Gene 540 (2014) 226–231

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Combined effects of collagen type I alpha1 (*COL1A1*) Sp1 polymorphism and osteoporosis risk factors on bone mineral density in Turkish postmenopausal women

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ARTICLE INFO

Article history:
Accepted 14 February 2014
Available online 22 February 2014

Keywords:
Collagen
Gene polymorphism
Bone mineral density
Osteoporosis risk factors
Postmenopausal women

ABSTRACT

Identification of risk factors for osteoporosis has been essential for understanding the development of osteoporosis. The collagen type I alpha1 (*COL1A1*) gene is suggested to be implicated in reduced bone mineral density (BMD) in osteoporosis. In the present study, the investigation of the effects of Sp1 polymorphic variants of *COL1A1* gene on BMD values, and the determination of the association between *COL1A1* Sp1 gene variants and osteoporosis risk factors in the context of gene–environment interaction in Turkish postmenopausal women were aimed. For the detection of *COL1A1* Sp1 polymorphism, PCR-RFLP techniques have been used. BMD for lumbar spine (L1–L4) and hip (femoral neck and total hip) was measured by DXA. This study was carried out using a sample of 254 postmenopausal women. We observed a trend decrease in BMD values in the subjects with "ss" genotype having lower BMD of lumbar spine, femoral neck and total hip than those with "SS" and "Ss" genotype, however the differences did not reach statistical significance ($P > 0.05$). We also found that the frequencies of the BMD under mean values at the femoral neck (57.5%) and total hip (76.2%) increased considerably in the subjects carrying "Ss/ss" genotypes in combination of having family history of osteoporosis (61.5% for femoral neck) and smoking history (90.0% for total hip). This population-based study indicates that *COL1A1* Sp1 polymorphism may contribute to the development of osteoporosis in combination of osteoporosis risk factors in Turkish postmenopausal women.

1. Introduction

Osteoporosis is a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture (NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis and Therapy, 2001). Bone mineral density (BMD), an important risk factor for osteoporosis, is under strong genetic control with heritability estimates ranging from 0.5 to 0.9 (Arden et al., 1996; Pocock et al., 1987; Slemenda et al., 1991), although many environmental factors, such as dietary intakes and physical activities play a crucial role in BMD (Wang et al., 2007). Several genes have been identified that may be involved in determining bone mineral density, of which one the most important candidate genes for predisposition to osteoporosis is the collagen type I alpha1 *(COL1A1)*.

Human bones constitute of bone mineral and bone matrix; the ratio is about 2:1. The main component of bone mineral is Ca; the main component of bone matrix is collagen. Osteoporosis is mainly due to the loss of Ca and collagen degradation (Li et al., 2010). *COL1A1* gene encodes the α1(I) protein chain of type I collagen, the major protein of bone (Mann et al., 2001). It is a heterotrimer protein consisting of two α1 chains one α2 chain. These are encoded by two different genes located on chromosomes 17 and 7 in humans (Rodrigues et al., 2008). Grant et al. (1996) identified a single nucleotide polymorphism (G → T) affecting a binding site for the transcription factor Sp1 in the *COL1A1* gene. Extensive studies have been performed on the molecular mechanism by which the Sp1 polymorphism might predispose to osteoporosis. The T allele has a higher affinity for Sp1 protein binding than the wild-type G allele, and transcription from the T allele is three fold higher than the G allele (Ralston, 2010). The T allele of the *COL1A1* Sp1 polymorphism increases *COL1A1* gene transcription, which leads to increased collagen α1 protein production, an abnormal ratio of α1 to α2 collagen chains which raises the possibility that some of the collagen

Abbreviations: BMD, bone mineral density; BMI, body mass index; COL1A1, collagen type I alpha1; DXA, dual energy X-ray absorptiometry; HWE, Hardy–Weinberg equilibrium; PCR-RFLP, polymerase chain reaction–restriction fragment lenght polymorphism.

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http://dx.doi.org/10.1016/j.gene.2014.02.028
0378-1119/

may be present in the form of homotrimers composed of three collagen α1(I) chains ([α1(I)$_3$]), instead of the normal heterotrimers ([α1(I)$_2$α2(I)]) (Mann et al., 2001). Overall effects this polymorphism can be observed as a subtle defect in bone mineralization and reduced bone strength, leading to an increased risk of fracture on bone.

The relationships between *COL1A1* Sp1 polymorphism and BMD were investigated among various populations. Previous studies have shown associations between *COL1A1* Sp1 polymorphism, low BMD, osteoporosis (Falcón-Ramírez et al., 2011; Gerdhem et al., 2004; Grant et al., 1996; Haris et al., 2000; MacDonald et al., 2001; Uitterlinden et al., 1998; Yazdanpanah et al., 2007) and increased fracture risk (Keen et al., 1999a; Mann and Ralston, 2003; McGuigan et al., 2001; Mezquita-Raya et al., 2002; Uitterlinden et al., 2001), while some have not reached statistical significance (Ashford et al., 2001; Hubacek et al., 2006; Lidén et al., 1998; Wynne et al., 2002). Studies regarding the association of collagen gene variations with BMD values in Turkish postmenopausal women are scarce (Efesoy et al., 2011; Erdogan et al., 2011; Simsek et al., 2008; Tural et al., 2013).

