

Original Article

No association between polymorphisms and haplotypes of *COL1A1* and *COL1A2* genes and osteoporotic fracture in postmenopausal Chinese women

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Aim: To study whether genetic polymorphisms of *COL1A1* and *COL1A2* genes affected the onset of fracture in postmenopausal Chinese women.

Methods: SNPs in *COL1A1* and *COL1A2* genes were identified via direct sequencing in 32 unrelated postmenopausal Chinese women. Ten SNPs were genotyped in 1252 postmenopausal Chinese women. The associations were examined using both single-SNP and haplotype tests using logistic regression.

Results: Twenty four (4 novel) and 28 (7 novel) SNPs were identified in *COL1A1* and *COL1A2* gene, respectively. The distribution frequencies of 2 SNPs in *COL1A1* (rs2075554 and rs2586494) and 3 SNPs in *COL1A2* (rs42517, rs1801182, and rs42524) were significantly different from those documented for the European Caucasian population. No significant difference was observed between fracture and control groups with respect to allele frequency or genotype distribution in 9 selected SNPs and haplotype. No significant association was found between fragility fracture and each SNP or haplotype. The results remained the same after additional corrections for other risk factors such as weight, height, and bone mineral density.

Conclusion: Our results show no association between common genetic variations of *COL1A1* and *COL1A2* genes and fracture, suggesting the complex genetic background of osteoporotic fractures.

Keywords: *COL1A1*; *COL1A2*; polymorphism; bone fracture; osteoporosis

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Introduction

Osteoporotic fracture is the most serious clinical consequence of osteoporosis. It is a major cause of the public health burden associated with osteoporosis due to the high rates of morbidity and mortality in the elderly and the tremendous health care costs^[1, 2]. The incidence of osteoporotic fracture is increasing rapidly in Asian countries^[3, 4]. The incidence for all osteoporotic fractures and hip fractures was 635/100 000 and 123/100 000 person-years in southern Chinese men, respectively^[5]. Therefore, the elucidation of ways to prevent osteoporotic fracture is an important goal. The most common osteoporotic fracture sites are the hip, wrist and spine^[6].

Most studies demonstrate a heritability of 50%–80% for bone mineral density (BMD), but fracture heritability has been estimated to be less than 50%^[7, 8]. The risk for osteoporotic frac-

ture is complex and is regulated by both environmental and genetic factors. To date, most genetic studies have focused on surrogate phenotypes of osteoporotic fracture, such as BMD, bone size, bone geometry parameters and bone turnover markers, to search for genes that underlie the susceptibility to osteoporotic fracture^[9]. Although the measurement of BMD has been the best method to identify the risk for osteoporotic fracture, a meta-analysis for the predictive ability of the measurement for hip fracture was reported a false positive rate of 15% and a detection rate of only 50%^[10]. Another study found that the association between genetic variations and wrist fracture was largely independent of BMD^[8]. Many studies have focused on the relationship between candidate genes and BMD, but some studies have found that candidate genes are associated with an increased risk for osteoporotic fracture independently of BMD^[11, 12], and the genetic correlation between these surrogate phenotypes and osteoporotic fracture is very low^[8, 13]. The underlying genetic factors that cause susceptibility to osteoporotic fracture are generally unknown;

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therefore, the underlying genetic associations with osteoporotic fracture were assessed in the present study. Due to low fracture heritability^[7, 13] and difficulty associated with recruiting a large number of individuals with the same fracture type, until now, few studies have performed a direct genetic evaluation of osteoporotic fracture using a case-control method.

Considering the important role of collagen type I in the bone matrix, genes that are related to type I collagen are potential candidates for osteoporotic fracture. Type I collagen acts by binding to two collagen chains, α_1 and α_2 , which are encoded by the *COL1A1* and *COL1A2* genes, respectively^[14]. There are common polymorphisms in *COL1A1*: Sp1 (rs1800012) in intron 1, -1997G/T (rs1107946) and -1663in/de1T (rs2412298) have been extensively studied because of their association with the risk for osteoporosis^[12, 15-18]. Of note, the Sp1 polymorphism is associated with a modest reduction of BMD and a significant increase in the risk for osteoporotic fracture, particularly vertebral fracture^[12, 16, 18]. A recent meta-analysis of clinical studies has confirmed that the Sp1 polymorphism is associated with a higher prevalence of fractures among heterozygote (Ss) and homozygote (ss) in comparison to wild type individuals (SS) and that *COL1A1* "s" allele has a stronger association with the risk for fracture than with BMD^[19]. However, the Sp1 polymorphism has not been found in the Chinese population^[20, 21]. The relationship between *COL1A2* genotypes and osteoporosis is less well characterized than that between *COL1A1* genotypes and osteoporosis. A number of studies have found an association between GT-repeat polymorphisms, *Msp* I, *Pvu* II, and *Eco*R I of the *COL1A2* gene and BMD^[22, 23] or bone size^[24]. However, there are few studies selected osteoporotic fracture as a direct phenotype. Therefore, additional data are required to confirm the potential role of collagen type I genes in the pathogenesis of osteoporotic fracture. In the present study, we aimed to investigate whether the genetic polymorphisms in the *COL1A1* and *COL1A2* genes affected the onset of osteoporotic fracture independently of clinical risk factors in postmenopausal Chinese women.

