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Original Article

Polymorphisms of genes in the OPG/RANKL/RANK pathway in the Mongols of Inner Mongolia China: relationship to other populations

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Abstract: The regulation of bone remodeling is controlled by the cellular interactions between osteoblasts and osteoclasts in which the OPG/RANKL/RANK pathway plays a paramount role. The aim of this study was to assess whether exists differences between polymorphisms of the *TNFSF11*, *TNFRSF11A* and *TNFRSF11B* genes in the OPG/RANKL/RANK pathway and different populations. In the present study, we genotyped 41 single-nucleotide polymorphisms (SNPs) in the *TNFSF11*, *TNFRSF11A*, and *TNFRSF11B* genes in the Mongols and Han populations of Northwest China. Mongols compared with five other populations: the Chinese Han in Beijing, China (CHB), the Japanese in Tokyo, Japan (JPT), a northern and western European population (CEU), the Yoruba in Ibadan, Nigeria (YRI) and Northwest Han Chinese using chi-squared tests and haplotype analysis. We determined that Mongols differed from CHB, CEU, JPT, YRI and Northwest Han Chinese in 1, 19, 1, 17 and 1 selected SNP genotypes after Bonferroni correction ($P < 0.05/40*6$), respectively. The rs3826617 in *TNFRSF11A* gene was significantly different compared with three ethnic populations (CHB, JPT and YRI). The rs8092023 in the *TNFRSF11A* gene just showed a significant difference compared with that in Northwest Han Chinese. Differences of polymorphisms of *TNFSF11* and *TNFRSF11B* genes just were found in the CEU and YRI compared with that in Mongols. Haplotype analysis also demonstrated differences between the Mongols and the other five populations. Our results illustrate for the first time the differences of polymorphisms of the *TNFSF11*, *TNFRSF11A*, and *TNFRSF11B* genes between Mongols and five other populations and may provide available information regarding genetic polymorphisms in the OPG/RANKL/RANK pathway of different populations and osteopathy-association studies.

Keywords: Ethnic difference, *TNFSF11*, *TNFRSF11A*, *TNFRSF11B*, Mongols, genetic polymorphism

Introduction

Osteopathy, bone-related disease, is a public health problem worldwide, causing serious damage to human health in the forms of diseases such as osteoporosis, osteonecrosis and osteosarcoma [1-4]. Structural deterioration of bone tissue is characterized by low bone mineral density (BMD), leading to an increased risk of fractures that occur mostly at the hip, spine, and wrist [5]. It has been reported that the heritability of BMD is between 50% and 85% at the spine and hip, influenced by multiple genes and environmental factors [6]. The balance between bone resorption and formation is controlled by the cellular interactions between osteoblasts and osteoclasts in which the OPG/RANKL/RANK pathway plays a crucial role as a determinant of BMD [7].

Osteoprotegerin (OPG), also called tumor necrosis factor receptor superfamily member 11B, is encoded by the *TNFRSF11B* gene. Receptor activator of nuclear factor kappa-B ligand (RANKL), also called tumor necrosis factor superfamily member 11, is encoded by the *TNFSF11* gene. Receptor activator of nuclear factor kappa-B (RANK), also called tumor necro-

sis factor receptor superfamily member 11A, is encoded by the *TNFRSF11A* gene. OPG, which is produced by osteoblasts as a decoy receptor for RANKL, prevents RANKL from binding to RANK and bone resorption. RANKL is synthesized in a membranous or soluble form by osteoblastic lineage cells, some cancer cells and immune cells. RANK is expressed on the surface of precursor osteoclasts and stimulates bone resorption through osteoclastogenesis and the activation of multinucleated mature osteoclasts [8, 9]. According to several candidate gene association studies, genome-wide association studies (GWASs), and meta-analyses, any variation of this pathway that results in an imbalance between bone formation and resorption [10-12]. However, we did not discover previous reports regarding differences between polymorphisms in the genes of the OPG/RANKL/RANK pathway and different populations.