Many potential risk factors for osteoporosis such as low body mass index (BMI) and age at menopause, smoking and family history of osteoporosis have also been identified in several studies. Smoking (Bjarnason and Christiansen, 2000; Rapuri et al., 2000), low BMI and age at menopause (Buttros Dde et al., 2011) and family history of osteoporosis (Keen et al., 1999b; Peris et al., 2002) were associated with decreased BMD values and osteoporosis. However, the combined effects of those and *COL1A1* Sp1 genotypes on BMD were not investigated so far. Therefore, in the present study, we aimed to investigate the association between the Sp1 polymorphism of the *COL1A1* gene and BMD in combination of osteoporosis risk factors in Turkish postmenopausal women.

2. Materials and methods

2.1. Subjects

The cohort of this study comprised 254 Turkish women (58.64 ± 7.72 mean aged), attending the Uskudar State Hospital in Istanbul, Turkey. All of the subjects were postmenopausal, which is defined as an absence of menstruation of at least a year. The participants received a detailed, standardized questionnaire including questions regarding the osteoporosis risk factors, such as menopausal status and age, smoking history, alcohol intaken, family history of osteoporosis, medication use and other medical conditions. Demographic and morphometric characteristics were also recorded. In attempt to successfully control the subjects characteristics and eliminate the confounding factors, we excluded those having conditions, diseases, and/or treatments known to interfere with bone metabolism, such as malignancies, endocrinologic disorders (hypo/hyperparathyroidism, hyperthyroidism, Cushing's syndrome), severe liver or gastroenteral diseases, skeletal diseases (Paget's disease, osteogenesis imperfecta, osteomalacia and rheumatoid arthritis) and current pharmacological treatment with corticosteroids, anabolic androgenic steroids, estrogens or estrogen-related molecules, anticonvulsants before enrollment. The study protocol was approved by the Local Ethical Commitee of Istanbul University, Istanbul Medical Faculty (Protocol No: 2006/2145) and written, informed consent was obtained from each participant prior to giving their blood sample.

2.2. BMD measurement

BMD for lumbar spine (L1–L4) and hip (femoral neck and total hip) was measured by GE-Lunar DPX Pro (GE Healthcare, Madison, WI, USA) Pencil Beam DXA densitometer. All DEXA scans were performed by the same technician and analyzed according to software (encore version 2005, 9.30.044) provided by the manufacturer. Briefly, subjects were positioned in the scanner according to standard procedures and remained motionless for approximately 10 minutes during scanning. The instrument was calibrated daily according to the manufacturer's instructions. BMD was expressed as grams per centimeter square (g/cm^2) and T scores which indicate the standard deviations of individiaul BMD determinations compared to those of young.

2.3. Genotyping

Blood specimens were collected in tubes containing EDTA, and DNA samples were extracted from whole blood with salting out procedure (Miller et al., 1988). Sp1 polymorphism of the *COL1A1* gene was determined in duplicate by polymerase chain reaction and restriction fragment lenght polymorphism (PCR-RFLP) using the primers and methods described by Grant et al. (1996) and Lei et al. (2003). The primers (MBI Fermentas, Lithuania) used for PCR to amplify *COL1A1* gene fragments were as follows; forward primer 5′-TAACTTCTGGACTA TTTGCGGACTTTTTGG-3′ and reverse primer 5′-GTCCAGCCCTCATCCT GGCC-3′ for the Sp1 restriction site. PCR reactions were carried out in a final volume at 25 μL containing 10X reaction buffer (KCl), 1 mM of each nucleotide (dATP, dCTP, dGTP and dTTP) (MBI Fermentas, Lithuania), 1.5 mM $MgCl_2$, 25 picomolar of each primer, 0.3 U of Taq DNA polymerase (MBI Fermentas, Lithuania) and 50 ng of DNA. Thermal profiles for amplification of *COL1A1* gene fragments consisted of an initial denaturating step of 5 min at 94 °C followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 62.5 °C for 1 min and extension at 72 °C for 1 min with a final extension step for 5 min at 72 °C. *COL1A1* Sp1 genotypes were amplified specific PCR product in a DNA thermal cycler (GeneAmp 9700 PCR System; Applied Biosystems, CA, USA). PCR products were then digested with restriction endonuclease Msp20I (MscI; MBI Fermentas, Lithuania). Digestion products were seperated by agarose gel electrophoresis and stained with ethidium bromide to visualize the fragmented DNA. Genotypes for *COL1A1* Sp1 polymorphism were classified as SS, Ss and ss (Uppercase letters represent absence and lowercase letters represent the presence of restriction sites). Automated Sequencing was used to confirm the results of *COL1A1* Sp1 polymorphism for a subset of 20 representative samples. Results between PCR and DNA sequencing analysis were 100 % concordant.

2.4. Statistical analysis

The analyses were performed using SPSS software for Windows, version 13.0. Clinical laboratory data were expressed as mean ± SD. Mean values were compared between different genotype groups by unpaired Student's *t*-test and one-way ANOVA. Chi-square test was used for comparing genotype distributions in relation to categorical variables and for Hardy–Weinberg Equilibrium (HWE). A p-value of less than 0.05 was considered to be statistically significant. Multivariate analysis was performed with binary logistic regression (Forward: LR). The odds ratios (OR) and the confidence intervals (CI) were calculated to estimate the relative risk. This analysis was used to identify association of *COL1A1* Sp1 polymorphism among several independent osteoporosis risk factors. In the logistic regression model, L1–L4 BMD ≤ 0.98, femoral neck ≤ 0.84 and total hip ≤ 0.90 were used as the dependent variables. Model included *COL1A1 Sp1* polymorphism, BMI, age at menopause, smoking history and family history of osteoporosis as independent variables.

