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Association of T869C gene polymorphism of transforming growth factor-β1 with low protein levels and anthropometric indices in osteopenia/osteoporosis postmenopausal Thai women

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ABSTRACT. Osteoporosis is the most common metabolic bone disease; it is an important health problem among postmenopausal women. We evaluated the association of three polymorphisms, T869C, C-509T and G915C, of the TGF-β1 gene with bone mineral density (BMD) serum TGF-β1 levels in 278 postmenopausal female osteopenia/osteoporosis subjects and 95 postmenopausal female control subjects. Serum TGF-β1 levels were significantly lower in osteopenia/osteoporosis subjects than in control subjects. Serum TGF-β1 levels

of the CT+CC (T869C) genotype group were significantly lower in osteopenia/osteoporosis subjects than in control subjects (11.3 *vs* 15.8 ng/mL). There was a significant difference in the CT+CC (T869C) genotype frequencies between the osteopenia/osteoporosis and control subjects (74.18 *vs* 60.22%; OR = 1.90, 95%CI = 1.16-3.12). In the age group of more than 50 years, subjects with the TC+CC genotype of T869C polymorphism had significantly increased risk of osteopenic/osteoporotic bones at L1 (OR = 2.36, 95%CI = 1.37-4.07), L2 (OR = 1.71, 95%CI = 1.01-2.90), L3 (OR = 2.21, 95%CI = 1.23-3.98), L4 (OR = 1.74, 95%CI = 1.00-3.03) and the femoral neck (OR = 1.80, 95%CI = 1.04-3.12). The CT+CC genotype of the T869C polymorphism of the TGF-β1 gene was found to be associated with lower serum TGF-β1 in osteopenia/osteoporosis subjects and increased risk of osteopenic and osteoporotic fracture at L1-4, femoral neck and total hip in postmenopausal Thai women. Logistic regression analysis showed that T869C polymorphism is a significant risk factor for osteopenia/osteoporosis. We concluded that T869C polymorphism of the TGF-β1 gene has an impact on decreased serum TGF-β1 levels and influences susceptibility to osteopenia/osteoporosis in Thai women.

Key words: Osteopenia/osteoporosis; Transforming growth factor β1; Serum TGF-β1; Bone mineral density

INTRODUCTION

Osteoporosis is a common skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture (Huang and Kung, 2006). Osteoporosis has increased rapidly and must now be considered an important health problem among postmenopausal Thai women. The prevalence of femoral neck and lumbar spine osteoporosis in Thai women aged between 40-80 years was 13.6 and 19.8% in 2000-2001, respectively (Pongchaiyakul et al., 2008). Multiple genetic as well as environmental factors have long been recognized to contribute to both osteoporosis and its associated phenotypes, including bone mineral density (BMD) (Williams and Spector, 2006).

The transforming growth factor β1 (TGF-β1) gene is located on chromosome 19q13. TGF-β1 is by far the most abundant among the three isoforms of TGF-β, both in bone and serum. It is produced by osteoblasts as an inactive propeptide and incorporated into newly formed bone matrix. Released during bone resorption and activated by the acidic microenvironment caused by the osteoclast, TGF-β1 inhibits the activity of the osteoclasts and stimulates proliferation and differentiation of preosteoblasts. The overall effect on bone remodeling of TGF-β1 plays an important role in bone formation. This important role in bone turnover makes the TGF-β1 gene a candidate for mediating the genetic influence on BMD and risk of fracture (Yoshiji, 2000; Langdahl et al., 2003; Kanaan and Kannan, 2006).