Materials and methods

Subjects

The study was approved by the Ethics Committee of the Shanghai Jiao Tong University Affiliated Sixth People's Hospital. All of the subjects included in the study were recruited from a local population in Shanghai City by the Department of Osteoporosis and Bone Diseases, Shanghai Jiao Tong University Affiliated Sixth People's Hospital from 2006 to 2009. All of the subjects signed informed consent documents before participating in the project. After signing, the subjects were assisted with the completion of a structured questionnaire that included information regarding anthropometric variables, lifestyle and medical history. A physical checkup and routine serum examination were performed, and all of the subjects were found to be in good health. Secondary causes of osteoporosis were excluded. Postmenopausal women who had experienced early menopause (before 45 years of age) and those who had undergone ovariectomy or were receiving

estrogen replacement therapy or antiosteoporotic drugs were excluded.

Volunteers were recruited from communities in Xuhui district, Shanghai, China and cases of vertebral fractures that were detected by radiographs were screened. Among patients who were seeking medical treatment in the outpatient department, Colles' and hip fractures (including femoral neck fractures and trochanter fractures) were found on the radiographs. The diagnosis was performed by orthopedic surgeons and radiologists according to the information provided by X-rays. In total, 1252 unrelated subjects (627 cases of fragility fracture in the case group and 625 cases in the control group) of postmenopausal Han women between the ages of 45 and 80 years were selected for inclusion in the study. The inclusion criteria for fragility fracture cases were as follows: (i) age <80 years and onset age >45 years; (ii) minimal fractures, usually due to falls from standing height or less; (iii) fracture site location in the lumbar spine, hip and radius; (iv) a diagnosis of osteoporotic fracture identified and confirmed by radiographs that were interpreted by orthopedic surgeons or radiologists and by the Genant semiquantitative method to assess vertebral fractures^[25]. There were 668 fracture times in the fracture group (Table 1): 419 cases were vertebral fractures (composition ratio of 62.7%), 156 cases were Colles' fractures (composition ratio of 22.4%) and 93 cases were hip fractures (composition ratio of 13.9%). All of the subjects with a fracture were considered to have primary osteoporosis. The inclusion criteria for control subjects were an age <80 years, an onset age >45 years and a lack of any fracture history. The exclusion criteria were same as those described in our previously reported study^[26].

Table 1. Details of the fracture sites in the case subjects.

Fracture site	Age (years)	No of fractures
Vertebral fracture (including thoracic and lumbar regions)	70.9±9.0	419
Hip fracture	70.6±9.7	93
Colles' fracture	68.8±9.2	156

BMD measurements

The BMD (g/cm²) of the left proximal femur (if the individual had a left hip fracture, the right proximal femur was measured) including the total hip, the femoral neck, the trochanter, and Ward's triangle were measured using dual-energy X-ray absorptiometry (DXA) on a Hologic QDR 2000 (Hologic, Bedford, MA, USA) and a Lunar Prodigy GE densitometer (Lunar Corp, Madison, WI, USA). Both of the scanners were calibrated daily, and the coefficient of variability (CV) values for the DXA measurements at L1-4, the total hip, and the femoral neck were 0.9%, 0.8%, and 1.93%, respectively, for the Hologic QDR 2000^[26] and 1.39%, 0.7%, and 2.22%, respectively, for the Lunar Prodigy^[27]. The long-term reproducibility of the DXA

data during the trial, which was based on phantom measurements that were repeated weekly, was 0.45%. Height and weight were measured using standardized equipment.

Sequencing analysis of the *COL1A1* and *COL1A2* genes

Genomic DNA was isolated from peripheral blood leukocytes using conventional methods. Single nucleotide polymorphisms (SNP) screening was conducted by DNA sequencing of the entire gene sequence in 32 unrelated healthy postmenopausal Chinese women using the ABI Prism 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). The primers used for the amplification and sequencing analysis were designed based on GenBank sequences (reference sequence of *COL1A1* mRNA: NM_000088.3 and *COL1A2* mRNA: NM_000089.3). Sequence variants were verified using the Phred/Phrap/Consed package and Polyphred^[28,29].