3. Results

3.1. The demographic characteristics, BMD status and COL1A1 genotypes and allele distribution

The demographic characteristics, BMD status and the genotype and allele frequencies of *COL1A1* Sp1 polymorphism of the study population were presented in Table 1. The genotype frequencies were 53.94 % for "SS", 41.73 % for "Ss" and 4.33 % for "ss" genotypes and were consistent with the Hardy–Weinberg Equilibrium ($P > 0.05$).

Table 1
Characteristics, BMD status and *COL1A1* genotype and allele frequencies of the study population.

	Study population
Number	254
Age	58.64 ± 7.72
Age at menopause (years)	46.71 ± 5.04
BMI (kg/m^2)	30.14 ± 5.06
Family history of osteoporosis (%)	41.8
Smoking history (%)	9.0
Lumbar spine (L1–L4) BMD (g/cm^2)	0.98 ± 0.15
Femoral neck BMD (g/cm^2)	0.84 ± 0.11
Total hip BMD (g/cm^2)	0.90 ± 0.12
***COL1A1* Sp1**	
Genotypes	
SS	137 (53.94 %)
Ss	106 (41.73 %)
ss	11 (4.33 %)
Alleles	
S	380 (74.8 %)
s	128 (25.2 %)

BMI: Body mass index, BMD: Bone mineral density.
The results were shown as mean ± SD except where noted.

3.2. Association of COL1A1 polymorphism with the BMD values

The association of *COL1A1* genotypes with BMD values was shown in Table 2. We observed a trend decrease in BMD values in the subjects with "ss" genotype having lower BMD of lumbar spine, femoral neck and total hip compared to those with "SS" and "Ss" genotypes, however, the differences did not reach statistical significance ($P > 0.05$).

3.3. Effects of the COL1A1 genotypes on BMD values in combination of osteoporosis risk factors

To assess whether *COL1A1* Sp1 polymorphism had any effect on BMD values in combination of osteoporosis risk factors, we divided the subjects into groups according to their BMI, menopause ages, smoking history, family history of osteoporosis and the carriage of different *COL1A1* genotypes (Table 3). Statistical analysis was performed using unpaired Student's *t*-test.

Subjects in BMI ≤ 27 group had lower BMD values of lumbar spine, femoral neck and total hip than the values of those in BMI > 27 group ($P < 0.001$). The differences remained significant when we divided the two groups as having "Ss/ss" genotypes ("s" allele) and "SS/Ss" genotypes ("S" allele), respectively ($P < 0.001$) (Table 3).

Subjects in age at menopause ≤ 50 group had lower BMD values of total hip compared to the values of those in age at menopause > 50 group, the difference was closely tied to statistical significance ($P = 0.057$). However, we didn't observe combined effects of *COL1A1* genotype groups with low age of menopause on BMD values ($P > 0.05$) (Table 3).

Subjects with smoking history had lower BMD values of total hip compared to the values of those with no smoking history, however, the difference was not statistically significant ($P = 0.115$). We observed an increase in the significance of the difference in the "Ss/ss" genotype group ($P = 0.078$), whereas there was a decrease in the significance of the difference in the subjects with "SS/Ss" genotypes ($P = 0.129$) (Table 3).

The difference between the BMD values of the subjects with and without family history of osteoporosis did not change considerably ($P > 0.05$), whereas there was a trend increase in the significance in the subjects with "Ss/ss" genotypes, however, the difference was not statistically significant, either ($P > 0.05$) (Table 3).

We also evaluated the combined effects of *COL1A1* genotypes and osteoporosis risk factors on BMD values by using chi-square (χ^2) test (Table 4). Therefore, we grouped the subjects according to the mean BMD values of lumbar spine (≤0.98 and >0.98), femoral neck (≤0.84 and >0.84) and total hip (≤0.90 and >0.90) of the study group.

In BMI ≤ 27 group, the frequencies of having BMD values of lumbar spine, femoral neck and total hip under the mean scores were higher than those in BMI > 27 group as expected ($P < 0.001$). The differences remained significant in "Ss/ss" and "SS/Ss" genotype groups ($P < 0.001$). However, there was a trend decrease in the significance of the difference in the frequency of femoral neck BMD values in "Ss/ss" genotype group ($P = 0.017$) (Table 4).

In age at menopause ≤ 50 group, the frequencies of having BMD values of lumbar spine, femoral neck and total hip under the mean scores were higher than those in age at menopause > 50 group, however, the differences weren't statistical significant ($P > 0.05$). We also didn't observe a significant difference among the "Ss/ss" and "SS/Ss" genotype groups ($P > 0.05$) (Table 4).

Table 2
Association of *COL1A1* Sp1 polymorphism with the BMD values.

Genotypes	BMD (g/cm^2)		
	Lumbar spine (L1–L4)	Femoral neck	Total hip
SS	0.97 ± 0.13	0.84 ± 0.11	0.90 ± 0.11
Ss	0.99 ± 0.16	0.84 ± 0.12	0.91 ± 0.13
ss	0.96 ± 0.12	0.83 ± 0.09	0.88 ± 0.10

The results were shown as mean ± SD. Statistical analyses were performed by using one-way ANOVA analysis for comparing all three genotype groups.

Table 3
Effects of the *COL1A1* genotypes on BMD values in combination of osteoporosis risk factors.

