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ORIGINAL ARTICLE

Genetic variants of vitamin D, estrogen α, parathyroid and collagen type I alpha receptor gene and its influence on circulating serum osteocalcin in postmenopausal osteoporosis: A cohort study

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Abstract
Osteocalcin is an abundant, highly conserved bone specific protein and the serum levels of OC have been used as a biochemical marker of bone turnover. The genetic variation of certain candidate genes impacts osteocalcin levels in the postmenopausal period and may predispose some women to high bone turnover. To identify the genes influencing variation in serum OC levels, we investigated the polymorphisms of Vitamin D, Estrogen α, Parathyroid and Collagen Type I *alpha* Receptor genes and its association with bone turnover evaluated by serum osteocalcin in postmenopausal women from south India. The polymerase chain reaction-restriction fragment length polymorphism strategy was used to detect the polymorphisms at all the four gene receptors (i.e., for VDR, ERα, PTH and COLIA1) in 300 postmenopausal women from South India. Serum osteocalcin levels were measured by immunoassay (ELISA).The serum osteocalcin levels for the *Apa* I polymorphisms showed varied results, in which, subjects in the control group with "GG" genotype and the osteopenic group with "TT" genotype of the *Apa*I polymorphism had a significantly higher serum osteocalcin concentration ($p < 0.05$). The *BstBI*-AA group in controls had a significantly higher level of serum osteocalcin, this suggests a higher state of bone turnover in the AA genotype. The outcome of this study proposes the probability of a small impact of the VDR- *Apa*I (GG) genotype, the VDR-*Taq*I (TT) genotype and the (AA) genotype of the PTH-*BstB*I polymorphism indicating a higher rate of bone turnover in the healthy postmenopausal women.

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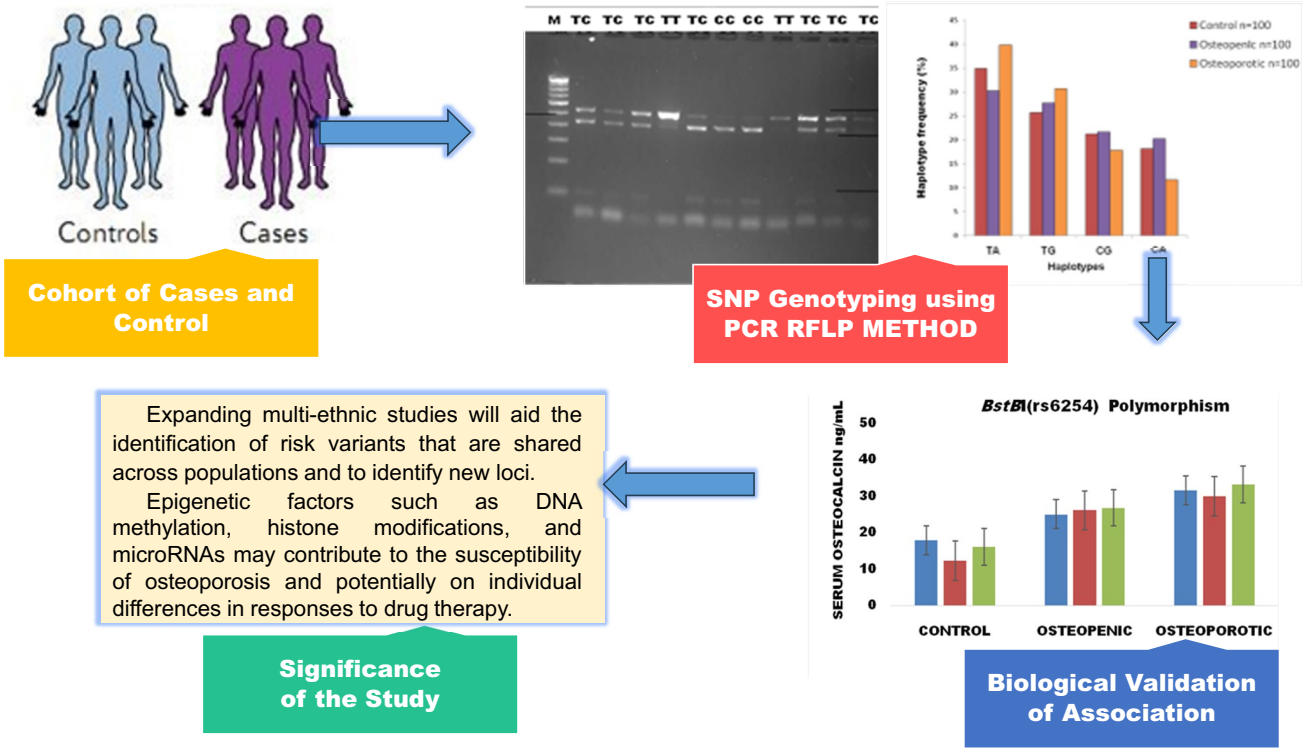
Graphical Abstract