Ethnic Mongols form one of the 55 ethnic minorities officially recognized by the People's Republic of China. According to the 2010 census, there are approximately 5.8 million people classified as ethnic Mongols living in China, accounting for 60% of all Mongols worldwide. Most of them live in Inner Mongolia, Northeast China, Xinjiang, etc. The Mongol population in China is over twice that of the sovereign state of Mongolia (http://en.wikipedia.org/wiki/Mongols_in_China).

The aim of this study was to determine whether exist differences in the single-nucleotide polymorphisms (SNPs) of the *TNFSF11*, *TNFRSF11A*, and *TNFRSF11B* genes between Mongols and five other ethnic populations: [the Chinese Han in Beijing, China (CHB), the Japanese in Tokyo, Japan (JPT), a northern and western European population (CEU), the Yoruba in Ibadan, Nigeria (YRI) and Northwest Han Chinese] and provide information regarding genetic polymorphisms in the OPG/RANKL/RANK pathway of different populations and osteopathy-association studies.

Material and methods

Ethics statement

The protocol in this study was completed in accordance with the principles of the Declaration of Helsinki and was ratified by the Ethical Committee of the International Mongolia Medicine Hospital. The participants all provided signed informed consent.

Study participants

A random sample of 200 Mongols (100 males and 100 females; mean age 44.2 ± 11.6) and 200 Northwest Han Chinese (100 males and 100 females; mean age 46.8 ± 12.6) who were healthy and unrelated were recruited between September and December 2014 from the medical examination center of the International Mongolia Medicine Hospital. All of the participants were randomly chosen among individuals whose ancestors have lived in the region for at least three generations and were self-identified as Mongol and Chinese Han. According to the recruitment standards, we surveyed the subjects using a self-designed questionnaire including demographic factors such as name, age, sex, race, place of origin, telephone number, address and potential risk factors including smoking, dietary conditions and alcohol consumption. Meanwhile, we also excluded subjects with chronic diseases of the kidney, heart, liver and brain by detailed exclusion criteria.

tagSNP selection

For the studied genes, tagging SNPs were selected from the International HapMap Project (http://www.hapmap.org/). The SNPs were selected based on the following criteria: (1) validation status, (2) degree of heterozygosity [minor allele frequencies (MAFs) > 0.1], (3) binned by the algorithm such that the pairwise linkage disequilibrium (LD) exceeds a threshold r^2 (r^2 = 0.8) and (4) requirement for tag-SNPs [13]. All of these SNPs were authenticated using the NCBI (http://www.ncbi.nlm.nih.gov/SNP/) and HapMap databases. Finally, a total of 41 SNPs were selected in three genes (17 in *TNFRSF11A*, 11 in *TNFSF11*, and 13 in *TNFRSF11B*).

Genotyping

Genomic DNA was isolated from blood samples using an extraction kit (GoldMag, China) and was stored at -20°C. The DNA concentration was measured by spectrometry (DU530 UV/VIS spectrophotometer; Beckman Instruments, Fullerton, CA, USA). We designed a Multiplexed SNP Mass EXTEND™ assay using Sequenom MassARRAY® Assay Design 4.0 Software and Sequenom MassARRAY® RS1000 to genotype