Studies on the association between several polymorphisms of the TGF-β1 gene and BMD have been reported. Yamada et al. (1998) described that, in postmenopausal Japanese

women, a thymine (T) to cytosine (C) polymorphism at nucleotide position 29 of the coding region (position 869 relative to the transcription initiation site) of the TGF-β1 gene, which results in the substitution of leucine (Leu) for a proline (Pro), is significantly associated with both BMD at the lumbar spine and reduced rate of bone loss. Yamada et al. (2001) reported the association of a cytosine (C) to thymine (T) polymorphism at nucleotide position -509 in the promoter region, alone or in combination with T869C of the TGF-β1 gene, with BMD and osteoporosis in Japanese women. Grainger et al. (1999) found that the C-509T polymorphism at the promoter region of the TGF-β1 gene was associated with the serum TGF-β1 level. The G915C (Arg25Pro) polymorphism of the TGF-β1 gene gives rise to an Arg→Pro substitution at amino acid residue 25 in the signal peptide sequence (Wood et al., 2000). The G915C polymorphism was reported to be present during the *in vitro* production of TGF-β1 (Awad et al., 1998). Langdahl et al. (1997) showed that the polymorphism of TGF-β1 in intron 4 has a one-base deletion (713-8delC) associated with low BMD and an increased bone turnover in Danish women.

In Thailand, TGF-β1 gene polymorphism data have not yet been studied. In this paper, we were interested in analyzing the associations of the three polymorphisms, T869C, C-509T and G915C of the TGF-β1 gene with BMD, serum TGF-β1 level in osteopenia and osteoporosis postmenopausal Thai women as compared with control subjects.

MATERIAL AND METHODS

Subjects

This study was performed with Thai volunteers: 278 postmenopausal women, who were detected to have osteopenia (BMD T-score of the lumbar spine or total hip was ≤-1 and ≤-2.5 g/cm^2) and osteoporosis (BMD T-score of the lumbar spine or total hip was ≤-2.5 g/cm^2), and 95 postmenopausal female control subjects (BMD T-score of the lumbar spine or total hip was ≥-1.0 g/cm^2) (World Health Organization, 2003). All subjects were evaluated at the Menopausal Clinic, Outpatient Department, General Practice Section of Department of Obstetrics and Gynecology, Ramathibodi Hospital, Bangkok. Exclusion criteria were diabetes mellitus, hypertension, cardiovascular disease, disorders known to affect bone metabolism, and unwillingness to participate. All subjects were apparently in good health and gave informed consent to participate in the study. Physical examinations were conducted by the same medical doctor throughout the study. The age, marital status, place of origin, lifestyle, anthropometric, drinking and smoking habits were assessed through standardized questionnaires. This study was approved by the Ethics Committee of the Faculty of Tropical Medicine and Faculty of Medicine at Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.

Measurement of BMD

BMD (g/cm^2) was measured at the lumbar spine (L1-4), total hip, femoral neck, trochanter, and Ward's triangle using dual-energy X-ray absorptiometry (DEXA) (Lunar Prodigy®; GE Healthcare, Lunar, USA) by a single experienced technician. Quality control was done by daily calibration and phantom scans. The coefficient of variation for phantom scans was 0.6% *in vivo*, and these values were 1.2 and 1.6% at lumbar spine 2-4 and femoral neck, respectively.

Measurement of biochemical markers

Ten milliliters of venous blood samples was taken early in the morning, after an overnight fast. Serum and blood samples were stored at -20°C until used. Serum calcium level was measured by using a Dimension RxL Max (Siemens Healthcare Diagnostics, USA). Serum osteocalcin level was measured by immunoradiometric assay kit (IRMA; Diagnostic Systems Laboratories, Inc., USA). Serum TGF-β1 level was measured by enzyme-linked immunosorbent assay kit (ELISA; IBL, Hamburg, Germany). The detection minimum of this assay was 1.9 pg/mL, and the intra-assay and interassay CVs were 1.0 and 7.5%, respectively.