Keywords Postmenopausal · Osteoporosis · Serum osteocalcin · Polymorphisms

Introduction

Osteoporosis is a typical metabolic bone disorder with a strong hereditary impact. It is portrayed by decline in bone mass and defects in bone tissue which weaken bone strength and lead to increased risk of bone fragility [14, 15]. Osteoporosis inflicts 33% of women and one out of eight men beyond the age of 50 [17]. In adolescence there is a high pace of bone turnover in which formation surpasses resorption. In young adulthood, formation and resorption are in exact equilibrium, but with aging there is an overall deficit of bone. Susceptibility to osteoporosis results from a wide range of genetic variations and their interactions with environmental factors [23]. Bone biomarkers produced from the bone remodeling measure includes bone development biomarkers, bone resorption biomarkers and regulators of bone turnover. Distinguishing of bone metabolism has been explored with the biomarkers of catalysts, proteins and by-products during the bone remodeling process [5]. Diverse biomarkers are as of now available for explicit and sensitive evaluation of the pace of bone development and bone resorption. These biomarkers are important to give the early assessment of osteoporosis when the BMD assessment does not offer adequate information to make the conclusion [20]. Osteocalcin has regularly been utilized as a serum marker for bone formation and act in the bone matrix to control mineralization. Osteocalcin may likewise be engaged with the enrollment and differentiation of osteoclasts. Morrison and collaborators observed that a typical allelic variation in a gene encoding the vitamin D receptor had a strong affiliation to bone mineral density and bone turnover estimated as serum osteocalcin [18]. Recognizing genetic risk factors for osteoporosis depends upon previous information, for example, the known association of a specific gene in aspects of osteoporosis, e.g., bone metabolism. This gene is then referred to as a "candidate gene". In this way, polymorphisms must be identified in the candidate gene that leads to contrasts in the functional levels of the encoded protein. Sequence analysis of a "candidate" osteoporosis gene in various individuals will recognize sequence variants, yet additionally a few databases are currently accessible that contain this data (e.g., NCBI dbSNP, HapMap, 1000Genomes, and a few more specialized databases). Some DNA sequence variations will be simply polymorphic (unknown polymorphisms), while others will have functional consequences for the activity of the protein

encoded (functional polymorphisms). Clearly, it relies upon the gene and the kind of polymorphisms in the population. Polymorphisms of interest are normally first tested in population- based or case- control "association studies", to assess the phenotype of interest at the population level. Testing of individual candidate genes to what their contribution is to osteoporosis risk is a valid analysis. Once that has been established, the interaction or multiplicative impacts of a few genes will be examined and finally, gene-environment interactions can be contemplated. Linkage and genetic mapping studies are generally performed for analyzing complex traits and disease. There are two principle subtypes of linkage analysis: parametric (determining a model of inheritance in a family) and nonparametric (no inheritance model) [21]. The most recent strategy has been more generally utilized for examination of complex traits. Numerous genome-wide linkage studies have been performed on Bone Mineral Density (BMD) and other related phenotypes of osteoporosis [24], however even in huge meta-analysis, linkage studies didn't yield any genome-wide significant loci for BMD [12], perhaps in light of the fact that basic variations controlling BMD have modest impacts which are hard to be distinguished by traditional linkage investigations [31]. Given the failure of linkage studies, researchers turned their focus to candidate gene investigations. However, results frequently gave off an impression of being non-replicative, most likely because of the statistical power, sample size, lack of standardized phenotype and genotype, limited number of gene variants evaluated and challenges in matching with cases and controls. Essentially, GWASs have distinguished numerous genome-wide significant loci. The significant advantage of GWASs over candidate gene investigations for common diseases is that they may lead to the identification of new susceptible genes and pathways. The VDR gene encodes the vitamin D receptor (Fig. 1) which plays a crucial role in calcium homeostasis and bone metabolism. Vitamin D is essential for calcium absorption, and its receptor, VDR, mediates its effects. Numerous studies have investigated the association between VDR polymorphisms (*Apa*I, *Taq*I, *Bsm*I) and bone mineral density (BMD) or osteoporosis risk. These polymorphisms have been linked to variations in VDR activity and its impact on calcium absorption and bone turnover. For example, the "*Bsm*I B allele" has been associated with lower BMD and increased osteoporosis risk in Caucasian populations [17]. Estrogen plays a crucial role in bone health by inhibiting bone resorption. Polymorphisms in the estrogen type I alpha gene, particularly *Pvu*II and *Xba*I, may affect estrogen receptor function. Research has examined the association between estrogen receptor gene polymorphisms and osteoporosis risk. Variants like *Pvu*II and *Xba*I have been linked to differences in estrogen receptor expression and function, potentially impacting bone density and fracture risk [27]. The parathyroid hormone (PTH) is crucial for maintaining calcium levels in the blood and bone remodeling. Polymorphisms in genes related to PTH regulation may influence bone metabolism. Some studies have explored the association between parathyroid gene polymorphisms and bone health. The *BstB*I polymorphism in the parathyroid gene has been studied for its potential influence on PTH levels and, consequently, bone density [2]. Collagen type I is a major component of bone matrix, and mutations or polymorphisms in the COL1A1 gene can affect collagen production, leading to bone disorders. The COL1A1 gene has been extensively studied in the context of osteoporosis

