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Original Article

Sclerostin as a Genetic Determinant of Trabecular Bone Score in Postmenopausal Women: The Bushehr Elderly Health (BEH) Program

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Abstract

Background: Sclerostin, a protein encoded by the *SOST* gene, is an important genetic risk factor for osteoporosis in postmenopausal women. This study was conducted on the Iranian postmenopausal women, to investigate the association between this gene and the Trabecular Bone Score (TBS) as a novel index used for assessing osteoporosis.

Methods: The present study, conducted in 2024, was performed on 1071 women aged 60 years and older who participated in the Bushehr Elderly Health (BEH) program. The associations between seven independent Single Nucleotide Polymorphisms (SNPs) within the *SOST* gene and mean TBS of L1 to L4 were examined using the additive, dominant, and recessive models. Genetic risk scores (GRS) were calculated for each postmenopausal woman based on the coefficient regressions derived from the additive and dominant models. The relationship between the GRS quartiles and TBS was evaluated using a linear regression model.

Results: After adjusting for age and Body Mass Index (BMI), the associations between the rs2023794-C and TBS were significant in the additive (β = 0.03, P= 4.7×10^{-5}, PFDR= 0.0003) and dominant (β = 0.032, P= 5×10^{-5}, PFDR= 0.0003) models. The GRS derived from both additive and dominant models were related to TBS ($P<0.05$). For the additive model GRS, TBS showed an average increase of 0.022 score for the fourth quartile in comparison with the first quartile, adjusted for age, BMI, type 2 diabetes mellitus (T2DM), and smoking status ($P=0.001$).

Conclusion: *SOST* gene is associated with TBS and may have implications for personalized medicine. Targeting sclerostin through *SOST* could offer a therapeutic approach in managing osteoporosis in high-risk postmenopausal women.

Keywords: Trabecular bone score; *SOST* gene; Sclerostin; Osteoporosis; Postmenopausal women

Introduction

Osteoporosis is a prevalent bone disease in postmenopausal women (1). Older women are the most susceptible to osteoporotic fractures, with the highest incidence occurring among those aged 95 years and older, reaching an age-specific rate of 17,465 per 100,000 population (2). More than half of elderly women in Iran are affected by osteoporosis, with the age-standardized prevalence estimated to be 62.7% (3).

The Trabecular Bone Score (TBS) is a novel complementary technique for osteoporosis evaluation and reflects the trabecular microarchitecture, including the number, thickness, separation, and connectivity of trabeculae. TBS measures variation from one pixel to the adjacent pixel in gray levels of the lumbar spine dual-energy X-ray absorptiometry (DXA) image (4-6). Bone Mineral Density (BMD) testing is the gold standard for the diagnosis of osteoporosis, but osteoporotic fractures may occur in individuals with normal BMD (7, 8).

Osteoporosis is a Complex condition; however, genetic factors are among the most important risk factors of developing postmenopausal osteoporosis (PMOP) (9, 10). Estrogen receptors which are encoded by the genes including *ESR1* and *ESR2* have a crucial role in developing PMOP (11, 12). Besides the estrogen receptor genes, sclerostin which is encoded by the *SOST* gene has a significant impact on PMOP. Sclerostin is an emerging therapeutic target for osteoporotic fractures in postmenopausal women. Romosozumab, an FDA-approved humanized monoclonal antibody sclerostin inhibitor, is utilized in the treatment of osteoporosis in postmenopausal women at high risk of fractures by targeting sclerostin (13-15). Sclerostin is a glycoprotein that inhibits the Wnt signaling pathway, which is essential for bone formation (16, 17). Variations in the *SOST* gene can lead to altered levels of sclerostin, affecting the balance between bone formation and resorption. High levels of sclerostin inhibit osteoblast activity, leading to decreased bone formation, while also promoting osteoclast activity, which results in increased bone resorption. This imbalance can contribute to the development of osteoporosis and increased fracture risk in postmenopausal women (18-20).

Understanding the role of the *SOST* gene and sclerostin in bone metabolism is vital for uncovering new therapeutic strategies for PMOP. To the best of our knowledge, no prior study has explored the potential impact of *SOST* on TBS. Given the high prevalence of PMOP, this investigation was performed to identify association between *SOST* gene and TBS within the Iranian postmenopausal women.