Table 1. Basic characteristics of the selected SNPs

SNP ID	Genes	Chr	Position	Role	Allele		Allele frequencies		HWE
					A	B	A (%)	B (%)	*P* value
rs3134053	TNFRSF11B	8	119946141	Intron	T	C	35.8	64.2	0.2173
rs11573896	TNFRSF11B	8	119947430	Intron	A	T	18.6	81.4	0.6383
rs1485286	TNFRSF11B	8	119950668	Intron	T	C	47.2	52.8	0.5661
rs3102725	TNFRSF11B	8	119951005	Intron	A	G	11.8	88.2	1
rs1905786	TNFRSF11B	8	119951692	Intron	T	C	27.9	72.1	1
rs1032128	TNFRSF11B	8	119951773	Intron	G	A	51.4	48.6	0.8822
rs3134056	TNFRSF11B	8	119952212	Intron	G	A	36.2	63.8	0.2819
rs3134058	TNFRSF11B	8	119954108	Intron	G	A	40.5	59.5	0.0568
rs11573856	TNFRSF11B	8	119954995	Intron	A	G	9.0	91.0	0.1992
rs11573849	TNFRSF11B	8	119956378	Intron	T	G	23.5	76.5	0.1663
rs3102731	TNFRSF11B	8	119959389	Intron	A	G	12.5	87.5	0.7455
rs11573828	TNFRSF11B	8	119959813	Intron	T	C	23.3	76.7	0.2273
rs1564861	TNFRSF11B	8	119965909	Promoter	C	A	36.9	63.1	0.3659
rs7999416	TNFSF11	13	43136346	Promoter	G	A	32.7	67.3	0.5211
rs12585014	TNFSF11	13	43140559	Intron	A	G	32.7	67.3	0.5211
rs7325635	TNFSF11	13	43145319	Intron	A	G	41.7	58.3	0.11
rs9525641	TNFSF11	13	43148024	Intron	C	T	42.7	57.3	0.2472
rs4941433	TNFSF11	13	43159135	Intron	T	G	30.8	69.2	0.8685
rs2148073	TNFSF11	13	43163799	Intron	G	C	30.4	69.6	0.7384
rs4356365	TNFSF11	13	43164171	Intron	T	C	30.8	69.2	0.8685
rs346589	TNFSF11	13	43166284	Intron	A	G	30.7	69.3	0.8691
rs2200287	TNFSF11	13	43168660	Intron	T	C	25.5	74.5	0.5766
rs875625	TNFSF11	13	43174100	Intron	G	A	45.6	54.4	0.7727
rs931273	TNFSF11	13	43178583	Intron	A	G	31.2	68.8	0.742
rs3826617	TNFRSF11A	18	59991878	Promoter	C	G	12.5	87.5	1
rs8092023	TNFRSF11A	18	59994784	Intron	A	T	24.9	75.1	1
rs4941125	TNFRSF11A	18	59997684	Intron	G	A	31.0	69.0	0.3253
rs7235803	TNFRSF11A	18	60000379	Intron	G	A	31.0	69.0	0.3253
rs12969154	TNFRSF11A	18	60006143	Intron	A	G	35.5	64.5	0.7524
rs12956925	TNFRSF11A	18	60013640	Intron	A	G	18.7	81.3	0.6434
rs4289084	TNFRSF11A	18	60015995	Intron	C	G	19.7	80.3	0.6541
rs7236060	TNFRSF11A	18	60023282	Intron	G	A	38.0	62.0	0.2304
rs3810024	TNFRSF11A	18	60025375	Intron	T	C	26.0	74.0	2.372E-50*
rs17069876	TNFRSF11A	18	60027078	Intron	A	G	15.3	84.7	0.7873
rs1805034	TNFRSF11A	18	60027241	Coding exon	C	T	41.2	58.8	0.6621
rs4524035	TNFRSF11A	18	60028244	Intron	G	A	43.0	57.0	0.6652
rs8083511	TNFRSF11A	18	60028655	Intron	C	A	40.5	59.5	0.8838
rs17069898	TNFRSF11A	18	60029281	Intron	G	A	44.7	55.3	1
rs12959396	TNFRSF11A	18	60039309	Intron	G	T	18.5	81.5	0.8174
rs9646629	TNFRSF11A	18	60051199	Intron	C	G	48.7	51.3	0.3245
rs884205	TNFRSF11A	18	60054857	Downstream	T	G	28.3	71.7	0.1577

SNP, single-nucleotide polymorphism. A/B stands for minor/major alleles on the control sample frequencies. HWE, Hardy-Weinberg equilibrium. The SNPs are excluded at HWE $P < 0.001$*. *$P < 0.001$, statistical significance.

the SNPs using the protocol recommended by the manufacturer [14, 15]. Data management and analysis were performed using Sequenom Typer 4.0 Software [16].

