Iraqi Journal of Biotechnology, 2024, Vol. 23, No. 2, 112-118

The Role of Parathyroid Hormone Receptor 1 Gene Polymorphism at rs1138518 SNP in the Incidence of Osteoporosis in a Sample of Iraqi Women

[1]Bushra A. Jassam Al-Juaifari, [2] Essam F. Alwan Al-Jumaili, [3]Ali A. Mahdi

[1]Al-Hikma University College
[2]Institute of Genetic Engineering and Biotechnology, University of Baghdad
[3]College of Health and Medical Technologies

Received: September 17, 2023 / Accepted: October 29, 2023 / Published: October 30, 2024

Abstract: Osteoporosis is a medical condition characterized by the weakening of bones, which makes them fragile and more prone to fractures. It is often associated with bone mineral density (BMD) reduction and changes in bone microstructure. Osteoporosis is influenced by a combination of genetic, diet and lifestyle factors, specific genetic variants can contribute to an individual's risk of developing the condition. The *PTHR*1 gene encodes the parathyroid hormone receptor1, which plays a role in calcium homeostasis and bone remodeling. Genetic variations within this gene, including rs1138518, have been studied in relation to bone health. The study aimed to detect the specific relationship between the rs1138518 variant on the *PTHR1* gene and osteoporosis in a sample of Iraqi women their ages between (20-50) years old. A total number of 120 samples: two group of (60) women diagnosed with osteoporosis and (60) apparently healthy control. The DNA was extracted for both groups and the *PTH1R* SNP (rs1138518) gene were detected by qRT-PCR. The result showed there was high significant difference between patient and control in genotypes of mutant allele (AA) (51.67% vs. 13.33%, respectively; odds ratio =1.376; p-value =0.0001**, and that A allele frequency was significantly increased in patients compared to control (0.67 vs. 0.33) and the wild allele T showed decreased frequency in patients (0.22 vs. 0.78). The genotype of rs1138518SNP was showed significant difference between patient and control in PO4 (p-value= 0.05*). The TA genotype of rs1138518 SNP was effective in increasing the serum level of MMP-9 in osteoporosis patients (4006.13 ±131.30) compared TT or AA genotype (3495.12 ±174.01 and 3581.09 ±95.10, respectively; p=0.017*). This study was concluded there is an A allele – related risk factor for osteoporosis in Iraqi women, while the wild allele T might have a protective effect.

Key words: *PTHR1*gene, Genotype, MMp-9, qRT-PCR, osteoporosis.

Corresponding author: (Email: bushra.jassam@hiuc.edu.iq)

Introduction

Osteoporosis is a metabolic bone sickness. It happens when the pace of bone resorption surpasses the pace of bone development, prompting a decline in bone thickness and expanded hazard of cracks. Low calcium admission and vitamin D3 insufficiency emphatically associated with the commonness of osteoporosis (1). Parathyroid hormone plays a crucial role in regulating calcium and phosphate levels in the body and maintain calcium homeostasis increasing calcium absorption in the intestines, reabsorption in the kidneys, and releasing calcium from bone when blood calcium levels are low. This last action, releasing calcium from bone, is where the connection between PTH and osteoporosis comes into play (2). PTH

and vitamin D3 are the two foremost regulators of mineral metabolism and are essential for maintaining bone health (3). Therefore, many researches that has studied the importance of vitamin D because of its role in regulating various physiological functions in the body (4). Parathyroid hormone receptor 1 *(PTH1R*) is a protein that plays a crucial role in regulating calcium and bone turnover homeostasis in the body. It is a class B1 G-protein-coupled receptor (GPCR) found on the surface of target cells, particularly in the bones and kidneys. *PTH1R* is primarily activated by parathyroid hormone and parathyroid hormone-related protein. Parathyroid hormone (PTH) regulates calcium and phosphate homeostasis in bone and kidney while parathyroid hormone-related protein (*PTHrP*) modulates cell proliferation and differentiation in developing bone and other tissues (5). When parathyroid hormone (PTH) binds to the *PTH1R* receptor, it initiates a signaling cascade that ultimately leads to an increase in calcium levels in the bloodstream. This is accomplished by promoting the release of calcium from bones, reabsorption of calcium in the kidneys, and activation of vitamin D, which enhances calcium absorption from the intestines. Excessive PTH production or *PTH1R* activity can result in hyperparathyroidism, characterized by elevated blood calcium levels. This condition may lead to kidney stones, bone loss, and other health problems (6). The parathyroid hormone receptor 1 (*PTH1R*) gene is a human gene located on the short arm of chromosome 3 at position 21.31. Mutations or variations in the *PTH1R* gene can lead to various skeletal and calcium metabolism disorders (7). MMP-9 is an enzyme that belongs to the matrix metalloproteinase family. It plays a role in the breakdown of extracellular matrix components, particularly collagen. MMP-9 is involved in various physiological and pathological processes, including tissue remodeling, wound healing, and inflammation (8). The Research has suggested that rs1138518 T/A SNP is associated with variations in the *PTH1R* gene, and may be associated with variations in bone mineral density and susceptibility to certain bone-related conditions, such as osteoporosis (3). This study aimed to detect the specific relationship between the rs1138518 variant on the *PTHR1* gene and osteoporosis in a sample of Iraqi women.

