RESEARCH ARTICLE

Genotype Combination of rs1042044 and rs6458093 in *GLP-1R* as A Genetic Risk for Osteoporosis in Postmenopausal Iraqi Women

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Received date: Jul 21, 2025; Revised date: Aug 26, 2025; Accepted date: Sep 11, 2025

Abstract

BACKGROUND: Many genetic factors are known to be related to osteoporosis, and currently the role of the glucagon-like peptide-1 receptor (*GLP-1R*) gene in bone health has been studied intensively. Some variation of this gene, such as rs1042044 and rs6458093, are known to be linked to metabolic diseases and lower bone mineral density, however their specific contribution to osteoporosis remains largely unexplored. Therefore, this study was conducted to investigate the combined genotypic effect of rs1042044 and rs6458093 as a genetic risk factor for osteoporosis in postmenopausal Iraqi women.

METHODS: Blood samples from 75 osteoporosis patients and 75 healthy controls, aged 45-85, were collected. DNA was extracted, and a region of *GLP-1R* gene was amplified by polymerase chain reaction (PCR) and sequenced using the Sanger method to identify polymorphisms. Serum parathyroid hormone (PTH) levels were also measured with chemiluminescent immunoassay (CLIA) methods.

RESULTS: Significant differences were observed for age, menopausal age, and PTH levels ($p<0.001$), but not for Body Mass Index (BMI). While individual SNPs (rs1042044 and rs6458093) showed no significant association with osteoporosis, a specific genotype combination of rs1042044 A and rs6458093 G was found to be a highly significant risk factor for the disease (OR=21.85, $p=0.026$). Linkage Disequilibrium analysis showed a D' value=0.85 and $R^2=0.45$ between the two SNPs.

CONCLUSION: Co-occurrence of the 'A' allele at rs1042044 and the 'G' allele at rs6458093 creates a highly susceptible genetic risk factor for osteoporosis, highlighting a potential novel biomarker for disease risk and providing a benchmark for future studies.

KEYWORDS: osteoporosis, postmenopausal, GLP1R, PTH, SNPS

Indones Biomed J. 2025; 17(5): 426-35

Introduction

Osteoporosis, defined by decreased bone mineral density (BMD) and microarchitectural degradation of bone tissue, markedly elevates fracture risk, especially in women who have gone through menopause.(1) The frequency of lower BMD is rising in the Middle East and North Africa (MENA) area, mostly attributable to aging populations, unhealthy habits, and insufficient information regarding bone health.(2)

Postmenopausal women encounter distinct issues regarding bone health, primarily due to the hormonal alterations linked to menopause.(3) Several risk factors for osteoporosis in postmenopausal women include estrogen insufficiency, age, familial genetic factors, smoking, metabolic illnesses, and pharmacological adverse effects.(4) Yet, osteoporosis is mostly caused by estrogen insufficiency. Following menopause, the ovaries produce less estrogen, starving the body of a versatile hormone that regulates the structure of bone by acting on osteoblasts (bone-forming cells) and osteoclasts (bone-resorbing cells).(5,6) Estrogen

plays a crucial role in the processes of bone development, fracture repair and maintaining bone health by improving the transformation of stem cells (primarily mesenchymal cells) into mature osteoblasts, which are specialized cells responsible for forming new bone tissue.(7) Yet, the hindering of bone resorption is accomplished by the effect of estrogen that suppress the formation of osteoclast and activate their apoptosis; therefore, osteoanabolic and anti-osteoclastic actions are diminished in the woman's body when estrogen levels are low, which results in continuous bone loss and destruction.(8)