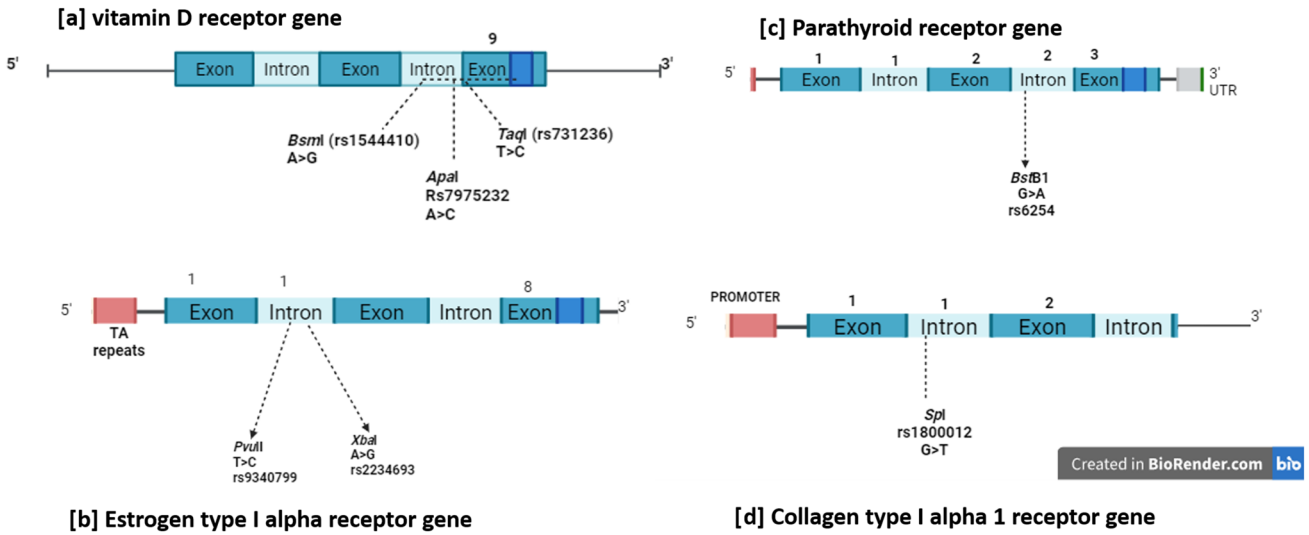


Fig. 1 Schematic overview of the polymorphism of the candidate genes

and bone diseases. Polymorphisms like the *Sp*I polymorphism have been associated with variations in bone density and fracture risk in some populations [30]. In summary, these five genes were chosen for testing in osteoporosis and bone metabolism studies because of their biological relevance to bone health and the existing body of research indicating potential associations between specific gene variants (polymorphisms) and bone-related phenotypes, such as bone mineral density, fracture risk, and calcium homeostasis. Prior studies have provided valuable insights into how these genetic variations may contribute to the development and progression of osteoporosis, making them important candidates for further investigation in clinical and genetic studies [19]. The aim of the current study was to examine the polymorphisms of the vitamin D receptor (VDR), estrogen receptor (ER) and parathyroid and collagen type I receptor gene polymorphisms in association with biochemical markers of bone turnover evaluated by serum osteocalcin in postmenopausal women from south India.

Methods

Study subjects

The present case–control study consisted of randomly selected 300 South Indian women in the post menopausal age group who belong to the south Indian population. A standard questionnaire was used to collect information regarding age, medical conditions; medications used risk factors, family history of osteoporosis together with dietary and lifestyle habit. The subjects who had various endocrinological disorders such as hyperthyroidism and hypo- and hyperparathyroidism, chronic disorders of the liver and kidney and other skeletal diseases (Paget's disease, osteogenesis imperfecta and rheumatoid arthritis) along with few other subjects using medications that were known to affect bone density and metabolism (such as calcium supplements, corticosteroids, anticonvulsants, hormone replacement therapy and heparin) or those with an unusual gynaecological history such as bilateral oophorectomy, irregular cycles or premature menopause before the age of 40 were excluded from this study. The stratified random sampling method was used in this study. The selection of the study group being restricted only to postmenopausal women, all of whom were to be without any of the above mentioned major risk factors posed a limiting factor. Thus, reducing the sample size to achieve 80% statistical power. Hong et al. [11], suggested that the dominant model required the smallest sample size (90 cases and 90 controls) to achieve 80% power when compared to other genetic models, under the following assumptions of 5% disease prevalence, 5% MAF, complete LD (D' = 1), 1:1 case-to-control ratio, and 5% type I error rate (α) and odds ratios of heterozygotes at 2 and odds ratios of rare homozygotes at 3.

Estimation of serum osteocalcin

Blood samples were obtained using standard venipuncture technique for DNA processing and measurement of blood chemistries. Serum was separated from clotted blood by centrifugation and then stored at − 80 °C until assayed. Serum concentrations of intact OC were measured in duplicate with enzyme-linked immunosorbent assay (ELISA) using the Human osteocalcin/Bone gla protein (OT/BGP) ELISA kit (Bioassay Technology Laboratory, China), in a micro titer strip well format using monoclonal antibody directed against distinct epitopes of human osteocalcin.

