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Evaluation of ZBTB40 Rs6426749, BMD and BMI in Iraqi women with osteoporosis

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Varieties within the genomic region of 1p36.12 have already been linked with osteoporosis in genome-wide association studies (GWASs), even though the functional implications behind these non-coding variants remain uncertain. We highlighted rs6426749 as a probable causative variant for osteon at 1p36.12 predicated on comprehensive genomic analyses and epigenetic evaluations. For sampling, fifty (50) patients with osteoporosis were admitted to Baghdad Teaching Hospital, and 40 healthy subjects were performed in Baghdad Teaching Hospital/Bone Density Screening Unit/ Baghdad - Iraq between September 2020 January 2021. We used nested PCR of the Genome-wide association studies of the primers designed by the primer3 program. For rs6426749 G/C genotyping, The results of osteoporosis patients (women) and the healthy group was studied according to bone mineral density. The results showed a significant difference between the two study groups in bone mineral density and body mass index. The mean of patient groups was mean±SD (0.6824±0.08285), and control groups were mean±SD (1.066±0.06876). Then we divided the patients into three groups according to their age, and from the age of 30 to 44, they were called pre-menopause, from the age of 45 to 55, they were called Menopause, and from the age of 56 to 70, they were called Post menopause, and we compared these three groups with the control. The results showed that there was statistically significant P-Value <0.0001 differences.

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KEYWORDS

Osteoporosis; Osteoporosis; BMD; BMI.

Introduction

Osteoporosis is a disease characterized by a reduction in bone mass and disruption of bone architecture, leading to impaired skeletal strength and increased susceptibility to fractures [1]. It is a significant public health problem. It is estimated to affect 200 million women worldwide and causes more than 8.9 million fractures annually [2,3]. Family history remains the strongest risk factor for the development of osteoporosis, and studies in animal models reinforce that this is a complex genetic disease [7,8]. At the other extreme, other sites were associated with markers that are not proven to play a role in bone biology. For particular, an unprecedented growth BMD GWAS[9] discovered 1p36.12, which many Snips Macro then reported. ZBTB40 (MIM: 612106), their next chromosome, seems to be more about 61 kb removed. It contains no recognized purpose or relation to bone chemistry [10, 11]. We then used reverse genetic methods to confirm that the previously under-characterized gene, ZBTB40, played a role in

osteoblast mineralization ex vivo. This work revealed that our methods efficiently find genes that impact osteoblast function [12].

Experimental method

Subjects and methods

Patients group

Fifty patient women with osteoporosis were admitting Baghdad Teaching Hospital with osteoporosis and 40 healthy populations. It Was conducted in Baghdad Teaching Hospital/Bone Density Examination Unit/in Baghdad-Iraq from September 2020 to January 2021.

Control group

Forty healthy volunteers. All patients and control groups were from the same ethnic group (Arabic).

Collection of the blood samples

10 mL of blood were obtained from each subject by vein puncture, 2 mL as put into ethylene damien tetra acetic acid (EDTA) tubes. The remaining 8 mL was pushed slowly into disposable tubes containing separating gel. Blood in the EDTA tubes was stored In -40 ˚C (deep freeze) to be used later in the genetic part of the study. In contrast, blood in the gel-containing tubes was allowed to clot at room temperature for 30 minutes and then centrifuged at 2000×g for approximately 15 minutes. Then, the sera were obtained and stored at -20 ˚C until analysis.

The criteria used to diagnose patients with osteoporosis

The selection of patients with osteoporosis was according to the bone mineral density (BMD) measurement using dual-energy X-ray absorptiometry (Dexa) machine in Baghdad teaching hospital in bone density examination unit. Osteoporosis was diagnosed by BMD at the spine for all patients. BMD is a static measure of bone composition, reflecting its “history” and detectable changes that take an extended period (years). The science of double X-ray project (Dexa) vertebrae measuring is being applied to establish an osteoporosis diagnostic test, forecast possible cod risk, AND follow clients by performing host controller judgments. Spatial and temporal bum is measured by weighing metal per cubic centimeter checked (gr/cm^2). Also, as a relation to the conceptual framework was developed. The estimated BMP for the child's age and gender (Z-Score) and (Young Normal) adults of the same-sex (T-Score).

Exclusion criteria

Patients with a history of bone disease, metabolic or endocrine disorders such as diabetes mellitus hyperthyroidism. Hyperparathyroidism, renal disease, liver disease, medications known to affect bone metabolism (E.G. anticonvulsants, corticosteroids, heparin sodium) were excluded, as shown in Table 1.