Osteoporosis is a complex condition; however, genetic factors are among the most important risk factors of developing postmenopausal osteoporosis (PMOP). (9) Despite the identification of over 500 loci linked to osteoporosis through genome-wide association studies (GWAS), a deeper understanding of the underlying molecular mechanisms is still needed to identify new PMOP targets and biomarkers.(10,11) Furthermore, it has been found that osteoporosis is associated with a large number of other single nucleotide polymorphisms (SNPs). (12) The glucagon-like peptide-1 receptor (*GLP-1R*) plays a significant role in several physiological processes, particularly in glucose metabolism and insulin secretion. (13) However, recent studies have explored its effects beyond glucose homeostasis, including its potential role in bone metabolism and osteoporosis.(14,15) The *GLP-1R* is expressed not only in pancreatic cells but also in bone cells, including osteoblasts (bone-forming cells) and osteoclasts (bone-resorbing cells).(16) A study investigates the role of *GLP-1R* signaling in osteoclasts and its potential effects on inhibiting osteoclast-genesis and reducing bone resorption. It provides evidence that *GLP-1R* activation can suppress osteoclast activity, leading to a reduction in bone loss and potentially contributing to bone preservation.(17) Variations in the *GLP-1R* gene (genetic polymorphisms) are still in early stages but may influence how the receptor functions and impacts bone metabolism. Some polymorphisms may enhance or impair *GLP-1R*'s effects on bone density.(18) Several *GLP-1R* SNPs was associated with bone health, including rs1042044, which has pro and contra regarding its effect on BMD and osteoporosis. For example, one study found that polymorphism of rs1042044 in *GLP-1R* has no effect on BMD (18), while another study found that the rs1042044 could be important in determining individual susceptibility to bone diseases like osteoporosis (19). However, polymorphism of rs1042044 has been related to other disease such as an increased risk of papillary thyroid cancer, and increase risk factor of gastrointestinal diabetes. (20) Meanwhile, another *GLP-1R* SNP, rs6458093, is known for its association with gestational diabetes mellitus (GDM) (21), which is similar with rs1042044 that is still related with metabolic disease.

Study with similar concept has also rarely been conducted in Iraq. With reference to what was mentioned above, the inconsistency of some studies regarding the relation of rs1042044 and rs6458093 with BMD; therefore, this study was performed to investigate the combined effect of these two polymorphisms (rs1042044 and rs6458093), rather than their individual effects, and whether or not they have any association with osteoporosis risk, especially in postmenopausal Iraqi women.

Methods

Subject Recruitment and Blood Sample Collection

Blood samples from 150 women, including 75 osteoporosis patients and 75 healthy controls, were collected since January 2024 to January 2025 from the government and private laboratories at Baghdad, Iraq. Osteoporosis subjects, aged between 45-85 years, were diagnosed with DEXA scan technique. While control subjects were chosen from healthy women with same age group. The blood sample taken from each subjects was divided into two parts; one of which was placed in a gel tube (free of anticoagulant) to obtain blood serum for the test of parathyroid hormone (PTH), and the other part was placed in a tube containing anticoagulant for DNA extraction. The protocol of this study was given by The Council of The Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq (No. 28S, 4/4/2024), and all study participants agreed to follow the study procedures.

DNA Extraction and Quantification

The DNA extraction was done by using DNeasy QIAamp DNA Blood Kit (QiAGEN, Hilden, Germany). A lysis buffer and Proteinase K were added to a blood sample to break open the cells and digest proteins. The resulting solution, called a lysate, was then loaded onto a DNeasy spin column. During centrifugation, the DNA was selectively bound to the column's membrane, while contaminants passed through. Remaining contaminants and salts were removed during a washing step that used an alcohol-based solution. Finally, the purified DNA was eluted from the spin column with AE buffer (10 mM Tris-Cl and 0.5 mM EDTA). Each DNA sample was quantified using qubit fluorometer Applied Biosystem (Waltham, MA, USA).

Polymerase Chain Reaction (PCR) Analysis and Genotyping of a Region within the *GLP-1R* Gene

A set of primers was designed to amplify a 775 bp region of the *GLP-1R* gene, with a forward primer (F) 5′-AAGCAGATAAAGTCCTTAGCA-3′ and a reverse primer (R) 5′-CTCCTCTTGATGGTGATGTG-3′. All reagents, including the PCR master mix, nuclease-free water, and primers, were purchased from New England Biolabs (Ipswich, MA, USA). The primers were prepared at a working concentration of 10 pmol/µL from a 100 pmol/µL stock solution, following a 10-fold dilution.(22) The optimal primer annealing temperature was determined to be 55 °C using the New England Biolabs online tool.