SNP selection and genotyping

According to the sequencing analysis of the *COL1A1* and *COL1A2* genes and specific criteria, ten SNPs in two genes were selected. SNPs in the two genes were selected based on the following criteria: (i) degree of heterozygosity (minor allele frequency (MAF) >10%); (ii) $r^2=0.8$; (iii) denoted as tagSNPs; (iv) amino acid mutations, missense mutations: rs1800215 (Ala1075Thr) and rs42524 (Pro549Ala), and synonymous mutations: rs1801182 (Thr29Thr) and rs1800248 (Gly1045Gly). Based on these criteria, we selected five SNPs in the *COL1A1* gene, including SNP 3 (rs2075554), SNP 7 (rs2586494), SNP 18 (rs1800215), SNP 19 (rs2586488) and SNP 23 (rs2277632), and five SNPs in *COL1A2*, including SNP 1 (rs1801182), SNP 9 (rs42517), SNP 18 (rs42524), SNP 19 (rs421587), and SNP 27 (rs1800248). Ten SNPs were genotyped using the TaqMan assay, for which the primer and probe sequences were optimized using the SNP Assay-by-Design service provided by Applied Biosystems. The genotype for every sample was named according to the ratio of the fluorescence intensities of the two dyes. The reactions were performed with a Mx3000P Real-Time PCR System (Stratagene, CA, USA) using 20 ng of genomic DNA in a 5- μ L reaction volume per well.

LD and haplotype analysis

Haplotypes were constructed from the population genotype data using the algorithm of Stephens with the Phase program version 2.0.2^[30]. The linkage disequilibrium block structure was examined using Haploview version 3.2^[31]. We examined Lewontin's D' and the LD coefficient r^2 between all of the pairs of biallelic loci. The genotype and haplotype frequencies were calculated for 32 unrelated subjects and 1252 postmenopausal women.

Statistical analysis

Statistical analyses were performed using SPSS version 11.0 (SPSS, Chicago, IL, USA). The allele frequencies for each SNP were calculated by counting the alleles, and the Hardy-Weinberg equilibrium was assessed by Chi-squared analysis.

Because of the different DXA devices that were used to

measure BMD in the 1252 subjects (some were measured by Hologic QDR 2000 and others by Lunar Prodigy GE), the absolute BMD values could not be compared. Therefore, we transformed the absolute value determined for the femoral neck BMD to a Z-score of the neck BMD using the BMD reference database for a previously reported Shanghai population^[26,27].

The age, height, weight, and femoral neck BMD (Z-score) for the nine genotypes in the two groups were analyzed by analysis of variance (ANOVA). Analysis of covariance (ANCOVA) was used to analyze the association of the nine genotypes or haplotypes with femoral neck BMD while simultaneously adjusting the height, weight, and years since menopause (YSM) as covariates. A Chi-squared analysis was used to test the differences among the nine SNP genotype frequencies between the case and control groups. By adjusting for age, height, weight, and YSM and BMD as risk factors, a logistic regression was used to analyze the association of the nine genotypes or haplotypes with the risk for osteoporotic fracture. $P<0.05$ was considered statistically significant in all of the analyses.

Results

Basic characteristics of the subjects

1252 postmenopausal women were included in the study: 627 osteoporotic fracture cases and 625 controls. The basic characteristics of the study subjects are shown in Table 2. Differences in age, height, weight, YSM and femoral neck BMD (Z-score) were found in the two groups according to a *t*-test analysis. The average age in the case group [(70.4 \pm 9.2) years] was older than that in the control group [(60.6 \pm 6.7) years] ($P<0.01$); the average height in the case group [(151.6 \pm 6.9) cm] was less than that in the control group [(155.3 \pm 5.6) cm] ($P<0.01$); the average weight in the case group [(55.7 \pm 9.7) kg] was lower than that in the control group [(57.6 \pm 8.3) kg] ($P<0.01$); the average femoral neck BMD was lower in the case group (Z-score: -0.42 \pm 1.07) than in the control group (Z-score: -0.08 \pm 0.97) ($P<0.01$); and YSM was longer in the case group [(16.9 \pm 7.1) years] than in the control group [(15.1 \pm 6.6) years] ($P<0.05$). No significant differences were observed between the two groups with respect to BMI ($P>0.05$).

SNP genotyping and linkage disequilibrium

SNPs were genotyped for a sample of 32 unrelated individuals who were randomly selected among 1252 subjects in our population, and 24 and 28 SNPs were identified in the *COL1A1* and *COL1A2* genes, respectively. Twenty SNPs in the *COL1A1* gene were found in dbSNP of the NCBI database, but four SNPs were novel. Twenty-one SNPs in the *COL1A2* gene were found in dbSNP of the NCBI database, but seven SNPs were novel. All of the SNPs demonstrated MAF of >1.0% in our population, and the genotype frequencies of these SNPs did not deviate from Hardy-Weinberg equilibrium ($P>0.05$; Tables 3 and 4). The frequencies of each SNP are shown in Tables 3 and 4. According to these genotype frequencies and the above mentioned criteria, five SNPs (rs2075554, rs2586494, rs1800215, rs2586488, and rs2277632) in the *COL1A1* gene and five SNPs

Table 2. Basic characteristics of the study subjects. Data are the mean±SD (range).