SNP genotyping

DNA was isolated for PCR amplification from the whole blood samples by non-enzymatic salting-out method [24]. The following polymorphisms of rs7975232, rs731236, rs1544410, rs2234693, rs9340799, rs6254 and rs1800012 was genotyped for all the subjects using PCR- restriction fragment length polymorphism (RFLP), as previously described by us in our earlier work [6].

Statistical analysis

All the analysis were carried out using the statistical package of Graph Pad Prism version 8. The association of genotype with serum osteocalcin was evaluated by analysis of variance (one-way ANOVA). Multiple linear regression analyses were performed to examine the interaction effects on the levels of serum osteocalcin variations among the SNPs. The subjects were divided into three groups based on their genotypes and each group was coded as "0", "1" and "2" respectively. The code "0" represents the homozygous genotype for the reference genotype, the code "1" represents the heterozygous genotype and code "2" represents the homozygous genotype for the variant allele. The p value < 0.05 was selected to define statistical significance.

Results

Baseline characteristics of the study subjects

The distribution of serum osteocalcin levels in the postmenopausal women is given in Fig. 2. The serum OC concentrations were measured in 300 subjects, ranging in age from 45 to 88 years. Values ranged from 2.20 ng/mL, the lower limit of detection of this assay, to 44.50 ng/mL. Mean values were

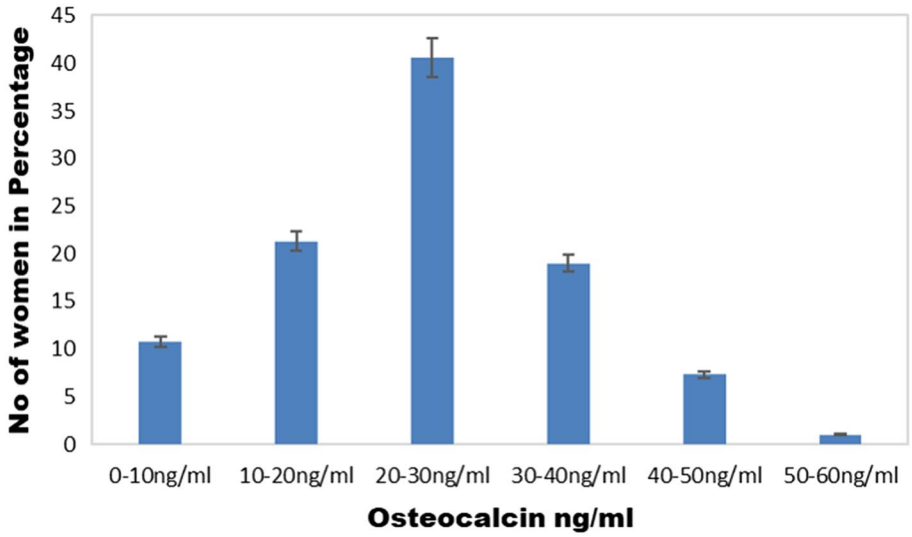


Fig. 2 Distribution of osteocalcin

Table 1 Mean (±SD) concentrations of serum osteocalcin according to age

Age	Osteocalcin ng/mL
45–50	23.15 ± 10.5 (99)[a]
50–55	25.66 ± 11.7 (48)
55–60	24.00 ± 9.7 (65)
60–65	22.37 ± 9.1 (37)
65–70	27.70 ± 13.3 (30)
70–75	24.94 ± 11.4 (14)
75–80	25.25 ± 15.0 (7)

[a]Number of subjects in parentheses

15.1 ± 7.8 ng/mL and 31.6 ± 9.8 ng/mL in healthy and osteoporotic postmenopausal women, respectively. The distribution of serum OC according to age is shown in Table 1. The highest mean of serum osteocalcin level was found among the age group 65–70 years (27.70 ± 13.3). The Anthropometric Characteristics of the Study Subjects are given in Table 2.

Association of VDR genotypes with serum osteocalcin levels

Analysis of the relationship between RFLPs and serum osteocalcin is shown in Table 3. The serum osteocalcin levels did not show any significant differences in their concentrations in subjects with different genotypes for *Bsm* I

Table 2 Anthropometric characteristics of the study subjects

Characteristics	Osteoporotic n = 100	Osteopenic n = 100	Control n = 100	*p* value
Age (in years)	59.3 ± 9.26	55.6 ± 8.17	55.4 ± 8.85	0.001*
Age of Menarche	12.1 ± 5.5	12.6 ± 7.8	12.5 ± 6.4	0.680
Age of menopause	56.7 ± 8.1	57.2 ± 7.9	56.8 ± 7.5	0.801
BMD (g/cm^2)	− 2.79 ± 1.69	− 1.45 ± 0.84	0.06 ± 0.91	< .00001*
Serum osteocalcin (ng/ml)	31.6 ± 9.8	25.9 ± 7.5	15.1 ± 7.8	< .00001*
BMI (Kg)/(m^2)	25.5 ± 6.06	26.01 ± 6.60	25.1 ± 5.31	0.602
Obesity assessment[#]				
Under weight	16.8 ± 1.2 (3%)	17.4 ± 0.7 (3%)	16.7 ± 1.4 (2%)	0.501
Normal	21.7 ± 1.7 (15%)	21.4 ± 1.8 (13%)	21.9 ± 1.7 (16%)	0.851
Overweight	26.9 ± 1.4 (10%)	27.3 ± 1.4 (11%)	27.1 ± 1.4 (7%)	0.621
Obese	35.2 ± 5.0 (6%)	35.0 ± 7.1 (7%)	33.1 ± 3.2 (7%)	0.399