Materials and methods

This study was conducted during the period from 1 June 2022 until the first of March 2023. The total numbers of samples are 120 were collected from Iraqi women aged between (20-50) years old from Al-Yarmouk Teaching Hospital /Baghdad include (60) patients were diagnosed with osteoporosis and (60) apparently healthy subject (control). Blood samples (5 ml) have been collected from each woman of both osteoporosis and healthy control. Two milliliters from blood were collected in tubes containing anticoagulant EDTA and were stored at 4^{o}C for genomic DNA extraction for genotyping.

SNP genotyping (rs1138518)

Genomic DNA was extracted using EasyPure® Genomic DNA extraction kits, following the manufacturer's instructions provided by TransGen Biotech in China. For quantitative real-time PCR with high-resolution melting analysis (qPCR-HRM), a Rotor gene Q Real-time PCR System from QIAGEN was employed. The HRM analysis involved a 0.2 °C scaling from 55 to 95 °C and was carried out in a 20 µl reaction volume.The quantitative real-time PCR

(qRT-PCR) targeting the Pin1 polymorphism was conducted using the Rotor-Gene® Q instrument from Qiagen in Germany. Each reaction included 3µl of DNA, 5µl of Nuclease-free water, 10 µl of 2x TransStart® Tip Green qPCR Super Mix from TransGen Biotech Co in China, and 1µl for each of the forward and reverse primers (10 µM) as listed in Table (1). The thermal profile for this procedure consisted of an initial enzyme activation step at 94 °C for 30 seconds (one cycle), followed by 40 cycles involving denaturation at 94 °C for 5 seconds, annealing at 58°C for 15 seconds, and extension at 72 °C for 20 seconds. The final dissociation stage ranged from 55 to 95 °C with duration of 5 seconds per degree.

Statistical analysis

The Statistical Analysis System-SAS (2018) program was used to affect different factors in study parameters. Chi-square test was used to significant comparison between percentage (0.05 and 0.01 probability). Estimate of Odd ratio and CI in this study. Least significant difference –LSD test and T-test were used to significant comparison between means in this study (SAS, 2018).

Table (1): The studies designed primers.

Primer	Sequence (5′→3′ direction)	primer size bp	Product size bp	Ta °C
PTHR1 gene (SNP Genotyping) **rs1138518**				
Forward	GAGACACCCCTCTTCACAGG	20	88	58
Reverse	CCTATGCCAACACTGTCTCC	20		

Result and discussion

From Table (2) the *PTH1R* rs1138518 SNP was observed to have three genotypes (TT, TA and AA) that were correspondent to two alleles, which were T and A. The statistical analysis showed that there was a significant difference between control and patients in the genotype of wild allele (TT) (70.00 vs 18.33% respectively; p=0.0001*) and the genotypes of hetero allele (TA) showed difference between patient and control (30.00 vs 16.67% respectively; odds ratio= 0.145; p=0.130). The result showed that there was a high significant difference between patient and control in the homozygous genotypes of mutant allele (AA) (51.67 vs 13.33%, the odds ratio of such association was 1.376, (p value= 0.0001**). When the comparison was made at the allele level, A allele frequency was high significantly increased in patients compared to control (0.67 vs. 0.22). In contrast, the wild allele (T) showed a significant decreased frequency in patients (0.33 vs. 0.78). These findings suggested that an A allele- related risk factor for osteoporosis in Ireqi women, while the wild allele (T) might have a protective effect.