	BMD (g/cm^2)		
	Lumbar spine (L1–L4)	Femoral neck	Total hip
BMI			
BMI ≤ 27	0.90 ± 0.12*	0.77 ± 0.08*	0.81 ± 0.09*
BMI > 27	1.00 ± 0.15	0.87 ± 0.11	0.93 ± 0.11
Ss + ss/BMI ≤ 27	0.90 ± 0.10*	0.78 ± 0.09*	0.82 ± 0.09*
Ss + ss/BMI > 27	1.02 ± 0.15	0.86 ± 0.12	0.94 ± 0.12
SS + Ss/BMI ≤ 27	0.90 ± 0.12*	0.77 ± 0.09*	0.81 ± 0.09*
SS + Ss/BMI > 27	1.00 ± 0.15	0.87 ± 0.11	0.94 ± 0.11
Age at menopause			
Age at menopause ≤ 50	0.97 ± 0.15	0.84 ± 0.11	0.89 ± 0.12
Age at menopause > 50	1.00 ± 0.15	0.85 ± 0.09	0.93 ± 0.11
Ss + ss/Age at menopause ≤ 50	0.99 ± 0.16	0.85 ± 0.12	0.91 ± 0.13
Ss + ss/Age at menopause > 50	0.99 ± 0.20	0.86 ± 0.09	0.94 ± 0.12
SS + Ss/Age at menopause ≤ 50	0.97 ± 0.15	0.83 ± 0.11	0.89 ± 0.12
SS + Ss/Age at menopause > 50	1.02 ± 0.15	0.85 ± 0.09	0.94 ± 0.10
Smoking history			
Smoking history (+)	0.97 ± 0.13	0.81 ± 0.10	0.86 ± 0.11
Smoking history (−)	0.98 ± 0.15	0.84 ± 0.11	0.91 ± 0.12
Ss + ss/Smoking history (+)	0.95 ± 0.12	0.80 ± 0.07	0.84 ± 0.08
Ss + ss/Smoking history (−)	0.99 ± 0.16	0.85 ± 0.12	0.92 ± 0.13
SS + Ss/Smoking history (+)	0.97 ± 0.13	0.81 ± 0.10	0.86 ± 0.12
SS + Ss/Smoking history (−)	0.98 ± 0.15	0.85 ± 0.11	0.91 ± 0.12
Family history of osteoporosis			
Family history (+)	0.98 ± 0.13	0.84 ± 0.11	0.90 ± 0.11
Family history (−)	0.98 ± 0.16	0.85 ± 0.12	0.91 ± 0.13
Ss + ss/Family history (+)	0.97 ± 0.13	0.84 ± 0.10	0.90 ± 0.11
Ss + ss/Family history (−)	1.01 ± 0.19	0.86 ± 0.13	0.93 ± 0.14
SS + Ss/Family history (+)	0.99 ± 0.14	0.84 ± 0.11	0.90 ± 0.11
SS + Ss/Family history (−)	0.98 ± 0.17	0.85 ± 0.12	0.91 ± 0.13

BMI: Body mass index, BMD: Bone mineral density. The results were shown as mean ± SD. Statistical analysis was performed using unpaired Student's *t* test. *, $P < 0.001$.
The specifications of the groups were done according to the osteoporotic risk factors (BMI, age at menopause, smoking history and family history of osteoporosis) and all variables were subdivided and analyzed according to the carriage of "Ss + ss" and "SS + Ss" genotypes.

Table 4
Effects of the *COL1A1* genotypes on mean BMD values in combination of osteoporosis risk factors.

	BMD (g/cm^2)					
	L1–L4 (n, %)		Femoral neck (n, %)		Total hip (n, %)	
	≤0.98	>0.98	≤0.84	>0.84	≤0.90	>0.90
BMI						
BMI ≤27	52 (82.5)***	11 (17.5)	48 (75.0)***	16 (25.0)	52 (81.3)***	12 (18.8)
BMI >27	81 (45.5)	97 (54.5)	82 (4.6)	102 (55.4)	71 (38.6)	113 (61.4)
Ss + ss/BMI ≤27	24 (80.0)***	6 (20.0)	21 (70.0)*	9 (30.0)	23 (76.7)***	7 (23.3)
Ss + ss/BMI >27	33 (39.8)	50 (60.2)	38 (44.7)	47 (55.3)	33 (38.8)	52 (61.2)
SS + Ss/BMI ≤27	50 (83.3)***	10 (16.7)	45 (73.8)***	16 (26.2)	50 (82.0)***	11 (18.0)
SS + Ss/BMI >27	77 (45.6)	92 (54.4)	75 (43.1)	99 (56.9)	66 (37.9)	108 (62.1)
Age at menopause						
Age at menopause ≤50	102 (57.3)	76 (42.7)	99 (55.0)	81 (45.0)	96 (53.3)	84 (46.7)
Age at menopause >50	21 (50.0)	21 (50.0)	22 (52.4)	20 (47.6)	17 (40.5)	25 (59.5)
Ss + ss/Age at menopause ≤50	41 (48.8)	43 (51.2)	43 (51.2)	41 (48.8)	41 (48.8)	43 (51.2)
Ss + ss/Age at menopause >50	12 (57.1)	9 (42.9)	10 (47.6)	11 (52.4)	8 (38.1)	13 (61.9)
SS + Ss/Age at menopause ≤ 50	100 (58.8)	70 (41.2)	93 (54.1)	79 (45.9)	93 (54.1)	79 (45.9)
SS + Ss/Age at menopause > 50	18 (46.2)	21 (53.8)	19 (48.7)	20 (51.3)	14 (35.9)	25 (64.1)
Smoking history						
Smoking history (+)	13 (65.0)	7 (35.0)	15 (71.4)	6 (28.6)	16 (76.2)*	5 (23.8)
Smoking history (−)	120 (54.3)	101 (45.7)	115 (50.7)	112 (49.3)	107 (47.1)	120 (52.9)
Ss + ss/Smoking history (+)	7 (77.8)	2 (22.2)	7 (70.0)	3 (30.0)	9 (90.0)**	1 (10.0)
Ss + ss/Smoking history (−)	50 (48.1)	54 (51.9)	52 (49.5)	53 (50.5)	47 (44.8)	58 (55.2)
SS + Ss/Smoking history (+)	12 (66.7)	6 (33.3)	13 (68.4)	6 (31.6)	14 (73.7)*	5 (26.3)
SS + Ss/Smoking history (−)	115 (54.5)	96 (45.5)	107 (49.5)	109 (50.5)	102 (47.2)	114 (52.8)
Family history of osteoporosis						
Family history (+)	54 (52.4)	49 (47.6)	61 (57.5)	45 (42.5)	57 (53.8)	49 (46.2)
Family history (−)	76 (58.0)	55 (42.0)	66 (49.3)	68 (50.7)	63 (47.0)	71 (53.0)
Ss + ss/Family history (+)	28 (56.0)	22 (44.0)	32 (61.5)	20 (38.5)	28 (53.8)	24 (46.2)
Ss + ss/Family history (−)	27 (45.8)	32 (54.2)	26 (44.1)	33 (55.9)	26 (44.1)	33 (55.9)
SS + Ss/Family history (+)	49 (51.0)	47 (49.0)	55 (55.6)	44 (44.4)	52 (52.5)	47 (47.5)
SS + Ss/Family history (−)	75 (59.5)	51 (40.5)	63 (48.8)	66 (51.2)	61 (47.3)	68 (52.7)