All values are expressed as Mean ± SE, ()—Values in are number of subjects in (%)

BMD bone mineral density, *BMI* body mass index

*$p < 0.05$ statistically significant for ANOVA

[#]WHO (1997) Standard Definition for Obesity, Obesity Prevention and the Global Epidemic-Report of a WHO Consultation on Obesity

Table 3 The relationship between the RFLPs and serum osteocalcin levels for *Apa*I, *Taq*I, *Bsm*I, *Pvu*II, *Xba*I and *Bsbt*I among the study groups using one way (ANOVA)

Variables	*Apa*I polymorphism								
	Controls			Osteopenic			Osteoporotic		
	TT	TG	GG	TT	TG	GG	TT	TG	GG
Serum osteocalcin	14.3 ± 7	14 ± 6.4	19.7 ± 11	27.9 ± 7.9	24.5 ± 6	22.3 ± 8.2	31.5 ± 10.5	31.6 ± 8.7	31.6 ± 11
p	0.02*			0.02*			0.9		
Variables	*Taq*I polymorphism								
	TT	TC	CC	TT	TC	CC	TT	TC	CC
Serum osteocalcin	17.8 ± 9.3	14.7 ± 7.2	12.6 ± 5.7	24.3 ± 7	26.8 ± 8	26 ± 7	31.6 ± 9.7	31.5 ± 9.1	32.2 ± 11
p	0.03*			0.4			0.8		
Variables	*Bsm*I polymorphism								
	AA	AG	GG	AA	AG	GG	AA	AG	GG
Serum osteocalcin	14.4 ± 7.1	15.6 ± 8.5	14.6 ± 6.5	27.9 ± 4.2	24.6 ± 8.1	28.2 ± 7.9	34.3 ± 11.1	29.9 ± 8.8	31.7 ± 10.3
p	0.7			0.08			0.1		
Variables	*Pvu*II POLYMORPHISM								
	TT	TC	CC	TT	TC	CC	TT	TC	CC
Serum osteocalcin	13.5 ± 7.1	16.5 ± 8.7	14.1 ± 6.5	26.5 ± 6.2	26.3 ± 8.0	24.2 ± 8.3	30.5 ± 7.6	30.8 ± 10.6	34.1 ± 10.4
P	0.3			0.5			0.3		
Variables	*Xba*I polymorphism								
	AA	AG	GG	AA	AG	GG	AA	AG	GG
Serum osteocalcin	15.5 ± 8.1	14.4 ± 7.0	15.8 ± 8.7	24.8 ± 5.6	26.3 ± 9.0	26.7 ± 6.9	32.6 ± 8.7	30.1 ± 11.8	25.7 ± 6.4
p	0.7			0.5			0.07		
Variables	*Bsbt*I polymorphism								
	GG	GA	AA	GG	GA	AA	GG	GA	AA
Serum osteocalcin	17.9 ± 9.4	12.3 ± 5.6	16.1 ± 7.5	25 ± 5.4	26.1 ± 8.4	26.8 ± 8.5	31.6 ± 11	30 ± 8.7	33.2 ± 9.2
p	0.007*			0.6			0.4		

COL1A1-*Sp*I polymorphism data is not shown since the TT genotype was not observed in the study population

*p value < 0.05 statistically significant-(One way ANOVA)

polymorphisms. However, osteocalcin levels for the *Apa* I polymorphisms showed varied results, in which, subjects in the control group with "GG" genotype and the osteopenic group with "TT" genotype of the *Apa*I polymorphism had a significantly higher serum osteocalcin concentration ($p < 0.05$). Similarly, for *Taq*I polymorphism, controls with wild type genotype "TT" shows significantly elevated levels ($p < 0.05$) than the osteopenic and the osteoporotic groups with no significant variations. It was observed that, among all three groups the subjects who were osteoporotic exhibited elevated concentrations of serum osteocalcin. There was no significant difference in the serum OC levels among the different genotypes. The combined genotypes for VDR- *Apa*I-*Taq*-*Bsm*I were analyzed for the association with serum osteocalcin levels. Among the combined genotypes only 26 groups were used for the analysis and the following six combined genotype groups GGCCAA, GGTCAA, GGTCGG, GGTTAG, GGT TGG, TGTTGG was not included in the calculations since the number was less than 5 in each group. The genotype "GGTTAA" was not observed in this study population. There were no significant association found between bone mineral density and serum osteocalcin with the combined genotypes. The combination genotype TTTCAA had the highest mean osteocalcin levels (31.0 ± 13.7) as seen in Table 4. In combination these genotypic differences are likely to affect VDR protein levels of function depending on the cell type, developmental stage and activation status.