Table (2): Genotype and allele frequency of rs1138518 T/A in patients and control groups

Genotype/ rs1138518 T/A	Patients No. (%)	Control No. (%)	Chi-Square ($\chi 2$)	P-value	O.R. (C.I.)
TT: Wild	11 (18.33%)	42 (70.00%)	18.13 **	0.0001	Ref. =1
TA: Hetro.	18 (30.00%)	10 (16.67%)	2.285 NS	0.130	0.145 (0.08-0.63)
AA: Mutant	31 (51.67%)	8 (13.33%)	14.40 **	0.0001	1.376 (0.86-2.51)
Total	60	60	--		
P-value	0.0001 **	0.0001 **	--		
Allele	Frequency				
T	40 (0.33)	94 (0.78)	0.0001 **		
A	80 (0.67)	26 (0.22)	0.0001 **		
**** (P≤0.01), NS: Non-Significant.**					

The gene for *PTHR1* is located in chromosome 3 and is expressed in osteocytes and osteoblasts. *PTHR1* gene encodes the parathyroid hormone receptor, which plays a critical role in regulating calcium and phosphate levels in the body. Abnormal expression, mutations or variations in the *PTH1R* gene can lead to various skeletal and calcium metabolism disorders (7). Several studies have tried to corelate the frequency distribution of polymorphism in patients with osteoporosis, this is the first study to confirm the association between the *PTHR1* rs1138518 SNP and premenopausal osteoporosis women.

Vilariño-Güell *et al*., 2007(9) and Abdi *et al*., 2021(3) are found association between the *PTHR1* rs1138518 SNP and postmenopausal osteoporosis patients, they found a significant difference in the distribution of the rs1138518 genotype among patient and control groups. The difference was primarily based on a higher frequency of heterozygote genotype.

Vilariño-Güell *et al*., (9) reported that common variants in *PTHR1*gene influence bone mineral density (BMD) in general population. They concluded that the association of *PTH1R* gene polymorphism on BMD was due to its role in attaining peak bone mass and not due to its effect on bone loss in old age.

Duncan et al., 1999(10) and Wynne *et al*., (11) found linkage of *PTHR1* gene to hip and spine BMD respectively.

Zhang *et al*., (12) failed to find any significant association between BMD and individual SNPs of *PTH1R*. Overall, these studies indicate PTHR1 gene variants to influence bone strength and hence osteoporosis risk.

Genotype and another parameter

The TA genotype of rs1138518 SNP was effective in increasing the serum level of MMP-9 in osteoporosis patients (4006.13 ±131.30 a) compared AA or TT genotype (3581.09 ± 95.10 and 3495.12 ± 174.01, respectively; p-value =0.017*) as showed in Table (7).

The result were listed in Table (6) showed significant difference between genotype of rs4986938 SNP in PO4 between patient and control group (p-value=0.050*). The result showed no significant difference between patient and control groups in genotype of rs4986938 SNP with PTH, VitD3 and Ca.

Table (3): Relationship of rs1138518 T/A in patients and control groups with PTH

Genotype of SNP (rs1138518)	Mean ± SE	
	Patients	Control
TT	74.13 ±5.29	46.94 ±1.52
TA	75.10 ±6.82	42.10±2.84
AA	72.40 ±4.46	44.75±3.54
P-value	0.932 NS	0.342 NS
NS: Non-Significant.		

Table (4): Relationship of rs1138518 T/A in patients and control groups with D3

Genotype of SNP (rs1138518)	Mean ± SE	
	Patients	Control
TT	17.00 ±1.32	22.20 ±1.14
TA	19.55 ±0.77	20.4 ±1.30
AA	18.55 ±0.73	21.5 ±2.32
P-value	0.242 NS	0.751 NS
NS: Non-Significant.		

Table (5): Relationship of rs1138518 T/A in patients and control groups with Ca

Genotype of SNP (rs1138518)	Mean ± SE	
	Patients	Control
TT	8.72 ±0.18	8.93 ±0.06
TA	8.63 ±0.17	8.98 ±0.12
AA	8.45 ±0.12	8.75 ±0.08
P-value	0.478 NS	0.434 NS
NS: Non-Significant.		