TABLE 1 The World Health Organization (WHO) classification of Osteoporosis

Normal	**BMD:** Is Within 1 SD of a Young Normal Adult (T-Score At -1.0 and Above)
Osteopenia	**BMD:** Is Between 1.0 and 2.5 SD Below That of a Young Normal Adult (T-Score Between -1.0 And -2.5)
Osteoporosis	**BMD:** Is 2.5 SD or More Below That of a Young Normal Adult (T-Score At or Below -2.5). Patients in This Group Who Have Already Experienced one or More Fractures are Deemed to Have Severe or Established Osteoporosis

Molecular analysis

DNA extraction

1. Collecting Blood
Collect whole blood in an anticoagulant tube (an EDTA tube is preferred) under sterile conditions (if used in the future). Ensure that the blood sample is at room temperature before beginning the protocol.
For frozen blood: to 200 µL of frozen blood pellet (kept on ice), add 200 µL of lysis solution (c1) (ds0010). The pellet with continuous pipetting and step 2 for proteinase k and rinse a treatment (optional). Incubate at 55 °C for 10 min and then proceed to step 4 of the protocol.
2. Add 20 µL of the Proteinase K solution (20 mg/mL) (DS0013) Into 2.0 mL Capped collection tube containing 200 µL of the whole blood. Vortex for 10-15 seconds to Ensure thorough mixing.
3. Lysis reaction add 200 µL of the lysis solution (c1) (ds0010) to the sample vortex thoroughly for a few seconds to obtain a homogenous mixture. Incubate at 55 °C for 10 min.
4. Prepare for binding; add 200 µL of ethanol (96-100%) to the lysate obtained from the above step to prepare lysate for binding to the spin column. Mix thoroughly by gentle pipetting.
5. Load Lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]
Transfer the lysate obtained from step 4 into the spin column provided centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 min. Discard the flow-through liquid and place the column in the same 2.0 mL assortment cylinder.
6. Prepare
(Make the mild detergent liquid as per the basic preparing directions.) In a spun, 50 mL of concentrated thoroughly rinse solutions were added to the column and centrifuge for 10 s at 6,500 X G (10,000 RPM). Re-use the same collection tube with a row before removing.
7. Rinse
(Make the washer fluid as per the basic procedure procedures)
To dry its line, l of dilution dissolution medium & centrifuged at 12,000-16,000 X G (13,300-16,000 RPM) or 15 seconds was more gently. If excess methanol is detected, discard the flow-through liquid and spin the blank article for a further minute at the same pace. Remove the columnar from the jet over the fluid filter flask and place it in a new uncapped 2.0 mL collection tube.
8. DNA elution
Peristaltic pump 120 L of eluted (ET) into the row without overflowing to the edges. Start growing at room temperature (15-25 °C) for 1 min. To evaporate your material quickly, centrifuged at 6000 X G (10,000 Mph) for 30 s. For such a good yield of genetic material, steps were repeated, yet approximately 140 eluted (ET).

Agarose gel electrophoresis

Agarose gel electrophoresis was adopted to confirm the presence of extracted DNA.

Preparation of agarose

The material used to prepare of gel electrophoresis is: 1X TBE buffer, loading dye, ethidium bromide (10 mg/mL).
1- 40 mL of 1X TBE was taken in a beaker.
2- 0.4 gm (for 1%) agarose is added to the buffer.
3- The solution was heated to boiling (using Micro Wave) until all the gel particles were dissolved.
4- One µL of ethidium bromide (10 mg/mL) was added to the agarose.
5- The agarose was stirred to get mixed and to avoid bubbles.
6- The solution was allowed to cool down at 50-60 ° C.

DNA loading

2 µL of loading dye were applied to each 5 µL DNA sample, and samples were added

carefully to the individual wells. Electrical power was turned on at 100 v/mAmp for 75 min. DNA moves from cathode to plus anode poles.

Nested-PCR application

For replicating a point to the point of a polymorphic transcription factor or generating a cDNA duplicate of an mRNA presence at extremely low frequency in a clinical material comprising a permanent group of cellular processes, nested polymerase chain reaction (PCR) is performed. Two successive PCR reactions are commonly used in PCR study

Human rs6426749 G/C genotyping

The primers designed by program For rs6426749 G/C genotyping, a set of primers was shown in Table 2.

