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The association between bone remodeling markers, genes polymorphisms with incidence of osteopenia among young Saudi female

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ABSTRACT

Osteopenia and osteoporosis, are prevalent skeletal systemic conditions, cause weaker bones and an increased risk of fragility fractures. This work is aimed to evaluate the relation between bone-remolding markers and genotypes of four single nucleotide polymorphisms in young Saudi females (rs2297480 of farnesyl diphosphate synthase (FDPS), rs3736228 of Low-density lipoprotein receptor-related protein 5 (LRP5), rs1234612 of sclerostin (SOST), and rs9934438 of Vitamin K epoxide reductase complex subunit 1 (VKORC1)). For this purpose, 750 premenopausal females aged 18 to 40 years old, either university students, postgraduates, or university employees were recruited and divided into three groups according to bone mineral density BMD (g/cm^2) divided by T score into osteoporosis (n = 12), osteopenia (n = 147), and normal (n = 591). Serum SOST, BALP, calcium, phosphate, ALP, albumin, beta-CTXs and human VDR levels were determined. TaqMan SNP Genotyping assays were used to genotype four polymorphisms using real-time PCR (applied biosystem). Results showed that BALP, CTX-1 and SOST were significantly higher in the osteoporosis and osteopenia groups than in the normal group. Bone mineral density readings were considerably lower in females with the GG genotype in FDPS rs2297480 and TT genotype in LRP5 rs3736228, which increase the risk for osteopenia by 3. 6-fold and 3. 06-fold than control respectively. Also, females with the TT genotype in LRP5 rs3736228 have decreased average values for Bone Mineral Density. In conclusion, the GG genotype of FDPS rs2297480 and the TT genotype of LRP5 rs3736228 was shown to be strongly associated with osteopenia in young Saudi females with low bone mineral density and SOST levels.

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Introduction

A prevalent systemic skeletal condition called osteoporosis (OP) causes weaker bones and a higher risk of fragility fractures (1). While secondary osteoporosis is brought on by specific clinical diseases that are potentially reversible, primary osteoporosis refers to bone loss brought on by the natural ageing process. Early fracture risk reduction and treatment of any underlying causes may prevent needless antiresorptive drug use (2).

In terms of genetics, the etiology of osteoporosis (OP) and fracture risk susceptibility has several factors, that include environmental effects in addition to genetic factors across several biologic processes. It is assumed that genetic factors account for 60% to 80% of accelerated bone loss (3, 4).

Genome-wide association studies (GWASs) have examined more than 66 loci representing bone mineral density (BMD), demonstrating the extremely polygenic character of BMD variance [3]. Even though there has been substantial progress in recent years in discovering candidate genes linked to BMD, fracture, and other relevant traits, the majority of genetic variations in different ethnic groups have yet to be discovered or confirmed.

Numerous single nucleotide polymorphisms (SNPs) in a number of genes have been linked to BMD thus far, although the evidence is ambiguous and contradictory. Each bone phenotype (density, quality, metabolic rate) results from the interplay of numerous genes, and despite the application of cutting-edge techniques, the "essential" gene, the one responsible for OP, has not yet been located (5, 6).

The Wnt signaling system, which is essential for bone growth during embryogenesis and has a double role in controlling bone mass by modulating both bone formation and resorption, is one of the most significant signaling routes in bones. The proteins involved in the proliferation, differentiation, and death of bone cells make up the Wnt pathway components (4). Low-density lipoprotein recep-

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tor-related protein 5/6 (LRP5/6), the construction of the multiprotein complex, is altered when cells are triggered through membrane receptors. This suppresses b-catenin, causing it to go to the nucleus where it starts the transcription of target genes (7). The membrane receptor of the Wnt signaling pathway, LRP5, has been linked to OP in the past by a genome-wide association analysis (8). Low bone mass and fractures are induced by LRP5 inactivation which is caused by the mutation of the osteoporosis pseudoglioma syndrome (9). Additionally, a number of naturally occurring Wnt signaling inhibitors, including the Dickkopf (DKK) and Sclerostin (SOST) proteins, silence LRP5/6 receptor signaling. The SOST gene produces the protein sclerostin, which binds to the LRP5/6 co-receptor to inhibit Wnt signaling in both osteoblasts and osteocytes (10, 11). SOST inactivating mutations are hypothesized to be the source of high bone mass disorders in sclerosteosis and van Buchem's disease (3).

Other significant genes that should be taken into consideration as target genes, such as farnesyl diphosphate synthase (FDPS) or geranylgeranyl pyrophosphate synthase (GGPS1), which keep the resorption activity of the osteoclasts, were revealed as a result of the identification of the mevalonate pathway as the target of the antiresorptive agents from the amino-bisphosphonates (N-BP) class (12). In addition to its hemostatic effect and implications in warfarin sensitivity, vitamin K also plays a significant role in maintaining bone strength. Mutations in the vitamin K epoxy reductase (VKORC1) gene may modify the gamma-carboxylation of osteocalcin and may influence BMD, making them worthy of study (13).

It is widely acknowledged that OP is a multifactorial complex disorder whose pathogenesis is caused by the collaboration of different genetic determinants controlling bone and mineral metabolism with "non-skeletal" risk factors (such as muscle strength, visual acuity, and balance), lifestyle choices, and environmental factors (14). But as of right now, no gene has been categorically identified as a key gene for OP.

The purpose of this study was to examine the association between bone remodeling indicators and the genotypes of four SNPs in young premenopausal Saudi females (FDPS rs2297480, LRP5 rs3736228, SOST rs1234612, and VKORC1 rs9934438).

Materials and Methods

This research was cross-sectional and observational that included 750 premenopausal females aged from 18 to 40 years old, either university students, postgraduates, or university employees (faculty or administrative staff).

