VDR and TNFSF11 polymorphisms are associated with osteoporosis in Thai patients

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Abstract. Determining molecular markers for osteoporosis may be valuable for improving the quality of life of affected elderly patients by aiding in early detection and disease management. In the present study, the association between single nucleotide polymorphisms (SNPs) of the vitamin D receptor (*VDR*) and tumour necrosis factor superfamily number 11 (*TNFSF11*) genes and the susceptibility of developing osteoporosis was investigated in a Thai female cohort. The study group consisted of 105 Thai postmenopausal patients diagnosed with osteoporosis and 132 healthy Thai postmenopausal female volunteers. DNA extracted from blood samples was used to genotype the *VDR* and *TNFSF11* genes using polymerase chain reaction-restriction fragment length polymorphism and sequencing analysis. For *VDR*, the frequencies of the genotypes TT, CT and CC for the *Taq*I SNP (rs731236) were 87.88, 11.36 and 0.76%, respectively, in the control group, while in the osteoporosis cohort were 92.38, 5.71 and 1.91%, respectively. For the *Fok*I SNP (rs2228570), the frequencies of the genotypes CC, CT and TT were 31.06, 55.30 and 13.64%, respectively, in the control group, and in the osteoporosis group were 29.52, 43.81 and 26.67%, respectively. For *Bsm*I SNP (rs1544410), the frequencies of the genotypes GG, GA and AA were 78.03, 18.94 and 3.03%, respectively, in control group, and in the osteoporosis group were 80.95, 18.10 and 0.95%, respectively. The significant risk of osteoporosis associated with the *Fok*I SNP was determined. The odds ratio (95% confidence interval) was 2.30 (1.14-4.69; P=0.01) among patients with osteoporosis with TT as the susceptibility genotype. For *TNFSF11*, the frequencies of the genotypes TT, CT and CC for the -290C>T SNP (rs9525641) in the control group were 36.36, 50.76 and 12.88%, respectively, while in the osteoporosis group were 31.43, 56.19 and 12.38%, respectively. For the -643C>T SNP (rs9533156), the frequencies of the genotypes TT, CT and CC in the control group were 35.61, 48.48 and 15.91%, respectively, while in the osteoporosis group were 32.38, 55.24 and 12.38%, respectively. For the -693G>C SNP (rs9533155), the frequencies of the genotypes CC, CG, and GG in the control group were 39.39, 46.97 and 13.64%, respectively, and in the osteoporosis group were 36.19, 53.33 and 10.48%, respectively. No significant associations of the *TNFSF11* SNPs with osteoporosis were determined; however, it was notable that the GCT haplotype of *TNFSF11* may be a protective haplotype for osteoporosis. Therefore, it was concluded that the SNP *Fok*I of *VDR* may be a potential molecular biomarker for the development of osteoporosis in Thai females.

Introduction

Osteoporosis is a disease associated with the human aging process, and consequently it primarily occurs in the elderly population (1). Bone fractures in patients with osteoporosis are caused by reduced bone strength, as well as changes to the structure of the bone, as these result in an inability to support body weight or put pressure on the bone, which may result in fracture (2). Between 1989 and 1992 the number of seniors globally, including in the Thai population, has notably increased, and consequently resulted in increased incidents of osteoporosis and bone fractures (3). Osteoporosis occurs more frequently in females compared with in males, due to the lack of the hormone oestrogen in postmenopausal women. Oestrogen deficiency is associated with increased bone resorption, which is augmented by osteoclasts, resulting in an imbalance between bone formation and resorption. This imbalance is the primary cause of osteoporosis (4). Oestrogen deficiency is not the only cause of osteoporosis; certain DNA alterations can also increase the risk of developing this disease. There are a number of genes involved in osteoclastogenesis (5); however, in the present study the focus was on vitamin D receptor (*VDR*) and tumour necrosis factor superfamily number 11 (*TNFSF11*), due to these genes