PCR was performed using an Applied Biosystems thermal cycler to generate a suitable product for DNA sequencing. To confirm successful amplification and the correct product size, the PCR product was evaluated by gel electrophoresis. A 1% agarose gel was prepared, and a 100 bp DNA ladder (New England Biolabs) was used to determine the size. The gel was run at 80 V for 80 minutes, and the presence of a single, distinct band of the expected size was confirmed under UV light.

Genotyping of the amplified region was accomplished using the Sanger sequencing method. After amplification, the PCR products were purified to remove excess that might interfere with the sequencing. Sequencing was performed in one direction on an ABI3730XL platform (Macrogen Corporation, Seoul, Korea), and the resulting sequences were analyzed using Geneious Prime software (Geneious, Auckland, New Zealand).

Estimation of PTH

Since increase PTH were known to accelerates bone loss and reduces BMD, therefore in this study, the measurement of PTH was performed using Diasorin LIAISON XL (Saluggia, Italy), which involved a fully automated, two-step chemiluminescent immunoassay (CLIA) method.(23) First, the serum was isolated from blood by centrifugation, then loaded onto the LIAISON XL analyzer along with the PTH reagent integral and calibrators. The instrument automatically pipetted samples and reagents, allowing PTH in the sample to bind to a solid-phase antibody and then to a labeled antibody, forming a "sandwich." After washing away the unbound material, starter reagents were added to generate a light signal directly proportional to the PTH concentration. The instrument measured this signal and calculated the PTH level using a built-in calibration curve. Quality control samples were run concurrently to ensure assay accuracy, and the final PTH results were reported.

Statistical Analysis

Several software and online tools were used in the statistical report of this study, the first was IBM SPSS version 28.0 (IBM Corporation, Armonk, NY, USA) to calculate mean, SE of mean, probability via student t-test, and second was WinEpi version 11.65 (http://www.winepi.net/uk/index.htm) to calculate the fisher's exact probability, odds ratio (OD), and 95% confidence intervals (CI), Pearson's chi-square for the categorical data; and third was SHEsis Plus online software (https://shesisplus.bio-x.cn/) to calculate Hardy-Weinberg equilibrium compatibility, genotypes' and alleles' construction, linkage disequilibrium (LD) and haplotype construction.(20)

Results

Characteristics of Osteoporosis and Controls Groups

A number of demographic and clinical characteristics were calculated for patients and controls, including age, menopausal age, body mass index (BMI) and PTH. The significant results were achieved with the variable of age ($p<0.001$) specifically at the age group 50–60 years ($p<0.001$). Other significant findings were menopausal age ($p<0.001$) specifically less than 10 years ($p<0.001$) and parathyroid hormone levels ($p<0.001$). While the result of BMI, no significant difference was recorded, as shown in Table 1.

PCR Amplification and Product Verification

As shown in Figure 1, a single, distinct band of the expected size 775 bp was observed for all samples, confirming successful amplification of the target gene region.

Genotype and Allele Frequency

Two single nucleotide polymorphisms were found in the sequence of 775 bp of *GLP-1R*. The first was rs1042044 (Leu260Phe) located on ch6:39073726, the second was rs6458093 (intron variant) located on chr6:39074049 as shown in Figure 2 and Figure 3, respectively.

Analysis of Hardy-Weinberg equilibrium indicated that the observed genotype frequencies were very close to the expected frequencies for both SNPs. This suggested that there were no significant factors like selection, mutation, migration, or non-random mating affecting these SNPs in a both groups (patients and control) that would cause a deviation from expected frequencies (Table 2). The distribution of genotypes and alleles in osteoporosis patients and control subjects, along with the chi-square (X^2) value,

Table 1. Characteristics of participants in the study.