The study was carried out in conformity with the Helsinki Declaration and the guidelines established by the College of Medicine, Taibah University's ethics committee (TU-20-016) on February 18, 2021 (Resolution No. TU-20-016). Before being included, each participant completed a consent form indicating their understanding of the nature and methodology of the study and their agreement to participate in genetic testing and the collection of clinical data. Those having a history of malignancy, osteometastasis, or metabolic bone illnesses (such as hyperparathyroidism, osteomalacia, or Paget disease), as well as those using medications that affect bone metabolism (such as anti-osteoporosis or vitamin K antagonists), were excluded from the study.

Demographic and clinical data were gathered by interviewing the participants: age, nationality, education level, job, name of college, body mass index (BMI), marital status, smoking history and age of menarche. Each participant was asked about their personal history of vitamin D deficiency and intake, osteoporosis, and bone fracture and their family history of bone fracture and osteoporosis. *Z* scores and *T* scores were determined for all participants. The T score evaluates the patient's bone density in relation to that of young, healthy people of the same sex. Osteoporosis is defined as a negative T score of 2. 5 or less at the femoral neck.

The Z score compares the patient's bone density to that of individuals who are the same patient's age and sex. A secondary cause of osteoporosis should be suspected if the negative Z score is 2. 5 (3). Participants in the study were separated into three groups: according to *T* score: osteoporosis (n = 12), osteopenia (n = 147), and normal (n = 591). Additionally, 5 ml of peripheral blood, 2 ml on an EDTA tube for genetic testing and 3 ml on a plain tube for biochemical analysis, was drawn from each study participant.

Biochemical determinations

Serum levels of albumin, calcium, phosphate, and alkaline phosphatase (ALP, a bone-formation marker) were detected by the automatic analyzer in local centers. Serum sclerostin (SOST) and bone alkaline phosphatase (BALP) levels were quantified using commercially available ELISA kits (Quantikine, R&D Systems, northeast Minneapolis, USA) according to the manufacturer's manuals enclosed in the assay kits. Serum levels of β carboxy telopeptide of type I collagen (β-CTX), a bone-resorption marker and vitamin D receptors were measured by a fully automatic electrochemiluminescence system (E170, Roche Diagnostics, Switzerland). The electrochemiluminescence immunoassay (ECLIA) using the Cobas E601 immunoassay analyzer was used to measure the serum levels of (PTH).

SNP genotyping

Using commercially available kits, genomic DNA was extracted from peripheral blood that had been withdrawn with EDTA (Quick gDNA MiniPrep Kit, Zymo Research, USA; PureLink Genomic DNA Mini Kit, Invitrogen, Thermo Fisher, USA). Using the real-time PCR method, we genotyped four SNPs (FDPS rs2297480, LRP5 rs3736228, SOST rs1234612, and VKORC1 rs9934438) in each participant using the real-time PCR technique. The manufacturer's instructions were followed during every step of the genotyping process. About 25 ng of genomic DNA, 10 ul of TaqMan Genotyping Master Mix (Applied Biosystems, Thermo Fisher, USA), 1. 25 ul of SNP assay kit (bought from Thermo Fisher, USA) comprising a particular primer and probe for each SNP, and nuclease-free water made up the reaction mixture with a total reaction volume 20 ul. The identical amplification regimen, which included a pre-stage of 30 seconds at 60 °C, a hold stage of 10 minutes at 95 °C, a PCR stage of 40 cycles, each consisting of 15 seconds at 95 °C and 1 minute at 60 °C, and a post-stage of 30 seconds at 60 °C, was used for all the genotyping. A real-time PCR system model 7500 was used for all of the investigations (Applied Biosystems, Thermo Fisher, USA).

Statistical analysis

With the aid of the IBM SPSS software package version 20. 0, data were fed into the computer and evaluated. (IBM Corp, Armonk, NY). Categorical data were shown as percentages and numbers. To compare the three groups, a chi-square test was used. Alternatively, a Monte Carlo adjustment test was used if less than five cases were expected in more than 20% of the cells. The Kolmogorov-Smirnov and Shapiro-Wilk tests for continuous data were used to determine whether the data were normal. The range (minimum and maximum), mean, standard deviation, and median were used to express quantitative data. The Kruskal-Wallis test and Dunn's Test were employed post hoc to compare groups for quantitative variables that were abnormally distributed. ANOVA was used to compare the three study groups. Two groups were compared using the Mann-Whitney test for quantitative variables with abnormally dispersed distributions. The 5% level was used to determine the significance of the obtained data.

Results

Table 1, shows that age in osteopenia was significantly higher than normal (p<0. 05) while it was insignificantly different between osteopenia and osteoporosis (p>0. 05). Education level, job, name of college, weight, height, BMI, history of smoking, and age of menarche were significantly different among the studied groups (p>0. 05). It also shows that a history of vitamin D deficiency, history of vitamin D intake, current on vitamin D therapy, history

Table 1. Comparison of the three studied groups according to different parameters.