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encoding *VDR* and receptor activator of nuclear factor-κB ligand (RANKL), respectively (6). Vitamin D in 1,25(OH)2D3 form stimulates bone resorption by binding to *VDR*. *VDR* with vitamin D forms a heterodimer with retinoid X receptor, in order to regulate *TNFSF11* expression (7,8). RANKL binds to RANK receptor, which is expressed on osteoclast progenitor cells, resulting in osteoclast activation followed by enhanced bone resorption (9). There are a number of single nucleotide polymorphisms (SNPs) in *VDR* that are known to be associated with osteoporosis and studies have demonstrated the association between polymorphism of *VDR* and the disease (10-13). For example, Gross *et al* (10) determined that the *Fok*I polymorphism (C>T; rs2228570) of *VDR* was significantly correlated with decreased bone mineral density (BMD) at the lumbar spine (LS) and an increased rate of bone loss in the hip, resulting in osteoporosis in Mexican women. Singh *et al* (11) demonstrated that the T allele was a risk factor of osteoporosis and also determined an association between haplotype AGT [*Taq*I (T>C; rs731236), *Bsm*I (G>A; rs1544410) and *Fok*I, respectively] and the disease in Northwest India. In the present study, the *VDR* SNPs *Taq*I, *Fok*I and *Bsm*I were investigated. The other gene of interest was TNFSF11, which encodes RANKL (14,15). There are a number of SNPs that are located in the promoter of *TNFSF11* (16); for the present study the focus was on -290C>T (rs9525641), -643C>T (rs9533156) and -693G>C (rs9533155). A previous study demonstrated that there was a significant association between LS-BMD and these three SNPs of *TNFSF11* (17); however, to the best of our knowledge, there has been no study to indicate if there is significant association between osteoporosis and SNPs located on *VDR* and *TNFSF11* genes in a Thai female patient cohort. Therefore, the present study focused on assessing the association of the *VDR* SNPs *Taq*I, *Fok*I and *Bsm*I and *TNFSF11* SNPs 290C>T, -643C>T and -693G>C with the occurrence of osteoporosis.

Materials and methods

Study population. For the present study, 105 postmenopausal Thai female volunteers with osteoporosis aged 56-88 years (mean age, 73.1±8.9 years) and 132 healthy Thai postmenopausal female volunteers aged 41-88 years (mean age, 63.4±8.7) were recruited from Thammasat Hospital, Pathum Thani, and Ramathibodi Hospital, Bangkok, Thailand between May 2013 and January 2014. All osteoporosis subjects were confirmed by orthopaedic physicians from Thamasat and Ramathibodi Hospitals. BMD measurement at the LS and hip was performed by dual energy X-ray absorptiometry (DXA). LS-BMD of patients with osteoporosis was 0.71±0.11 g/cm^2 and the LS T-score was -2.59±1.00 g/cm^2, while the total hip BMD T-score was 0.61±0.09 g/cm^2 and the total hip T-score was 2.08±0.81 g/cm^2 (18). Previously defined reference values for LS-BMD and total hip BMD T-scores of osteoporosis in Thai women were used for classifying the BMD results and for diagnosis of osteoporosis in our sample using DXA (19). The present study was approved by the Ethical Committees of Ramathibodi Hospital and Thammasat Hospital, and informed consent was obtained from all participating subjects.

Sample collection and DNA extraction. Peripheral blood samples (5 ml) were collected from all subjects in EDTA tubes

(Corning Life Sciences, Tewksbury, MA, USA). The samples were centrifuged to obtain the buffy coat (1,000 x g for 10 min at room temperature). Genomic DNA was extracted from the buffy coat fraction by phenol/chloroform extraction (20).

Genotype analysis
VDR SNP genotyping. DNA extracted from osteoporosis and healthy Thai postmenopausal females was genotyped at the *Taq*I (rs731236), *Fok*I (rs2228570) and *Bsm*I (rs1544410) polymorphisms of *VDR* by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (21). Table I lists the primer sequences with annealing temperature and restriction enzymes used for genotyping. The expected fragments at *Taq*I were: 249 bp in the TT genotype; 148 and 101 bp in the CC genotype; and 249, 148 and 101 bp in the CT genotype. The expected fragments at *Fok*I were: 266 bp in the CC genotype; 184 and 63 bp in the TT genotype; and 266, 184 and 63 bp in the CT genotype. The expected fragments at *Bsm*I were: 283 bp in the AA genotype; 200 and 83 bp in the GG genotype; and 283, 200 and 83 bp in the GA genotype.