BMI: Body mass index, BMD: Bone mineral density. The results were shown as number of individuals and frequency. Statistical analysis was performed using *chi-square* test.
*, $P < 0.05$; **, $P < 0.01$ (FE: Fisher exact test); ***, $P < 0.001$.
The specifications of the groups were done according to the osteoporotic risk factors (BMI, age at menopause, smoking history and family history of osteoporosis) and all variables were subdivided and analyzed according to the carriage of "Ss + ss" and "SS + Ss" genotypes.

The frequency of having BMD values of total hip under the mean scores was higher in the subjects with smoking history than the frequency of those with no smoking history ($P = 0.011$). We observed an increase in the significance of the difference in the subjects with "Ss/ss" genotypes (Fisher exact test, $P = 0.007$), whereas there was a decrease in the subjects with "SS/Ss" genotypes ($P = 0.027$) (Table 4).

The frequencies of having BMD values of lumbar spine, femoral neck and total hip under mean scores were higher in the subjects with family history of osteoporosis than the frequencies of those without family history of osteoporosis, however, the differences were not statistically significant ($P > 0.05$). The frequency of having BMD values of femoral neck under the mean scores was higher in the subjects with "Ss/ss" genotypes and family history of osteoporosis compared to the frequency of those without family history of osteoporosis, the difference was closely tied to statistically significance ($P = 0.066$). No significant association was found between the subjects with "SS/Ss" genotypes, family history of osteoporosis and BMD values ($P > 0.05$) (Table 4).

Multivariate logistic regression analysis confirmed body mass index as the most important predictor for having BMD values of L1–L4, femoral neck and total hip above the mean scores among parameters such as *COL1A1* Sp1 polymorphism, age at menopause, smoking history and family history of osteoporosis (Table 5).

4. Discussion

To date, there had been several studies to enlighten the genetic basis of osteoporosis. Therefore, a large number of polymorphisms in multiple candidate genes have been investigated in various populations. The *COL1A1* Sp1 polymorphism has previously been found to be associated with decreased BMD and osteoporosis. Uitterlinden et al. (1998) found that postmenopausal women in Netherlands with "Ss" and "ss" genotype had lower BMD values at the lumbar spine and femoral neck compared to the women with 'SS' genotype. Haris et al. (2000) and MacDonald et al. (2001) have found that bone loss was significantly greater in "ss" homozygotes compared with "Ss" heterozygotes and "SS" homozygotes. "T" (s) allele was associated with low femoral neck BMD values in Caucasion postmenopausal women and in elderly Swedish women (Gerdhem et al., 2004; Yazdanpanah et al., 2007) and with a significant reduction in lumbar spine BMD and increased risk of total fracture in healthy women from northeast London (Keen et al., 1999a). In Mexican women, "ss" genotype was also associated with low BMD of lumbar spine in contrast to "SS" genotype (Falcón-Ramírez et al., 2011). BMD values at the lumbar spine and femoral neck were found to be lower in the "Ss" and "ss" genotype groups when compared with "SS" homozygotes in a meta-analysis study (Mann and Ralston, 2003). We could not find a statistical significant relation between *COL1A1* Sp1 genotypes and BMD, however we observed a trend decrease in the subjects with "ss" genotype having lower BMD values compared to those with "SS" and "Ss" genotypes. In Simsek et al.´s (2008) study with Turkish postmenopausal women, "Ss" heterozygosity was associated with lower BMD values of lumbar spine and femoral neck compared with "SS" homozygotes whereas Erdogan et al. (2011) have concluded that there was no association in terms of average BMD values with *COL1A1* Sp1 genotype and allele frequencies. In the two other studies with Turkish postmenopausal women, no statistical significant difference was found in *COL1A1* Sp1 genotype and allele frequencies of osteoporotic patients and controls (Efesoy et al., 2011; Tural et al., 2013), also in terms of BMI, smoking and menopause age between the genotypes (Tural et al., 2013). Efesoy et al. (2011) have also not found any association between the *COL1A1* Sp1 genotypes and BMD. Similarly, we also could not find a significant effect of *COL1A1* Sp1 genotypes on BMD either, we found combined effects of *COL1A1* genotypes and having osteoporosis risk

Table 5
Multivariate logistic regression analysis[a].