Table 4 Serum osteocalcin levels (ng/ml) in relation to *Apa*I, TaqI and *Bsm*I genotype combinations

S. no	Combined genotype	n (274)	Osteocalcin ng/ml	Bone mineral density g/cm^2
1	GGCCAG	5	22 ± 8.0	− 0.78 ± 1.9
2	GGCCGG (Variant)	5	15.15 ± 4.8	− 1.77 ± 1.3
3	GGTCAG	17	26.56 ± 13.1	− 1.92 ± 1.1
4	TGCCAA	**7**	24.4 ± 13.8	0.11 ± 2.0
5	TGCCAG	22	24.3 ± 10.7	− 1.76 ± 1.3
6	TGCCGG	6	26.6 ± 14.5	− 1.4 ± 2.1
7	TGTCAA	13	26 ± 7.6	− 1.28 ± 1.5
8	TGTCAG	30	20.8 ± 7.3	− 1.02 ± 1.4
9	TGTCGG	12	19.4 ± 10.5	− 1.33 ± 1.5
10	TGTTAA	10	21.9 ± 10.1	− 1.33 ± 2.3
11	TGTTAG	20	26.2 ± 10.8	− 1.81 ± 1.7
12	TTCCAA	12	29.7 ± 16	− 1.88 ± 1.6
13	TTCCAG	23	21.3 ± 9.6	− 1.41 ± 2.1
14	TTCCGG	6	25.1 ± 13.0	− 1.66 ± 1.2
15	TTTCAA	**10**	31.0 ± 13.7	− 2.04 ± 1.0
16	TTTCAG	37	24.2 ± 11.2	− 1.57 ± 1.5
17	TTTCGG	**10**	28.6 ± 8.2	**− 2.15 ± 1.4**
18	TTTTAA (Reference)	10	22.5 ± 5.8	− 1.07 ± 0.83
19	TTTTAG	20	22.6 ± 9.3	− 0.96 ± 2.1
20	TTTTGG	6	26.7 ± 13.4	− 1.0 ± 1.7
	F ratio	–	1.235	1.044
	p value (ANOVA)	–	0.2	0.4

GGCCAA, GGTCAA, GGTCGG, GGTTAG, GGTTGG, TGTTGG (6) genotypes were not included in the calculations since the number was less than 5 in each group. The genotype "GGTTAA" was not observed in this study population.

*p value ≤ 0.05 statistically significant

Association of ERα 1 genotypes with serum osteocalcin levels

The mean levels of osteocalcin in control, osteopenic and osteoporotic postmenopausal women are classified according to the genotypes in Table 3. There was no significant difference in the osteocalcin levels across the *PvuII* (controls TT = 13.5 ± 7.1, TC = 16.5 ± 8.7, CC = 26.5 ± 6.2; $p = 0.3$, osteopenic TT = 26.5 ± 6.2, TC = 26.3 ± 8.0, CC = 24.2 ± 8.3; $p = 0.5$, osteoporotic TT = 30.5 ± 7.6, TC = 30.8 ± 10.6, CC = 34.1 ± 10.4; $p = 0.3$) and *Xba*I polymorphisms (controls AA = 15.5 ± 8.1, AG = 14.4 ± 7.0, GG = 15.8 ± 8.7; $p = 0.7$, osteopenic AA = 24.8 ± 5.6, AG = 26.3 ± 9.0, GG = 26.7 ± 6.9; $p = 0.5$, osteoporotic AA = 32.6 ± 8.7, AG = 30.1 ± 11.8, GG = 25.7 ± 6.4; $p = 0.07$). The genotype combination did not have any significant association with serum osetocalin levels. The combined genotype TTAG showed (Table 5) the lowest level of OC (20.6 ± 8.7 ng/mL) whereas genotype CCAA revealed women with a higher serum osteocalcin level (28 ± 12.1 ng/mL).

Table 5 Serum osteocalcin levels (ng/ml) in relation to *Pvu*II and *Xba*I genotype combinations

Genotype	N (300)	Osteocalcin ng/ml
TTAA (reference)	44	25.5 ± 10
TTAG	39	20.6 ± 8.7
TTGG	14	24 ± 10
TCAA	46	24.9 ± 10.1
TCAG	61	24.9 ± 12.6
TCGG	38	21.7 ± 8.9
CCAA	**26**	28 ± 12.1
CCAG	19	23.2 ± 11.2
CCGG(Variant)	13	26.3 ± 11.7
F ratio	–	1.41
p value (ANOVA)	–	0.19

*p value ≤ 0.05 statistically significant

Association between PTH- *BstBI* genotypes with serum osteocalcin levels

The levels of serum osteocalcin a turnover marker examined, the levels of serum osteocalcin in controls were statistically higher in the PTH–GG group than in the PTH–GA and AA PTH; (GG = 17.9 ± 9.4, GA = 12.3 ± 5.6, AA = 16.1 ± 7.5; $p = 0.007$*). The mean levels of osteocalcin among the genotype groups in osteopenic and osteoporotic postmenopausal women did not have significant difference: (osteopenic GG = 25 ± 5.4, GA = 26.1 ± 8.4, AA = 26.8 ± 8.5; $p = 0.6$, osteoporotic GG = 31.6 ± 11.5, GA = 30 ± 8.7, AA = 33.2 ± 9.2; $p = 0.4$) as given in Table 3. Although there was no significant difference in the serum levels of osteocalcin among the three genotype groups in the osteopenic and osteoporotic women, the PTH-AA group in controls had a significantly higher level of serum osteocalcin, this suggests a higher state of bone turnover in the AA genotype [16].