Trait	Cases (n=627)	Controls (n=625)	P value
Age (year)	70.4±9.2 (46.0–82.5)	60.6±6.7 (45.0–81.8)	<0.001
Height (cm)	151.6±6.9 (131.0–170.5)	155.3±5.6 (135.6–169.0)	<0.001
Weight (kg)	55.7±9.7 (34.0–88.0)	57.6±8.3 (35.4–87.0)	<0.001
BMI (kg/cm ²)	24.2±3.7 (13.2–38.2)	23.9±3.1 (14.6–39.5)	0.107
Years since menopause (year)	16.9±7.1 (0.8–31.6)	15.1±6.6 (0.6–30.1)	0.030
Femoral neck BMD (Z-score)	-0.42±1.07 (-3.78–3.29)	-0.08±0.97 (-2.90–3.71)	<0.001

Table 3. Positions and frequencies of *COL1A1* gene polymorphisms in the Chinese population (n=32).

SNP name	Loci	SNP	SNP in dbSNP	Genotype frequencies (% , N)		
SNP1	471+195T>C	C/T	Novel	TT (71.8, 23)	CT (28.2, 9)	
SNP2		C/T	rs35231764	TT (71.8, 23)	CT (25, 8)	CC (3.2, 1)
SNP3		A/G	rs2075554	AG (53.1, 17)	GG (37.5, 12)	AA (9.4, 3)
SNP4		A/C	rs2075555	CC (43.8, 14)	CA (46.9, 15)	AA (9.4, 3)
SNP5		A/G	rs2141279	GG (56.3, 18)	AG (37.5, 12)	AA (6.2, 2)
SNP6		A/G	rs2734281	AG (53.1, 17)	AA (28.1, 9)	GG (18.8, 6)
SNP7		T/G	rs2586494	TT (34.4, 11)	GT (43.8, 14)	GG (21.8, 7)
SNP8		A/G	rs2253369	AG (43.8, 14)	AA (43.8, 14)	GG (12.4, 4)
SNP9		C/T	rs1007086	CT (46.9, 15)	TT (37.5, 12)	CC (15.6, 5)
SNP10		C/G	rs41316671	GG (71.8, 23)	CG (18.8, 6)	CC (9.4, 3)
SNP11		C/T	rs2696247	TC (43.8, 14)	TT (43.8, 14)	CC (12.4, 4)
SNP12	2028+27G>A	A/G	Novel	GG (93.8, 30)	GA (6.2, 2)	
SNP13		C/G	rs2075556	GC (43.8, 14)	GG (43.8, 14)	CC (12.4, 4)
SNP14		T/G	rs2075558	GG (75.0, 24)	TT (12.6, 4)	TG (12.4, 4)
SNP15	2559+27C>T	T/C	Novel	CC (93.8, 30)	CT (6.2, 2)	
SNP16		C/G	rs2075559	CG (46.9, 15)	CC (34.4, 11)	GG (18.7, 6)
SNP17	2949C>A	A/C	Novel	CC (93.8, 30)	CA (6.2, 2)	
SNP18		A/G	rs1800215	AG (53.2, 17)	GG (46.8, 15)	
SNP19		C/T	rs2586488	TC (43.8, 14)	TT (34.4, 11)	CC (21.8, 7)
SNP20		T/C	rs2277634	CC (93.8, 30)	CT (6.2, 2)	
SNP21		T/C	rs41316715	CC (90.6, 29)	CT (9.4, 3)	
SNP22		T/C	rs2277633	CC (93.8, 30)	CT (6.2, 2)	
SNP23		T/C	rs2277632	TC (53.1, 17)	TT (28.1, 9)	CC (18.8, 6)
SNP24		A/G	rs2249492	GG (37.5, 12)	AG (43.8, 14)	AA (18.7, 6)

(rs1801182, rs42517, rs42524, rs421587, and rs1800248) in the *COL1A2* gene were subsequently measured.

The data for ten SNPs in the *COL1A1* and *COL1A2* genes are summarized in Table 5. Among those SNPs, rs1800215 in the *COL1A1* gene deviated from Hardy-Weinberg equilibrium, as determined by chi-squared analysis ($\chi^2=85.63$, $P<0.001$), and therefore, this SNP was excluded from the subsequent statistical analysis. The genotype frequencies of the other nine SNPs did not deviate significantly from Hardy-Weinberg equilibrium ($P>0.05$). The LD patterns of *COL1A1* and *COL1A2* are shown in Figures 1 and 2. In the *COL1A1* gene, based on the D' values ($D'=0.87$), we identified one block with a high LD that ranged from intron 44 to intron 48 and included rs2586488 and rs2277632 (Figure 1A). When the haplotypes were reconstructed with the inclusion of rs2586488 and rs2277632, four potential haplotypes were obtained for the *COL1A1* gene

(Figure 1B). In the *COL1A2* gene, only a single LD block was identified based on the D' values ($D'=0.87$), and it ranged from exon 3 to exon 47 and included rs1801182, rs42517, rs42524, rs421587, and rs1800248 (Figure 2A). When the haplotypes were reconstructed with the inclusion of these five SNPs in the *COL1A2* gene, six possible haplotypes were obtained (Figure 2B).