	Osteoporosis (n = 12)	Osteopenia (n = 147)	Normal(n = 591)	Test of Sig.	p
Age (years)				H = 13. 290*	0. 001*
Median (min. – max.)	22 (20 – 37)	26 (18 – 35)	23 (18 – 40)		
Sig. betw. Grps	p_1 = 0. 066, p_2 = 0. 433, p_3 < 0. 001*				
Education Level Secondary	1 (8. 3%)	13 (8. 8%)	66 (11. 2%)		
Undergraduate	7 (58. 3%)	67 (45. 6%)	282 (47. 7%)	χ^2 = 1. 695	MCp = 0. 932
College graduate	4 (33. 3%)	62 (42. 2%)	225 (38. 1%)		
Postgraduate	0 (0%)	5 (3. 4%)	18 (3. 0%)		
Weight				H = 0. 907	0. 635
Median (min. – max.)	63 (39. 5 – 99)	59 (36 – 105)	60 (32 – 112)		
Height				H = 1. 575	0. 455
Median (min. – max.)	159. 5 (150 – 171)	157 (140 – 185)	159 (53 – 177)		
BMI (Kg/m²)				H = 0. 907	0. 635
Median (min. – max.)	23. 0 (16. 5 – 35. 9)	23. 5 (14. 7– 27. 6)	23. 67 (13. 6 –24. 3)		
Marital status Single	9 (75. 0%)	88 (59. 9%)	404 (68. 4%)		
Married	3 (25. 0%)	54 (36. 7%)	173 (29. 3%)	χ^2 = 7. 353	MCp = 0. 399
Divorced	0 (0%)	5 (3. 4%)	13 (2. 2%)		
Widow	0 (0%)	0 (0%)	1 (0. 2%)		
Smoker	4 (33. 3%)	56 (38. 1%)	190 (32. 1%)	χ^2 = 1. 873	0. 392
Age of menarche (years)				F = 2. 543	0. 079
Mean ± SD.	13. 25 ± 1. 42	12. 76 ± 1. 78	13. 10 ± 1. 68		
History of vitamin D deficiency	5 (41. 7%)	77 (52. 4%)	332 (56. 2%)	χ2 = 1. 589	0. 452
History of vitamin D intake	7 (58. 3%)	63 (42. 9%)	281 (47. 5%)	χ2 = 1. 691	0. 429
Currently on vitamin D therapy	3 (25. 0%)	14 (9. 6%)	73 (12. 4%)	χ2 = 2. 795	0. 247
History of bone fracture No	4 (33. 3%)	95 (64. 6%)	361 (61. 1%)		
Yes	3 (25. 0%)	30 (20. 4%)	108 (18. 3%)	χ^2 = 7. 309	MCp = 0. 108
Unknown	5 (41. 7%)	22 (15. 0%)	122 (20. 6%)		
History of osteoporosis No	4 (33. 3%)	97 (66. 0%)	357 (60. 4%)		
Yes	6 (50. 0%)	17 (11. 6%)	70 (11. 8%)	χ^2 = 12. 480*	MCp = 0. 011*
Unknown	2 (16. 7%)	33 (22. 4%)	164 (27. 7%)		
BMD (g/cm²				H = 368. 405*	<0. 001*
Median (min. – max.)	2. 20 (−4. 6 –2. 50)	1. 30 (−2. 80 –1)	0. 30 (−3. 4 – 3. 1)		
Comparison of groups	p_1 = 0. 209, p_2 < 0. 001*, p_3 < 0. 001*				

SD: Standard deviation; F: ANOVA test; χ^2: Chi-square test; MC: Monte Carlo. H: Kruskal–Wallis test; pairwise comparisons between each pair of groups was done using Dunn's Test for multiple comparisons post hoc. p: p-value for comparison of the studied groups. p_1: p-value for comparison of osteoporosis and osteopenia. p_2: p-value for comparison of osteoporosis and normal. p_3: p-value for comparison of osteopenia and normal. *: Statistically significant at $p \leq 0.05$

of osteoporosis and a family history of bone fracture was insignificantly different among the studied groups (p>0. 05) while a family history osteoporosis was significantly different among the groups (p<0. 05). T Score and Z Score were significantly lower in osteoporosis and osteopenia than normal (p<0. 05) and insignificantly different between osteoporosis and osteopenia (p>0. 05).

Table 2, shows that laboratory investigations, PTH (pg/ml), Calcium (mg/dl), Albumin (gm/dl), Phosphorus (mg/dl) and Alkaline phosphatase (IU/L) were insignificantly different among the studied groups (p>0. 05). BALP (ng/ml), CTX-1 (ng/ml) and SOST (pg/ml) were significantly higher in osteoporosis and osteopenia than normal (p<0. 05) and insignificantly different between osteoporosis and osteopenia (p>0. 05).

Table 3, explains that FDPS SNP rs2297480, LRPS SNP rs3736228, VKORC1 SNP rs9934438 and SOST SNP rs1234612 were insignificantly different among the groups (p>0. 05) according to Hardy-Weinberg equilibrium. Also, it shows that LRPS SNP rs3736228 TT genotypes frequency and FDPS SNP rs2297480 GG genotypes frequency were significantly higher in patients with osteoporosis and osteopenia than normal. while VKORC1 SNP rs9934438 and SOST SNP rs1234612 were matched among the three groups (p>0. 05).

Table 4, shows that LRPS SNP rs3736228 TT genotypes frequency and FDPS SNP rs2297480 GG genotypes frequency were significant predictors for osteoporosis and osteopenia.

Table 5 and 6, shows that FDPS SNP rs2297480 GG genotypes were significantly more frequent in older age, with short stature divorced female. Also, it shows that FDPS SNP rs2297480 TG genotypes were significantly more frequent in females with a history of vitamin D deficiency and intake with abnormal bone mineral density. And, it shows that LRPS SNP rs3736228 and FDPS SNP rs2297480 were not associated with different laboratory investigations except SOST (pg/ml) which was significantly associated with FDPS SNP rs2297480.

Discussion

Researchers have been looking at the involvement of genetic variables in the pathogenesis of bone loss for the past 20 years, but they have not yet found any conclusive information concerning the etiology of OP in this area.

Table 2. Comparison of the three studied groups according to different parameters.