TABLE 2 A set of rs6426749 G/C

Primer	Sequence	Temperture °C	GC%	Product Size
Forward Primer inner	5´GAATTCATACTGGCTGCTGTGC´3	62.7	50	225/153
Reverse Primer inner	3´AGTTGTCCATTGGCCTGCAC´5	62.4	55	225/153
Forward Primer outer	5´CCATGCCTTAGTTTCTCCATCTGT´3	62.9	45.8	336
Reverse Primer outer	3´AAGCTTCAGTTTTCTGGCATGAGT´5	61.2	41.7	336

Reconstituting and diluting primers

1. Titer plate, enhance, blend in the subsequent command

- Master mix 10 μL
- Outer forward 1 μL
- Outer reverse 1 μL
- Template DNA 3 μL
- H_20 5 μL

2. Topping yours reflects a balance with such a drop (20 L) of light paraffin if the heating kilter does not have a warmed lid.

3. Insert the vials or the tiny titer platform in the hot pulsar using hot start elaboration. Use the denaturation, annealing, and polymerization timings and degrees given in Table 3 to complete another stage of loudness.

4. Cast aside a fraction (5 to 10 L) again from the process for more evaluation

5. Make the second round of amplification, combine the right elements inside a hygienic 0.5-mL narrow acceleration pipe or microscopic assay dish well:• Master mix 10 μL

- Inner forward 1 μL
- Inner reverse 1 μL
- DNA PCR product 1 μL
- H_2O 7 μL

TABLE 3 nested-PCR cycling program

Steps	Temp. (°C)	Time sec	Cycle
Initial Denaturation	95	60	
DNA Denaturation	95	20	35cyc
Annealing	62	20	
Extension	72	20	

Note: put it in the machine on the fifth cycle, turn it off, and start adding 1 micro of a mixture of outer forward and outer revers.

6. If a thermal process called mitosis does not have a warm cover, add 1 mL (state and) of light mineral oil to the starting materials.
7. Within the heat reverse transcription system, insert the tubes or the microtiter plate. Use the denaturation, annealing, and polymerization times and temperatures listed in the adjacent Table 4 again for the next round and replication:

TABLE 4 Nested-PCR cycling program

Steps	Temp. (°C)	Time sec	Cycle
Initial denaturation	95	60	40
DNA denaturation	95	20	
Annealing	58	20	
Extension	72	20	

8. Chromatography polyacrylamide gel to evaluate data (5–10 L) from that (from Steps 4) nor third waves of replication.

Results and discussion

Distribution of osteoporosis patients and control group according to bone mineral density (BMD)

The results of osteoporosis patients (women) and the healthy group were studied according to bone mineral density (Table 5 and Figure 1). The results showed significant differences in the association between the two study groups in bone mineral density. The mean of patient groups was mean±SD (0.6824±0.08285), and control groups were mean±SD (1.066±0.06876). Then, we divided the patients into three groups according to their age, and from the age of 30 to 44, they were called pre from the age of 45 to 55, they were called Meno, and from the age of 56 to 70, they were called a post, and we compared these three groups with the control. The results showed statistically significant P-Value <0.0001differences between the three study groups in bone mineral density with healthy controls Table 6 and Figure 2. The mean of patient groups for pre was mean±SD (0.6693±0.08243), and control groups were mean±SD (1.066±0.06876), the mean for meno was mean±SD (0.6925±0.07379) and control groups were mean±SD (1.066±0.06876) and mean of patient groups for the post was mean±SD (0.6760± 0.09626), and control groups were mean±SD (1.066±0.06876). Previous studies showed osteoporosis group demonstrated higher BMD values at each site decreased with increasing age for men and women aged 40 years and older. However, lumbar spine BMD values increased with age for women older than 75 years. After adjustment for demographic variables, BMD values at the lumbar spine, femoral neck, and total femur decreased by 0.129 g/cm^2, 0.151 g/cm^2, and 0.137 g/cm^2, respectively [15].

TABLE 5 Distribution of osteoporosis patients and control groups according to bone mineral density (BMD)

Variable	Study Groups Mean ± SD		P Values
	Patients	Control	
BMD	0.6824±0.08285	1.066±0.06876	0.0001**

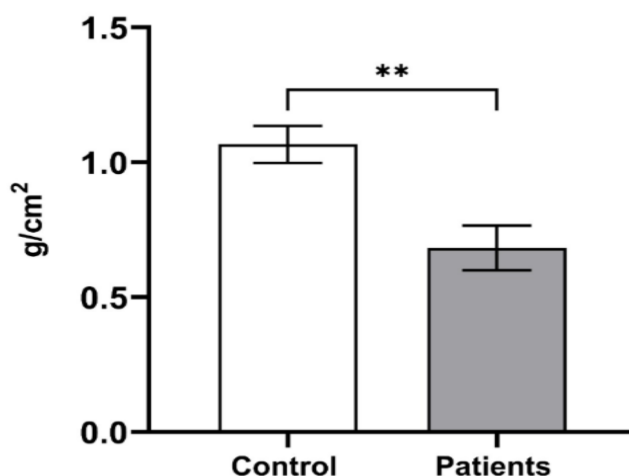

FIGURE 1 Distribution of osteoporosis patients and control groups according to bone mineral density (BMD)