Association between SNP and haplotype and BMD in postmenopausal women

There were no differences in the femoral neck BMD values (Z-score) determined for the 9 SNP genotypes according to an ANOVA analysis (data not shown). The results for the association between the *COL1A1* and *COL1A2* genotypes and BMD were determined by ANCOVA. Following an adjustment for height, weight and YSM as covariates, no significant results

Table 4. Positions and frequencies of COL1A2 gene polymorphisms in the Chinese population (n=32).

SNP name	Loci	SNP	SNP in dbSNP	Genotype frequencies (% , N)		
SNP1		C/T	rs1801182	TT (46.9, 15)	CT (40.6, 13)	CC (12.5, 4)
SNP2	96+112A>G	A/G	Novel	AA (93.8, 30)	AG (6.2, 2)	
SNP3	97-119C>G	C/G	Novel	CC (84.4, 27)	CG (15.6, 5)	
SNP4		A/G	rs4729131	GG (46.9, 15)	GA (46.9, 15)	AA (6.2, 2)
SNP5		C/T	rs1800222	TT (46.9, 15)	CT (40.6, 13)	CC (12.5, 4)
SNP6		A/T	rs405946	AA (78.1, 25)	AT (22.9, 7)	
SNP7		A/G	rs406226	AA (78.1, 25)	AG (22.9, 7)	
SNP8		C/T	rs28417792	CT (43.8, 14)	CC (43.8, 14)	TT (12.4, 4)
SNP9		A/G	rs42517	AG (46.9, 15)	AA (46.9, 15)	GG (6.2, 2)
SNP10	739-96T>C	C/T	Novel	TT (93.8, 30)	CT (6.2, 2)	
SNP11		C/T	rs42518	TT (78.1, 25)	CT (22.9, 7)	
SNP12		A/G	rs28754326	GG (93.8, 30)	GA (6.2, 2)	
SNP13		C/T	rs42519	TT (75, 24)	CT (25, 8)	
SNP14		A/G	rs2521206	AG (46.9, 15)	AA (28.1, 9)	GG (25.0, 8)
SNP15		T/C	rs52520	TT (78.1, 25)	CT (22.9, 7)	
SNP16		A/G	rs34026686	GG (78.1, 25)	AG (22.9, 7)	
SNP17		A/G	rs412777	AA (50.0, 16)	AG (50.0, 16)	
SNP18		C/G	rs42524	GG (75, 24)	CG (25, 8)	
SNP19		A/G	rs421587	AA (71.9, 23)	AG (28.1, 9)	
SNP20		A/G	rs2301643	GG (78.1, 25)	AG (22.9, 7)	
SNP21		T/G	rs1800238	GG (43.8, 14)	GT (37.5, 12)	TT (19.7, 6)
SNP22	2296-82C>T	C/T	Novel	CC (93.8, 30)	CT (6.2, 2)	
SNP23		A/G	rs42530	AA (53.1, 17)	AG (40.7, 13)	GG (6.2, 2)
SNP24	3105+15G>C	C/G	Novel	GG (93.8, 30)	CG (6.2, 2)	
SNP25	3105+43G>A	A/G	Novel	GG (78.1, 25)	AG (22.9, 7)	
SNP26		C/T	rs13590724	TT (78.1, 25)	CT (22.9, 7)	
SNP27		C/T	rs1800248	CC (75, 24)	CT (25, 8)	
SNP28	3712-13C>T	C/T	Novel	CC (93.8, 30)	CT (6.2, 2)	

Table 5. Characteristics of the analyzed SNPs. MAF: minor allele frequency in the whole sample.

Gene name	SNP name	Allele	Location	Amino acid change	MAF (Hapmap-HCB)	MAF (Hapmap-CEU)	MAF (This study)
COL1A1	rs2075554	A/G	Intron 11	None	0.411	0.190	0.335
	rs2586494	A/G	Intron 16	None	0.467	0.175	0.5
	rs1800215	A/C	Exon 44	Ala1075Thr	0.000	0.026	0.287
	rs2586488	A/G	Intron 44	None	0.467	0.353	0.458
	rs2277632	A/G	Intron 48	None	0.378	0.208	0.371
COL1A2	rs1801182	C/T	Exon 3	None	0.289	0.008	0.381
	rs 42517	A/G	Intron 12	None	0.329	0.276	0.27
	rs 42524	G/C	Exon 28	Pro549Ala	0.056	0.202	0.068
	rs 421587	A/G	Intron 28	None	0.193	0.308	0.165
	rs1800248	T/C	Exon 47	None	0.102	0.125	0.103

were obtained for the association between each SNP and the femoral neck BMD (data not shown). Using ANCOVA to analyze the association of the haplotypes with femoral neck BMD, we failed to detect any differences in femoral neck BMD among the various haplotypes (data not shown).