Dependent variable	Independent variables		Exp(B) (OR)	P value	95% CI for Exp(B)
L1–L4 ≤ 0.98	***COL1A1* Sp1 Polymorphism**	**s Allele**		NS	
		S Allele (Ref.)	1		
	BMI	**BMI ≤ 27**	0.221	0.000	0.107–0.458
		BMI > 27 (Ref.)	1		
	Age at menopause	**Age at menopause ≤ 50**		NS	
		Age at menopause > 50 (Ref.)	1		
	Smoking history	**Smoking history (+)**		NS	
		Smoking history (−) (Ref.)	1		
	Family history	**Family history (+)**		NS	
		Family history (−) (Ref.)	1		
Femoral neck ≤ 0.84	***COL1A1* Sp1 Polymorphism**	**s Allele**		NS	
		S Allele (Ref.)	1		
	BMI	**BMI ≤ 27**	0.267	0.000	0.134–0.534
		BMI > 27 (Ref.)	1		
	Age at menopause	**Age at menopause ≤ 50**		NS	
		Age at menopause > 50 (Ref.)	1		
	Smoking history	**Smoking history (+)**		NS	
		Smoking history (−) (Ref.)	1		
	Family history of osteoporosis	**Family history (+)**		NS	
		Family history (−) (Ref.)	1		
Total hip ≤ 0.90	***COL1A1* Sp1 Polymorphism**	**s Allele**		NS	
		S Allele (Ref.)	1		
	BMI	**BMI ≤ 27**	0.164	0.000	0.079–0.340
		BMI > 27 (Ref.)	1		
	Age at menopause	**Age at menopause ≤ 50**		NS	
		Age at menopause > 50 (Ref.)	1		
	Smoking history	**Smoking history (+)**		NS	
		Smoking history (−) (Ref.)	1		
	Family history of osteoporosis	**Family history (+)**		NS	
		Family history (−) (Ref.)	1		

BMI: Body mass index, NS: Not significant.
[a] , All participants are included (n = 254).

factors (smoking history and family history of osteoporosis) on BMD values. We did not find such a relation like Simsek et al. (2008) in our study group and we are unable to give a precise explanation for that. We presume that this difference may stem from different geographic background and number of subjects between the two studies. Furthermore, our results of genotype and allele distributions of *COL1A1* Sp1 polymorphism show a remarkable difference from their study. This situation can affect the results of the interactions between *COL1A1* Sp1 genotypes and BMD values.

There are previous reports showing no relationship between *COL1A1* Sp1 genotypes and BMD in postmenopausal women from Sweden (Lidén et al., 1998), UK (Ashford et al., 2001), Czech population (Hubacek et al., 2006), Ireland (Wynne et al., 2002), Belgium (Aerssens et al., 1998), Denmark (Heegaard et al., 2000), Finland (Välimäki et al., 2001), China (Lau et al., 2004), and Serbia (Trajkovic et al., 2010), Netherlands (Pluijm et al., 2004). No significant association was found between *COL1A1* Sp1 genotypes and BMD in twin pairs of white adult American women by Hustmyer et al. (1999).

Smoking history, low BMI, age at menopause and family history of osteoporosis also exert significant influences on bone loss in osteoporosis. Smoking was associated with low BMD (Bjarnason and Christiansen, 2000; Rapuri et al., 2000) and increased risk of any fracture (Kanis et al., 2005). Time of menopause, smoking and family history of osteoporosis/fracture were found to be clinical indicators of risk for osteoporosis, whereas high BMI proved to be a protective factor (Buttros Dde et al., 2011; Keen et al., 1999b; Peris et al., 2002). Although the association between these factors and low BMD/osteoporosis have been reported in several studies, the combined effects of those and *COL1A1* genotypes on BMD were not investigated so far.

To evaluate the interaction between *COL1A1* Sp1 variants and osteoporosis risk factors, we subdivided our study group according to the presence/absence of *COL1A1* Sp1 variants and risk factors, and analyzed combined effects of these parameters on BMD values. We observed that the mean BMD values decreased considerably in the subjects carrying "Ss/ss" genotypes in combination of having family history of osteoporosis and smoking history. However, we could not find a significant combined effect of the *COL1A1* genotypes and BMI and age at menopause on BMD values.

The main limitation in this report is relatively small sample size, consequently, statistical power to examine multiple interactions as we have done. Furthermore, the frequencies of "ss" genotype of Sp1 polymorphism is rarely observed in this study. We believe that further studies with a higher number of subjects with the contributions of previous studies may be necessary to conclude with greater certainty of the association between *COL1A1* Sp1 polymorphism, risk factors and predisposition to osteoporosis.