Methods

Participants

This study was conducted using the data of the Bushehr Elderly Health (BEH) program. BEH is a population-based longitudinal cohort study conducted in Bushehr, a southern province of Iran. It began in 2013, and initially 3000 participants aged 60 years and above were selected using the multistage cluster random sampling method. The inclusion criteria for participants included age ≥60, residency in Bushehr for at least one year prior to the study, no plans to leave the city for two years after the study initiation, and the physical and mental ability to participate in the study. After 2.5 years, 2772 individuals were eligible for the second stage of the first phase, which focused on musculoskeletal disorders. The design and methodology of the BEH are described in details elsewhere (21, 22).

Genotyping and quality control

All 3000 participants in the BEH study underwent genotyping using the Illumina GSA Arrays "Infinium iSelect 24x1 HTS Custom Beadchip Kit". However, after excluding individuals who did not participate in the second stage of BEH study, lacked TBS data, or were removed during quality control, a total of 1071 postmenopausal women

were investigated in this study. Single Nucleotide Polymorphisms (SNPs) that violated Hardy-Weinberg equilibrium ($P<10^{-6}$) or had a minor allele frequency ≤0.01 as well as insertion-deletion (indel) polymorphisms were excluded.

Outcome measures

The phenotype investigated in the present study was the mean TBS of L1 to L4 (TBS L1-L4). The evaluation of TBS was conducted using TBS iNsight® software version 2.2, which was installed on a DXA machine (Discovery WI, Hologic Inc, USA). TBS iNsight is a specialized software integrated into the DXA workstation to perform a bone texture assessment of lumbar spine examinations in adult individuals.

SNP selection

SOST is located on chromosome 17q21.31, its position on the chromosome is from 41,831,106 to 41,836,159 (GRCh37) (16). To ensure that potential primer regions were adequately covered, we included a genomic region of approximately ±2 kb around the gene. Following quality control measures, a total of 22 variants remained in the BEH study genomic database. From these SNPs, seven independent SNPs with low linkage disequilibrium (r2≤.2) were chosen for the analysis.

Statistical analysis

A generalized linear model (GLM) was used for the additive, dominant, and recessive models. The coding labels for the additive model were defined based on the minor allele count with A representing the minor allele: BB = 0, AB = 1, and AA = 2. The dominant (AA + AB versus BB), and recessive models (AA versus AB + BB) were also investigated. The significance threshold was set at a False Discovery Rate (FDR) <0.05.

Two separate genetic risk scores (GRS) were calculated based on the results from the additive and dominant models. Risk scores for each individual were determined using regression coefficients and allele values obtained from both models. To calculate an individual's GRS, allele values were assigned to a numerical value based on whether they represented the effect allele or not (0, 1, and 2 for the additive model; 0 and 1 the dominant model). These values were then multiplied by their corresponding beta coefficients for each genetic model. The sum of these products yielded the GRS for that individual. The association between GRS and TBS was investigated using multiple linear regression analysis, adjusted for age, Body Mass Index (BMI), type 2 diabetes mellitus (T2DM), and smoking status. QC processes were carried out using PLINK version 2 software. The relationships between SNPs and TBS in different genetic models were examined using R 4.4.0 with the SNPassoc package. Additionally, R version 4.4.0 was used to perform linear regression analyses and to visualize data.

Ethics

BEH Program which we used its data was approved by Ethics Committee under code B-91–14-2. The participants in the original study have signed a written informed consent to participate in the study and allow the researchers to use the data. In addition, the current study received approval from the Research Ethics Committee at Tehran University of Medical Sciences with the reference code IR.TUMS.SPH.REC.1400.237.

Results

In this study, 1071 elderly women were included after undergoing a quality control process. The mean age and BMI of the participants were 69.2±6.4 years and 28.65±5.34 kg/m² respectively. In terms of smoking habits, 35% of the women were current smokers, and 34.8% were diagnosed with T2DM. Additionally, the mean TBS was 1.241 with a standard deviation of 0.087 (Table 1).

Seven independent SNPs within or ±2kb around the *SOST* gene with a r2≤0.2 are presented in Table 2, three of them were 3-prime untranslated variants.