	Osteoporosis (*n* = 12)	Osteopenia (*n* = 147)	Normal (*n* = 591)	Test of Sig.	*p*
		PTH (pg/ml)			
Mean ± SD.	20. 09 ± 10	21. 46 ± 13. 55	22. 93 ± 13. 28	H = 3. 184	0. 204
Median (min. – max.)	17. 5 (8. 3 – 41. 5)	19. 7 (3 – 98)	21. 2 (3 – 98)		
		Calcium (mg/dl)			
Mean ± SD.	9. 13 ± 0. 96	9. 27 ± 0. 63	9. 45 ± 3. 54	H = 0. 864	0. 649
Median (min. – max.)	9. 3 (7. 1 – 10. 5)	9. 3 (7 – 10. 5)	9. 4 (7 – 9. 4)		
		Albumin (gm/dl)			
Mean ± SD.	4. 62 ± 0. 26	4. 61 ± 0. 35	4. 65 ± 0. 33	H = 2. 385	0. 304
Median (min. – max.)	4. 6 (4. 10 – 5)	4. 6 (3. 53 – 5. 4)	4. 7 (3. 6 – 5. 4)		
		Phosphorus (mg/dl)			
Mean ± SD.	3. 57 ± 0. 68	3. 49 ± 0. 60	3. 43 ± 0. 66	F = 0. 657	0. 519
Median (min. – max.)	3. 7 (2. 6 – 4. 4)	3. 5 (2. 3 – 5. 3)	3. 5 (2. 4 – 5. 4)		
		Alkaline phosphatase (IU/L)			
Mean ± SD.	59. 42 ± 12. 55	59. 16 ± 14. 79	59. 24 ± 16. 22	H = 0. 150	0. 928
Median (min. – max.)	61 (33 – 81)	60 (49 – 98)	59 (28 – 120)		
		BALP (ng/ml)			
Mean ± SD.	25. 3 ± 11. 6	24. 4 ± 10. 0	17. 2 ± 9. 9	H = 57. 034*	<0. 001*
Median (min. – max.)	28. 1 (6. 6 – 40)	26. 0 (14 – 45)	14. 9 (6 – 41)		
Comparison of groups	$p_1 = 0.916$, $p_2 = 0.016^*$, $p_3 < 0.001^*$				
		CTX-1 (ng/ml)			
Mean ± SD.	15. 5 ± 6. 8	16. 9 ± 7. 0	10. 1 ± 6. 1	H = 132. 497*	<0. 001*
Median (min. – max.)	15. 8 (4 – 28. 9)	16. 1 (4. 1 – 31. 8)	9. 6 (7 – 63. 6)		
Comparison of groups	$p_1 = 0.718$, $p_2 = 0.001^*$, $p_3 < 0.001^*$				
		VDR (ng/ml)			
Mean ± SD.	14. 02 ± 4. 05	13. 01 ± 4. 34	13. 12 ± 5. 19	H = 1. 980	0. 372
Median (min. – max.)	14. 20 (9 – 23. 8)	12. 10 (7 – 34)	11. 8(6 – 38)		
		SOST (pg/ml)			
Mean ± SD.	157. 2 ± 24. 5	162. 6 ± 29. 1	130. 8 ± 12. 3	F = 210. 222*	<0. 001*
Median (min. – max.)	162 (123 – 198)	167 (99– 232)	131(80– 192)		
Comparison of groups	$p_1 = 0.548$, $p_2 < 0.001^*$, $p_3 < 0.001^*$				

SD: Standard deviation; F: ANOVA test; H: Kruskal–Wallis test. *p*: *p*-value for comparing the studied groups. *: Statistically significant at $p \leq 0.05$

Table 3. Comparison of the three studied groups according to genotyping.

	Osteoporosis (n = 12)	Osteopenia (n = 147)	Normal (n = 591)
		rs3736228	
CC	4 (33. 3%)	81 (55. 1%)	376 (63. 6%)
CT	6 (50. 0%)	50 (34. 0%)	191 (32. 3%)
TT	2 (16. 7%)	16 (10. 9%)	24 (4. 1%)
Comparison of groups		$^{MC}p_1$ = 0. 286, $^{MC}p_2$ = 0. 032*, $^{MC}p_3$ = 0. 007*	
		rs9934438	
GG	4 (33. 3%)	31 (21. 1%)	170 (28. 8%)
GA	6 (50. 0%)	78 (53. 1%)	279 (47. 2%)
AA	2 (16. 7%)	38 (25. 9%)	142 (24. 0%)
Comparison of groups		$^{MC}p_1$ = 0. 613, $^{MC}p_2$ = 0. 867, p_3 = 0. 170	
		rs2297480	
TT	5 (41. 7%)	78 (53. 1%)	351 (59. 4%)
TG	4 (33. 3%)	52 (35. 4%)	219 (37. 1%)
GG	3 (25. 0%)	17 (11. 6%)	21 (3. 6%)
Comparison of groups		$^{MC}p_1$ = 0. 408, $^{MC}p_2$ = 0. 014*, p_3 < 0. 001*	
		rs1234612	
TT	4 (33. 3%)	57 (38. 8%)	239 (40. 4%)
TC	5 (41. 7%)	69 (46. 9%)	256 (43. 3%)
CC	3 (25. 0%)	21 (14. 3%)	96 (16. 2%)
Comparison of groups		$^{MC}p_1$ = 0. 557, $^{MC}p_2$ = 0. 685, p_3 = 0. 699	

^{HW}**p**: p-value of chi-square for goodness of fit for Hardy-Weinberg equilibrium. MC: Monte Carlo. p_1: p-value for comparing osteoporosis and osteopenia groups. p_2: p-value for comparing osteoporosis and normal groups. p_3: p-value for comparing osteopenia and normal groups.

Table 4. The odds ratio of osteoporosis and osteopenia vs. normal.

	Osteoporosis vs. normal		Osteopenia vs. normal	
	p_1	**OR_1 (LL – UL 95%C. I)**	**p_2**	**OR_2 (LL – UL 95%C. I)**
rs3736228				
CC®				
CT	0. 097	2. 953 (0. 823 – 10. 589)	0. 311	1. 215 (0. 820 – 1. 800)
TT	0. 021*	7. 833 (1. 366 – 44. 934)	0. 001*	3. 095 (1. 573 – 6. 088)
rs9934438				
GG®				
GA	0. 890	0. 914 (0. 254 – 3. 285)	0. 067	1. 533 (0. 970 – 2. 423)
AA	0. 557	0. 599 (0. 108 – 3. 316)	0. 151	1. 468 (0. 869 – 2. 478)
rs2297480				
TT®				
TG	0. 713	1. 282 (0. 341 – 4. 827)	0. 739	1. 068 (0. 724 – 1. 577)
GG	0. 003*	10. 029 (2. 243 – 44. 835)	<0. 001*	3. 643 (1. 837 – 7. 226)
rs1234612				
TT®				
TC	0. 820	1. 167 (0. 310 – 4. 397)	0. 541	1. 130 (0. 763 – 1. 674)
CC	0. 419	1. 867 (0. 410 – 8. 499)	0. 760	0. 917 (0. 527 – 1. 595)

