our findings. Finally, causal relationship between *RANK* polymorphisms and OA risk was not certified in the present study. Therefore, well-designed studies with larger sample size based on multiple centers should be performed in the future. In vivo and in vitro researches should be designed to explore functional roles of *RANK* gene in OA etiology and potentially related molecular mechanisms. Besides, polymorphism distributions vary dramatically between different populations and the association of *RANK* polymorphisms with OA risk observed in this study might be not applicable in other population.

In conclusion, the C allele of rs1805034 polymorphism may be a potential risk factor for knee OA, especially for moderate grade. These results may help us to understand the mechanism of knee OA.

Author contributions

Conceptualization: Changcheng Wang, Li Luo, Dongyi Li.
Data curation: Changcheng Wang, Li Luo, Fengde Tian, Dongyi Li.
Formal analysis: Changcheng Wang, Li Luo, Fengde Tian, Ning An, Yao Zhang, Ruihu Hao, Peng Xiao.
Funding acquisition: Yao Zhang, Zihao Zhou, Lin Guo.
Investigation: Yao Zhang, Ruihu Hao, Dongyi Li.
Methodology: Ruihu Hao.
Writing – original draft: Ning An, Zihao Zhou, Peng Xiao, Lin Guo.
Writing – review & editing: Changcheng Wang, Fengde Tian, Ning An, Zihao Zhou, Peng Xiao, Lin Guo.

References

[1] Rahmati M, Nalesso G, Mobasheri A, et al. Aging and osteoarthritis: central role of the extracellular matrix. Ageing Res Rev 2017;40: 20–30.
[2] Bartley EJ, Palit S, Staud R. Predictors of osteoarthritis pain: the importance of resilience. Curr Rheumatol Rep 2017;19:57.
[3] Pereira D, Ramos E, Branco J. Osteoarthritis. Acta medica portuguesa 2015;28:99–106.
[4] Johnson VL, Hunter DJ. The epidemiology of osteoarthritis. Best practice & research. Clin Rheumatol 2014;28:5–15.
[5] Palazzo C, Nguyen C, Lefevre-Colau MM, et al. Risk factors and burden of osteoarthritis. Ann Phys Rehabil Med 2016;59:134–8.
[6] D'Adamo S, Cetrullo S, Minguzzi M, et al. MicroRNAs and autophagy: fine players in the control of chondrocyte homeostatic activities in osteoarthritis. Oxid Med Cell Longev 2017;2017:3720128.
[7] Kalaitzoglou E, Griffin TM, Humphrey MB. Innate immune responses and osteoarthritis. Curr Rheumatol Rep 2017;19:45.

[8] Zhang Y, Zeng C, Wei J, et al. Associations of cigarette smoking, betel quid chewing and alcohol consumption with high-sensitivity C-reactive protein in early radiographic knee osteoarthritis: a cross-sectional study. BMJ Open 2016;6:e010763.
[9] Liu Y, Zhang H, Liang N, et al. Prevalence and associated factors of knee osteoarthritis in a rural Chinese adult population: an epidemiological survey. BMC Public Health 2016;16:94.
[10] Kang B, Zhao F, Zhang X, et al. Association between the interaction of SMAD3 polymorphisms with body mass index and osteoarthritis susceptibility. Int J Clin Exp Pathol 2015;8:7364–70.
[11] Su SL, Yang HY, Lee HS, et al. Gene-gene interactions between TGF-beta/Smad3 signalling pathway polymorphisms affect susceptibility to knee osteoarthritis. BMJ Open 2015;5:e007931.
[12] Nielsen FK, Egund N, Jorgensen A, et al. Assessment of subchondral bone marrow lesions in knee osteoarthritis by MRI: a comparison of fluid sensitive and contrast enhanced sequences. BMC Musculoskelet Disord 2016;17:479.
[13] Perez-Sayans M, Somoza-Martin JM, Barros-Angueira F, et al. RANK/RANKL/OPG role in distraction osteogenesis. Oral Surg Oral Med Oral Pathol Oral Radiol Endodontics 2010;109:679–86.
[14] Takegami N, Akeda K, Yamada J, et al. RANK/RANKL/OPG system in the intervertebral disc. Arthritis Res Ther 2017;19:121.
[15] Warren JT, Zou W, Decker CE, et al. Correlating RANK ligand/RANK binding kinetics with osteoclast formation and function. J Cell Biochem 2015;116:2476–83.
[16] Zhou XW, Liu YC, Jian XC, et al. Expressions of RANK, RANKL, and osteoprotegerin in male rats at different ages. Nan Fang Yi Ke Da Xue Xue Bao = J Southern Med Univ 2011;31:1539–42.
[17] Zhou S, Thornhill TS, Meng F, et al. Influence of osteoarthritis grade on molecular signature of human cartilage. J Orthop Res 2016;34: 454–62.
[18] Mohamed RH, El-Shahawy EE. Relationship between RANK and RANKL gene polymorphisms with osteoporosis in rheumatoid arthritis patients. Genet Test Mol Biomarkers 2016;20:249–54.
[19] Ruyssen-Witrand A, Degboe Y, Cantagrel A, et al. Association between RANK, RANKL and OPG polymorphisms with ACPA and erosions in rheumatoid arthritis: results from a meta-analysis involving three French cohorts. RMD open 2016;2:e000226.
[20] Luyten FP, Denti M, Filardo G, et al. Definition and classification of early osteoarthritis of the knee. Knee Surg Sports Traumatol Arthrosc Off J ESSKA 2012;20:401–6.
[21] Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957;16:494–502.
[22] Gianfrancesco F, Rendina D, Di Stefano M, et al. A nonsynonymous TNFRSF11A variation increases NFkappaB activity and the severity of Paget's disease. J Bone Mineral Res Off J Am Soc Bone Miner Res 2012;27:443–52.
[23] Tyrovola JB. The "Mechanostat Theory" of frost and the OPG/RANKL/RANK system. J Cell Biochem 2015;116:2724–9.
[24] Chen X, Wang Z, Duan N, et al. Osteoblast-osteoclast interactions. Connect Tissue Res 2017;1–9.
[25] Wang Y, van Assen AHG, Reis CR, et al. Novel RANKL DE-loop mutants antagonize RANK-mediated osteoclastogenesis. FEBS J 2017; 284:2501–12.
[26] Assmann G, Pfoehler C, Simon P, et al. Genetic variations in the genes encoding receptor activator nuclear factor kappa B (RANK), receptor activator nuclear factor kappa B ligand (RANKL) and osteoprotegerin (OPG) in patients with psoriasis and psoriatic arthritis: a case-control study. J Dermatol 2011;38:519–23.
[27] Assmann G, Koenig J, Pfreundschuh M, et al. Genetic variations in genes encoding RANK, RANKL, and OPG in rheumatoid arthritis: a case-control study. J Rheumatol 2010;37:900–4.