Association between collagen type I alpha 1-Sp1 polymorphism genotypes with serum osteocalcin levels

The mean levels of serum osteocalcin did not have significant differences among the genotype for the Collagen Type I gene receptor as given in Table 3 for the study groups; (controls GG = 15 ± 8, GT = 14 ± 7; p = 0.3, osteopenic GG = 26 ± 7, GT = 25 ± 7; $p = 0.6$, osteoporotic GG = 31 ± 9, GT = 32 ± 10; $p = 0.5$).

Gene-to-gene interactions of genotypes with respect to osteocalcin by multiple linear regression

The results of multiple linear regression analysis in postmenopausal women with respect to the gene interaction model among the *VDR*, *ER α1, PTH* and *COLIA1* genotypes for serum osteocalcin observed a significant interaction between *Taq*I × *Bsm*I (Adjusted $R^2 = 0.831$, $p = 0.04$*). No other interaction models were found to be significant as seen in Table 6.

Discussion

The subjects for this study comprised of South Indian postmenopausal women, to evaluate the polymorphisms of the VDR, ER α1, PTH and COLIA1 genes in association with serum osteocalcin levels. Osteocalcin comprises 25% of the non-collagenous matrix proteins and is manufactured by the bone cells [1, 19]. To determine whether allelic variation in the VDR receptor gene is the cause of the variation in serum osteocalcin levels. Three polymorphisms namely *Apa*I, *Taq*I and *Bsm*I was investigated among the 300 postmenopausal women and the results revealed an increased level of serum osteocalcin in healthy postmenopausal women for *Apa*I (GG) and *Taq*I (TT) polymorphisms. Meanwhile, the ApaI (TT) genotype shows a fundamentally raised degree of serum osteocalcin in osteopenic women. However, the mean serum osteocalcin level was significantly higher in the group of postmenopausal osteoporotic women than in control women. These differences may indicate a higher rate of bone turnover in healthy postmenopausal women [12]. Such differences in serum osteocalcin concentrations in healthy and osteoporotic women were not found to be consistent (Table 3). This may be due to the small sample size or the absence of age matching between the allelic subgroups. In a recent report the TT of *Taq*I genotype was associated with low levels of OC in overweight and obesity type 2 diabetes mellitus subjects [25]. In contrast to the present study, the findings of the Sheehan et al. [28]suggests that in healthy Irish adults with the tt VDR genotype have higher rates of bone turnover than those with Tt or TT VDR genotypes and, therefore, may have a higher risk of low osteocalcin.

Table 6 Gene-to-gene interactions of genotypes with respect to osteocalcin by multiple linear regression

Variables	t	*p*	AdjR^2	95% confidence interval
Trait (Osteocalcin)				
Intercept	5.75	< 0.0001****	0.090	16.69–34.05
*Apa*I	1.0	0.31	0.912	− 2.909 to 8.916
*Taq*I	0.53	0.59	0.886	− 3.614 to 6.304
*Bsm*I	0.50	0.61	0.886	− 4.273 to 7.242
*Pvu*II	0.34	0.73	0.903	− 6.625 to 4.650
*Xba*I	0.39	0.69	0.900	− 6.186 to 4.124
*BstB*I	0.95	0.34	0.893	− 7.105 to 2.466
Sp1	0.26	0.79	0.929	− 13.93 to 10.65
Best two-way gene–gene interaction models identified by multiple linear regression				
*Taq*I * *Bsm*I	1.98	0.04*	0.831	− 5.580 to − 0.0276

Adj.R2 is the proportion of variation of the dependent variable explained by the regression model. Other numbers in the table are the partial regression coefficients for the parameters of each factor

*$p < 0.05$

****$p < .0001$

The association of ER α genotypes and serum osteocalcin levels was also assessed in this study. Osteocalcin being a distinct bone turnover marker assuming a significant part in bone redesigning raises the likelihood that the ESR genotypes characterized by XbaI and PvuII might be of importance in deciding the variability of bone rebuilding and the level of reaction to retreatment by estrogen. Our results did not show any significant association between genotypes and serum osteocalcin. However Rapuri et al. [23] reported that the change in bone remodelling markers was significantly higher in women with CC or GG genotypes. Sowers et al. [24] reports that women homozygous for the ER α gene variant without an *Xba*I restriction site (AA) and *Pvu*II restriction site (TT) had significantly lower osteocalcin levels. Estrogen receptor 1 plays a significant part in the support of the skeletal framework which has been demonstrated in trial mice, from which the gene was erased from the particular bone cells and their precursors. Absence of the estrogen receptor in osteoblast precursor cells affected the periosteum, while their lack in differentiated osteoblasts, osteoclasts, and osteocytes occur in reduced cancellous bone mass [3]. In the assessments on ERα gene XbaI polymorphism and COL1A1gene Sp1 polymorphism, it was accounted for that there was no differentiation as far as normal OC levels, genotype, and allele frequencies among the groups. A review in elderly women by Koren et al. [27] reports that the Px haplotype of the ERα gene receptor and the "s" allele of the COLIA1 sp1 polymorphism might be engaged with causing the phenotypic articulation of higher circulating levels of PTH and higher bone turnover, which, thus, may prompt bone loss.