Table 1: Descriptive characteristics of the Iranian postmenopausal women

Variable	*Descriptive statistics (Number=1071)*
Age, Mean (SD*) (yr)	69.2 (6.4)
BMI, Mean (SD) (kg/m2)	28.65 (5.34)
T2DM, Frequency (%)	
Yes	371 (34.8)
No	694 (65.2)
Smoking, Frequency (%)	
Never	500 (46.9)
Current smoker	374 (35)
Former smoker	193 (18.1)
TBS** L1-L4, Mean (SD)	1.241 (0.087)

*Standard deviation, ** Trabecular bone score

Table 2: Independent single nucleotide polymorphisms of *SOST* gene in the Iranian postmenopausal women

rsID	*Position**	*Allele1/Allele2*	*MAF***	*Functional consequence*
rs141066600	41829684	T/C	0.013	Downstream gene variant
rs17881550	41831443	C/G	0.401	3-prime untranslated variant
rs17886183	41831706	T/C	0.023	3-prime untranslated variant
rs75901553	41831844	A/G	0.023	3-prime untranslated variant
rs117857467	41836456	A/G	0.028	Upstream transcript variant
rs2023794	41837660	C/T	0.062	Upstream transcript variant
rs2342311	41838593	T/C	0.059	Upstream gene variant

*GRCh37, **Minor Allele Frequency

The results of additive, dominant, and recessive models are presented in Table 3. The association between the rs2023794-C SNP and TBS remained significant after adjusting for age (β = .032, P= 2.4×10^{-5}, PFDR= .0001), as well as age and BMI (β = .03, P= 4.7×10^{-5}, PFDR= .0003). On average, each copy of the C allele was associated with an increase in TBS of approximately 0.03 units for this SNP. The rs2023794-C SNP also showed a significant association with TBS in the dominant model; having one or two copies of the C allele increased TBS by 0.033 units when adjusted for age (β = 0.033, P=2 .5×10^{-5}, PFDR= .0001). No significant associations were found between SNPs and TBS under the recessive model.

As depicted in Fig. 1, there is an increasing pattern in TBS across GRS quartiles derived from the seven investigated SNPs. In other words, as the GRS (calculated based on the additive and dominant models) increased, a corresponding increase in TBS was observed. By conducting an ANOVA analysis, we observed that the differences between TBS means in the four quartile groups of GRS were statistically significant for both the additive and dominant models (P<0.05).

Table 3: Relationship between single nucleotide polymorphisms of *SOST* gene with trabecular bone score in the Iranian postmenopausal women

Variable		***Model 1****				***Model 2***			
rsID	Effect Allele	B	CI***, 0.95%	*P*	P_{FDR}	B	CI, 0.95%	*P*	P_{FDR}
Additive									
rs141066600	T	0.009	(-0.02, 0.04)	0.5	0.8	0.007	(-0.022, 0.037)	0.6	0.6
rs17881550	C	0.004	(-0.002, 0.011)	0.2	0.7	0.004	(-0.003, 0.011)	0.2	0.4
rs17886183	T	-0.015	(-0.04, 0.01)	0.2	0.7	-0.016	(-0.041, 0.008)	0.2	0.4
rs75901553	A	0.01	(-0.013, 0.034)	0.3	0.8	0.011	(-0.011, 0.035)	0.3	0.4
rs117857467	A	0.01	(-0.011, 0.032)	0.3	0.7	0.009	(-0.012, 0.03)	0.4	0.4
rs2023794	C	0.032	(0.017,0.047)	2.4×10^{-5}	0.0001	0.03	(0.015, 0.045)	4.7×10^{-5}	0.0003
rs2342311	T	0.006	(-0.009, 0.022)	0.4	0.8	0.006	(-0.009, 0.022)	0.4	0.4
Dominant									
rs141066600	T	0.009	(-0.02, 0.04)	0.5	0.5	0.007	(-0.022, 0.03)	0.6	0.6
rs17881550	C	0.008	(-0.002, 0.018)	0.1	0.4	0.008	(-0.002, 0.018)	0.1	0.4
rs17886183	T	-0.016	(-0.04, 0.01)	0.2	0.5	-0.017	(-0.043, 0.009)	0.2	0.4
rs75901553	A	0.011	(-0.13, 0.035)	0.3	0.5	0.013	(-0.011, 0.036)	0.2	0.4
rs117857467	A	0.01	(-0.011, 0.033)	0.3	0.5	0.01	(-0.011, 0.032)	0.3	0.4
rs2023794	C	0.033	(0.017, 0.048)	2.5×10^{-5}	0.0001	0.032	(0.016, 0.046)	5×10^{-5}	0.0003
rs2342311	T	0.007	(-0.01, 0.02)	0.4	0.5	0.007	(-0.009, 0.023)	0.4	0.4
Recessive									
rs141066600	T	-	-	-	-	-	-	-	-
rs17881550	C	0.001	(-0.012, 0.015)	0.7	0.9	0.0008	(-0.012, 0.014)	0.9	0.9
rs17886183	T	-0.003	(-0.17, 0.16)	0.9	0.9	-0.018	(-0.18, 0.14)	0.8	0.9
rs75901553	A	0.004	(-0.16, 0.17)	0.9	0.9	0.0004	(-0.16, 0.16)	0.9	0.9
rs117857467	A	-0.001	(-0.17, 0.16)	0.9	0.9	-0.026	(-0.19, 0.13)	0.7	0.9
rs2023794	C	0.047	(-0.049, 0.14)	0.3	0.9	0.046	(-0.04, 0.14)	0.3	0.9
rs2342311	T	0.009	(-0.08, 0.1)	0.8	0.9	0.004	(-0.091, 0.099)	0.9	0.9