TABLE 6 Distribution of osteoporosis patients groups of three with control groups according to bone mineral density (BMD)

Groups	Age	No.of	mean±SD	P value
Control	30 to 50	40	33.67 ± 7.031	0.0001**
Pre menopause	30 to 44	9	26.51±5.034	0.0001**
Menopause	45 to 54	23	29.53± 5.368	0.0001**
Post menopause	55 to 70	18	28.51±4.108	0.0001**

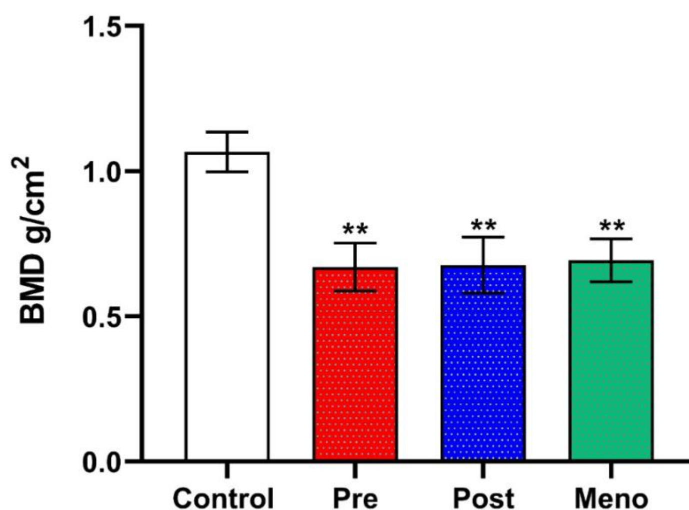

FIGURE 2 Distribution of osteoporosis patients groups of three with control groups

The outcomes for osteoporosis (women) and the healthy group were studied, bestowing the body frame catalog, as shown in Table 7 and Figure 3. The results exposed momentous variances flanked by the two training assemblages in the build quantity guide. Patient groups were mean ± SD (28.62 ± 4.913), and the control groups were the mean ± SD (33.67 ± 7.031). We also compared the three groups of patients. Results revealed a significant difference in P-value <0.0001differences between the three study groups in body mass index with healthy controls, as shown in Table 8 and Figure 4. The mean of patient groups for pre was mean's (26.51±5.034), and control groups

were means (33.67±7.031), the mean for men was mean±SD (29.53± 5.368), and control groups were meanness (33.67±7.031), and mean of patient groups for the post was mean SD (28.51±4.108), and control groups were mean±SD (33.67±7.031). Previous studies would include 313 menopausal ladies. We compared the age of patients in the various data analysis clusters based on their Weight. The maturity level of the control group (with such a Bag of 25), which consisted of ten cases out of a population of 313, is 46 to 87 years, with a mean SD of (58.910.10). Other groupings' age group and mean. The findings of the analysis comparing case subgroup (BMI 25) and control subjects (BMI 25) indicate that there have been no clinically significant variations in genders or average ± confidence interval (mean SD) across the 2 sets. Furthermore, there are still no variations in the Patients with osteoporotic value amongst patient categories (obese, obesity, or deadly prevalence of obesity) [16]. A multivariable assessment was done to eliminate misleading variables. In this model, ethnicity, age, and smoking were not connected to the bed. The only characteristics that remained uniquely related to bone loss are age since menstruation. It has been proven that having a higher BMI is protective (OR 14.80, p 0.001). Obese patients (BMI > 30) had a 92 percent lower risk of osteoarthritis, and fat women (Mass index 14–25–30) used to have 63 % less likely to die. The number of years due to hormonal changes is discovered to be a fracture risk factor (OR 14.04, p 0.001). Whenever the risk was broken down into subgroups, it rose after 20 years after menopause and then rose gradually each 5 years afterward when [17].