TNFSF11 SNP genotyping. All DNA samples were also genotyped at the -290C>T (rs9525641), -643C>T (rs9533156) and -693G>C (rs9533155) polymorphisms of *TNFSF11* by PCR-RFLP (19). The primer sequences and enzymes are given in Table I. The expected fragments at -290C>T were: 146 bp in the CC genotype; 124 and 22 bp in the TT genotype; and 146, 124 and 22 bp in the CT genotype. The expected fragments at -643C>T were: 498 bp in the CC genotype; 322 and 176 bp in the TT genotype; and 498, 322 and 176 bp in the CT genotype. The expected fragments at -693G>C were: 498 bp in the GG genotype; 375 and 123 bp in the CC genotype; and 498, 375 and 123 bp in the GC genotype.

Direct sequencing. To confirm the sequence of each genotype from PCR-RFLP, 12 samples per SNP were randomly selected for sequence analysis. Briefly, each PCR product was purified using a PCR purification kits (Qiagen GmbH, Hilden, Germany). Purified products were sent to AITbiotech Pte Ltd. (Singapore) for sequencing using forward primers of each genotype.

Statistical analysis. The deviation from Hardy-Weinberg equilibrium at the P<0.05 level was calculated by comparing χ^2 values between the expected and the observed values for genotype counts to evaluate the consistency of genotype frequencies for the normal controls. Allele and genotype frequency was compared between patients with osteoporosis and control subjects. Odds ratios (OR), 95% confidence intervals (CIs) and P-values were used as parameters to compare the frequency of an SNP with the risk of osteoporosis. The three SNPs of each gene were analysed in haplotype blocks. The PLINK v1.07 program (http://pngu.mgh.harvard.edu/purcell/plink/) was used to perform all statistical analysis including inheritance modelling (22). P<0.05 was considered to indicate a statistically significant difference.

Results

*Genotyping of VDR SNPs Taq*I, *Fok*I *and Bsm*I. The genotyping data is summarized in Table II. The frequencies of the

Table I. Primer sequences with annealing temperature and restriction enzymes used for genotyping.

Single nucleotide polymorphism	Primer sequence, 5'-3'	Enzyme for RFLP	Product size, bp
Vitamin D receptor gene			
rs731236	F: TGGTGGGATTGAGCAGTGAG	*Taq*I	Uncut: 249
			Cut: 101/148
	R: GTACTGCTTGGAGTGCTCCT		
rs1544410	F: AACCTGAAGGGAGACGTAGCA	*Bsm*I	Uncut: 283
			Cut: 200/83
	R: TTGTACCCTGCCCGCAAGAAA		
rs2228570	F: ACCAAGGATGCCAGCTGG	*Fok*I	Uncut: 266
			Cut: 19/63/184
	R: GCTTCTTCTCCCTCCCTTTC		
Tumor necrosis factor superfamily member 11 gene			
rs9533155	F: GCCACAGTTCTGAATAGAGG	*Bsa*JI	Uncut: 498
			Cut: 123/375
	R: GGATAAGGATTGCACCTCAG		
rs9533156	F: GCCACAGTTCTGAATAGAGG	*Tsp*RI	Uncut: 498
			Cut: 176/322
	R: GGATAAGGATTGCACCTCAG		
rs9525641	F: ATCCTAAGGAGGAAACCGAGAC	*Mse*I	Uncut: 146
			Cut: 124/22
	R: GGAGGTCCAAGAGATGGGTTTA		

genotypes TT, CT and CC for the *Taq*I SNP in the control group were 87.88, 11.36 and 0.76%, respectively, and the frequencies in the osteoporosis group were 92.38, 5.71 and 1.91%, respectively. For the *Fok*I SNP, the frequencies of the genotypes CC, CT and TT were 31.06, 55.30 and 13.64%, respectively, in the control group, and in osteoporosis group were 29.52, 43.81 and 26.67%, respectively. For the *Bsm*I SNP, the frequencies of the genotypes GG, GA and AA were 78.03, 18.94 and 3.03%, respectively, in control group, and in the osteoporosis group were 80.95, 18.10 and 0.95%, respectively. The distributions of the genotypes of all three SNPs in the control group were in Hardy-Weinberg equilibrium ($P > 0.05$).

