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Original Article

Relation of JAGGED 1 and collagen type 1 alpha 1 polymorphisms with bone mineral density in Chinese postmenopausal women

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Abstract: Osteoporosis is a complex disease characterized by low bone mineral density (BMD), which is determined by an interaction of genetics and environmental factors. Collagen type alpha 1 (COL1A) and JAGGED (JAG1) genes have been implicated in relation to BMD. The aim of this study was to investigate possible association among BMD and rs2273061 of JAG1, rs1107946 and rs1800012 of Col1A1 polymorphisms, as well as their haplotypes with BMD in postmenopausal Chinese women. A structured questionnaire for risk factors was recorded and BMD in lumbar spine and total hip was measured by dual-energy X-ray absorptiomety. Genomic DNA was obtained from 367 postmenopausal Chinese women. Genomic DNA was extracted from EDTA-preserved peripheral venous blood by phenol-chloroform extraction method and analyzed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). As a result, the rs1800012 polymorphism of COL1A1 showed an association with BMD of the lumbar spine under a dominant model. Besides, haplotype analysis of COL1A1 gene showed that G-G haplotype presented higher BMD in lumbar spine. No significant association between genotypes and alleles distributions of the rs1107946 polymorphism of COL1A1 and rs2273061 polymorphism of the JAG1 was found. In conclusion, our results suggest that the rs1800012 polymorphism of the COL1A1 and one haplotype were significantly associated with lumbar spine BMD variations in Chinese postmenopausal women.

Keywords: Bone mineral density, polymorphism, JAG1, COL1A1, haplotypes, Chinese postmenopausal women

Introduction

Osteoporosis (OMIM166710) is a common skeletal disorder characterized by low bone mineral density (BMD) and microarchitectural deterioration of bone tissue which increases the susceptibility to fracture and is one of causes of diminished quality of life among postmenopausal women [1]. Multiple factors influence BMD, however, genetic components play an important role in its pathogenesis with heritability > 60% [2]. There are several genes involved in the genetic determination of BMD. In this regard, a large number of polymorphisms in multiple candidate genes have been investigated in relation to BMD variations.

Finding a genotype that is a marker for those at risk of lower BMD could enable targeted therapy before osteoporosis develops. *Collagen type 1 alpha 1 gene (COL1A1)* is one of these genes that have been implicated in osteoporosis. This gene encodes the 1 (I) protein chain of type I collagen, which makes up 90% of the organic matrix that has a role in bone mineralization and gives flexibility to the bone [3]. Plenty of association studies have been performed between *COL1A1* and BMD variation and osteoporosis. One of the most studied single nucleotide polymorphisms (SNP) is the Sp1 transcription factor binding sites rs1800012 (+ 1245 G > T), located in the first intron of the *COL1A1* gene, an important region for the regulation of collagen transcription [4]. This polymorphism in the *CoL1A1* gene have been found to alter bone strength by altering binding affinity for the transcription factor Sp1. Presence of the T allele leads to abnormal production of the α1 collagen chain in comparison to the α2 collagen chain, which has an adverse effect on bone

Table 1. Single nucleotide polymorphisms (SNPs) investigated in this study and PCR primers for the genotyping of COL1A1 and JAG1

Gene	Region	rs number	Primer pairs	Enzyme	Genotype (bp)
Col1A1	promoter	rs1107946	F: 5'CACCCTGCCCTAGACCAC-3'	Eco31I	TT: 293
			R: 5'GAAAATATAGAGTTTCCAGAG-3'		TG: 293, 217, 76
					GG: 217, 76
Col1A1	Intron 1	rs1800012	F: 5'CTGGACTATTTGCGGACTTTTTGG-3'	Msc1	TT: 260
			R: 5'GTCCAGCCCTCATCCTGGCC-3'		TG: 280, 260
					GG: 280
JAG1	Intron 3	rs2273061	F: 5'TACCCATTAAAGAAGGTAAActAGT-3'	Spe I	AA: 309
			R: 5'CACGCGGTCTGATACTCAAAGTG-3'		AG: 309, 289
					GG: 289