DNA extraction and genotyping

DNA was extracted from the peripheral leukocytes in EDTA-treated whole blood using a Flexi Gene DNA kit (Qiagen, Hilden, Germany). The genotypes of the T869C and G915C polymorphisms of the TGF-β1 were amplified by polymerase chain reaction-restriction fragment length polymorphim (PCR-RFLP) method (Wood et al., 2000). PCR was done on a Gene Amp PCR system 9700 (Applied Biosystem, USA). Primers (forward primer sequence: 5'-TTCCCTCGAGGCCCTCTA-3' and reverse primer sequence: 5'-GCCGCAGCTTGGA CAGGATC-3') were used to amplify a portion of the T869C and G915C sequences from 100 ng genomic DNA in a 50-µL reaction containing PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl), 2.0 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphates, 1 µM of each primer, and 1 U *Taq* DNA polymerase. Amplification was performed by denaturation at 96°C for 10 min, 35 cycles of denaturation at 96°C for 75 s, annealing at 62°C for 75 s, extension at 73°C for 75 s, and final extension at 73°C for 5 min. After amplification, the 294-bp PCR products were digested with *Msp*A1I in a 25-µL reaction containing 20 µL PCR products, 2.5 µL 10X buffer (Bio Basic Inc., Canada; supplied by the manufacturer), and 10 U *Msp*A1I for 4 h at 37°C, electrophoresis on 4% Seakem®LE agarose (BioWhittaker Molecular Application, Rockland, ME, USA) and visualized by ethidium bromide staining. The PCR product (294 bp) with the T allele of the T869C genotype was digested to four fragments (161, 67, 40, and 26 bp (less visible)), and the PCR product with the C allele of the T869C genotype was digested to five fragments (149, 67, 40, 26, and 12 bp (less visible)) in length relative to the size DNA markers. For G915C polymorphism, 20 µL of the 294-bp PCR product was digested with 10 U *Bgl*I for 4 h at 37°C, electrophoresis on 4% Seakem®LE agarose (BioWhittaker Molecular Application) and visualized by ethidium bromide staining. The PCR product (294 bp) with the G allele of the G915C genotype was digested to three fragments (131, 103 and 60 bp), and the PCR product with the C allele of the G915C genotype was digested to two fragments (131 and 163 bp) in length relative to the size DNA markers. The genotype of the C-509T polymorphism of the TGF-β1 was amplified by PCR-RFLP method (Shu et al., 2004). The primers of PCR for C-509T were 5'-GAGCAATTCTTACAGGTGTCTGC-3' (forward) and 5'-GAGGGTGTCAGTGGGAG GAG-3' (reverse). The PCR was done on a Gene Amp PCR system 9700 (Applied Biosystem). Each 30-µL PCR mixture contained 100 ng genomic DNA, 1 µM of each primer, 2.0 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphates, and 1 U *Taq* DNA polymerase. Amplification was performed by denaturation at 96°C for 15 min, 35 cycles of denaturation

at 94°C for 30 s, annealing at 62°C for 30 s, extension at 72°C for 45 s, and a final extension at 72°C for 8 min. After amplification, the 81-bp PCR products were digested with *Eco*81I in a 25-μL reaction containing 20 μL PCR products, 2.5 μL 10X buffer (Bio Basic Inc.; supplied by the manufacturer), and 10 U *Eco*81I for 4 h at 37°C, electrophoresis on 4% Seakem®LE agarose (BioWhittaker Molecular Application) and visualized by ethidium bromide staining. The 81-bp PCR product with the C allele was digested to two fragments (42 and 39 bp), and the PCR product with the T allele could not be digested by *Eco*81I in length relative to the size DNA markers.

Statistical analysis

The statistical software program SPSS 10.0 for Windows (SPSS Inc., Chicago, IL, USA) was used to analyze the individual parameters, which were detected in control and osteopenia/osteoporosis subjects and were compared using the Mann-Whitney U-test (two-tailed). The statistical differences between groups in genotypic frequencies were assessed by the Pearson chi-square test. A P value <0.05 was considered to be statistically significant. The results are reported as median, range, and 95% confidence interval (95%CI) for median as calculated by the statistical computer software package MINITAB (Ryan et al., 1985).

RESULTS

The median, range, and 95%CI for age, anthropometric parameters, biochemical markers, and BMD of bone in osteopenia/osteoporosis and control subjects are shown in Table 1. There were no statistical significant differences between the osteopenia/osteoporosis and control subjects for serum osteocalcin and calcium. All anthropometric data, except height, showed significant differences between the osteopenia/osteoporosis and control subjects. Serum TGF-β1 levels in osteopenia and osteoporosis subjects were found to be significantly lower than in control subjects. The BMD of lumbar spine 1-4 (L1-4), hip, and radius were significantly higher in control subjects.