Genotyping of TNFSF11 SNPs -290C>T, -643C>T and -693G>C. As displayed in Table II, the frequencies of the genotypes TT, CT and CC for -290C>T SNP in the control group were 36.36, 50.76 and 12.88%, respectively, and in the osteoporosis group were 31.43, 56.19 and 12.38%, respectively. For -643C>T SNP, the frequencies of the genotypes TT, CT and CC in the control group were 35.61, 48.48 and 15.91%, respectively, and in the osteoporosis group were 32.38, 55.24 and 12.38%, respectively. For -693G>C SNP, the frequencies of the genotypes CC, CG, and GG in the control group were 39.39, 46.97 and 13.64%, respectively, and in the osteoporosis group were 36.19, 53.33 and 10.48%, respectively. The distributions of the genotypes of all three SNPs in the control group were in Hardy-Weinberg equilibrium ($P > 0.05$).

SNP analysis of the VDR gene at TaqI, FokI and BsmI. The allele frequencies are displayed in Table II and all genotyping data are displayed in Table III. The data indicated that there

was a significant risk of osteoporosis associated with the *Fok*I SNP; with TT as the susceptibility genotype, the OR (95% CI) was 2.30 (1.14-4.69; P=0.01) in patients with osteoporosis, while with CT as the susceptibility genotype the OR (95% CI) was 0.63 (0.36-1.09; P=0.08). The OR (95% CI) of the minor T allele as the susceptibility allele for *Fok*I SNP among patients with osteoporosis was 1.34 (0.92-1.97; P=0.11). For the *Taq*I SNP, with CC as the susceptibility genotype the OR (95% CI) was 2.54 (0.18-7.26; P=0.41) in patients with osteoporosis, while with CT as the susceptibility genotype, the OR (95% CI) was 0.47 (0.16-1.36; P=0.13). The OR (95% CI) of the minor C allele as the susceptibility allele was 0.73 (0.30-1.72; P=0.43). For the *Bsm*I SNP, with AA as the susceptibility genotype the OR (95% CI) was 0.31 (0.01-2.98; P=0.26) in patients with osteoporosis; whereas, the OR (95% CI) of GA, as the susceptibility genotype, was 0.95 (0.46-1.92; P=0.89). The OR (95% CI) of the minor allele A, as the susceptibility allele, was 0.78 (0.42-1.44; P=0.40).

SNP analysis of the TNFSF11 gene at -290C>T, -643C>T and -693G>C. For the -290C>T SNP, the OR (95% CI) of CC, as the susceptibility genotype, was 0.96 (0.41-2.20; P=0.91), while the OR (95% CI) of CT, as the susceptibility genotype, was 1.24 (0.72-2.15; P=0.41) in the patients with osteoporosis. The OR (95% CI) of the minor C allele, as the susceptibility allele, was 1.10 (0.74-1.62; P=0.62). For the -643C>T SNP, the OR (95% CI) of CC, as the susceptibility genotype, was 0.75 (0.33-1.67; P=0.44), while the OR (95% CI) of CT, as the susceptibility genotype, was 1.31 (0.76-2.27; P=0.30). The OR (95% CI) of the minor C allele, as the susceptibility allele, was 0.99 (0.67-1.46; P=0.97). For the -693G>C SNP, the OR (95% CI) of GG, as the susceptibility genotype, was 0.74 (0.31-1.75;

Table II. Summary of genotypes.