F: forward primer, R: reverse primer.

composition and mechanical strength [4]. Another important polymorphism in the promoter region of COL1A1 gene, the PCOL2 variance rs1107946 (-1997G/T), was first found significantly associated with lumbar spine BMD and femoral neck BMD in a cohort of Spanish postmenopausal women by Garcia-Giralt et al. [5]. Liu et al. revealed a strong interaction between PCOL2 and Sp1 variance in a study of elderly Caucasian females [6]. Bustamante et al. reported that *COL1A1*-1997 G > T and 1245 G > T polymorphisms are associated with increased BMD for lumbar spine [7]. Association studies of these polymorphisms have been conducted in different populations, but no consistent results are reached.

Another important candidate gene for predisposition to osteoporosis is the *JAGGED 1* (*JAG1*) gene, which has been reported involved in bone formation. This gene encodes a cell surface protein called Jagged1, one member of the Delta/Serrate domain (DSL) family, which is the ligand binding to Notch receptors. JAG1 is expressed in osteoblastic cells in vivo and in vitro during bone regeneration and its activation is also associated with increased bone mineral deposition [8]. Jagged1 protein is implicated in trabecular and endosteal osteoblasts, but not in periosteal osteoblasts, and its expression level increased with intermittent parathyroid hormone treatment [9]. A genome-wide association analysis identified that the rs2273061 polymorphism localized in intron 3 of the JAG1 gene was associated with the variations of BMD and osteoporotic fractures in European descent and Asian populations. Additionally, this polymorphism affects the expression of the gene in vitro [10].

The aim of this study is to investigate if there is a possible association between rs1800012 and rs1107946 polymorphisms and their haplotypes of the COL1A1 gene as well as rs2273061 polymorphism of the JAG1 gene with the presence of BMD variations in Chinese Han postmenopausal women.

Subjects and methods

Subjects

367 Chinese Han population postmenoapusal women from Department of Gynecology of Affiliatied, Hospital of Weifang Medical College were included in this study. All subjects were interviewed using a standard questionnaire, including body mass index (BMI), years since menopause, menarche age, tobacco and alcohol use, physical activity and estrogen replacement therapy. All women involved in this study were unrelated individuals and considered postmenopausal if they had not had a menstrual period within the 12 months prior to the examination. The study was approved by the Hospital of Weifang Medical College. Informed consent was obtained from all women.

BMD measurement

Dual-energy X-ray absorptiometry (Norland EXCELL, USA) was used to assess BMD (in grams per square centimeter) at total hip (TH) (trochanter, Ward's area and femoral neck) and lumbar spine(LS) (L2-L4) according to manufacturer's instruction with the patient lying supine on the imaging table. Both machines were calibrated daily. *T* score was used to analyze BMD data.

Table 2. General characteristics of 367 Chinese postmenopausal women

Variable	Mean ± SD
Age (years)	60.15 ± 7.32
Height (m)	155.30 ± 0.41
Weight (kg)	60.53 ± 0.25
BMI (kg/m^2)	25.09 ± 2.65
BMD lumbar spine (g/cm^2)	0.848 ± 0.138
BMD total hip (g/cm^2)	0.911 ± 0.136
Years since menopause	14.7 ± 7.4
Tobacco use, n (%)	29 (7.9%)
Alcohol intake, n (%)	7 (1.9%)
Physical activity, n (%)	121 (33.1%)
Estrogen replacement therapy, n (%)	50 (13.7%)

BMI: body mass index, BMD: body mineral density, SD: standard deviation.

Genotyping

Peripheral venous blood samples were obtained from all subjects, and genomic DNA was isolated using the phenol-chloroform extraction method from EDTA-preserved peripheral venous blood. Genotyping of COL1A1 and JAG1 polymorphisms were determined by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis. PCR amplification was performed in 2720 Thermal Cycler (Applied Biosystems, USA) and PCR products were digested by restriction enzymes. The primer sequences, restriction enzymes, and fragment lengths are presented in **Table 1**.