Age, anthropometric parameters, biochemical markers, and BMD were compared according to the T869C polymorphism of the TGF-β1 gene in osteopenia/osteoporosis and control subjects as shown in Table 2. In all the genotype polymorphisms (TT, CT+CC), there were significant differences in age and anthropometric data (except height) in osteopenia/osteoporosis and control subjects. To determine whether the presence of the CT+CC genotype of the T869C polymorphism affects the serum TGF-β1 levels of osteopenia/osteoporosis subjects compared with the control group, the serum TGF-β1 levels of osteopenia/osteoporosis subjects were compared with those of control subjects. There were significantly lower serum TGF-β1 levels in osteopenia/osteoporosis subjects than in control subjects in the CT+CC genotype of T869C polymorphisms ($P < 0.001$).

The serum TGF-β1 levels of the osteopenia/osteoporosis subjects were compared with those of control subjects in the C-509T polymorphism of the TGF-β1 gene. There were significantly lower serum TGF-β1 levels in the osteopenia/osteoporosis group than in control subjects in the CT+TT genotype of the C-509T polymorphism ($P = 0.003$) (Table 3). No significant difference was observed between the control and osteopenia/osteoporosis subjects of the serum TGF-β1 levels in the G915C polymorphism (data not shown).

Table 1. Median, range, and 95% confidence interval (CI) for age, body mass index (BMI), anthropometric variables, serum osteocalcin, serum TGF-β1, calcium, and bone mineral density (BMD) of osteopenia/osteoporosis and control subjects.

	Control (N = 95) Median (range)	95%CI	Osteopenia/osteoporosis (N = 278) Median (range)	95%CI	P value*
Age (years)	53.0 (36.0-72.0)	52.0-54.5	58.5 (41.0-75.0)	58.0-59.8	<0.001
Weight (kg)	59.4 (43.6-95.0)	57.5-61.6	54.5 (37.5-95.0)	53.0-55.3	<0.001
Height (cm)	155.0 (146.0-173.0)	155.0-157.0	155.0 (138.0-170.0)	154.0-156.0	0.200
BMI (kg/m^2)	24.56 (19.35-39.54)	23.43-25.56	22.63 (15.41-33.41)	22.20-23.05	<0.001
Waist (cm)	81.5 (64.5-111.0)	79.5-83.0	77.0 (54.0-108.0)	76.0-78.0	0.001
Hip (cm)	99.0 (86.0-125.0)	97.7-100.0	95.0 (75.0-119.0)	94.5-96.0	<0.001
Arm circumference (cm)	28.00 (19.0-41.0)	27.5-29.3	27.0 (14.9-36.5)	27.0-27.5	<0.001
Triceps skin fold (mm)	23.0 (11.0-39.0)	22.0-25.0	21.0 (9.40-33.0)	20.1-22.0	0.002
Subscapular skin fold (mm)	27.0 (11.0-40.0)	25.0-30.0	24.0 (9.5-40.0)	23.0-25.1	0.005
Serum osteocalcin (ng/mL)	6.3 (0.9-27.8)	4.9-7.5	6.1 (0.1-27.1)	5.5-6.7	0.363
Serum TGF-β1 (ng/mL)	13.5 (1.6-45.4)	11.4-15.8	11.1 (1.4-62.5)	10.7-11.6	0.001
Calcium (mg/dL)	9.4 (8.5-10.6)	9.3-9.5	9.3 (7.8-10.8)	9.3-9.4	0.155
Lumbar spine 1 BMD (g/cm^2)	1.079 (0.758-1.404)	1.007-1.118	0.901 (0.420-1.363)	0.879-0.913	<0.001
Lumbar spine 2 BMD (g/cm^2)	1.158 (0.850-1.485)	1.110-1.176	0.937 (0.496-1.401)	0.900-0.950	<0.001
Lumbar spine 3 BMD (g/cm^2)	1.262 (0.917-1.569)	1.192-1.322	1.008 (0.690-1.561)	0.980-1.024	<0.001
Lumbar spine 4 BMD (g/cm^2)	1.223 (0.923-1.656)	1.200-1.400	0.989 (0.710-1.552)	0.980-1.040	<0.001
Femoral neck BMD (g/cm^2)	0.926 (0.712-1.224)	0.857-0.990	0.775 (0.289-1.670)	0.761-0.788	<0.001
Hip total BMD (g/cm^2)	1.054 (0.788-1.322)	0.999-1.077	0.856 (0.229-1.098)	0.844-0.869	<0.001
Radius total BMD (g/cm^2)	0.558 (0.467-0.584)	0.541-0.569	0.459 (0.562-0.961)	0.450-0.470	<0.001