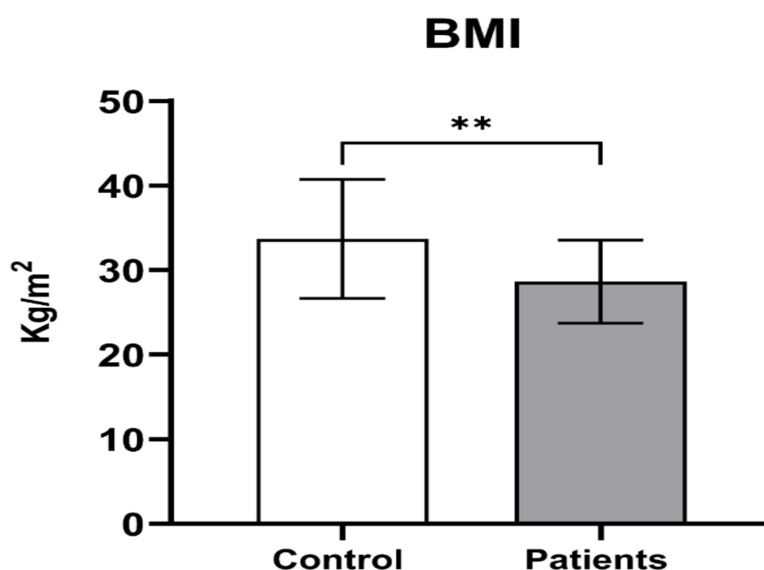

FIGURE 3 Distribution of osteoporosis patients

TABLE 7 Distribution of osteoporosis patients and control groups according to body mass index (BMI)

Variable	Study Groups Mean ± SD		P Values
BMI	Patients 28.62 ± 4.913	Controls 33.67 ± 7.031	0.0001**

TABLE 8 Distribution of osteoporosis patients groups of three with control groups

Groups	Age	No.of	mean±SD	P value
Control	30 to 50	40	33.67 ± 7.031	0.0001**
Pre	30 to 44	9	26.51±5.034	0.0001**
Meno	45 to 54	23	29.53± 5.368	0.0001**
Post	55 to 70	18	28.51±4.108	0.0001**

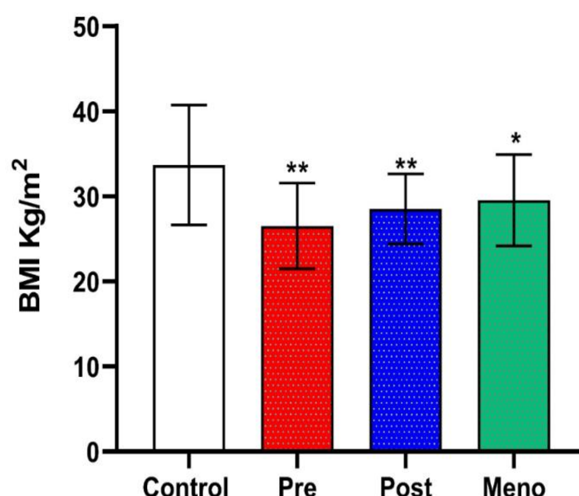

FIGURE 4 Distribution of osteoporosis patients groups of three with control groups

Genotype rs6426749 G/C gene polymorphism between patients and control

To detect the genotype polymorphism (rs6426749), the PCR was done for a target region that contains the targeted SNP. The amplified fragment, a single strand result of the desired product with a molecular weight of 336 bp, appeared sharp in the agarose gel representing the outer band. Genotyping of rs6426749. As shown in Figures 5 and 6, the samples possessing the GG genotype appeared with a band of 225 bp, while the samples with the CC genotype showed a single 153 bp band, while the GC heterozygous genotype showed two bands, 225 bp, and 153bp. These are results for patients, but for healthy people, the results show GG and GC and do not show CC, unlike patients.

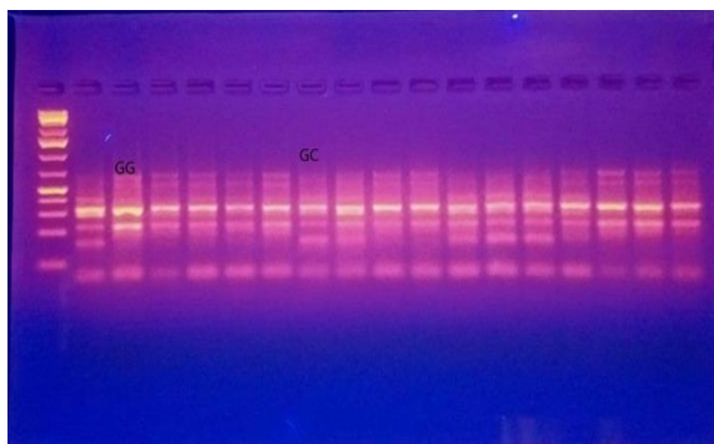

FIGURE 5 Electrophoresis pattern of amplification refractory mutation system–polymerase chain reaction (nested--PCR) for detection of ZBTB40 gene (rs6426749) in the control group. Homozygous: GG genotype (225 bp), homozygous: and heterozygous: GC genotype (225bp +153bp)