Variables	Osteoporosis Subjects	Control Subjects	Probability (*p*-value)
Age, mean±SE (years)	60.04±0.81	52.21±0.81	<0.001*
<50	49.56±0.24	48.36±0.21	>0.05
50–60	56.16±0.50	52.67±0.54	<0.001*
61–70	65.07±0.47	65.20±1.16	>0.05
>70	73.0±0.78	73.75±0.95	>0.05
Menopausal Age, mean±SE (years)	48.73±0.28	47.28±0.25	<0.001*
<10	48.77±0.43	47.12±0.26	<0.001*
10–19	48.85±0.40	48.0±1.07	>0.05
≥20	48.0±0.79	48.75±1.11	>0.05
BMI, mean±SE (kg/m^2)	28.17±0.51	27.28±0.51	>0.05
18.5–24.9	19.93±0.90	21.05±0.67	>0.05
25–29.9	26.30±0.26	26.22±0.27	>0.05
≥30.0	31.15±0.33	30.73±0.33	>0.05
PTH, mean±SE (pg/mL)	56.22±1.60	43.06±1.88	<0.001*

*Statistically significant.

OR with 95% CI, and the p-value for each comparison were shown in Table 3.

Genetic Model Association of *GLP-1R* SNPs and Osteoporosis

None of the tested genetic models showed a statistically significant association (p<0.05 and 95% CI not crossing 1) between *GLP-1R* SNPs and osteoporosis. Although the dominant model in both SNPs had the lowest Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC), still the association was not significant as shown in Table 4.

The investigation the genetic predisposition to osteoporosis based on combinations of two specific SNPs: rs1042044 and rs6458093 was shown in Table 5. The "A-A" combination (rs1042044 A/rs6458093 A) serves as the reference genotype.

A genotype combination of (rs1042044 C/rs6458093 G) was found in 34.84% of the study population while a combination of (rs1042044 C/rs6458093 A) was less common, which occurred in 15.16% of individuals and both combinations were not considered as a risk at p>0.05. On the contrary, a combination of (rs1042044 A/rs6458093 G) was the least frequent (5 subjects, 3.16%). However, it showed a strong and statistically significant association with an increased risk of osteoporosis (OR=21.85; 95% CI: 1.50 - 318.42; p=0.026). This meant that individuals carrying this specific genotype combination had a substantially higher likelihood of having osteoporosis compared to the reference group.

Association of the rs1042044 and rs6458093 Haplotype with Osteoporosis

The results of a haplotype analysis, which examines combinations of alleles (haplotypes) from the two SNPs (rs1042044 and rs6458093) and their association with osteoporosis were shown in Table 6.

The haplotype AA (rs1042044 A/rs6458093 A) frequencies in both groups were very similar (0.48 in cases, 0.466 in controls) with no significant association at adjusted and unadjusted p-values were greater than 0.05. Haplotype CG (rs1042044 C/rs6458093 G) showed a trend towards an increased risk of osteoporosis with borderline statistically significant at the unadjusted level (Fisher's p=0.069, Pearson's p=0.053), but it did not reach statistical significance after correcting for multiple comparisons in which p<0.05 (*e.g.*, Holm=0.15, FDR_BH=0.071). Haplotype CA (rs1042044 C/rs6458093 A) showed a trend

Figure 1. Agarose gel electrophoresis of PCR-amplified DNA from the *GLP-1R* gene. Lanes M contain a 100 bp DNA ladder. Lanes 1-10 show a single, distinct band of the expected size (~775 bp) in the amplified products.