BMI = body mass index; TGF-β1 = transforming growth factor-beta 1 gene. *Mann-Whitney U-Wilcoxon rank sum W-test (two-tailed). Statistical significant difference between osteopenia/osteoporosis and control subjects, at $P < 0.05$.

Table 2. Characteristics of osteopenia/osteoporosis and control subjects according to the T869C polymorphism of the TGF-β1 gene.

	TT			CT+CC		
	Osteopenia/osteoporosis (N = 71) Median (range)	Control (N = 37) Median (range)	P value*	Osteopenia/osteoporosis (N = 204) Median (range)	Control (N = 56) Median (range)	P value*
Age (years)	60.0 (41.0-70.0)	54.0 (40.0-67.0)	0.000	58.0 (41.0-75.0)	52.0 (36.0-72.0)	<0.001
Weight (kg)	54.5 (40.9-95.0)	62.7 (44.0-95.0)	0.001	54.5 (37.5-92.8)	58.6 (43.6-89.7)	<0.001
Height (cm)	155.0 (142.0-169.0)	156.0 (146.0-167.0)	0.121	155.0 (138.0-170.0)	155.0 (146.0-173.0)	0.875
BMI (kg/m^2)	22.88 (17.9-33.41)	25.55 (19.56-39.54)	0.003	22.46 (15.41-32.88)	23.93 (19.35-33.91)	0.002
Serum osteocalcin (ng/mL)	5.6 (0.2-16.6)	5.6 (1.2-24.8)	0.758	6.3 (0.1-19.6)	6.8 (0.9-27.8)	0.192
Serum TGF-b1 (ng/mL)	11.0 (1.5-62.5)	10.1 (4.3-45.4)	0.832	11.3 (1.4-51.8)	15.8 (1.6-44.0)	<0.001
Calcium (mg/dL)	9.4 (8.8-10.4)	9.4 (8.6-10.0)	0.560	9.30 (7.8-10.8)	9.4 (8.5-10.6)	0.190

BMI = body mass index; TGF-β1 = transforming growth factor-beta 1 gene; TT = wild type; CT = heterozygous; CC = homozygous. *Mann-Whitney U-Wilcoxon rank sum W-test (two-tailed). Statistical significant difference between osteopenia/osteoporosis and control subjects, at $P < 0.05$.

Table 3. Characteristics of osteopenia/osteoporosis and control subjects according to the C-509T polymorphism of the TGF-β1 gene.

	CC			CT+TT		
	Osteopenia/osteoporosis (N = 6) Median (range)	Control (N = 4) Median (range)	P value*	Osteopenia/osteoporosis (N = 270) Median (range)	Control (N = 91) Median (range)	P value*
Age (years)	60.0 (50.0-67.0)	47.0 (39.0-52.0)	0.024	58.5 (41.0-75.0)	53.0 (36.0-72.0)	<0.001
Weight (kg)	56.0 (53.7-68.6)	57.7 (49.5-69.5)	0.670	54.5 (37.5-95.0)	59.4 (43.6-95.0)	<0.001
Height (cm)	155.5 (149.0-161.0)	155.5 (149.0-162.0)	0.914	155.0 (138.0-170.0)	155.0 (146.0-173.0)	0.183
BMI (kg/m^2)	22.51 (22.03-28.55)	23.06 (20.34-31.30)	0.670	22.67 (15.41-33.41)	24.55 (19.35-39.54)	<0.001
Serum osteocalcin (ng/mL)	5.7 (3.2-6.6)	7.4 (0.9-14.4)	0.513	6.2 (0.1-27.1)	6.1 (1.0-27.8)	0.354
Serum TGF-b1 (ng/mL)	10.7 (7.0-39.1)	33.4 (15.4-38.2)	0.343	11.2 (1.4-62.5)	13.2 (1.6-45.4)	0.003
Calcium (mg/dL)	9.3 (9.2-9.8)	9.4 (9.4-9.6)	0.663	9.3 (7.8-10.8)	9.4 (8.5-10.6)	0.178

BMI = body mass index; TGF-β1 = transforming growth factor-beta 1 gene; CC = wild type; CT = heterozygous; TT = homozygous. *Mann-Whitney U-Wilcoxon rank sum W-test (two-tailed). Statistical significant difference between osteopenia/osteoporosis and control subjects, at $P < 0.05$.