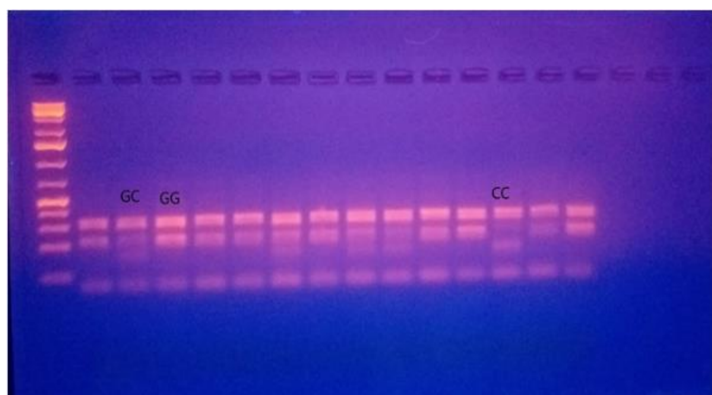

FIGURE 6 Electrophoresis pattern of amplification refractory mutation system–polymerase chain reaction (nested--PCR) for detection of ZBTB40 gene (rs6426749) in the Patients group. Homozygous: GG genotype (225bp), homozygous: CC genotype (153bp), and heterozygous: GC genotype (225bp+153bp)

Genotype frequencies of polymorphism rs6426749

The frequency of genotype GG was in patients 29(58%) in control 27(68%). This increment was with an odd ratio recorded for this relation as 1.0, and the G allele showed a highly significant increment (<0.0001) of frequency in patients (0.62) than in control (0.83), and the odd ratio for this relation was (0.0013). In contrast, the GC genotype showed a frequency in control 13(32%) than in patients 4(8%) with odd ratio equal to 0.2865 (C.I. 0.09440 to 0.9429). On the other hand, the genotype CC shows up in patients 17(34%) in control (0%) with an odd ratio equal to 32.6271 (C.I. 1.8708 to 569.0262) A, while significant with CC genotype(p>0.0003) we observe that in Table 8.

TABLE 8 Genotype distribution of ZBTB40 gene(rs6426794) GG &GC and CC polymorphism in control and patients

rs6426794 Genotype Frequency (%)						
Genotype	**Control n=40**	**Patients n=50**	**p-value**	**Chi-square**	**Odds Ratio**	**95% Cl**
GG	27 (68%)	29			1.0	
GC	13 (32%)	4	0.0403	4.204	0.2865	0.09440 to 0.9429
CC	0 (0%)	17	0.0003**	13.01	32.6271	1.8708 to 569.0262
Chi-squared	1.5059	34.4633				
p-value	0.4710 NS	<0.0001**				
Allele frequency (%)						
Allele	**Control n=80**	**Patients n=100**	**p-value**	**Chi-square**	**Odds Ratio**	**95% Cl**
G C	0.83 (67) 0.17 (13)	0.62 (62) 0.38 (38)	0.0013 **	10.35	3.159	1.512 to 6.197

NS=Non-significant, *significant at p value≤0.05, **significant at p value≤0.01

We also compared control and the three groups of patients with rs6426749. The results for the first group of patients pre menopause with the control are shown in Table 9 rs6426749 G/C for the two study groups that appeared with controls. Compared GG genotype.

The frequency of genotype GG was between control and patients was in patients 8(73%) in control 27(68%). This increment was with an odd ratio recorded for this relation as 1.0, and the G allele showed a non-significant increment (<0.8295) of frequency in patients (0.82) than in control (0.83). The odds ratio for this relation was (1.145). while the GC genotype showed a frequency in control 13(32%) than in patients 2(18%) with odd ratio equal to 0.5192 (C.I 0.1008 to 2.473) were non-significant P-value 0.4404. On another hand the genotype CC show up in patients 1(9%) in control 0(0%) with odd ratio equal to 9.7059 (C.I 0.3609 to 261.0017) in GC genotype, while non-significant with GG genotype(p>0.079).

We also compared control and the three groups of patients with rs6426749. The results for the first group of patients' menopause with the control are shown in -Table 10 rs6426749 G/C for the two study groups that appeared controls. Compared to GG genotype between control and patients, The frequency of genotype GG was in patients 10(48%) in control 27(68%). This increment was with odd ratio recorded for this relation as 1.0, the G allele showed a non-significant increment (<0.2661) of frequency in patients (0.75) than in control (0.83), and the odds ratio for this relation was (1.718). While the GC genotype showed a frequency in control 13(32%) than in patients 0(0%) with an odd ratio equal to 0.097(C. I 0.005 to 1.7822) were significant P-value 0.03. On the other hand, the genotype CC shows up in patients 11(52%) in control 0(0%) with an odd ratio equal to 60.2381(C. I 3.2509 to 1116.206) while significant with CC genotype(p0.0001).