OR_1: Odds ratio for osteoporosis and normal. OR_2: Odds ratio for osteopenia and normal. CI: Confidence interval; LL: Lower limit; UL: Upper Limit. ®: reference group. *: Statistically significant at $p \leq 0.05$

Table 5. Relation between rs3736228, rs2297480 and demographic data.

	rs3736228			rs2297480		
	CC (*n* = 461)	**CT (*n* = 247)**	**TT (*n* = 42)**	**TT (*n* = 434)**	**TG (*n* = 275)**	**GG (*n* = 41)**
Age						
Median (min. – max.)	23 (18 – 37)	24 (19 – 40)	24 (19 – 35)	24 (18 – 37)	23 (18 – 40)	29 (20 – 40)
H (*p*)		**1. 586 (0. 453)**			**9. 168* (0. 010*)**	
Education Level						
Secondary Education	56 (12. 1%)	20 (8. 1%)	4 (9. 5%)	47 (10. 8%)	28 (10. 2%)	5 (12. 2%)
Undergraduate education	217 (47. 1%)	114 (46. 2%)	25 (59. 5%)	196 (45. 2%)	143 (52. 0%)	17 (41. 5%)
College graduate	173 (37. 5%)	106 (42. 9%)	12 (28. 6%)	177 (40. 8%)	98 (35. 6%)	16 (39. 0%)
Postgraduate Education	15 (3. 3%)	7 (2. 8%)	1 (2. 4%)	14 (3. 2%)	6 (2. 2%)	3 (7. 3%)
χ^2 (*p*)		**6. 483 (0. 371)**			**6. 452 (0. 374)**	
Name of college						
Ibn Sina National College	258 (57. 1%)	120 (48. 8%)	28 (68. 3%)	228 (53. 3%)	158 (58. 5%)	20 (48. 8%)
Tibah	194 (42. 9%)	126 (51. 2%)	13 (31. 7%)	200 (46. 7%)	112 (41. 5%)	21 (51. 2%)
χ^2 (*p*)		**7. 559* (0. 023*)**			**2. 507 (0. 286)**	
Weight						
Median (min. – max.)	60 (32 – 119)	61 (35 – 112)	58. 5 (38 – 105)	60 (32 – 112)	59 (36 – 133)	61. 50 (38 – 96)
H (*p*)		**1. 069 (0. 586)**			**0. 305 (0. 858)**	
Height						
Median (min. – max.)	159 (53 – 195)	159 (53 – 176)	156. 5(145-170)	159 (140 – 195)	159 (53 – 177)	157 (53 – 171)
H (*p*)		**2. 570 (0. 277)**			**7. 011* (0. 030*)**	
BMI (Kg/m^2)						
Median (min. – max.)	23. 51 (13. 67 –24. 3)	23. 8 (13. 67 –24. 3)	24. 45 (17. 35 – 36. 67)	23. 67(13. 67 – 37. 47)	23. 44(14. 27 –24. 3)	25. 27(16. 59 –24. 28)
H (*p*)		**2. 404 (0. 301)**			**0. 854 (0. 652)**	
Marital status						
Single	305 (66. 2%)	167 (67. 6%)	29 (69. 0%)	284 (65. 4%)	197 (71. 6%)	20 (48. 8%)
Married	143 (31. 0%)	74 (30%)	13 (31. 0%)	136 (31. 3%)	75 (27. 3%)	19 (46. 3%)
Divorced	13 (2. 8%)	5 (2. 0%)	0 (0%)	13 (3. 0%)	3 (1. 1%)	2 (4. 9%)
Widowed	0 (0%)	1 (0. 4%)	0 (0%)	1 (0. 2%)	0 (0%)	0 (0%)
χ^2 (^{MC}p)		**3. 892 (0. 792)**			**13. 330* (0. 028*)**	
Smoker	148 (32. 1%)	89 (36. 0%)	13 (31. 0%)	143 (32. 9%)	87 (31. 6%)	20 (48. 8%)
χ^2 (*p*)		**1. 230 (0. 541)**			**4. 788 (0. 091)**	
Age of menarche						
Mean ± SD.	12. 98 ± 1. 75	13. 15 ± 1. 62	12. 93 ± 1. 61	13. 04 ± 1. 76	13. 03 ± 1. 62	13. 02 ± 1. 60
F(*p*)		**0. 843(0. 431)**			**0. 011(0. 989)**	
History of vitamin D deficiency	249 (54. 0%)	144 (58. 3%)	21 (50. 0%)	235 (54. 1%)	158 (57. 5%)	21 (51. 2%)
χ^2 (*p*)		**1. 681 (0. 431)**			**1. 022 (0. 600)**	
History of vitamin D intake	215 (46. 6%)	118 (47. 8%)	18 (42. 9%)	188 (43. 3%)	146 (53. 1%)	17 (41. 5%)
χ^2 (*p*)		**0. 361 (0. 835)**			**6. 954* (0. 031*)**	
Currently on vitamin D therapy	58 (12. 6%)	30 (12. 2%)	2 (4. 8%)	49 (11. 3%)	37 (13. 6%)	4 (9. 8%)
χ^2 (*p*)		**2. 248 (0. 325)**			**1. 023 (0. 600)**	
Family history of bone fracture						
No	293 (63. 6%)	140 (56. 7%)	27 (64. 3%)	262 (60. 4%)	179 (65. 1%)	19 (46. 3%)
Yes	88 (19. 1%)	47 (19. 0%)	6 (14. 3%)	86 (19. 8%)	45 (16. 4%)	10 (24. 4%)
Unknown	80 (17. 4%)	60 (24. 3%)	9 (21. 4%)	86 (19. 8%)	51 (18. 5%)	12 (29. 3%)
χ^2 (*p*)		**5. 737 (0. 220)**			**6. 056 (0. 195)**	
Family history of osteoporosis						
No	290 (62. 9%)	144 (58. 3%)	24 (57. 1%)	260 (59. 9%)	173 (62. 9%)	25 (61. 0%)
Yes	58 (12. 6%)	31 (12. 6%)	4 (9. 5%)	55 (12. 7%)	31 (11. 3%)	7 (17. 1%)
Unknown	113 (24. 5%)	72 (29. 1%)	14 (33. 3%)	119 (27. 4%)	71 (25. 8%)	9 (22. 0%)
χ^2 (*p*)		**3. 047 (0. 550)**			**1. 784 (0. 775)**	