In conclusion, we could not find a significant association between *COL1A1* Sp1 genotypes and low BMD. However, we found that there were combined effects of *COL1A1* genotypes and having family history of osteoporosis and smoking history on low BMD values. Therefore, we suggest that *COL1A1* Sp1 polymorphism may affect BMD status and the development of osteoporosis in combination of osteoporosis risk factors in Turkish postmenopausal women.

Conflict of interest

Authors declare that no competing interests exist.

Acknowledgment

The present work was supported by a grant from Istanbul University Scientific Research Projects (Project No: T-68/15122006 and UDP-13847).

References

Aerssens, J., Dequeker, J., Peeters, J., et al., 1998. Lack of association between osteoarthritis of the hip and gene polymorphisms of VDR, COL1A1, and COL2A1 in postmenopausal women. Arthritis and Rheumatism 41, 1946–1950.

Arden, N.K., Baker, J., Hogg, C., et al., 1996. The heritability of bone mineral density, ultrasound of the calcaneus and hip axis length: A study of postmenopausal twins. Journal of Bone and Mineral Research 11, 530–534.

Ashford, R.U., Luchetti, M., McCloskey, E.V., et al., 2001. Studies of bone density, quantitative ultrasound, and vertebral fractures in relation to collagen type I alpha 1 alleles in elderly women. Calcified Tissue International 68, 348–351.

Bjarnason, N.H., Christiansen, C., 2000. The influence of thinness and smoking on bone loss and response to hormone replacement therapy in early postmenopausal women. The Journal of Clinical Endocrinology and Metabolism 85, 590–596.

Buttros Dde, A., Nahas-Neto, J., Nahas, E.A., et al., 2011. Risk factors for osteoporosis in postmenopausal women from southeast Brazilian. Revista Brasileira de Ginecologia e Obstetrícia 33, 295–302.

Efesoy, A., Yılmaz, O., Erden, G., et al., 2011. Relationship of the vitamin D receptor and Collagen Iα1 gene polymorphisms with low bone mineral density and vertebral fractures in postmenopausal Turkish women. Turkish Journal of Rheumatology 26, 295–302.

Erdogan, M.O., Yıldız, H., Artan, S., et al., 2011. Association of estrogen receptor alpha and collagen type I alpha 1 gene polymorphisms with bone mineral density in postmenopausal women. Osteoporosis International 22, 1219–1225.

Falcón-Ramírez, E., Casas-Avila, L., Miranda, A., et al., 2011. MSp1 polymorphism in collagen I α1 gene is associated with osteoporosis in lumbar spine of Mexican women. Molecular Biology Reports 38, 2987–2992.

Gerdhem, P., Brändström, H., Stiger, F., et al., 2004. Association of the collagen type 1 (COL1A1) Sp1 binding site polymorphism to femoral neck bone mineral density and wrist fracture in 1044 elderly Swedish women. Calcified Tissue International 74, 264–269.

Grant, S.F., Reid, D.M., Blake, G., et al., 1996. Reduced bone density and osteoporosis associated with a polymorphic Sp1 binding site in the collagen type I alpha 1 gene. Nature Genetics 14, 203–205.

Haris, S.S., Patel, M.S., Cole, D.E., et al., 2000. Associations of the collagen type Ialpha1 Sp1 polymorphism with five-year rates of bone loss in older adults. Calcified Tissue International 66, 268–271.

Heegaard, A., Jorgensen, H.L., Vestergaard, A.W., et al., 2000. Lack of influence of collagen type Ialpha1 Sp1 binding site polymorphism on the rate of bone loss in a cohort of postmenopausal danish women followed for 18 years. Calcified Tissue International 66, 409–413.

Hubacek, J.A., Weichetova, M., Bohuslavova, R., et al., 2006. No associations between genetic polymorphisms of TGF-beta, PAI-1, and COL1A1, and bone mineral density in Caucasian females. Endocrine Regulations 40, 107–112.

Hustmyer, F.G., Liu, G., Johnston, C.C., et al., 1999. Polymorphism at an Sp1 binding site of COL1A1 and bone mineral density in premenopausal female twins and elderly fracture patients. Osteoporosis International 9, 346–350.

Kanis, J.A., Johnell, O., Oden, A., et al., 2005. Smoking and fracture risk: a meta-analysis. Osteoporosis International 16, 155–162.

Keen, R.W., Woodford-Richens, K.L., Grant, S.F., et al., 1999a. Association of polymorphism at the type I collagen (COL1A1) locus with reduced bone mineral density, increased fracture risk, and increased collagen turnover. Arthritis and Rheumatism 42, 285–290.

Keen, R.W., Hart, D.J., Arden, N.K., et al., 1999b. Family history of appendicular fracture and risk of osteoporosis: a population-based study. Osteoporosis International 10, 161–166.

Lau, E.M., Choy, D.T., Li, M., et al., 2004. The relationship between COLI A1 polymorphisms (Sp 1) and COLI A2 polymorphisms (Eco R1 and Puv II) with bone mineral density in Chinese men and women. Calcified Tissue International 75, 133–137.

Lei, S.F., Deng, F.Y., Liu, X.H., et al., 2003. Polymorphisms of four bone mineral density candidate genes in Chinese populations and comparison with other populations of different ethnicity. Journal of Bone and Mineral Metabolism 21, 34–42.

Li, Y., Zhao, Y., Tang, R., et al., 2010. Preventive and therapeutic effects of antler collagen on osteoporosis in ovariectomized rats. African Journal of Biotechnology 9, 6437–6441.