PCR Amplification of the 293 bp fragment encompassing the CoL1A1 rs1107946 (-1997 G/T) polymorphic site was performed in 10 μl, 1× buffer containing 10 mM Tris HCl, PH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 240 mM dNTPs, 200 ng of template DNA, 0.5 μM of each primer, and 1.25 U Taq DNA polymerase (TransGen Biotech, Beijing, China). After initial denaturation at 94°C for 5 min, amplification was performed by 35 cycles of denaturation at 94°C for 40 s, annealing at 56°C for 30 s, and extension at 72°C for 30 s. Final extension was allowed to proceed at 72°C for 10 min.

Amplification of the 254 bp fragment encompassing the CoL1A1 + 1245 G > T polymorphic site (rs1800012) was performed in 10 μl, 1× buffer containing 10 mM Tris HCl, PH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 250 mM dNTPs, 200 ng of template DNA, 0.5 μM of each primer, and 1.25 U Taq DNA polymerase (TransGen Biotech, Beijing, China). After initial denaturation at 94°C for 5 min, amplification was performed by 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min. Final extension was allowed to proceed at 72°C for 10 min.

Amplification of the 309 bp fragment encompassing the JAG1 + A > G polymorphic site (rs2273061) was performed in 10 μl, 1× buffer containing 10 mM Tris HCl, PH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 250 mM dNTPs, 200 ng of template DNA, 0.5 μM of each primer, and 1.25 U Taq DNA polymerase (TransGen Biotech, Beijing, China). After initial denaturation at 94°C for 5 min, amplification was performed by 35 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 30 s, and extension at 72°C for 1 min. Final extension was allowed to proceed at 72°C for 10 min.

After the amplification, PCR reaction products were separated on a 2-3% agarose gel with TAE buffer. 3 μL aliquots of products were digested with Eco31I, Msc1 and Spe I restriction endonucleases (TAKARA) respectively for 12 h at 37°C and subjected to electrophoresis on 10% polyacrylamide gels, with 1× TBE running buffer stained with ethidium bromide and detected under UV light. Samples of known genotype were run as controls and a DNA-free water negative control in each batch.

Statistical analysis

Quantitative variables were summarized as mean and standard deviation and qualitative variables as relative frequencies for all subjects. One-way analysis of covariance (ANCOVA) was used for continuous variables and χ^2 test for categorical variables. Deviations from Hardy-Weinberg equilibrium were test using χ^2 test. Data analysis was performed using SPSS16.0 software (SPSS, Chicago, IL, USA). $P < 0.05$ was regarded as statistically significant. Linkage disequilibrium (LD) among polymorphisms was tested using Haploview 4.2 [11].

Results

The main demographic and clinical features of 367 postmenopausal women are shown in **Table 2**. The two SNPs of *COL1A1* (rs1107946 and rs1800012) and the SNP of *JAG1* (rs2273061) genotype and allele frequencies

Table 3. Hardy-Weinberg equilibrium, genotype, and allele frequencies for the two SNPs of COL1A1and JAG1in postmenopausal Chinese women

SNP	Genotype	N	Frequency (%)	HWE
rs1107946 of CoL1A1	GG	196	53.4	P = 0.16
	GT	137	37.3	
	TT	34	9.3	
	G	529	72.1	
	T	205	27.9	
rs1800012 of CoL1A1	GG	255	69.5	P = 0.14
	GT	97	26.4	
	TT	15	4.1	
	G	608	82.8	
	T	126	17.2	
rs2273061 of JAG1	AA	127	127	P = 0.47
	AG	172	172	
	GG	68	68	
	A	426	58.1	
	G	308	41.9	

HWE: Hardy-Weinberg equilibrium.