*Adjusted for age **Adjusted for age and BMI ***Confidence Interval

The linear regression analysis revealed that, the risk scores calculated based on the additive and dominant models were related to TBS. For the additive model GRS, TBS showed an average increase of 0.022 score for the fourth quartile compared to the first quartile, after adjusting for age, BMI, T2DM, and smoking status (β = 0.022, *P*=0.001). In the dominant model, association was significant for both the third quartile (β = 0.02, *P*= 0.01) and fourth quartile (β = 0.024, *P*= 0.0001), compared with the first quartile. After adjusting for all covariates, TBS increased by 0.02 and .024 respectively for these quartiles (Table 4).

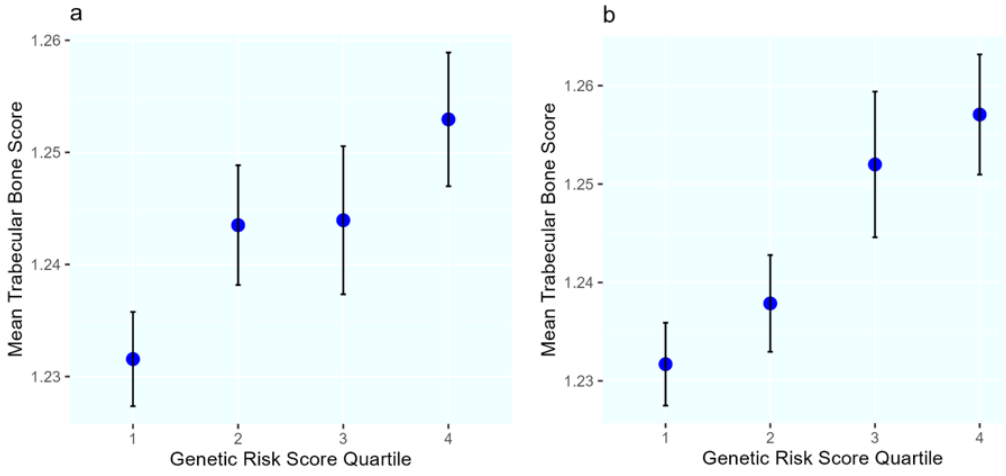

Fig. 1: Mean trabecular bone scores among the quartiles of the genetic risk score derived from a) additive model and b) dominant model in the Iranian postmenopausal women

Table 4: Relationship between GRS derived from the *SOST* gene with TBS in the Iranian postmenopausal women

Variable		***Model 1****			***Model 2*****	
GRS***	B	CI****, 0.95%	*P*	B	CI, 0.95%	*P*
Additive GRS						
2	0.011	(-0.001, 0.024)	0.08	0.011	(-0.001, 0.024)	0.07
3	0.011	(-0.003, 0.027)	0.1	0.011	(-0.003, 0.026)	0.1
4	0.023	(0.01, 0.03)	0.001	0.022	(0.009, 0.035)	0.001
Dominant GRS						
2	0.005	(-0.006, 0.018)	0.3	0.005	(-0.006, 0.018)	0.3
3	0.02	(0.003, 0.036)	0.01	0.02	(0.003, 0.037)	0.01
4	0.026	(0.012, 0.04)	0.0001	0.024	(0.011, 0.038)	0.0001

*Adjusted for age **Adjusted for age, BMI, T2DM, and smoking status ***GRS ****Confidence Interval