Table (6): Relationship of rs1138518 T/A in patients and control groups with PO4

Genotype of SNP (rs1138518)	Mean ± SE	
	Patients	Control
TT	5.15 ±0.28 a	3.63 ±0.09
TA	4.50 ±0.16 b	3.59 ±0.15
AA	4.68 ±0.10 ab	3.34 ±0.12
P-value	0.050*	0.425 NS
* (P<0.05), NS: Non-Significant. Means having with the different letters in same column differed significantly		

Table (7): Relationship of rs1138518 T/A in patients and control groups with MMP-9

Genotype of SNP (rs1138518)	Mean ± SE	
	Patients	Control
TT	3495.12 ±174.01 b	1849.89 ±36.55
TA	4006.13 ±131.30 a	1783.46 ±95.69
AA	3581.09 ±95.10 b	1805.21 ±78.11
P-value	0.017*	0.705 NS
* (P<0.05), NS: Non-Significant. Means having with the different letters in same column differed significantly		

In osteoporosis patients, there is often an imbalance in bone remodeling, with excessive bone resorption compared to bone formation, PTH is one of the hormones that can stimulate bone resorption (13). While MMP-9 is highly expressed in osteoclasts and important for extracellular matrix degradation during bone resorption as well as bone remodeling (14), the direct relationship between PTH and MMP-9 is complex and not fully understood. However, some studies suggest that PTH can indirectly influence the activity of

MMP-9 through its effects on osteoblasts and osteoclasts. Elevated PTH levels can stimulate osteoclasts (15) which may increase the production and activity of MMP-9, potentially contributing to bone degradation.

Bolton *et al*., 2009(16) found that increased circulating MMP-9 in patients with chronic obstructive pulmonary disease (COPD) was related to the presence of osteoporosis and not to lung function. MMP-9 may be a biomarker of increased bone resorption.

Many previous studies found that MMP9 levels were significantly associated with increased bone resorption in osteoporosis (17).

Sabry *et al*., 2021 (18) found that MMP-9 plays a regulatory role in bone formation and is considered a potential molecular link linking atherosclerosis and osteoporosis.

In osteoporosis, the focus is primarily on calcium levels rather than phosphate levels. Calcium and phosphate are both critical minerals for bone health, and they work together to maintain bone strength and density (19). Typically, when calcium levels in the blood are low, the body may compensate by increasing phosphate levels. This occurs because the parathyroid hormone (PTH) stimulates the release of calcium from bones and increases its absorption from the intestines (20). As a result, phosphate levels may rise as part of this regulatory process.

Abdi *et al*., (3) found that genotypes of *PTHR1* rs1138518 may play a role in regulating circulating D3 level.

Conclusion

Genetic variation of *PTHR1* gene, including rs1138518 T/A SNP increased risk of osteoporosis and it's may vary among different populations. TA genotype of rs1138518 SNP associated with increased MMP-9 levels. Elevated MMP-9 levels can lead to increased degradation of the extracellular matrix in bone tissue, which, in turn, may contribute to the development or progression of osteoporosis. This study concludes that the A allele was associated with an increased risk to developed osteoporosis in Iraqi premenopausal women, while the wild allele T might have a protective effect.

Reference

1. Voulgaridou, G.; Papadopoulou, S.; Detopoulou, P.; Tsoumana, D.; Giaginis, C.; Kondyli, F., *et al*. (2023). Vitamin D and Calcium in Osteoporosis, and the Role of Bone Turnover Markers: A Narrative Review of Recent Data from RCTs. Diseases, 11(1): 29.
2. Crooks, M. and Bendall, S. (2021). Parathyroid hormone and vitamin D: from benchto bedside. Orthopaedics and Trauma, 35(5): 267-273
3. Abdi, S.; Almiman, A. A.; Ansari, M.; Alnaami, A.; Mohammed, A.; Aljohani, N., *et al*. (2021). *PTHR1* Genetic Polymorphisms Are Associated with Osteoporosis among Postmenopausal Arab Women. BioMed Research International, (1): 1-8
4. Al-Baidani, S. J. (2018). Study of vitamin D receptor gene polymorphisms and cytogenetic change in a sample of Iraqi infertile women with polycystic ovary syndrome. Doctorate thesis, Institute of Genetic Engineering and Biotechnology for Postgraduate Studies. University of Baghdad.
5. Vilardaga, J. P.; Romero, G.; Friedman, P. A. and Gardella, T. J. (2011). Molecular basis of parathyroid hormone receptor signaling and trafficking: a family B GPCR paradigm. Cellular and Molecular Life Sciences, 68: 1–13.
6. Rodriguez, M.; Salmeron, M.; Martin-Malo, A.; Barbieri, C.; Mari, F.; Molina, R., *et al*. (2016). A new data analysis system to quantify associations between biochemical parameters of chronic kidney disease-mineral bone disease. PLoS One, 11(1).
7. Csukasi, F.; Bosakova, M.; Barta, T.; Martin, J. H.; Arcedo, J.; Barad, M., *et al*., (2022). Skeletal diseases caused by

mutations in *PTH1R* show aberrant differentiation of skeletal progenitors due to dysregulation of DEPTOR. Frontiers Cell Developmental Biology, 10