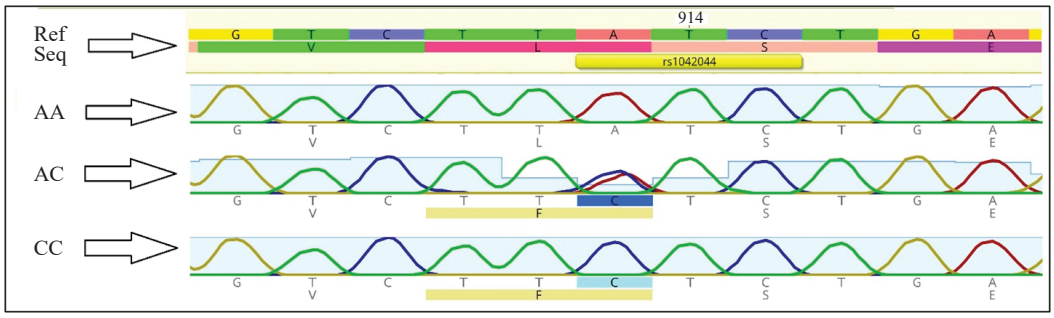

Figure 2. A chromatogram of DNA sequencing focusing on a region containing SNP (rs1042044) A>C with three genotypes (AA, AC and CC).

towards a protective effect against osteoporosis, but it did not reach statistical significance after correcting for multiple comparisons at the unadjusted level (Fisher's p=0.071, Pearson's p=0.05) and adjusted p-values were below 0.05 (*e.g.*, Holm=0.15, FDR_BH=0.071). The OR of 0.52 means individuals with this haplotype were roughly half as likely to have osteoporosis compared to the reference.

LD plots specifically showing the D' value between rs1042044 and rs6458093 which was found to be 0.85 (Figure 4A). Furthermore, the R^2 value was 0.45 (Figure 4B), signifying a moderate level of correlation between the alleles of rs1042044 and rs6458093. This suggested that while these SNPs were strongly linked, one did not perfectly predict the other's genotype, and that some independent variation exists.

Discussion

The current study results showed that significant differences are consistent with the known epidemiology of osteoporosis (Table 1). Age was a highly significant differentiating factor, with osteoporosis patients being significantly older than controls. This finding aligns with the progressive decline in BMD that occurs with aging, which increases susceptibility to osteoporosis and highlights the need for specific treatments to improve bone health and prevent fractures.(24)

Similarly, menopausal age was significantly different between groups, with osteoporosis patients having a higher mean menopausal age. This finding aligns with the well-established understanding that hormonal changes, particularly estrogen deficiency following menopause, accelerate bone loss.(25-27) The absence of significant differences in older menopausal duration groups (10-19 and ≥20 years) might reflect a plateauing of the menopausal effect or smaller sample sizes within those specific subgroups.

Notably, PTH levels were significantly higher in osteoporosis patients compared to controls. Elevated PTH levels can lead to increased bone resorption and are a known contributor to bone loss, especially in conditions like primary or secondary hyperparathyroidism.(28) This finding further supports the physiological mechanisms contributing to osteoporosis in our patient cohort which is correspondent to another previous study results that provided significant insights into the intricate mechanisms of PTH-induced as a risk of osteoporosis.(29)

While very low BMI is often considered a risk factor for osteoporosis, the results of this study did not find BMI

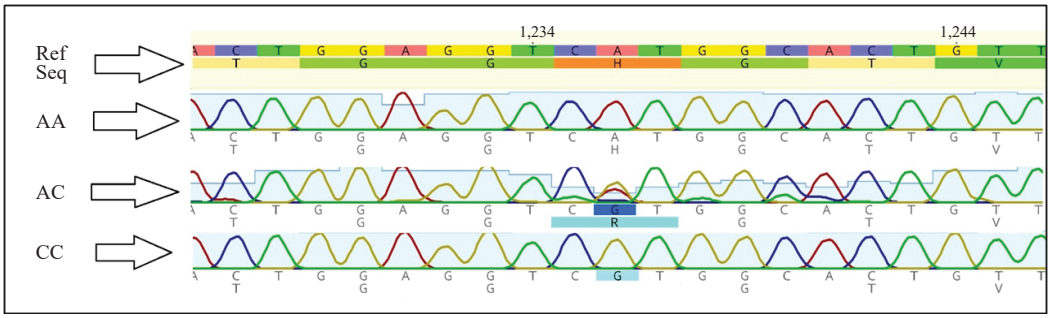

Figure 3. A chromatogram of DNA sequencing focusing on a region containing SNP (rs6458093) A>G with three genotypes (AA, AG and GG).