Of the three polymorphisms of the TGF-β1 gene, there was a statistically significant difference between the T869C polymorphism of osteopenia/osteoporosis and control subjects (P = 0.012; odds ratio (OR) = 1.90) as shown in Table 4. The frequencies of the CT+CC genotype of T869C polymorphisms were 74.18% for osteopenia/osteoporosis and 60.22% for control subjects. In C-509T and G915C polymorphisms, there were no statistically significant differences between the osteopenia/osteoporosis and control subjects (P = 0.286; OR = 1.98 *vs* P > 0.05; OR = 1.64, respectively).

Table 4. Frequencies of the TGF-β1 gene polymorphisms (T869C, C-509T, and G915C) in osteopenia/osteoporosis and control subjects.

Genotype of genes	Osteopenia/osteoporosis [N (%)]	Control [N (%)]	Odds ratio (95%CI)	P value*
T869C				
CT+CC	204 (74.18)	56 (60.22)	1.90	0.012
TT	71 (25.82)	37 (39.78)	(1.16-3.12)	
C-509T				
CT+TT	270 (97.83)	91 (95.79)	1.98	0.286
CC	6 (2.17)	4 (4.21)	(0.54-7.17)	
G915C				
GC	5 (1.89)	1 (1.17)	1.64	>0.05
GG	259 (98.11)	85 (98.83)	(0.19-14.24)	

*P value ≤0.05 was considered to be statistically significant. T869C genotype: TT = wild type; CT = heterozygous; CC = homozygous. C-509T genotype: CC = wild type; CT = heterozygous; TT = homozygous. G915C genotype: GG = wild type; GC = heterozygous.

After adjusting for age (>50 years), subjects with the CT+CC genotype of the T869C polymorphism had increased risk of osteopenic/osteoporotic bones than normal bones at L1-4, femoral neck (P = 0.001; OR = 2.36), (P = 0.033; OR = 1.71), (P = 0.006; OR = 2.21), (P = 0.049; OR = 1.74), (P = 0.036; OR = 1.80) respectively, except at total hip (P = 0.090; OR = 1.78) and total radius (P = 0.365; OR = 1.30) (Table 5).

Table 5. Association between BMD (T-score) at lumbar spine 1-4, femoral neck, total hip, total radius and the T869C genotype of the TGF-β1 gene after adjustment for age >50 years.

Position of bones	T869C genotype			
	CT+CC [N (%)]	TT [N (%)]	Odds ratio (95%CI)	P value*
Lumbar spine 1 BMD (T-score)				
Osteopenic/osteoporotic	119 (55.35)	31 (34.44%)	2.36	0.001
Normal BMD	96 (44.65)	59 (65.56%)	(1.37-4.07)	
Lumbar spine 2 BMD (T-score)				
Osteopenic/osteoporotic	120 (55.56)	38 (42.22%)	1.71	0.033
Normal BMD	96 (44.44)	52 (57.78%)	(1.01-2.90)	
Lumbar spine 3 BMD (T-score)				
Osteopenic/osteoporotic	90 (41.67)	22 (24.44%)	2.21	0.006
Normal BMD	126 (58.33)	68 (75.56%)	(1.23-3.98)	
Lumbar spine 4 BMD (T-score)				
Osteopenic/osteoporotic	95 (43.98%)	28 (31.11%)	1.74	0.049
Normal BMD	121 (56.02%)	62 (68.89%)	(1.00-3.03)	
Femoral neck BMD (T-score)				
Osteopenic/osteoporotic	99 (46.48%)	29 (32.58%)	1.80	0.036
Normal BMD	114 (53.52%)	60 (67.42%)	(1.04-3.12)	
Total hip BMD (T-score)				
Osteopenic/osteoporotic	60 (28.04%)	16 (17.98%)	1.78	0.090
Normal BMD	154 (71.96%)	73 (82.02%)	(0.92-3.46)	
Total radius BMD (T-score)				
Osteopenic/osteoporotic	125 (58.14%)	46 (51.69%)	1.30	0.365
Normal BMD	90 (41.86%)	43 (48.31%)	(0.77-2.20)	