8. Huang H. (2018). Matrix Metalloproteinase-9 (MMP-9) as a Cancer Biomarker and MMP-9 Biosensors: Recent Advances. Sensors, 18(10): 3249.
9. Vilariño-Güell, C.; Miles, L. J.; Duncan, E. L.; Ralston, S. H.; compston, J. E.; Cooper, C., *et al*. (2007). *PTHR1* polymorphisms influence BMD variation through effects on the growing skeleton, Calcified Tissue International, 81(4): 270–278.
10. Duncan, E. L.; Brown, M. A.; Sinsheimer, J.; Bell, J.; Carr, A. J.; Wordsworth, B. P. *et al*. (1999). Suggestive linkage of the parathyroid receptor type 1 to osteoporosis. Journal of Bone and Mineral Research, 14(12): 1993–1999.
11. Wynne, F.; Drummond, F. J.; Daly, M.; Brown, M.; Shanahan, F.; Molloy, M. G., *et al*. (2003). Suggestive linkage of 2p22-25 and 11q12-13 with low bone mineral density at the lumbar spine in the Irish population. Calcified Tissue International, 72(6): 651–658.
12. Zhang, Y. Y.; Liu, P. Y.; Lu, Y.; Xiao, P.; Liu, Y.; Long, J., *et al*. (2006). Tests of linkage and association of *PTH/PTHrP* receptor type 1 gene with bone mineral density and height in Caucasians. Journal of Bone and Mineral Metabolism, 24(1): 36–41.
13. Florencio-Silva, R.; Sasso, G.; Sasso-Cerri, E.; Simoes, M. and Cerri, P. (2015). Biology of Bone Tissue: Structure, Function, and Factors That Influence Bone Cells. BioMed Research International, 421746.
14. Vrieze, E.; Sharif, F.; Metz, J. R.; Flik, G. and Richardson, M. (2011). Matrix metalloproteinases in osteoclasts of ontogenetic and regenerating zebrafish scales. Bone, 48: 704–712.
15. Tominaga, N.; Yonaha, T.; Yamanouchi, M.; Sumi, H.; Taki, Y.; Shibagaki, Y., *et al*. (2021). Bone responsiveness to parathyroid hormone is negatively associated with parathyroid hormone-lowering drug use in patients undergoing hemodialysis: a cross-sectional study. BMC Nephrology, 22:275
16. Bolton, C. E.; Stone, M. D.; Edwards, P. H.; Duckers, J. M.; Evans, W. D., *et al.* (2009). Circulating matrix metalloproteinase-9 and osteoporosis in patients with chronic obstructive pulmonary disease. Chronic Respiratory Disease, 6(2): 81-7.
17. Kim, K.; Punj, V.; Kim, J-M.; Lee, S.; Ulmer, T. S.; Lu, W., *et al*. (2016).MMP-9 facilitates selective proteolysis of the histoneH3 tail at genes necessary for proficient osteoclastogenesis. Genes Development, 30(2): 208–19.
18. Sabry, M.; Mostafa, S.; Rashed, L.; Abdelgwad, M.; Kamar, S. and Estaphan, S. (2021). Matrix metalloproteinase 9 a potential major player connecting atherosclerosis and osteoporosis in high fat diet fed rats. PLoS One, 16(2): 0244650.
19. Civitelli, R. and Ziambaras, K. (2011). Calcium and phosphate homeostasis: concerted interplay of new regulators. Journal of Endocrinological Investigation, 34(7): 3–7.
20. Al-Mahdawi, F. K. I.; Sultan, A. S. and Al-Wandi, N. (2018). Evaluation parathyroid hormone function and some minerals in chronic renal failure. Journal of Pharmaceutical Sciences Research, 10(1): 96–99.