Table 2. Hardy-Weinberg equilibrium and genotype frequencies.

SNP		Osteoporosis Subjects (n=75)		Control Subjects (n=75)	
		Observed	Expected	Observed	Expected
rs1042044	AA	17 (22.6%)	17.8 (23.7%)	23 (30.6%)	19.8 (26.4%)
	AC	39 (52.0%)	37.5 (50.0%)	31 (41.3%)	37.5 (50.0%)
	CC	19 (25.4%)	19.8 (76.3%)	21 (28.1%)	17.8 (23.6%)
	HWE analysis	X^2=0.125; p>0.05		X^2=2.239; p>0.05	
rs6458093	AA	26 (34.6%)	25.8 (34.4%)	34 (45.3 %)	32 (42.6 %)
	AG	36 (48.0%)	36.4 (48.5%)	30 (40.0%)	34 (45.3 %)
	GG	13 (17.4%)	12.8 (17.1%)	11 (14.7 %)	9 (12.1 %)
	HWE analysis	X^2=0.007; p>0.05		X^2=1.025; p>0.05	

to be a distinguishing characteristic in this particular cohort. This might suggest that in our population, the effects of age, menopausal status, and PTH levels outweighed or masked any potential BMI effect, a finding that is consistent with studies confirming that BMI may not fully represent the actual fat effect.(30)

The current genetic analysis began by confirming that both rs1042044 and rs6458093 were in Hardy-Weinberg Equilibrium in both patient and control groups (Table 2), indicating that Iraqi women population is genetically stable and suitable for association studies. Direct analysis of individual SNP genotype and allele frequencies (Table 3) for rs1042044 and rs6458093 showed no statistically significant associations with osteoporosis. Although some findings suggested a trend, such as the rs1042044 AA genotype appearing to be a protective factor, the 95% CI consistently crossed 1, indicating no significant effect. These results are contrary to other studies that have investigated a significant correlation between rs1042044 and various biological effects.(20,31,32) This discrepancy may be due to differences in human populations' evolutionary histories, which lead to variations in allele frequencies across ethnic groups, or because a SNP's effect might be dependent on environmental exposures that differ between populations.(33)

The genetic model analysis (Table 4) showed dominant, recessive, codominant, and overdominant models for each SNP. Consistent with the individual genotype analysis, none of the tested genetic models demonstrated a statistically significant association with osteoporosis. While the dominant models for both SNPs showed the lowest AIC and BIC values, suggesting a better model fit, the lack of statistical significance indicates that neither SNP, when analyzed individually, acts as a strong independent predictor of osteoporosis in our cohort. Despite the lack of significant individual SNP associations, a crucial finding emerged from the combined genotype analysis (Table 5). There were a strong and statistically significant association between the rs1042044 A/rs6458093 G genotype combination and a substantially increased risk of osteoporosis. This unique

Table 3. Association analysis of *GLP-1R* (rs1042044 and rs6458093) and osteoporosis.

SNPs	Genotypes/ Alleles	Osteoporosis Subjects	Control Subjects	X^2	OR (95% CI)	*p*- value
rs1042044	AA	17 (22.7%)	23 (30.7%)	1.227	0.66 (0.32-1.37)	0.036
	AC	39 (52.0%)	31 (41.3%)	1.714	1.54 (0.81-2.92)	0.252
	CC	19 (25.3%)	21 (28.0%)	0.136	0.87 (0.42-1.79)	0.854
	A	73 (48.7%)	77 (51.3%)	0.213	0.90 (0.57-1.41)	0.729
	C	77 (51.3%)	73 (48.7%)	0.213	1.11 (0.71-1.75)	0.729
rs6458093	AA	26 (34.7%)	34 (54.3%)	1.778	0.64 (0.33-1.23)	0.243
	AG	36 (48.0%)	30 (40.0%)	0.974	1.38 (0.73-2.63)	0.411
	GG	13 (17.3%)	11 (14.7%)	0.198	1.22 (0.51-2.91)	0.824
	A	88 (58.7%)	98 (65.3%)	1.415	0.75 (0.47-1.20)	0.284
	G	62 (41.3%)	52 (34.7%)	1.415	1.33 (0.83-2.12)	0.284