SD: Standard deviation; F: ANOVA test; H: Kruskal–Wallis test. χ^2: chi-square test; MC: Monte Carlo. *p*: *p*-value for comparing rs3736228 and rs2297480with different parameters. *: Statistically significant at $p \leq 0.05$

Table 6. Relation between rs3736228, rs2297480, BMD and laboratory data.

	rs3736228			rs2297480		
	CC (*n* = 461)	**CT (*n* = 247)**	**TT (*n* = 42)**	**TT (*n* = 434)**	**TG (*n* = 275)**	**GG (*n* = 41)**
			BMD (g/cm²)			
Median (min. – max.)	0 (−3. 9 – 2. 7)	−0. 1 (−4. 6 3. 1)	−0. 75 (−3. 6 – 2. 7)	−0. 10 (−3. 9 – 3. 1)	0. 0 (−3. 10 – 2. 70)	−0. 90 (−4. 60 – 2. 0)
H(*p*)		**5. 009 (0. 082)**			**7. 921* (0. 019*)**	
			PTH (pg/ml)			
Median (min. – max.)	37. 6 (6. 3 – 142)	36. 3 (6 – 126. 9)	40. 6 (11. 1 – 116. 2)	38 (6– 142. 3)	35. 1 (6. 7 – 128. 5)	40. 70 (8. 70 – 111)
H (*p*)		**2. 246 (0. 325)**			**3. 806 (0. 149)**	
			Calcium (mg/dl)			
Median (min. – max.)	9. 4 (7 – 10. 7)	9. 4 (7 – 9. 4)	9. 4 (7 – 10. 50)	9. 4 (7 – 9. 4)	9. 40 (7 – 10. 50)	9. 30 (7 – 10. 50)
H (*p*)		**0. 567 (0. 753)**			**4. 146 (0. 126)**	
			Albumin (gm/dl)			
Median (min. – max.)	4. 7 (3. 54 – 5. 4)	4. 7 (3. 45 – 5. 20)	4. 7 (3. 50 – 5. 10)	4. 7 (3. 6– 5. 30)	4. 7 (3. 50 – 5. 40)	4. 6 (2. 32 – 5. 40)
H (*p*)		**1. 377 (0. 502)**			**2. 778 (0. 249)**	
			Phosphorus (mg/dl)			
Mean ± SD.	3. 44 ± 0. 64	3. 48 ± 0. 65	3. 29 ± 0. 70	3. 47 ± 0. 66	3. 42 ± 0. 65	3. 42 ± 0. 57
F(*p*)		**1. 577 (0. 207)**			**0. 433 (0. 648)**	
			Alkaline phosphatase (IU/L)			
Median (min. – max.)	59 (4 – 108)	60 (26 – 120)	59 (6. 20 – 98)	60 (6. 20 – 120)	59 (8. 1 – 108)	57 (14– 97)
H (*p*)		**0. 938 (0. 626)**			**1. 402 (0. 496)**	
			BALP (ng/ml)			
Median (min. – max.)	16 (5. 7 – 45)	15. 55 (6. 5 – 40)	17. 5 (12 – 39)	15. 7 (5. 7 – 45)	16. 50 (7. 5 – 41)	18 (5 – 40)
H (*p*)		**3. 719 (0. 156)**			**1. 764 (0. 414)**	
			CTX-1 (ng/ml)			
Median (min. – max.)	10. 50 (6. 5 – 63. 62)	10. 20 (5. 1 – 47. 2)	11. 77 (7. 9– 29. 50)	10. 29 (1. 5 – 63. 6)	10. 39 (6. 5 – 58. 5)	12. 10 (4. 4 – 26. 4)
H (*p*)		**2. 136 (0. 344)**			**4. 526 (0. 104)**	
			VDR (ng/ml)			
Median (min. – max.)	11. 90 (5. 43 – 38)	12 (2. 68 – 34)	11. 35 (3. 75 – 35)	12 (2. 68 – 38)	12 (5. 70 – 38)	12. 20 (5. 4 – 23. 8)
H (*p*)		**0. 474 (0. 789)**			**0. 477 (0. 788)**	
			SOST (pg/ml)			
Mean ± SD.	136. 8 ± 20. 6	137. 5 ± 22	144. 4 ± 26. 2	136. 4 ± 20. 87	137. 8 ± 21. 7	145. 85 ± 23. 8
F(*p*)		**1. 672 (0. 433)**			**3. 706* (0. 025*)**	

SD: Standard deviation; F: ANOVA test; H: Kruskal–Wallis test. χ^2: chi-square test; MC: Monte Carlo. *p*: *p*-value for comparing rs3736228 and rs2297480with different parameters. *: Statistically significant at $p \leq 0.05$

In the present study, patients were categorized according to their *T* scores into three groups: osteoporosis (*n* = 12 (1. 6%)), osteopenia (*n* = 147 (19. 6%)), and normal (*n* = 591 (78. 8%)).

Osteoporosis is defined by the World Health Organization (WHO) as having a T score that is 2. 5 standard deviations (SD) or more below the mean BMD of young adults. Hip bone density was assessed using a DXA scan in the National Health and Nutrition Examination Survey (NHANES), which was conducted from 1988 to 1994 on men and women over the age of 50. Men were more likely than women to have hip osteopenia and osteoporosis, with prevalence rates of 18% and 2% versus 56% and 16%, respectively (15). Also, Rai et al. reported that 54% of premenopausal women had osteopenia, and 8% had osteoporosis (16).