*P value ≤0.05 was considered to be statistically significant. T869C genotype: TT = wild type; CT = heterozygous; CC = homozygous. BMD = bone mineral density (T-score): osteopenic (T-score ≤ -1); osteoporotic (T score ≤ -2.5).

Logistic regression and OR for possible associations between the osteopenia/osteoporosis and TGF-β1 gene polymorphism at positions T869C and age-adjusted, serum TGF-β1 and body mass index (BMI)-adjusted are shown in Table 6. Two variables, gene polymorphism at positions T869C (OR = 1.97, P = 0.030) and age (>50 years) (OR = 1.15, P < 0.001), were considered risk factors for osteopenia/osteoporosis. On the other hand, serum TGF-β1 level and BMI (≥25 kg/m^2) were considered to be protective factors of this disease.

Table 6. Logistic regression analysis when osteopenia/osteoporosis was used as dependent variable and serum TGF-β1, age-adjusted, BMI-adjusted and the TGF-β1 gene polymorphism at the T869C genotype were taken as independent variables.

Variables	β	Odds ratios Exp (β)	(95%CI)	P value
T869C	0.678	1.97	1.07-3.63	0.030*
Serum TGF-β1	-0.031	0.97	0.95-0.99	0.012*
Age (>50 years)	0.142	1.15	1.10-1.21	<0.001*
BMI (≥25 kg/m^2)	-1.228	0.29	0.16-0.53	<0.001*

*P value ≤0.05 was considered to be statistically significant. CI = confidence interval; BMI = body mass index.

DISCUSSION

Many studies have attempted to elucidate the functions of TGE-β1, and reported that TGF-β1 may be an important regulator of the development of osteopenia in mice (Gazit et al., 1998). Thus, functional polymorphisms of TGF-β1 may affect BMD and the risk of fracture.

This is the first reported study, to our knowledge, to assess the three polymorphisms, T869C, C-509T, G915C, of the TGF-β1 gene in postmenopausal Thai women. We found two polymorphisms in the first exon, T869C and G915C polymorphisms. Only the CT+CC genotype of the T869C polymorphism was associated with low serum TGF-β1 levels and increased risk of osteopenic and osteoporotic fracture at lumbar spine and femoral neck. In this study, lower serum TGF-β1 levels were found in osteopenia/osteoporosis subjects rather than in control subjects in the CT+CC genotype of the T869C polymorphism (Table 2). Therefore, it could be concluded that the CT+CC genotype of the T869C polymorphism was the cause of lower serum TGF-β1 in osteopenia and osteoporosis among postmenopausal Thai women evaluated in this study.

The T869C polymorphism of the TGF-β1 gene codes for the substitution of the amino acid leucine for proline at position 10 of the protein in the signal peptide sequence (Derynck et al., 1985, 1987). The T869C polymorphism associated with the serum level of TGF-β1 suggests that the Leu^{10}→Pro substitution may affect the function of the signal peptide, possibly influencing intracellular trafficking or export efficiency of the preprotein. However, it remains unclear whether the differences in the circulating concetration of TGF-β1 among individuals with different TGF-β1 genotypes are reflected in the concentrations of this cytokine in the microenvironment of bone (Yamada et al., 2001).

After adjustment for age >50, subjects with the CT+CC genotype of the T869C polymorphism had increased risk of osteopenic and osteoporotic bones than normal bones at L1-4 and femoral neck (Table 5). At 50 years for women, the lifetime risk of hip fracture, vertebral fracture, and Colles' fracture were 17.5, 16, and 16%, respectively (Hunter and Sambrook, 2000). Women at age 50 with osteopenia and osteoporosis were between 37-50 and 13-18%,

respectively (Looker et al., 1997). Therefore, this study used age 50 as the cut-off for calculating the statistical difference in the T869C polymorphism frequency between ages <50 and >50 and the association between lumbar spine 1-4, femoral neck, total hip, total radius, and age in the two groups.