Table 4. Genetic model analysis between *GLP-1R* SNPs and osteoporosis.

SNPs	Genotypes/ Alleles	Osteoporosis Subjects	Control Subjects	OR (95% CI)	*p*-value	AIC	BIC
rs1042044							
Codominant	AA	17 (22.7)	23 (30.7)	1.0	0.34	173.4	191.4
	AC	39 (52.0)	31 (41.3)	0.50 (0.20-1.27)			
	CC	19 (25.3)	21 (28.0)	0.65 (0.23-1.87)			
Dominant	AA	17 (22.7)	23 (30.7)	1.0	0.17	171.7	186.7
	AC/CC	58 (77.3)	52 (69.3)	0.55 (0.23-1.31)			
Recessive	AA/AC	56 (74.7)	54 (72.0)	1.0	0.98	173.5	188.6
	CC	19 (25.3)	21 (28.0)	1.01 (0.43-2.40)			
Over-dominant	AA-CC	36 (48.0)	44 (58.7)	1.0	0.22	172	187.1
	AC	39 (52.0)	31 (41.3)	0.62 (0.29-1.33)			
rs6458093							
Codominant	AA	26 (34.7)	34 (54.3)	1.0	0.42	173.8	191.9
	AG	36 (48.0)	30 (40.0)	0.61 (0.26-1.41)			
	GG	13 (17.3)	11 (14.7)	0.57 (0.19-1.71)			
Dominant	AA	26 (34.7)	34 (45.3)	1.0	0.19	171.8	186.9
	AG-GG	49 (65.3)	41 (54.7)	0.60 (0.27-1.30)			
Recessive	AA-AG	62 (82.7)	64 (85.3)	1.0	0.55	173.2	188.2
	AA	13 (17.3)	11(14.7)	0.57 (0.19-1.71)			
Over-dominant	AA-GG	39 (52.0)	45 (60.0)	1.0	0.40	172.8	187.9
	AG	36 (48.0)	30 (40.0)	0.72 (0.34-1.23)			

finding suggests that while each SNP individually might not confer significant risk, their co-occurrence creates a highly susceptible genetic background for osteoporosis. The very high OR, although accompanied by a wide CI due to its low frequency, indicates a substantial risk increase for individuals with this specific genotype, which highlights the importance of analyzing interacting genetic loci rather than individual SNPs in isolation, a concept that has been proven in studies of other complex diseases such as cancer and diabetes. (18,32,34) In contrast, other combined genotypes, such as rs1042044 C/rs6458093 G and rs1042044 C/rs6458093 A, did not show statistically significant associations, though the former hinted at a protective trend and the latter at an increased risk trend.

The results of current haplotype analysis (Table 6), examining co-inherited allele combinations, largely mirrored the findings from the individual SNP and genetic model analyses. The most common haplotype, AA (rs1042044 A/rs6458093 A), showed no significant association with osteoporosis. The CG haplotype (rs1042044 C/rs6458093 G) displayed a trend towards increased risk (OR=1.599), and the CA haplotype (rs1042044 C/rs6458093 A) a trend towards a protective effect (OR=0.52). However, after applying multiple comparison corrections, none of these trends reached statistical significance. It is important to reconcile the very strong genotype combination association (Table 5) with the non-significant haplotype findings. Haplotype analysis typically considers alleles on the same

Table 5. Association of rs1042044 and rs6458093 genotype assortments with a risk of osteoporosis.

rs1042044	rs6458093	Frequency	Percentage	OR (95% CI)	*p*-value
A	A	70	0.4684	1.00	-
C	G	52	0.3484	0.57 (0.30 - 1.06)	0.08
C	A	23	0.1516	1.87 (0.78 - 4.50)	0.16
A	G	5	0.0316	21.85 (1.50 - 318.42)	0.026

Table 6. Association of *GLP-1R* SNPs (rs1042044 and rs6458093) haplotypes with the risk of osteoporosis.