Gene	SNP	Allele Major	Allele Minor	Group	Genotype, n (%)			Allele frequency		OR minor allele (95% CI)	OR major allele (95% CI)	P-value (OR minor allele)
VDR												
	rs731236	T	C		TT	CT	CC	T	C			
				Control	116 (87.88)	15 (11.36)	1 (0.76)	0.94	0.06	Ref.	Ref.	-
				Osteoporosis	97 (92.38)	6 (5.71)	2 (1.91)	0.95	0.05	0.73 (0.30-1.72)	1.38 (0.58-3.31)	0.43
	rs1544410	G	A		GG	GA	AA	G	A			
				Control	103 (78.03)	25 (18.94)	4 (3.03)	0.88	0.13	Ref.	Ref.	-
				Osteoporosis	85 (80.95)	19 (18.10)	1 (0.95)	0.90	0.10	0.78 (0.42-1.44)	1.29 (0.69-2.39)	0.40
	rs2228570	C	T		CC	CT	TT	C	T			
				Control	41 (31.06)	73 (55.30)	18 (13.64)	0.59	0.41	Ref.	Ref.	-
				Osteoporosis	31 (29.52)	46 (43.81)	28 (26.67)	0.51	0.49	1.34 (0.92-1.97)	0.74 (0.51-1.09)	0.11
TNFSF11												
	rs9533155	C	G		CC	CG	GG	C	G			
				Control	52 (39.39)	62 (46.97)	18 (13.64)	0.63	0.37	Ref.	Ref.	-
				Osteoporosis	38 (36.19)	56 (53.33)	11 (10.48)	0.63	0.37	1.00 (0.68-1.48)	1.00 (0.67-1.48)	0.99
	rs9533156	T	C		TT	CT	CC	T	C			
				Control	47 (35.61)	64 (48.48)	21 (15.91)	0.60	0.40	Ref.	Ref.	-
				Osteoporosis	24 (32.38)	58 (55.24)	13 (12.38)	0.60	0.40	0.99 (0.67-1.46)	1.01 (0.68-1.48)	0.97
	rs9525641	T	C		TT	CT	CC	T	C			
				Control	48 (36.36)	67 (50.76)	17 (12.88)	0.62	0.38	Ref.	Ref.	-
				Osteoporosis	33 (31.43)	59 (56.19)	13 (12.38)	0.60	0.40	1.10 (0.74-1.62)	0.91 (0.62-1.34)	0.62

OR, odds ratio; CI, confidence interval; SNP, single nucleotide polymorphism; VDR, vitamin D receptor; TNFSF11, tumor necrosis factor superfamily member 11.

Table III. OR (95% CI) of genotypic frequency of SNPs at VDR and TNFSF11 in osteoporosis patients and control subjects.

SNP	Genotype	OR (95% CI)	P-value
VDR			
rs731236	CC	2.54 (0.18-7.26)	0.41
	CT	0.47 (0.16-1.36)	0.13
	TT	1.67 (0.67-4.48)	0.25
rs15444410	AA	0.31 (0.01-2.98)	0.26
	GA	0.95 (0.46-1.92)	0.89
	GG	1.20 (0.60-2.38)	0.58
rs2228570	TT	2.30 (1.14-4.69)	0.01
	CT	0.63 (0.36-1.09)	0.08
	CC	0.93 (0.51-1.69)	0.80
TNFSF11			
rs9533155	GG	0.74 (0.31-1.75)	0.46
	CG	1.29 (0.75-2.23)	0.33
	CC	0.87 (0.50-1.53)	0.61
rs9533156	CC	0.75 (0.33-1.67)	0.44
	CT	1.31 (0.76-2.27)	0.30
	TT	0.85 (0.49-1.54)	0.60
rs9525641	CC	0.96 (0.41-2.20)	0.91
	CT	1.24 (0.72-2.15)	0.41
	TT	0.80 (0.45-1.43)	0.43

OR, odds ratio; CI, confidence interval; SNP, single nucleotide polymorphism; *VDR*, vitamin D receptor; *TNFSF11*, tumor necrosis factor superfamily member 11.

Table IV. Haplotypes analysis of single nucleotide polymorphisms in *VDR* (rs731236, rs1544410, rs2228570) and *TNFSF11* (rs9533155, rs9533156 and rs9525641) indicating protective and risk haplotypes for osteoporosis.

	Haplotype frequency		
Haplotype	Osteoporosis	Control	P-value
VDR			
CAT	0.193	0.026	0.618
TAT	0.021	0.017	0.793
TGT	0.443	0.369	0.105
CAA	0.005	0.037	0.020
TAA	0.058	0.044	0.510
TGA	0.455	0.506	0.270
TNFSF11			
GCC	0.363	0.349	0.755
CCC	0.024	0.023	0.930
CTC	0.014	0.011	0.773
GCT	0.005	0.019	0.175
CCT	0.009	0.011	0.837
CTT	0.584	0.586	0.967

VDR, vitamin D receptor; *TNFSF11*, tumor necrosis factor superfamily member 11.