This result is consistent with many previous studies, which report the association between the T869C polymorphism, the C-509T polymorphism of the TGF-β1 gene and serum TGF-β1 level, and BMD (Langdahl et al., 1997; Yamada et al., 1998, 2001). The first report by Yamada et al. (1998) stated that there was association between the CC genotype of the T869C polymorphism and higher serum TGF-β1 level, and higher BMD at lumbar spine, observed in postmenopausal Japanese women. Hinke et al. (2001) showed the TT genotype of this polymorphism was associated with a higher serum TGF-β1 level, higher BMD at the lumbar spine, and the femoral neck in postmenopausal German women. Langdahl et al. (2003) showed that the CC genotype of the T869C polymorphism was associated with higher bone mass at the total hip in Danish women. Lau et al. (2004) observed that the TC genotype of this polymorphism was associated with lower bone mass at the total hip region but there were no significant differences of the serum TGF-β1 levels in postmenopausal southern Chinese women. In contrast, Ziv et al. (2003) reported that the TGF-β1 Leu10Pro polymorphism was not associated with BMD at the lumbar spine or hip, and fracture risk in white women in the United States.

The logistic regression (Table 6) obtained the similar results. T869C polymorphism, serum TGF-b1, age-adjusted, and BMI-adjusted showed a significant independent predictor to osteoporosis BMD. T869C polymorphism and age (>50 years) had a risk effect whereas serum TGF-b1 and BMI (≥25 kg/m^2) was found to be an indication of protection against osteoporosis. In contrast, Barrera et al. (2004) reported high BMI against femoral neck osteoporosis in healthy elderly subjects.

Although the level of serum TGF-β1 in the CT+TT genotype of the C-509T polymorphism in osteopenia/osteoporosis subjects was found to be lower than in control subjects (Table 3), there were no significant differences found in the C-509T polymorphism in osteopenia/osteoporosis subjects as compared with control subjects (Table 4). In contrast, Grainger et al. (1999) reported that the TT genotype of the C-509T polymorphism in the promoter region was associated with higher serum TGF-β1 levels. The reason for this apparent discrepancy is not clear. It might be attributed to the T869C polymorphism being in strong linkage disequilibrium to the C-509T polymorphism in the promoter region of the TGF-β1 gene (Grainger et al., 1999; Yamada et al., 2001). Yamada et al. (2001) reported that the C-509T polymorphism in the promoter region, alone or in combination with the T869C polymorphism of the TGF-β1 gene, was associated with L2-L4 BMD and osteoporosis in Japanese women.

This difference might be caused by genetic background in the subjects studied, environmental exposures, study design and analysis, or a difference in linkage disequilibrium between these two polymorphisms or other TGF-β1 polymorphisms. In this study, when combining all three polymorphisms of TGF-β1 we did not evaluate their association. Therefore, the association between combining T869C and C-509T polymorphisms of the TGF-β1 gene with BMD and fracture risk should be evaluated in further studies.

In conclusion, the CT+CC genotype of the T869C polymorphism of the TGF-β1 gene is associated with lower serum TGF-β1 in osteopenia and osteoporosis subjects and increased risk of osteopenic and osteoporotic fracture at L1-4 and femoral neck in postmenopausal Thai women. Moreover, we found that T869C polymorphism and age were also significantly asso-

ciated with increased risk of osteopenia and osteoporosis. On the other hand, serum TGF-β1 level and BMI (≥25 kg/m^2) are the protective factors of this disease. This study is the first report, to our knowledge, of the T869C polymorphism of the TGF-β1 gene in Thais, thereby providing a genetic basis for the TGF-β1 gene research in humans. Further studies with larger sample sizes are needed to confirm these findings and should evaluate the association between combining T869C and C-509T polymorphisms of the TGF-β1 gene with BMD and fracture risk.

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