Table 4. Bone mineral density in Chinese postmenopausal women in different genotype subgroups of the COL1A1and JAG1genes

SNP	Genotype	LS (g/cm^2)		TH (g/cm^2)	
		Mean	SD	Mean	SD
rs1107946 of CoL1A1	GG	0.856	0.143	0.919	0.128
	GT	0.848	0.128	0.906	0.136
	TT	0.851	0.139	0.913	0.141
rs1800012 of CoL1A1	GG	0.863	0.142	0.907	0.136
	GT	0.833	0.129*	0.912	0.132
	TT	0.831	0.133*	0.909	0.140
	GT TT	0.834	0.132*	0.911	0.137
rs2273061 of JAG1	AA	0.856	0.135	0.902	0.137
	AG	0.845	0.144	0.899	0.129
	GG	0.860	0.140	0.911	0.142

LS: lumbar spine, TH: total hip.

are presented in **Table 3**. Hardy-Weinberg equilibrium test was performed for the polymorphisms under study and all these three genotype frequencies were in Hardy-Weinberg equilibrium.

For rs1107946 and rs1800012 of COL1A1, There was no significant difference in TH BMD between Col1A1 genotypes. At the LS, there was a significant difference in BMD between Sp1 polymorphism GG genotypes and GT genotypes (P = 0.027). There was also a significant difference in BMD between GG and TT genotypes (P = 0.019). The Sp1 polymorphism was significantly associated only with lumbar spine BMD under the dominant model (GG vs. GT + TT); P = 0.017 (**Table 4**).

Moreover, data from the genotyping analysis of the two SNPs of COL1A1 (rs1107946 and rs1800012) demonstrated the presence of four haplotypes: G-G, T-G, G-T and T-T with frequencies of 60.7%, 27.6%, 11.7 and 0.03% respectively (**Figure 1**). The rare TT haplotype was excluded due to low frequencies. A significant association was observed between G-G haplotype and BMD at the lumbar spine (P = 0.021). The two SNPs of COL1A1 were at high linkage disequilibrium (D' = 0.79). However, the TH BMD was not significantly associated with any haplotypes.

On other hand, genotype and allele distributions of rs2273061 polymorphism of the JAG1 showed no significant differences under any model tested.

Discussion

In this study, we have analyzed the association of two polymorphisms of the COL1A1 gene (rs1800012 and rs1107946) and their haplotypes as well as the rs2273061 polymorphism of the JAG1 gene with the presence of BMD variations in Chinese postmenopausal women. There was no difference in age, BMI, or smoking status when analyzed by genotypes, allowing comparisons between genotypes to be conducted. We found that the rs1800012 polymorphism and G-G haplotype of COL1A1 were associated with BMD variations in lumbar spine.

One of the most intensively investigated polymorphism of COL1A1 affects a Sp1 binding site within intron 1 at 1245 bp relative to the transcription start site. COL1A1 gene 1245 G > T has been associated with low BMD and an increased risk of osteoporotic fracture in several studies [4, 12]. Several association studies about this SNP and its relation to BMD have