Table 2. Genotype frequencies in Mongols compared with five other populations

SNP ID	Genes	Allele		Mongol genotype frequencies				*p* values against five populations (after Bonferroni correction)				
		A	B	AA	AB	BB	SUM	CHB	CEU	JPT	YRI	NCH
rs3134053	TNFRSF11B	T	C	21	101	78	200	4.96E-02	1.56E-06	4.22E-01	8.62E-04	4.10E-02
rs11573896	TNFRSF11B	T	C	133	58	8	199	2.07E-01	5.52E-01	2.26E-01	9.85E-01	1.13E-01
rs1485286	TNFRSF11B	T	G	57	93	46	196	8.78E-01	7.17E-06	1.35E-01	9.09E-03	2.69E-01
rs3102725	TNFRSF11B	T	G	2	43	154	199	9.90E-01	4.88E-02	6.65E-01	5.64E-01	3.33E-01
rs1905786	TNFRSF11B	A	G	15	81	103	199	2.38E-01	1.31E-08	8.57E-01	2.80E-01	8.32E-01
rs1032128	TNFRSF11B	T	C	47	92	42	181	1.22E-01	9.83E-07	2.32E-02	5.83E-03	8.36E-02
rs3134056	TNFRSF11B	A	G	77	100	22	199	3.23E-01	6.05E-01	2.36E-02	1.49E-06	6.25E-02
rs3134058	TNFRSF11B	T	C	26	110	64	200	9.75E-02	4.46E-05	5.93E-01	2.38E-04	1.21E-01
rs11573856	TNFRSF11B	A	G	167	30	3	200	4.42E-02	3.35E-01	2.31E-02	3.67E-04	1.14E-03
rs11573849	TNFRSF11B	T	C	113	80	7	200	3.52E-02	3.54E-05	6.98E-01	6.50E-08	4.00E-03
rs3102731	TNFRSF11B	G	C	152	46	2	200	4.55E-01	8.34E-02	7.40E-01	4.42E-02	3.20E-01
rs11573828	TNFRSF11B	A	T	111	77	7	195	1.10E-02	7.80E-05	7.37E-01	6.42E-05	3.83E-03
rs1564861	TNFRSF11B	A	G	76	99	24	199	2.91E-01	8.49E-01	5.97E-01	2.40E-05	9.44E-02
rs7999416	TNFSF11	A	G	19	93	88	200	2.19E-01	3.17E-06	3.34E-01	2.58E-04	6.44E-01
rs12585014	TNFSF11	G	A	88	93	19	200	7.57E-01	7.38E-05	1.75E-01	5.81E-05	6.44E-01
rs7325635	TNFSF11	T	C	62	109	29	200	1.28E-01	2.15E-01	4.59E-01	1.25E-09	4.39E-02
rs9525641	TNFSF11	C	G	61	107	32	200	2.51E-01	6.96E-02	5.14E-01	1.83E-02	5.38E-02
rs4941433	TNFSF11	A	G	95	87	18	200	4.18E-01	4.26E-05	2.52E-01	6.59E-04	6.81E-01