The three groups were comparable regarding demographic features except for age, as females with osteopenia were older than those in the normal group, while the osteopenia and osteoporosis groups were matched. As in younger individuals, bone buildup is greater than bone loss, but with aging, bone resorption exceeds formation even in healthy persons, and bone density decreases throughout life by less than 1% per year (17).

Recent NHANES data revealed a significant rise in the prevalence of osteopenia and osteoporosis in both gen-

ders, as age-specific increases in the prevalence of low bone density (15).

Our results revealed also that 50% of females with osteoporosis and 11. 6% of females with osteopenia had family histories of these conditions. Risk factors for osteoporosis include fractures and positive family histories of the disease. They are cited as one of the most significant risk factors in several research (18).

In terms of biochemical analysis, we found that although the difference between the osteoporosis and osteopenia groups was minor, BALP, CTX-1 (ng/ml), and SOST (pg/ml) were considerably greater in the osteoporosis and osteopenia groups than in the normal group.

A screening biomarker for osteoporosis, BALP is a marker for the creation of new bone (16). Hydrolyzing phosphate esters at the osteoblast cell surface to provide a high phosphate concentration for bone remodeling, contributes to the process of bone mineralization. Thus, BALP levels signify periods of active bone production and bone development (16).

Among various bone-turnover markers, serum C-terminal telopeptide of type I collagen (CTX-I) was shown to have a positive relationship with fracture, as documented in a recent meta-analysis by Tian et al. (19).

During the bone-formation phase of bone remodeling, osteoblasts secrete osteocalcin, also known as bone gamma-carboxyglutamic acid-containing protein (BGLAP) (20). In osteoporosis, calcium and phosphorus are generally deficient, and a calcium-dependent biomarker called osteocalcin has a strong affinity for the hydroxyapatite-containing bone matrix that is necessary for bone mineralization. Osteoporosis causes a reduction in the production of hydroxyapatite crystals, which raises serum levels of osteocalcin (21). Singh et al. documented a negative correlation between serum osteocalcin levels and BMD grading, and these results are comparable with ours (22).

In terms of genotyping, our results revealed that the frequency of the LRPS SNP rs3736228 TT allele is significantly associated with osteoporosis, osteopenia, and *Z* score.

It is well known that Wnt pathway genes play a significant role in skeletal homeostasis (23). Numerous studies have previously connected adult lumbar spine BMD to polymorphisms in the LRP5 gene. In Maya-Mestizo women, Canto-Cetina et al. discovered a strong correlation between changes in all BMD sites and the rs3736228 polymorphism (24). It's interesting to note that neither the Mexican (26), Chinese (27), nor Slovenian (25) populations showed a significant connection of the same SNP with BMD.

Ciubean et al. showed that OP was linked with the LRP5 rs3736228 CC and CT genotypes (p = 0. 05 and p = 0. 041, respectively) (3). Additionally, BMD values in the femoral neck and total hip of postmenopausal women with the CC genotype are considerably lower (both p 0. 05).

Intriguingly, the LRP5 rs3736228 CC genotype tended to have greater BMD values than the TT genotype in all BMD locations in an Italian sample (28). Additionally, Markatseli et al. observed that the presence of the CT/TT genotype is linked with poorer lumbar spine BMD in a sample of Greek peri- and postmenopausal women (29). The homozygous group carrying the minor T allele had the lowest BMD scores, whereas the homozygous group carrying the major C allele had higher BMD, on analysis of rs37362288 genotypes in relation to BMD in adult Japanese women (30).

One of the crucial enzymes in the mevalonate pathway, FDPS, was found to be the primary biochemical target of N-BPs. We observed that the frequency of the FDPS SNP rs2297480 GG frequency was significantly associated with osteopenia.

According to Ciubean et al, Osteoporosis and the presence of the main allele T are significantly associated (p = 0. 005). Additionally, the lumbar spine and total hip BMD values were considerably lower in genotype TT of the rs2297480 SNP (both p 0. 05) (3). A suitable explanation for this difference may be the different ethnicities and populations studied: those authors studied only postmenopausal Romanian women, whereas we tested only premenopausal Saudi women.

In another study on Saudi women, The VDR rs731236 gene showed that CC allele carriers had a significant risk of osteopenia. The AA genotype of rs11568820 showed lower levels of physical activity, bone mineral density, Z scores, serum osteocalcin, phosphorus, and parathyroid hormones (31). Also, a studies on postmenopausal women found an association of RANK polymorphisms with osteopenia showing its clinical importance in the diagnosis and prognosis of bone diseases (32,33).

The GG genotype of FDPS rs2297480 and the TT genotype of LRP5 rs3736228 might be risk factors for Osteopenia among young Saudi females. Also, the GT genotype of FDPS rs2297480 is more frequent with low bone mineral density and SOST levels.

Limitations

Because only Saudi women were included in the study, it is not obvious whether the findings apply to women of other ethnicities. This is the study's primary limitation. A larger sample size is needed for genetic research in order to acquire acceptable statistical power, hence the cohort size was modest. Furthermore, because volunteers rather than the public were used in the study's clinical design, there is still a chance for bias.

Institutional review board statement

The study was carried out in conformity with the Helsinki Declaration and the guidelines established by the College of Medicine, Taibah University's ethics committee (TU-20-016) on February 18, 2021 (Resolution No. TU-20-016).

Informed consent statement

Before being included, each participant completed a consent form indicating their understanding of the nature and methodology of the study and their agreement to participate in genetic testing and the collecting of clinical data

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Conflict of Interest

None.