P=0.46), while of CG, as the susceptibility genotype, was 1.29 (0.75-2.23; P=0.33). The OR (95%) of the minor allele G, as the susceptibility allele, was 1.00 (0.68-1.48; P=0.99).

Model of inheritance at the VDR locus. Models of inheritance of each SNP in VDR were determined as follows: for the *Taq*I SNP, when the mode of inheritance was dominant, the OR (95% CI) of CC or CT was 0.60 (0.22-1.56; P=0.255) and when the mode of inheritance was recessive, the OR (95% CI) of CC was 2.54 (0.18-7.87; P=0.414); for the *Bsm*I SNP, when the mode of inheritance was dominant, the OR (95% CI) of GG or GA was 3.25 (0.34-7.54; P= 0.264) and when the mode of inheritance was recessive, the OR (95% CI) of GG was 1.20 (0.60-2.38; P= 0.581); and for the *Fok*I SNP, when the mode of inheritance was dominant, the OR (95% CI) of TT or CT was 1.00 (0.59-1.95; P= 0.799) and when the mode of inheritance was recessive, the OR (95% CI) of TT was 2.30 (1.14-4.69; P=0.012; data not shown).

Model of inheritance at the TNFSF11 locus. Models of inheritance of each SNP in *TNFSF11* were determined as follows: for the -693G>C SNP, when the mode of inheritance was dominant, the OR (95% CI) of GG or CG was 1.15 (0.65-2.02; P=0.614) and when the mode of inheritance was recessive, the OR (95% CI) of GG was 0.74 (0.31-1.75; P=0.462); for the -643C>T SNP, when the mode of inheritance was dominant,

the OR (95% CI) of CC or CT was 1.15 (0.65-2.06; P=0.604) and when the mode of inheritance was recessive, the OR (95% CI) of CC was 0.75 (0.33-1.67; P=0.443); and for the -290C>T SNP, when the mode of inheritance was dominant, the OR (95% CI) of CC or CT was 1.25 (0.70-2.23; P=0.427) and when the mode of inheritance was recessive, the OR (95% CI) of CC was 0.96 (0.41-2.20; P=0.909; data not shown).

Haplotype analysis. Haplotype analysis of SNPs *Taq*I, *Bsm*I and *Fok*I in *VDR* was conducted (Table IV). The frequency of CAA in the osteoporosis group was 0.48%, while in the control group was 3.71% (P=0.02). The frequency of CAT in the osteoporosis group was 19.3%, while in the control group was 2.6% (P=0.618). Haplotype analysis of SNPs -693G>C, -643C>T and -290C>T in *TNFSF11* was also conducted (Table IV). The frequency of GCT in the osteoporosis group was 0.5%, while in the control group was 2% (P=0.180).

Discussion

There are numerous factors associated with osteoporosis, including age, nutrition, hormones and genetics (23,24). A number of studies have demonstrated association between SNPs in a number of genes and osteoporosis, including the SNP *Fok*I of *VDR* (rs2228570) and rs2324851 (C>T) of *TNFSF11* (11,25). In the present study, the association of 3 SNPs in *VDR* (*Taq*I, *Bsm*I and *Fok*I) and of 3 in *TNFSF11* (-693G>C, -643C>T and -290C>T) with osteoporosis was evaluated. It was determined that in *VDR*, the TT genotype of the *Fok*I SNP was a risk factor of osteoporosis, while there was no significant

association of the SNPs *Taq*I and *Bsm*I with osteoporosis. This data was similar to previous reports, in which BsmI genotype frequency was higher in Turkish and Caucasian populations, compared with in Asian populations; for example, Indian and Thai (11,26). The presents results are similar to the study by Singh *et al* (11), which demonstrated that allele T for *Fok*I SNP was a risk factor of osteoporosis, but also that there was a significant association, while the present study indicated that allele T had no significant association. There was also contrast in the results on inheritance mode between the present data and the study by Singh *et al* (11). In the present study, it was determined that TT was recessive in the osteoporosis group, while Singh *et al* reported TT as dominant. These observations indicated that SNP *Fok*I may be a risk factor of osteoporosis in Asian populations, for example Thai and Indian.