rs2148073	TNFSF11	A	G	17	87	95	199	4.25E-01	1.08E-04	2.89E-01	9.95E-01	5.87E-01
rs4356365	TNFSF11	G	A	18	87	95	200	8.80E-01	3.51E-04	1.20E-01	9.82E-02	6.81E-01
rs346589	TNFSF11	G	A	95	86	18	199	4.41E-01	7.83E-05	2.38E-01	1.97E-02	6.96E-01
rs2200287	TNFSF11	C	T	108	79	11	198	6.45E-01	1.15E-01	9.06E-01	1.08E-01	5.79E-02
rs875625	TNFSF11	C	A	56	99	39	194	4.99E-01	2.85E-02	2.56E-01	2.10E-01	2.85E-01
rs931273	TNFSF11	G	T	93	89	18	200	1.76E-01	3.31E-06	2.98E-01	1.44E-16	5.42E-01
rs3826617	TNFRSF11A	C	G	3	44	153	200	3.44E-37	–	4.50E-32	3.27E-48	6.74E-01
rs8092023	TNFRSF11A	G	A	112	75	12	199	4.02E-03	5.86E-01	1.13E-02	5.87E-02	2.22E-06
rs4941125	TNFRSF11A	A	G	92	92	16	200	2.65E-02	2.63E-01	6.08E-01	1.04E-12	5.58E-03
rs7235803	TNFRSF11A	C	G	92	92	16	200	1.28E-02	4.47E-02	6.08E-01	1.67E-10	5.58E-03
rs12969154	TNFRSF11A	A	G	80	85	25	190	7.13E-01	1.17E-12	9.66E-01	1.17E-12	8.33E-01
rs12956925	TNFRSF11A	G	A	133	59	8	200	2.65E-03	1.14E-01	1.44E-01	1.37E-02	6.26E-03
rs4289084	TNFRSF11A	A	T	9	61	130	200	2.34E-02	2.08E-04	8.47E-01	2.44E-06	3.51E-01
rs7236060	TNFRSF11A	G	A	33	86	81	200	8.54E-02	2.97E-01	2.97E-02	9.12E-04	8.48E-02
rs17069876	TNFRSF11A	T	C	143	51	5	199	5.98E-03	2.10E-05	7.65E-01	7.57E-07	4.45E-01
rs1805034	TNFRSF11A	G	A	32	100	67	199	5.22E-01	1.18E-02	1.39E-01	4.72E-01	2.42E-02
rs4524035	TNFRSF11A	G	A	63	102	35	200	4.68E-01	5.92E-19	1.46E-01	4.73E-04	5.07E-04
rs8083511	TNFRSF11A	C	T	70	98	32	200	8.66E-01	3.81E-08	1.46E-01	1.93E-01	5.90E-04
rs17069898	TNFRSF11A	C	A	61	98	40	199	7.76E-01	8.62E-01	2.75E-01	4.11E-05	2.24E-02
rs12959396	TNFRSF11A	G	A	132	62	6	200	2.06E-01	4.23E-10	4.35E-01	3.14E-01	1.36E-01
rs9646629	TNFRSF11A	T	G	51	93	56	200	2.46E-01	5.06E-01	9.71E-01	8.58E-07	9.85E-01
rs884205	TNFRSF11A	G	A	20	71	105	196	9.07E-02	4.86E-01	7.41E-01	6.05E-11	4.12E-01