SNP and haplotype analyses for osteoporotic fractures

Using Chi-squared analysis, no differences were observed between the case and control groups with respect to the nine SNP genotype frequencies (Table 6). The same method was used to evaluate differences between the case and control

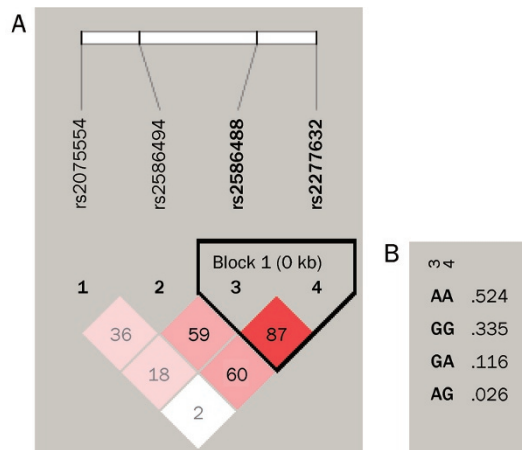


Figure 1. LD patterns for the *COL1A1* gene in 1252 subjects. Squares in red indicate a strong LD. Values in the cells represent the pairwise degrees of LD indicated by $D' \times 100$ when $D' < 1$. (A) 1 to 4 represent rs2075554, rs2586494, rs2586488, and rs2277632, respectively. (B) Haplotype frequencies for the *COL1A1* gene.

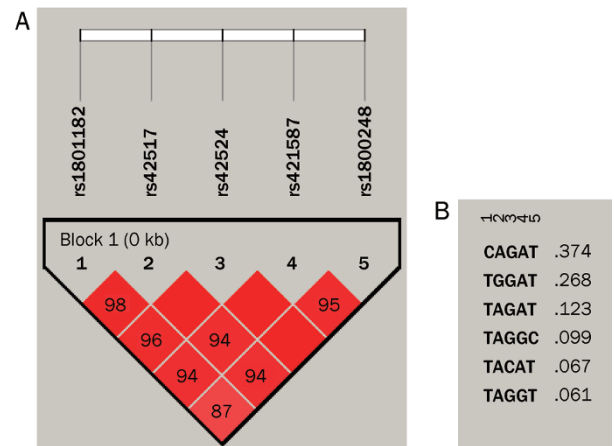


Figure 2. LD patterns for the *COL1A2* gene in 1252 subjects. Squares in red indicate a strong LD. Values in the cells represent the pairwise degrees of LD indicated by $D' \times 100$ when $D' < 1$. (A) 1 to 5 represents rs1801182, rs42517, rs42524, rs421587, and rs1800248, respectively. (B) Haplotype frequencies for the *COL1A2* gene.

Table 6. Frequencies of the nine SNPs.

Gene name	SNP		Genotype, N (%)		χ^2	P value
			Cases	Controls		
<i>COL1A1</i>	rs2075554	AA	80 (12.8)	72 (11.5)	0.486	0.784
		AG	266 (42.6)	267 (42.8)		
		GG	279 (44.6)	285 (45.7)		
	rs2586494	AA	155 (24.7)	166 (26.6)	0.898	0.638
		AC	305 (48.7)	304 (48.6)		
		CC	167 (26.6)	155 (24.8)		
	rs2586488	AA	193 (30.8)	182 (29.1)	0.705	0.703
		AG	296 (47.2)	310 (49.6)		
		GG	138 (22.0)	133 (21.3)		
	rs2277632	AA	258 (41.1)	248 (39.7)	0.329	0.848
		AG	279 (44.5)	284 (45.4)		
		GG	90 (14.4)	93 (14.9)		
<i>COL1A2</i>	rs1801182	CC	89 (14.2)	101 (16.1)	1.658	0.437
		CT	297 (47.4)	276 (44.2)		
		TT	240 (38.3)	248 (39.7)		
	rs42517	AA	336 (53.7)	337 (53.9)	1.321	0.517
		AG	246 (39.3)	234 (37.4)		
		GG	44 (7.0)	54 (8.6)		
	rs42524	GG	543 (86.7)	545 (87.2)	2.319	0.314
		CG	81 (12.9)	74 (11.8)		
		CC	2 (0.3)	6 (1.0)		
	rs421587	AA	435 (69.5)	441 (70.6)	0.820	0.664
		AG	163 (27.8)	163 (26.1)		
		GG	93 (2.7)	21 (3.4)		
	rs1800248	TT	509 (81.3)	497 (79.5)	0.833	0.659
		TC	108 (17.3)	120 (19.2)		
		CC	9 (1.4)	8 (1.3)		

groups with respect to the nine SNP allele frequencies, but no differences were detected (data not shown). Following

an adjustment for age, height, weight, YSM, and BMD as risk factors, we failed to find any significant associations between

osteoporotic fracture and each SNP according to a logistic regression (data not shown). We further investigated the relationship between various haplotypes and osteoporotic fracture using logistic regression. No significant association was found between any of the haplotypes and osteoporotic fracture (Table 7). However, height, weight and femoral neck BMD (Z-score) were found to be risk factors for osteoporotic fracture (Table 7). Meanwhile, we investigated the relationship between various haplotypes and vertebral, wrist and hip fracture using logistic regression, respectively. Also no significant association was found between any of the haplotypes and various sites fracture (data not shown).