Discussion

A candidate gene approach was used to investigate the relationship between genetic variants of *SOST* gene and TBS in postmenopausal women. In this study, the rs2023794 SNP within the *SOST* gene, was related to TBS. This SNP was related to bone density in Chinese women. Zhang et al. revealed that postmenopausal Chinese women with the CC genotype of the rs2023794 have higher BMD values compared with individuals with other genotypes (23). Sclerostin is a small glycoprotein expressed by the *SOST* gene. Sclerostin is primarily produced by osteocytes, the most abundant cells found in mature bone tissue. Lima et al. for the first time reported a relationship between high serum sclerostin and high TBS in Berardinelli-Seip congenital lipodystrophy patients (24). In a study involving postmenopausal women, researchers found a significant relationship between the serum sclerostin levels and TBS (25).

The initial evidence of the impact of this gene and sclerostin on bone structure was observed for the BMD phenotype. Balemans et al. explored that mutation in this gene and demonstrated increased bone density in sclerosteosis patients (26). Another study showed that mutation in the *SOST* gene severely impairs the biological function of sclerostin by reducing its levels in the extracellular environment and diminishing its ability to bind to

LRP5. Consequently, this loss of function leads to a disruption in the antagonistic activity of sclerostin on canonical Wnt signaling (27). Sclerostin normally acts as an inhibitor of bone formation. When sclerostin is absent or dysfunctional, the inhibitory effect on osteoblasts is reduced, allowing for increased activity and formation of new bone. Sclerostin deficiency leads to increased bone density and strength (20).

After menopause, estrogen levels decrease, leading to an imbalance between bone formation and resorption. This results in accelerated bone loss and an increased risk of osteoporosis in postmenopausal women (28-30). Considering the functional impact of sclerostin on bone formation and osteoporosis, it was initially recognized as a potential therapeutic target for treating osteoporosis. Clinical trials have shown promising results for monoclonal antibody sclerostin inhibitors in postmenopausal women with osteoporosis. It has been found to significantly reduce the risk of vertebral and nonvertebral fractures compared to a placebo or other osteoporosis treatments. Additionally, it has demonstrated rapid gains in bone density within the first year of treatment. A randomized clinical trial conducted by McClung et al. assessed the efficacy of romosozumab in 419 postmenopausal women with low BMD. According to the results, the use of romosozumab was found to be associated with increased BMD and bone formation, as well as lower levels of bone resorption in postmenopausal women with low bone mass (31).

Romosozumab and blosozumab are monoclonal antibodies are used in the treatment of postmenopausal osteoporosis by targeting sclerostin. They work to increase bone formation and reduce the risk of fractures and do this by preventing sclerostin from inhibiting Wnt signaling, which promotes the differentiation and function of osteoblasts. This ultimately leads to an increase in BMD. Several meta-analysis studies performed on randomized clinical trials have shown an improved effect of monoclonal antibody sclerostin inhibitors in the treatment of osteoporotic patients (32-35). Singh et al. in a meta-analysis study conducted on a total of 6,137 patients in romosozumab group and 5,732 patients in control group showed that monoclonal antibody significantly reduced the incidence of vertebral fractures, nonvertebral fractures, and clinical fractures. Also, BMD was significantly increased in the romosozumab treated groups at lumbar spine, total hip, and femoral neck (32). Given its efficacy demonstrated in clinical trials, romosozumab represents a valuable therapeutic option for postmenopausal women at risk for or diagnosed with osteoporosis.

One of the limitations of this study was the small sample size of postmenopausal women. Additionally, the GRS calculation encompassed only seven SNPs within the *SOST* gene. Nonetheless, this research was the first investigation into the relationship between this gene and TBS within the Iranian postmenopausal women.

Conclusion

SOST gene and the presence of sclerostin appears to have a negative impact on TBS, indicating a potential detrimental effect on trabecular bone quality. Furthermore, inhibition of sclerostin has been suggested as a potential therapeutic target for improving bone strength and reducing fracture risk. Overall, these findings highlight the significant influence of sclerostin on trabecular bone health and the potential implications for managing osteoporosis and related conditions. Considering the potential impact of sclerostin on TBS, it is suggested to conduct randomized clinical trials to further investigate the effect of monoclonal antibody sclerostin inhibitors on TBS. This would provide more robust evidence regarding the efficacy and safety of these inhibitors in improving trabecular bone quality and potentially reducing fracture risk.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that they have no competing interests.

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