The degree of osteocalcin as turnover marker was also analyzed in PTH- *BstB*I polymorphism among the controls, osteopenic and osteoporotic postmenopausal women. It was noticed that the osteocalcin levels within the controls was statistically higher in the PTH–AA group than in the PTH– GA and GG groups (Table 3). However, the degree of osteocalcin was not significantly different between the genotypes for the osteopenic and osteoporotic women. Similar results were observed with no association was observed in

Arab postmenopausal women with serum levels of osteocalcin and PTHR1 gene polymorphisms [27]. Although there was no significant difference in the serum levels of osteocalcin among the three genotype groups in the osteopenic and osteoporotic women, the PTH-AA group in controls had a significantly higher level of serum osteocalcin, this suggests a higher state of bone turnover in the AA genotype among the control group.

This study also demonstrated a significant association of SNP combination *Taq*I (rs731236) T > C and *Bsm*I (rs1544410) A > G in the VDR gene with serum osteocalcin (Table 6). Marcia et al. [36] reported on the polymorphisms in VDR, COLIA1, osteocalcin and osteonectin genes which were related with change in BMD with time and the serum markers of osteocalcin. In any case, they revealed a significant gene- gene collaboration impact with the ER genotypes and the VDR *Bsm*I genotype. The biological and functional proof would be needed to confirm the intriguing impact of polymorphisms on the circulating levels of osteocalcin. Additionally, to the best of our knowledge we were the first to demonstrate the influence of gene–gene interactions in *VDR*, *ER α1, PTH* and *COLIA1* genes on the circulating levels of serum osteocalcin in postmenopausal women. To further investigate the genetic interactions between these genes, the number of the studied SNPs should be increased and confirmed in the larger cohorts.

Serum OC levels are viewed as heritable in different populaces like the Mexican American populace [22], Australian [16] and United Kingdom [8]. How the hereditary determinants of serum OC may be or in which pathways they could act isn't clear. For example, at younger ages serum OC could transcendently reflect skeletal improvement and serum OC levels may be emphatically impacted by genes related with bone formation. Nonetheless, as ages increases, serum OC may transcendently reflect bone turnover rates and OC levels might be strongly impacted by genes influencing osteoblast or osteoclast function. There are no evident candidate genes that could impact bone turnover. A few affiliation investigations of serum OC levels and gene variations have been performed to identify candidate genes, including type I collagen alpha 1 [7], the estrogen receptor a gene [10, 16] and the Vitamin D receptor gene and reported to have no association accounted for in this population. This study reports no association among age and BMI to any of these gene variations in our study. It is conceivable that the overall impacts of various genes on phenotypic variation differ among populaces, and results acquired from the south Indian population may not be generalized to other population. Many cross-sectional information in a large group of postmenopausal women showed that serum osteocalcin increases at the menopause and consequently declines with increasing age [4, 35, 37]. The high levels of osteocalcin in the postmenopausal women are credited to the lack of estrogen, an observation which associates to similar reports [10], which demonstrate a strong correlation between the estrogen inadequacy and the raised osteocalcin levels. The low estrogen levels at the menopause leads to a lower absorption of calcium, bringing about low serum calcium concentrations and a higher osteoclastic resorption of the bone, thus increasing bone turnover, which in turn contributes as risk factors of osteoporosis. Serum osteocalcin is a accurate marker of the bone turnover, especially when the resorption and bone formation are coupled. It is also considered as a specific marker of bone development when the formation and resorption are uncoupled [34].

In conclusion it is noticed that genetic factors are most likely not a significant determinant in explaining the variation of the circulating serum osteocalcin levels in postmenopausal women. The outcome of this study proposes the probability of a small impact of the VDR-*Apa*I (GG) genotype, the VDR-*Taq*I (TT) genotype and the (AA) genotype of the PTH-*BstB*I polymorphism indicating a higher rate of bone turnover in the healthy postmenopausal women. This impact, if present, would be an important clinical risk factor for osteoporosis after menopause. In contrast, our previous study in the same population supports the significant role of VDR-*Bsm*I (rs1544410) and PTH-*BstB*I (rs6254) polymorphisms and its significant allelic association with bone mineral density either individually or in different combinations pertaining to osteoporosis susceptibility among postmenopausal women. The genetic effects on premenopausal bone turnover, peak bone mass and environmental mechanisms will probably play a significant part in mediating postmenopausal bone turnover. The high rate of bone turnover after menopause is probably responsible for the increased rate of bone loss, our results indicate that genetic factors regulate bone development within a group of normal subjects and demonstrate strongly that more focus ought to be coordinated towards the regulation of bone turnover and in understanding the molecular mechanisms underlying the genetic impact on postmenopausal osteoporosis.

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Author contributions CF devised and performed the experiments and data analysis; JT designed and PAS supervised and managed all studies. All the authors contributed towards writing the manuscript.

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Availability of data and materials The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Conflict of interest Authors declare that they have no conflict of interest.

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