NCH: Northwest Han Chinese population.

Statistical analyses

Hardy-Weinberg Equilibrium (HWE) and chi-squared tests were performed using Microsoft Excel and the SPSS 19.0 statistical package (SPSS, Chicago, IL, USA). Validation of each variant frequency in Mongols and Northwest Han Chinese was tested for departure from

HWE ($P < 0.001$) using an exact test. We calculated the genotype frequencies of Mongols, Northwest Han Chinese and Mongols compared with the other five populations (data from HapMap: http://hapmap.ncbi.nlm.nih.gov) separately using chi-squared test. All *p* values obtained in this study were two-sided, and Bonferroni's multiple adjustment was applied to the level of significance, which was set at $P < 0.00021$ (0.05/40*6). We performed haplotype interaction analysis on the genes containing the significant SNPs. The linkage disequilibrium structure was examined using Haploview 4.2.

Results

We successfully sequenced 41 SNPs in three genes (*TNFSF11*, *TNFRSF11A* and *TNFRSF11B*) genotyped from 200 Mongols and 200 Northwest Han Chinese in the study. Basic characteristics of the selected SNPs, including the SNP ID, gene, chromosome, position, role, allele, allele frequencies and HWE are shown in **Table 1**. We ignored rs3810024 with a *P* value < 0.001 because of the small probability that their deviation from HWE could be explained by chance.

The genotype frequencies in Mongols and identified significant variants in Mongols compared with the five other populations are shown in **Table 2**. We determined that Mongols differed from CHB, CEU, JPT, YRI and Northwest Han Chinese in 1, 19, 1, 17 and 1 selected SNP genotypes after Bonferroni correction ($P < 0.05/40*6$), respectively. The rs3826617 in the *TNFRSF11A* gene was significantly different compared with that in three ethnic populations (CHB, JPT and YRI). The rs8092023 in the *TNFRSF11A* gene showed a significant difference compared with that in Northwest Han Chinese. We also determined that there was a difference in the polymorphisms of the *TNFSF11* and *TNFRSF11B* genes in CEU and YRI compared with those in Mongols, but not in CHB, JPT and Northwest Han Chinese in the present study.

We identified five LD blocks and four LD blocks in Mongols and Northwest Han, respectively. Three LD blocks were identified in CHB and JPT, and two LD blocks were identified in CEU and YRI in the *TNFRSF11A* gene (**Figure 1**). The block identified in the five other populations excluded YRI and consisted of two complete LD markers (rs4941125 and rs7235803) with a D' value equal to 1. The block identified in Mongols, JPT, CEU and YRI excluded CHB and Northwest Han, and also consisted of two complete LD markers (rs8083511 and rs17069898) with a D' value equal to 1.

Discussion

In recent years, studies of *TNF/TNFR* superfamily members have been responsible for elucidating previously unrecognized linkages between the immune system and other biological systems, as well as previously unrealized networks controlling various disease conditions [17]. According to previous massive reports, the OPG/RANKL/RANK signaling pathway played a crucial role in bone metabolism and osteopathy development had been confirmed, including inherited bone diseases, acquired bone pathologies, osteoimmunology and osteonecrosis [8, 9, 18, 19]. Any variation of this pathway results in a negative balance between bone formation and resorption.

In the current study, we found that 1, 19, 1, 17 and 1 variants differed in CHB, CEU, JPT, YRI and Northwest Han Chinese, respectively, after Bonferroni correction ($P < 0.05/40*6$). The rs3826617 in the *TNFRSF11A* gene was significantly different compared with that in three ethnic populations (CHB, JPT and YRI). No significant difference was found in rs3826617 between Mongols and the Northwest Han Chinese population, implying that the samples may be from the same region. The rs8092023 in *TNFRSF11A* showed a significant difference compared with that in Northwest Han Chinese. In the haplotype analysis, the block consisted of two complete LD markers (rs8083511 and rs17069898) in the *TNFRSF11A* gene similar to that identified in Mongols, JPT, CEU and YRI and excluded Chinese Han (Beijing and Northwest region). According to the above interesting findings, polymorphisms and biofunction in the *TNFRSF11A* gene should mostly focus on the relationship between different ethnic populations and osteopathy based on the OPG/RANKL/RANK pathway.

The *TNFRSF11A* gene encodes RANK, a type I, 616-amino acid, homo-trimerizing transmembrane protein containing four extra-cellular cysteine-rich pseudorepeats. Trimerization was