References

1. Sözen T, Özışık L, Başaran N. An overview and management of osteoporosis. Eur J Rheumatol 2017; 4: 46–56.
2. Sheu A, Diamond T. Bone mineral density: testing for osteoporosis. Aust Prescr. 2016; 39: 35–9.
3. Ciubean A. D, Ungur R. A, Irsay L. et al. Polymorphisms of FDPS, LRP5, SOST and VKORC1 genes and their relation with osteoporosis in postmenopausal Romanian women. PLoS One 2019; 14: e0225776.
4. Rivadeneira F, Mäkitie O. Osteoporosis and bone mass disorders: from gene pathways to treatments. Trends Endocrinol Metab 2016; 27:262–81.
5. Ralston S. H, Uitterlinden AG. Genetics of osteoporosis. Endocr Rev 2010; 31: 629–62.
6. Zofkova I, Nemcikova P, Kuklik M. Polymorphisms associated with low bone mass and high risk of atraumatic fracture. Physiol Res 2015; 64: 621–31.
7. Angers S, Moon RT. Proximal events in Wnt signal transduction. Nat Rev Mol Cell Biol 2009; 10: 468–77.
8. Richards J. B, Rivadeneira F, Inouye M. et al. Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study. Lancet 2008; 371:1505–12.
9. Levasseur R, Lacombe D, de Vernejoul MC. LRP5 mutations in osteoporosis-pseudoglioma syndrome and high-bone-mass disorders. Joint Bone Spine 2005; 72:207–14.
10. Mafi Golchin M, Heidari L, Ghaderian SM. et al. Osteoporosis: a silent disease with complex genetic contribution. J Genet Genomics 2016; 43: 49–61.
11. Winkler DG, Sutherland MK, Geoghegan JC, et al. Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. Embo J 2003; 22: 6267–76.
12. Seshacharyulu P, Rachagani S, Muniyan S. et al. FDPS cooperates with PTEN loss to promote prostate cancer progression through modulation of small GTPases/AKT axis. Oncogene 2019; 38:5265–80.
13. Holzer G, Grasse A. V, Zehetmayer S. et al. Vitamin K epoxide reductase (VKORC1) gene mutations in osteoporosis: a pilot study. Transl Res 2010; 156: 37–44.
14. Marini F, Masi L, Marcucci G. et al. Genetics of Osteoporosis. Multidisciplinary Approach to Osteoporosis; Springer 2018; pp. 25–44. Springer Nature Switzerland AG, Gwerbestrasse 11, 6330 Cham Switzerland
15. Alswat KA. Gender disparities in osteoporosis. J Clin Med Res 2017; 9:382–7.
16. Rai AD, Sherpa ML, Singh A. et al. Bone alkaline phosphatase and urine hydroxyproline assay in pre and postmenopausal women in the State of Sikkim and its correlation with bone mineral density. J Midlife Health 2021; 12: 304–9.
17. Demontiero O, Vidal C, Duque G. Aging and bone loss: new insights for the clinician. Ther Adv Musculoskelet Dis 2012;4: 61–76.
18. Bijelic R, Milicevic S, Balaban J. The influence of non-preventable risk factors on the development of osteoporosis in postmenopausal women. Mater Sociomed 2019; 31:62–5.
19. Tian A, Ma J, Feng K. et al. Reference markers of bone turnover for prediction of fracture: a meta-analysis. J Orthop Surg Res 2019; 14:68.
20. Hamdi RA. Evaluation of serum osteocalcin level in Iraqi postmenopausal women with primary osteoporosis. J Fac Med Baghdad 2013; 55:166–9.
21. Jagtap VR, Ganu JV, Nagane NS. BMD and serum intact osteocalcin in postmenopausal osteoporosis women. Indian J Clin Biochem 2011;26 :70–3.
22. Singh S, Kumar D, Lal AK. Serum osteocalcin as a diagnostic biomarker for primary osteoporosis in women. J Clin Diagn Res 2015;9:Rc04-7.
23. Harada S, Rodan G. A. Control of osteoblast function and regulation of bone mass. Nature 2003; 423: 349–55.
24. Canto-Cetina T, Polanco Reyes L, González Herrera L. et al. Polymorphism of LRP5, but not of TNFRSF11B, is associated with a decrease in bone mineral density in postmenopausal Maya-Mestizo women. Am J Hum Biol 2013; 25: 713–8.
25. Mencej-Bedrac S, Prezelj J, Kocjan T. et al. Analysis of association of LRP5, LRP6, SOST, DKK1, and CTNNB1 genes with bone mineral density in a Slovenian population. Calcif Tissue Int 2009; 85:501–6.
26. Falcón-Ramírez E, Casas-Avila L, Cerda-Flores R. M. et al. Association of LRP5 haplotypes with osteoporosis in Mexican women. Mol Biol Rep 2013; 40: 2705–10.
27. Zhou P. R, Liu H. J, Liao E. Y. et al. LRP5 polymorphisms and response to alendronate treatment in Chinese postmenopausal women with osteoporosis. Pharmacogenomics 2014; 15: 821–31.
28. Massart F, Marini F, Bianchi G. et al. Genetic predictors of skeletal outcomes in healthy fertile women: the Bonturno study. Joint Bone Spine 2013; 80:414–9.
29. Markatseli AE, Hatzi E, Bouba I. et al. Association of the A1330V and V667M polymorphisms of LRP5 with bone mineral density in Greek peri- and postmenopausal women. Maturitas 2011; 70:188–93.
30. Ezura Y, Nakajima T, Urano T. et al. Association of a single-nucleotide variation (A1330V) in the low-density lipoprotein receptor-related protein 5 gene (LRP5) with bone mineral density in adult Japanese women. Bone 2007;40: 997–1005.
31. Taha IM, Allah AM, El Tarhouny S. Association of Vitamin D gene polymorphisms and bone mineral density in healthy young Saudi females. Curr Mol Med 2019;19(3):196-205.
32. Azeez S, Jafar S, Aziziaram Z, Fang L, Mawlood A, Ercisli M. Insulin-producing cells from bone marrow stem cells versus injectable insulin for the treatment of rats with type I diabetes. Cell Mol Biomed Rep 2021; 1(1): 42-51. doi: 10.55705/cmbr.2021.138888.1006
33. Abdi S, Bukhari I, Ansari MG, BinBaz RA, Mohammed AK, Hussain SD, Aljohani N, Al-Daghri NM. Association of polymorphisms in RANK and RANKL genes with osteopenia in Arab postmenopausal women. Disease Markers. 2020 Dec 9;2020. https://doi.org/10.1155/2020/1285216