Haplotype	Case (Frequency)	Control (Frequency)	Chi^2 (p-value)	Fisher's (p-value)	Pearson's (p-value)	OR [95% CI]	Holm	SidakSS	SidakSD	FDR_BH	FDR_BY
AA	72 (0.48)	70 (0.466)	0.053	0.907	0.817	1.054 [0.67~1.659]	0.817	0.998	0.817	0.817	1
CG	61 (0.406)	45 (0.300)	3.734	0.069	0.053	1.599 [0.991~2.578]	0.150	0.196	0.143	0.071	0.148
CA	16 (0.106)	28 (0.186)	3.835	0.071	0.05	0.52 [0.268~1.008]	0.150	0.186	0.143	0.071	0.148

chromosome, while the highly significant "A-G" genotype combination suggests that the risk might stem from having specific alleles at these two loci, regardless of whether they are on the same chromosome or on opposite chromosomes. The LD analysis between the two SNPs provides crucial context for this finding.(35) A high D' value of 0.85 indicates a strong historical linkage, which supports the rationale for performing haplotype analysis and investigating combined genotype effects. However, the R^2 value of 0.45 signifies only a moderate correlation, suggesting that while the SNPs are linked, each may contribute independently or tag distinct, but correlated, genetic variations.(36,37) This moderate R^2 helps to explain why analyzing the specific two-SNP genotype combination yielded a highly significant result, even when individual SNPs and broader haplotypes did not. A conclusive link between GLP-1R gene SNPs and postmenopausal osteoporosis has yet to be established and requires further investigation.(38)

The current study has several limitations that should be considered. First, our sample size is relatively small, which, while sufficient to detect a significant association, may have limited the statistical power to identify other effects and resulted in wide CI for our OR. Second, while this study provides a novel insight into the genetic risk of osteoporosis in this previously unstudied population, its findings may not be generalizable to other ethnic groups due to variations in genetic backgrounds and allele frequencies. Third, the exact biological mechanism by which this combination confers risk remains to be elucidated. Therefore, a replication of this study in a larger and more diverse cohort would be crucial to confirm our findings and test their generalizability. Future studies should also consider performing functional analyses to investigate how the rs1042044 A and rs6458093 G alleles, either individually or in combination, affect GLP-1R gene expression or protein function. Finally, given the complex nature of osteoporosis, subsequent research should incorporate a broader range of demographic and lifestyle factors to investigate potential gene-environment interactions.

Figure 4. LD plots results. A: represent D' (D prime) value of 0.85; B: represent R^2 (R-squared) value of 0.45 (where SNP1 is rs1042044 and SNP2 is rs6458093).

Conclusion

In conclusion, the specific genotype combination of rs1042044 A and rs6458093 G emerged as a significant genetic risk factor, highlighting the potential importance of gene-gene interactions in osteoporosis susceptibility. This association, despite the moderate linkage disequilibrium between the two SNPs, suggests a complex genetic architecture involving these loci.

Acknowledgments

The researchers extend their thanks and appreciation to all participants in this research, both patients and healthy individuals, and we wish the patients a speedy recovery.

Authors Contribution

AKA was involved in concepting and planning the research, FA and AQA performed the data acquisition/collection calculated the experimental data and performed the analysis, AKA drafted the manuscript, designed the figures and aided in interpreting the results. All authors took parts in giving critical revision of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest or competing interests related to the content of this manuscript.

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