TABLE 9 Genotype distribution of ZBTB40 gene(rs6426749) GG and GC and CC polymorphism in control with pre-menopause.

rs6426794 Genotype Frequency (%)						
Genotype	**Control n=40**	**Pre n=11**	**p-value**	**Chi-square**	**Odds Ratio**	**95% Cl**
GG	27 (68%)	8 (73%)			1.0	
GC	13 (32%)	2 (18%)	0.4404 NS	0.5952	0.5192	0.1008 to 2.473
CC	0 (0%)	1 (9%)	0.0790 NS	3.086	9.7059	0.3609 to 261.0017
Chi-squared	1.5059	1.6636				
p-value	0.4710 NS	0.4352 NS				
Allele frequency (%)						
Allele	**Control n=80**	**Patients n=22**	**p-value**	**Chi-square**	**Odds Ratio**	**95% Cl**
G C	0.83 (67) 0.17 (13)	0.82 (18) 0.18 (4)	0.8295 NS	0.04636	1.145	0.3713 to 3.773

NS=Non-significant, *significant at p value≤0.05, **significant at p value≤0.01

TABLE 10 Genotype distribution of ZBTB40 gene(rs6426749) GG &GC and CC polymorphism in control with menopause

rs6426794 Genotype Frequency (%)						
Genotype	**Control n=40**	**Meno n=21**	**p-value**	**Chi-square**	**Odds Ratio**	**95% Cl**
GG	27 (68%)	10 (48%)			1.0	
GC	13 (32%)	0 (0%)	0.0361 *	4.392	0.0970	0.00053 to 1.7822
CC	0 (0%)	11 (52%)	<0.0001 **	18.35	60.2381	3.2509 to 1116.206
Chi-squared	1.5059	21.00				
p-value	0.4710 NS	<0.0001 **				
Allele frequency (%)						
Allele	**Control n=80**	**Meno n=42**	**p-value**	**Chi-square**	**Odds Ratio**	**95% Cl**
G C	0.83 (67) 0.17 (13)	0.75 (27) 0.25(9)	0.2661 NS	1.237	1.718	0.6908 to 4.412

NS=Non-significant, *significant at p value≤0.05, **significant at p value≤0.01

We also compared control and the three groups of patients with rs6426749. The results were for the first group of patients post menopause with the control presented in Table 11 rs6426749 G/C for the two study groups appeared genetics of fractures cases differed in our pas way with controls. Compared GG genotype. Genotype GG was in patients 11(61%) in control 27(68%). This increment was with an odd ratio recorded for this relation as 1.0, and the G allele showed a significant increment (<0.03) of frequency in patients (0.67) than in control (0.83), and the odd ratio for this relation was (2.577). While the GC genotype showed a frequency in control 13(32%) than in patients 2(11%) with odd ratio equal to 0.377 (C.I 0.075 to 1.842) were non-significant P-value 0.23. On the other hand, the genotype CC shows up in patients 5(28%) in control 0(0%) with an odd ratio equal to 26.304 (C.I 1.3417 to 515.7076) within contrast to of the Participants and also the Healthy controls, there has been no noteworthy disparity. (p<0.2340) in GC genotype, while significant with CC genotype (p≤ 0.002).

TABLE 11 Genotype distribution of ZBTB40 gene(rs6426749) GG &GC and CC polymorphism in control with post menopause

rs6426794 Genotype Frequency (%)						
Genotype	**Control n=40**	**Post n=18**	**p-value**	**Chi-square**	**Odds Ratio**	**95% Cl**
GG	27 (68%)	11 (61%)			1.0	
GC	13 (32%)	2 (11%)	0.2340 NS	1.416	0.3776	0.07596 to 1.842
CC	0 (0%)	5 (28%)	0.002 **	9.548	26.3043	1.3417 to `515.7076
Chi-squared	1.5059	2.250				
p-value	0.4710 NS	0.3247 NS				
Allele frequency (%)						
Allele	**Control n=80**	**Post n=36**	**p-value**	**Chi-square**	**Odds Ratio**	**95% Cl**
G C	0.83 (67) 0.17 (13)	0.67 (24) 0.33 (12)	0.0384 *	4.286	2.577	1.053 to 6.222

NS=Non-significant, *significant at p value≤0.05, **significant at p value≤0.01

Both U-2OS и recombinant mscs, the rs6426749-C gene had vastly greater reporter production even than the rs6426749-G gene. Erythrocytes' most remarkable significant change has been seen, which had a 2.27-fold increase. You wanted to see whether CREB1 was involved see whether CREB1 was involved in varied epigenetic regulation upregulation.