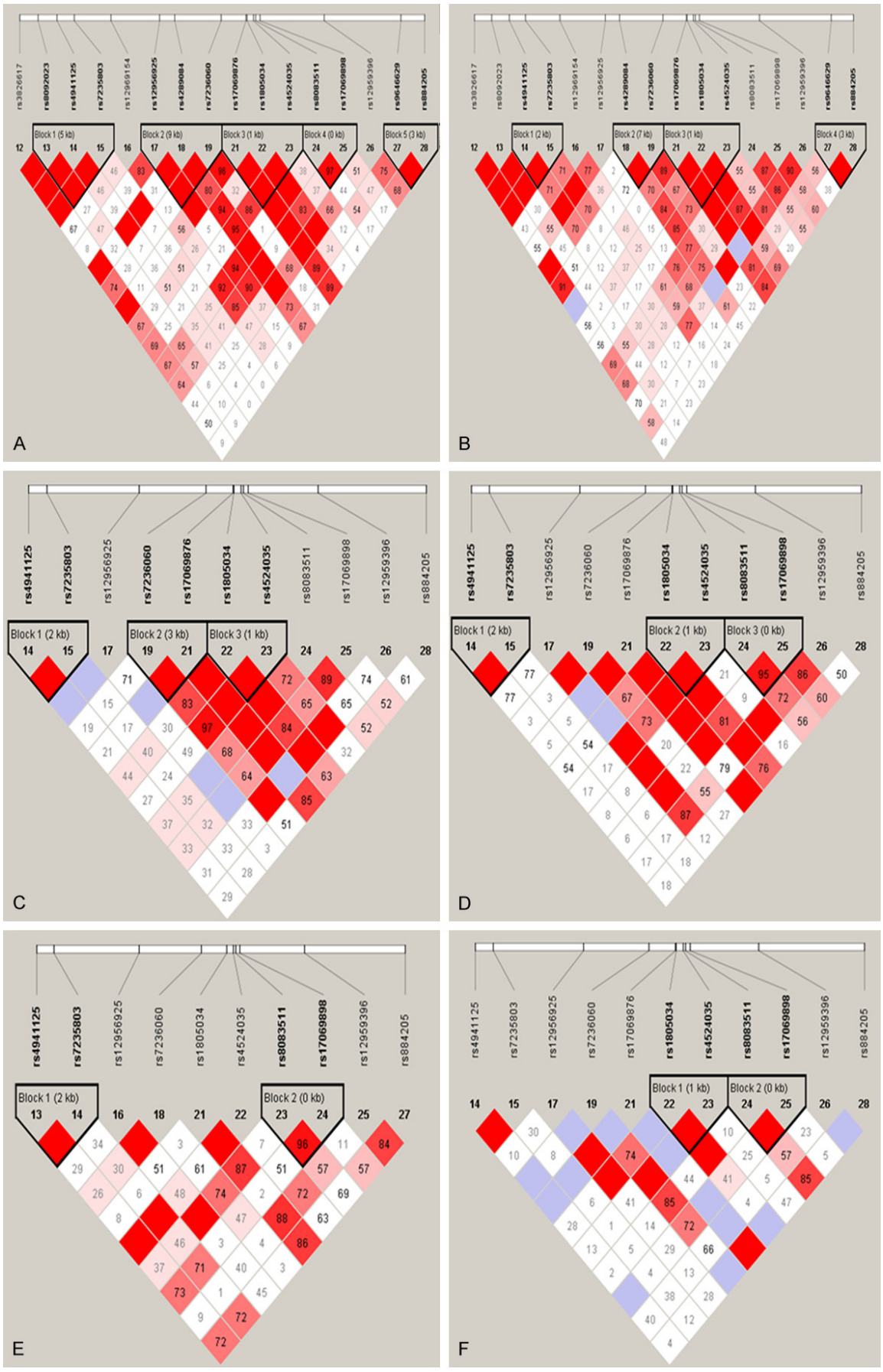
A
B
C
D
E
F

Figure 1. Linkage disequilibriumanalysis of the *TNFRSF11A* gene in each of the six populations. LD is displayed by standard color schemeswith bright red for very strong LD (LOD > 2, D' = 1), pink red (LOD > 2, D' < 1) and blue (LOD < 2, D' = 1) for intermediate LD, and white (LOD < 2, D' < 1) for no LD. A. Mongols; B. Northwest Han; C. CHB; D. JPT; E. CEU; F. YRI.