Lidén, M., Wilén, B., Ljunghall, S., Melhus, H., 1998. Polymorphism at the Sp 1 binding site in the collagen type I alpha 1 gene does not predict bone mineral density in postmenopausal women in Sweden. Calcified Tissue International 63, 293–295.

MacDonald, H.M., McGuigan, F.A., New, S.A., et al., 2001. COL1A1 Sp1 polymorphism predicts perimenopausal and early postmenopausal spinal bone loss. Journal of Bone and Mineral Research 16, 1634–1641.

Mann, V., Ralston, S.H., 2003. Meta-analysis of COL1A1 Sp1 polymorphism in relation to bone mineral density and osteoporotic fracture. Bone 32, 711–717.

Mann, V., Hobson, E.E., Li, B., et al., 2001. A COL1A1 Sp1 binding site polymorphism predisposes to osteoporotic fracture by affecting bone density and quality. The Journal of Clinical Investigation 107, 899–907.

McGuigan, F.E., Armbrecht, G., Smith, R., et al., 2001. Prediction of osteoporotic fractures by bone densitometry and COL1A1 genotyping: a prospective, population-based study in men and women. Osteoporosis International 12, 91–96.

Mezquita-Raya, P., Muñoz-Torres, M., de Dios Luna, J., et al., 2002. Performance of COL1A1 polymorphism and bone turnover markers to identify postmenopausal women with prevalent vertebral fractures. Osteoporosis International 13, 506–512.

Miller, S.A., Dykes, D.D., Poleskey, H.S., 1988. Simples salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research 16, 1215.

NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis and Therapy, 2001. Osteoporosis prevention, diagnosis and therapy. JAMA 285, 785–795.

Peris, P., Guañabens, N., Martínez de Osaba, M.J., et al., 2002. Clinical characteristics and etiologic factors of premenopausal osteoporosis in a group of Spanish women. Seminars in Arthritis and Rheumatism 32, 64–70.

Pluijm, S.M., van Essen, H.W., Bravenboer, N., et al., 2004. Collagen type I α1 Sp1 polymorphism, osteoporosis, and intervertebral disc degeneration in older men and women. Annals of the Rheumatic Diseases 63, 71–77.

Pocock, N.A., Eisman, J.A., Hopper, J.L., et al., 1987. Genetic determinants of bone mass in adults. A twin study. The Journal of Clinical Investigation 80, 706–710.

Ralston, S.H., 2010. Genetics of osteoporosis. Annals of the New York Academy of Sciences 1192, 181–189.

Rapuri, P.B., Gallagher, J.C., Balhorn, K.E., et al., 2000. Smoking and bone metabolism in elderly women. Bone 27, 429–436.

Rodrigues, A.M., Girão, M.J., da Silva, I.D., et al., 2008. COL1A1 Sp1-binding site polymorphism as a risk factor for genital prolapse. International Urogynecology Journal and Pelvic Floor Dysfunction 19, 1471–1475.

Simsek, M., Cetin, Z., Bilgen, T., et al., 2008. Effects of hormone replacement therapy on bone mineral density in Turkish patients with or without COL1A1 Sp1 binding site polymorphism. The Journal of Obstetrics and Gynaecology Research 34, 73–77.

Slemenda, C.W., Christian, J.C., Williams, C.J., et al., 1991. Genetic determinants of bone mass in adult women: A reevaluation of the twin model and the potential importance of gene interaction on heritability estimates. Journal of Bone and Mineral Research 6, 561–567.

Trajkovic, K., Perovic, M., Tarasjev, A., et al., 2010. Association of collagen type I alpha1 gene polymorphism with bone mineral density in osteoporotic women in Serbia. Journal of Women's Health 19, 1299–1303.

Tural, S., Kara, N., Alayli, G., et al., 2013. Association between osteoporosis and polymorphisms of the bone Gla protein, estrogen receptor 1, collagen 1-A1 and calcitonin receptor genes in Turkish postmenopausal women. Gene 515, 167–172.

Uitterlinden, A.G., Burger, H., Huang, Q., et al., 1998. Relation of alleles of the collagen type Ialpha1 gene to bone density and the risk of osteoporotic fractures in postmenopausal women. The New England Journal of Medicine 338, 1016–1021.

Uitterlinden, A.G., Weel, A.E., Burger, H., et al., 2001. Interaction between the vitamin D receptor gene and collagen type Ialpha1 gene in susceptibility for fracture. Journal of Bone and Mineral Research 16, 379–385.

Välimäki, S., Tähtelä, R., Kainulainen, K., et al., 2001. Relation of collagen type I alpha 1 (COLIA 1) and vitamin D receptor genotypes to bone mass, turnover, and fractures in early postmenopausal women and to hip fractures in elderly people. European Journal of Internal Medicine 12, 48–56.

Wang, C.L., Tang, X.Y., Chen, W.Q., et al., 2007. Association of estrogen receptor α gene polymorphisms with bone mineral density in Chinese women: a meta-analysis. Osteoporosis International 18, 295–305.

Wynne, F., Drummond, F., O'Sullivan, K., et al., 2002. Investigation of the genetic influence of the OPG, VDR (Fok1), and COL1A1 Sp1 polymorphisms on BMD in the Irish population. Calcified Tissue International 71, 26–35.

Yazdanpanah, N., Rivadeneira, F., van Meurs, J.B., et al., 2007. The −1997 G/T and Sp1 polymorphisms in the collagen type I alpha1 (COL1A1) gene in relation to changes in femoral neck bone mineral density and the risk of fracture in the elderly: the Rotterdam study. Calcified Tissue International 81, 18–25.