To eliminate the age differences that might be produced by false-negative results, we matched 627 cases in the fracture group to 625 cases in the control group. After matching the two groups according to age, 813 postmenopausal women among the 1252 cases were included (402 cases of fragility fracture in the case group and 411 cases in the control group). And we used the same method as the above statistical analyses to analyze association between polymorphisms and haplotypes in *COL1A1* and *COL1A2* genes and osteoporotic fracture in 813 postmenopausal women. No differences were found in neck BMD among the various SNPs or any haplotypes (data not shown). No significant association was found between polymorphisms or any of the haplotypes and osteoporotic fracture (data not shown).

Discussion

This study is the first to report the distribution by DNA sequencing of the entire *COL1A1* and *COL1A2* genes in healthy postmenopausal Chinese women. In the present study, we found four novel SNPs in the *COL1A1* gene and seven novel SNPs in the *COL1A2* gene and that the distribution frequencies of two SNPs in *COL1A1* (rs2075554 and rs2586494) and three SNPs in *COL1A2* (rs42517, rs1801182, and rs42524) were significantly different from those documented for the European Caucasian population shown in the HapMap database.

This result suggests that the allele distribution of the two gene frequencies differs among various ethnicities.

The extent to which genetic information can improve the prognosis of a fracture is largely unknown. Based on the importance of collagen type I in the bone matrix, we explored the associations of the *COL1A1* and *COL1A2* genes with osteoporotic fracture in postmenopausal Chinese women.

The *COL1A1* gene has been widely studied in previous reports, but inconsistent results have been obtained, even for the same allele. For example, some studies have found that the Sp1 polymorphism in the *COL1A1* gene contributes to susceptibility to vertebral fracture or BMD^[12, 15, 16, 18], but other studies have reported that there is no such association^[20, 21]. These discordant findings may be due to the inclusion of study subjects from populations with different ethnicities. Due to the lack of the Sp1 polymorphism in Chinese women^[20, 21], this SNP was not evaluated in the present study. Few studies have investigated the association among other SNPs (excluding the Sp1 polymorphism) in *COL1A1* and osteoporotic fracture or BMD^[16, 17]. Gareia-Giralt *et al*^[16] found two novel SNPs, -1997G/T (rs1107946), and -1663in/de1T (rs2412298), in *COL1A1* that were located in the upstream regulatory region of the gene in postmenopausal Spanish women; the authors suggested that -1997G/T had a significant association with BMD. Jin *et al*^[17] found that the -1997G/T, -1663in/de1T, and +1245G/T polymorphisms affected BMD and that the binding of several regulatory factors (Nmp4 and Osterix) regulated the transcription of *COL1A1*. In the present study, we evaluated four SNPs in the *COL1A1* gene (rs2075554, rs2586494, rs2586488, and rs2277632) and failed to find an association between single-SNP or haplotype analysis and osteoporotic fracture.

Until now, most studies have investigated the association between SNPs in *COL1A2* and BMD or other surrogate phenotypes^[22-24, 32]. To our knowledge, this is the first study to investigate the association of *COL1A2* with osteoporotic fracture. Our results revealed that there was no individual SNP

Table 7. Haplotype analyses to assess osteoporotic fractures. OR: odds ratio.

	Variant	B	SE	P value	OR	95% CI for OR	
						Lower	Upper
COL1A1	Height	-0.101	0.011	0.000	0.904	0.884	0.924
	Weight	0.022	0.008	0.008	1.022	1.006	1.038
	Femoral neck BMD (Z-score)	-0.349	0.070	0.000	0.705	0.615	0.809
	Haplo1-AA	0.019	0.341	0.922	1.001	0.520	1.917
	Haplo2-GG	0.145	0.244	0.587	1.135	0.702	1.878
	Haplo3-GA	0.128	0.199	0.530	1.147	0.778	1.693
	Haplo4-AG	0.104	0.206	0.521	1.127	0.759	1.606
COL1A2	Haplo1-CAGAT	-0.090	0.246	0.695	0.952	0.581	1.455
	Haplo2-TGGAT	-0.080	0.235	0.737	0.923	0.571	1.501
	Haplo3-TAGAT	0.020	0.120	0.867	1.020	0.806	1.291
	Haplo4-TAGGC	-0.023	0.090	0.807	0.975	0.823	1.160
	Haplo5-TACAT	-0.138	0.209	0.589	0.889	0.595	1.367
	Haplo6-TAGGT	-0.099	0.267	0.700	0.923	0.637	1.537