promoted by interaction with RANKL. The human *TNFRSF11A* gene is located on chromosome 18q22.1, and RANK mRNA has been detected in the thymus, liver, colon, mammary glands, prostate, pancreas, bone marrow, heart, lung, brain, skeletal muscle, kidney, liver, and skin [8, 18]. The *TNFRSF11A* gene plays a crucial role in various system disorders and the OPG/RANKL/RANK pathway. Familial expansile osteolysis and Paget's disease are rare autosomal dominant conditions characterized by enhanced bone remodeling and osteolytic lesions present in the long bones. Short in-frame duplications in exon 1 of the gene encoding RANK has been linked to familial expansile osteolysis and Paget's disease of the bone. It has been shown that these mutations disrupt the function of the RANK signal peptide, resulting in constitutive RANK activity [20]. P. Tu et al. suggested that genetic polymorphisms of the *TNFRSF11A* gene influence BMD mainly in the femoral neck, inducing osteoporosis in postmenopausal Chinese women [1]. In our study, we determined the polymorphisms of *TNFRSF11A* showed a significant difference in Mongols compared with that in the five other populations.

Meanwhile, differences were found in the polymorphisms of the *TNFSF11* and *TNFRSF11B* genes in CEU and YRI compared with Mongols, excluding CHB, JPT and Northwest Han Chinese among the Asian populations. Due to increased *TNFSF11* (RANKL) expression, patients receiving hormone ablation therapy for breast cancer (estrogen suppression) or prostate cancer (chemical or surgical castration for testosterone suppression) may also develop osteoporotic bone loss [21]. Lu et al. found rs2200287 and rs2148073 in the *TNFSF11*gene were associated with age at menarche and natural menopause in white women [22]. Dongetal. found rs12585014, rs7988338, and rs2148073 of *TNFSF11* were significantly associated with femoral neck compression strength index [23]. The *TNFRSF11B* (OPG) was reduced on the osteoclast surface and prevented ovariectomy-associated bone loss in the lumbar vertebrae, distal femur and femur neck [24, 25]. Rosh and el et al. also identified a few SNPs within or in the vicinity of *TNFRSF11B* (rs3134056, *TNFSF11* (rs2148073) and *TNFRSF11A* (rs4941125) femoral head and lumbar spine BMD in European men [26]. Differences in the expression of *TNFSF11* and *TNFRSF11B* are noted among Europeans and Africans in our study.

During the investigation, we determined the characteristic lifestyle of Mongols, such as dietary history and habits. Higher meat and salt intake and lower vegetable and fruit intake were commonly observed in the Mongol populations. In addition, alcohol consumption was a more popular habit among the 200 Mongols (males: 72.9%, females: 69.3%) who were regular alcohol drinkers. This lifestyle may be related to most Mongols living in the cold areas of the Northern hemisphere. According to previous reports, ethanol-induced skeletal damage seemed mainly dependent on the negative effects on bone formation. Lifestyle factors and trauma likely contributed to the high fracture incidence of alcohol abusers, and the OPG/RANKL ratio was significantly higher in alcoholics. OPG was positively correlated with daily alcohol assumption and with the indexes of liver cytolysis [27, 28].

However, intrinsic limitations still exist in our study. Our sample size is relatively not sufficient, thus, further investigation of the OPG/RANKL/RANK pathway related to gene polymorphisms in a larger Mongol population is necessary to ascertain the results obtained in the current study.

Mongols is a crucial ethnic group among the 55 ethnic minorities in China. Our results illustrate for the first time the differences of the polymorphisms of the *TNFSF11*, *TNFRSF11A* and *TNFRSF11B* genes between Mongols and five other populations and may provide available information regarding genetic polymorphisms in the OPG/RANKL/RANK pathway of different populations and osteopathy-association studies.

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Disclosure of conflict of interest

None.

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