*Taq*I and *Bsm*I are located near the 3'untranslated region of *VDR*, which may control its RNA stability (27). SNP *Fok*I is located on exon 2 and is a start codon polymorphism located 3 codons upstream of a second start codon (28). The change of ATG (allele T) to ACG (allele C) results in a *VDR* protein that is 3 amino acids shorter, which may affect ligand (vitamin D) affinity, and thus impact the ability of *VDR* to regulate gene expression (28); therefore, it is not surprising to determine significant association between *Fok*I and a number of diseases, including diabetes, thyroid diseases and osteoporosis (29,30). For haplotype analysis of *VDR*, it was determined that CAA was a significant protective haplotype, while CAT appeared to be a risk factor haplotype in osteoporosis. To observe the association between SNPs of *TNFSF11* and osteoporosis, numerous studies have focused on SNPs at the promoter of *TNFSF11* (16,17), since the expression of *TNFSF11* in encoding RANKL may be regulated by *VDR* with vitamin D and other transcription factors; therefore, the regulatory region of *TNFSF11* may be an important contributor in osteoporosis development. A number of SNPs in the *TNFSF11* promoter have been studied. In the current study, the focus was on 3 SNPs, -290C>T (rs9525641), -643C>T (rs9533156) and -693G>C (rs9533155), but a significant association with regard to osteoporosis was not determined; however, it was indicated that GCT may be a protective haplotype. Overall, it was notable that none of the tested SNPs of *TNFSF11* were associated with osteoporosis in Thai postmenopausal females.

A number of studies have demonstrated association of other *TNFSF11* SNPs and osteoporosis, including rs2277439 (A>G) and rs2324851 (C>T) in a Chinese cohort (25), and rs2277438 (A>G) in a Korean cohort (31). All of these SNPs are located in introns. Intron variants may also be enhancers that act on the gene they are located in, or may enhance the expression of numerous genes (32). Additionally, studies have demonstrated that other SNPs, including rs9594738 (C>T) and rs9533090 (C>T), located in the super-enhancer region for regulation of RANKL expression, were observed to be significantly associated with BMD in genome-wide association studies (33). It is possible that base pair change at these SNPs may affect the binding of the transcription factor, resulting in the regulation of RANKL expression. This particularly applies to rs9533090, where deletion of the region harboring this SNP could result in the reduction of RANKL expression (34). The SNP rs9533090 can be C or T; however, allele C has greater ability to recruit the transcription factor nuclear factor I C compared

with allele T, and may thus more efficiently elevate enhancer activity and increase RANKL expression (33); therefore, it is plausible to consider an association between rs9533090 and osteoporosis. It is important to study the association between rs9533090 and osteoporosis in any population; however, only a limited number of studies have investigated this (35,36). From findings aforementioned, it is possible that there is a significant association between other SNPs in *TNFSF11* and osteoporosis in the Thai female population. The SNP rs9533090 is notable in this regard and may be a risk factor for the development of osteoporosis. The present study indicated a significant association between osteoporosis and the *Fok*I SNP of *VDR*, with T appearing as a susceptibility allele in Thai females. The mode of inheritance of *Fok*I SNP was recessive. This preliminary data from the present study indicated that the *Fok*I SNP of *VDR* may be a molecular biomarker in Thai patients with osteoporosis. Thai females with the risk factor genotype TT of *Fok*I SNP should be cautioned with regard to lifestyle in order to prevent osteoporosis development. Overall, the present data may have value on a clinical basis and beneficial for early detection, prevention and management of osteoporosis in Thai females.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MT performed experiments, analysed data and wrote the first draft of the manuscript. NT acted as the clinician who diagnosed osteoporosis and collected the clinical samples. AW and RT analysed data. PY wrote the proposal for grants, designed the study, analysed data and revised the manuscript.

Ethics approval and consent to participate

The study was approved by the ethical committees of Ramathibodi Hospital (approval no. 04-54-44) and Thammasart Hospital (approval no. MTU-EC-OT-4-087/56) and informed consent was obtained from all participating subjects.

Consent for publication

All participating subjects consented to the publication of relevant data.

Competing interests

The authors declare that they have no competing interests.

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