or any of haplotypes in *COL1A2* associated with osteoporotic fracture. Lindahl *et al*^[23] discovered interactions between the *COL1A2* gene and BMD in older men from Sweden, the United Kingdom and Hong Kong ($n=2004$). Their results demonstrated that rs42524 was significantly associated with BMD and older men with a CC or GG genotype had a higher BMD than men who carried the CG genotype. In the present study, the same SNP was evaluated and failed to demonstrate any association with BMD or the risk for osteoporotic fracture in postmenopausal women. These inconsistent findings may be explained by the use of study subjects with different ethnicities and genders. Lau *et al*^[22] found that *Pvu* II and *Eco*R I in the *COL1A2* gene was associated with BMD in elderly men in Hong Kong, but they did not find any association between these two SNPs and BMD in 450 postmenopausal women. Lei *et al*^[24] analyzed *Msp* I in the *COL1A2* gene and osteoporosis according to another phenotype (bone size). Using the quantitative transmission disequilibrium test (QTDT) in Chinese population, they found that this SNP was associated with femoral neck bone size. These studies, which were performed in Chinese population, used BMD or bone size as the study phenotype, in contrast to the use of osteoporotic fracture as the phenotype in the present study. Therefore, our results cannot be compared with the above findings obtained for the Chinese population.

A shortcoming of association studies is the recognition of population admixtures that yield false-positive or false-negative genetic effects for complex traits, which can have significant adverse effects on the results^[33]. To eliminate the age stratification that might be produced by false-negative or false-positive results, we matched 402 cases in the fracture group to 411 cases in the control group. But we also did not find differences in neck BMD among the various SNPs or any haplotypes, and also failed to find significant association between polymorphisms or any of the haplotypes and osteoporotic fracture.

Although osteoporotic fracture is a heritable trait, many studies have also indicated that fracture is partly governed by genetic factors that are distinct from bone mineralization as measured by the BMD^[8, 10, 11, 13]. Tran *et al*^[34] also found that genetic profiling could enhance the predictive accuracy of fracture prognosis by comparison of clinical risk factors and 50 genes in 858 men and 1358 women with fragility fractures. Because fractures typically occur later in life, it is difficult to study the genetics of the osteoporotic fracture phenotype^[11, 13, 35]. Therefore, quantitative risk factors such as BMD, bone geometry parameters and bone turnover markers are used to predict the risk for osteoporotic fractures^[9, 10]. However, it is important to note that none of these surrogate phenotypes are ideal for a genetic study^[36], because these phenotypes could not entirely replace osteoporotic fractures. Furthermore, recent genome-wide association study (GWAS) showed that rs4355801 near the *TNFRSF11B* (osteoprotegerin) gene and rs3736228 in the *LRP5* gene were associated with BMD, only rs3736228 was associated with the risk of osteoporotic fractures identified in 314 075 SNPs in 6463 people^[37].

This study also did not find associations between SNPs in *COL1A1* and *COL1A2* genes and osteoporotic fracture or BMD^[37], and was accordant with our result.

One of the strengths of the present study was that the fracture data obtained for the case group was verified by hospital radiographs rather than comprising a collection of self-reported fractures. Another strength was that the SNPs in our study were gotten in the sequencing analysis of *COL1A1* and *COL1A2* genes in postmenopausal Chinese women. Our study has several limitations. First, because we selected the most informative SNPs, which demonstrated a high MAF and r^2 , some causal variants (such as rare SNPs) might have been overlooked. Second, a number of other risk factors were not taken into account, such as the fall frequency.

In conclusion, we tested the association between SNPs and haplotypes in *COL1A1* and *COL1A2* genes with osteoporotic fracture in postmenopausal Chinese women. Our results failed to find the association between each of the 9 SNPs and haplotypes in *COL1A1* and *COL1A2* genes and osteoporotic fracture. Although our findings must be confirmed in other populations, the present results suggest that the common genetic variants in *COL1A1* and *COL1A2* are not major contributors to the risk for osteoporotic fracture in postmenopausal Chinese women. To confirm whether the SNPs in *COL1A1* and *COL1A2* can be used as a genetic means of determining the risk for osteoporotic fracture requires a large number of sample cases from populations that comprise different ethnicities and ages. In addition, lower and rare SNPs information in these two genes needs further research.

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Author contribution

Wei-wei HU measured all SNPs, carried out all statistical analyses and drafted the manuscript. Zhen-lin ZHANG conceived and designed the study and revised the manuscript. Jin-wei HE guided the work of the genetics laboratory and guaranteed and confirmed the quality of the genetic data. Chun WANG, Hao ZHANG, and Hua YUE guided the work of the genetics laboratory. Jie-mei GU, Wen-zhen FU, Yun-qiu HU, Miao LI, and Yu-juan LIU carried out the field work including sample and data collection. Yao-hua KE coordinated the DNA sample collection. All authors read and approved the final manuscript.

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