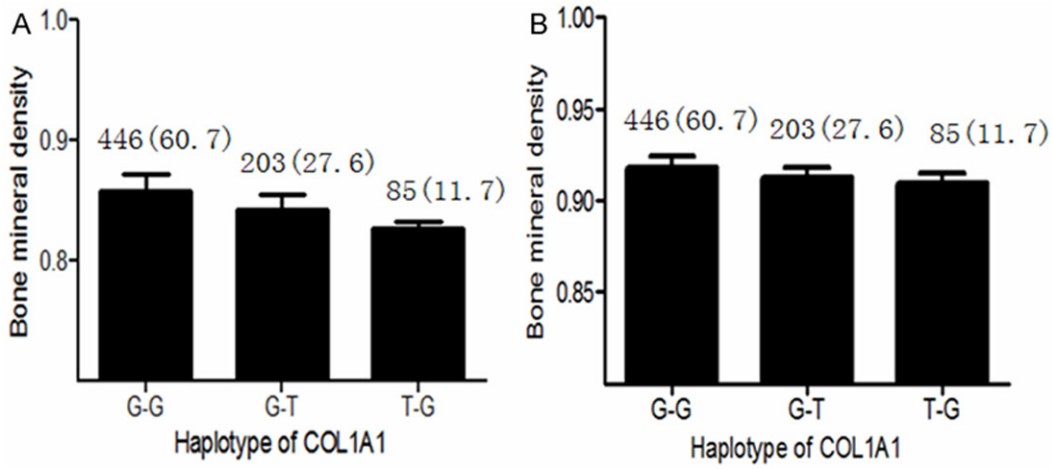

Figure 1. Bone mineral density in postmenopausal Chinese women in different haplotypes of the COL1A1gene. Numbers and frequency with their percentages of each haplotype is shown. A. Bone mineral density of lumbar spine in different haplotypes of the COL1A1gene. B. Bone mineral density of total hips in different haplotypes of the COL1A1gene.

been reported previously. Grant SF et al. investigated that GT heterozygotes at the polymorphic Sp1 site had significantly lower BMD than GG homozygotesin two populations of British women and BMD was lower still in T/T homozygotes [13]. A meta-analysis of 26 studies confirmed the association of the T allele of the 1245 G > T polymorphism with a modest reduction in BMD and a significant risk of osteoporotic fracture [14]. Bustamante et al. reported that COL1A1-1997 G > T and 1245 G > T polymorphisms are associated with increased BMD for lumbar spine [7]. Our results are in agreement with these published works. This study found a significantly lower BMD at the LS in postmenopausal women with GT (26.4%) genotypes and TT (4.1%) genotypes compared with GG genotypes (69.5%). There was a significant reduction in BMD of LS in women with TT and GT genotypes (0.834 g/cm^2) compared to GG genotype (0.863) on a dominant model, P = 0.017.

The PCOL2 variances located in the promoter region of COL1A1 gene was associated with BMD of the lumbar spine and femoral neck in postmenopausal Spanish women [5] and a strong interaction between PCOL2 and Sp1 variance was observed in a study of elderly Caucasian females [6]. These results were also confirmed in Caucasian women from the USA and from the UK and Japanese [6, 15]. However, we found no significant differences between BMD and PCOL2 variances. Our results are in accordance with those described in a population-based study of unrelated postmenopausal Mexican-Mestizo Women that showed no association between -1997 G > T alleles and BMD [16].

Regarding to linkage disequilibrium (LD) among the two SNPs of COL1A1in our population, we observed higher lumbar spine of BMD in G-G haplotype. We observed that these were in high LD (D' = 0.79) different from the values described by Mejía et al. for the Mexican-Mestizo women (D' = 0.84) [16] and values described by Liu et al. for the Caucasian population (D' = 0.918) [6]. In agreement with our results, both authors found a significant association only of the haplotype G-G with BMD.

GWAS of BMD variations performed by Kung et al. identified that the rs2273061 of JAG1 was associated with BMD in three independent cohorts of European descent [10]. In regard to results of the rs2273061 polymorphism, we didn't find any association of this SNP with BMD. Similar data were reported by Kung et al. in the Northern Chinese population [10]. Population diversity may explain these different results.

To our best knowledge, this is the first study to simultaneously examine the possible relationship between bone mineral density and JAG1 rs2273061, COL1A1 rs1107946 and rs180-0012 polymorphisms in Chinese postmeno-

pausal women from Han population. The limitation of our study is the small sample size. In conclusion, the rs1800012 polymorphism of COL1A1 showed an association with BMD of the lumbar spine under a dominant model. G-G haplotype of COL1A1 presented higher BMD in lumbar spine. By enlarging the study we hope that more explanatory and definitive results can be obtained. And further studies are also necessary in different populations.

Disclosure of conflict of interest

None.

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