Now final step was to see what effects CREB1 inhibition had. Impact of CREB1 sirna and CREB1 upregulation affect rs6426749 genotype transcriptional activity. A rs6426749-C alleles used to have a 15.4 percent decline in transcriptional activity in the U-2OS cell in the vicinity of CREB1 shRNA, but the rs6426749-G gene had no response. Co-loading of the CREB1 amplification vehicle by U-2OS cells led to a substantial spike in rs6426749 transcriptional regulation in a C intergenic direction [18].

Bone Mineral Density association with the rs6426749 G/C Genotype

rs6426749 G/C genotype was a study in women with osteoporosis as presented in Tables 12-14 and Figure 7. The results showed significant overtone among bone mineral density with t rs6426749 G/C (p <0.01) by comparing control and the three groups of patients.

Previous studies showed the relationship, respectively [19], between osteoporosis-in Southeast Asia generations, the China and Japanese people. n Korean and Japanese individuals, a range of 12 Mutations from 12 loci were linked to poor BMD (=-0.009 to -0.041, P=0.002-0.049). An aggregate of four Alleles across different locations was correlated to elbow mobility. CIs1.02-2.70, P=0.006-0.036 [20].

TABLE 12 Bone Mineral Density with the rs6426749 G/C genotype among control and the first group of patients pre menopause

Parameter	Polymorphism					
	GG		GC		CC	
	Control	Pre	Control	Pre	Control	Pre
Mean BMD	1.08	0.68	1.05	0.62	-	0.71
SD	0.07	0.08	0.05	0.03	-	-
P Value	<0.0001		<0.0001		<0.0001	

TABLE 13 Bone Mineral Density with the rs6426749 G/C genotype among control and the second group of patients' menopause

Parameter	Polymorphism					
	GG		GC		CC	
	control	Meno	Control	Meno	Control	Meno
Mean BMD	1.08	0.71	1.05	-	-	0.67
SD	0.07	0.07	0.05	-	-	0.08
P Value	<0.0001		<0.0001		<0.0001	

TABLE 14 Bone Mineral Density with the rs6426749 G/C genotype among control and the third group of patients post menopause

Parameter	Polymorphism					
	GG		GC		CC	
	control	Post	control	Post	Control	Post
Mean BMD	1.08	0.68	1.05	0.80	-	0.61
SD	0.07	0.06	0.05	0.17	-	0.09
P Cost	< 0.003		< 0.002		< 0.003	

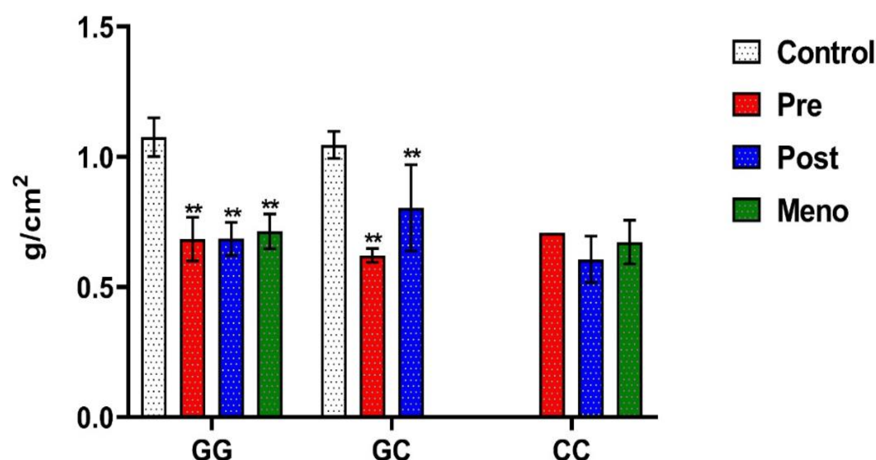

FIGURE 7 Bone mineral density and its association with the rs6426749 G/C genotype

Body Mass Index (BMI) and Its Association with the rs6426749 G/C Genotype

The genetic makeup of women with osteoporosis was also studied and appeared to be as shown in (Table 15, 16, 17) and (Figure 8). The results showed a significant association between BMI and the rs6426749 G/C genotype. The results were between the control group and the three groups of patients. The differences were significant for GG and GC either CC there are no significant differences.

TABLE 15 Body mass index with the rs6426749 G/C genotype among control and the first group of patients pre menopause.

Parameter	Polymorphism					
	GG		GC		CC	
	control	Pre	Control	Pre	Control	Pre
Mean	34.11	27.75	32.77	22.67	-	29.30
SD	7.69	4.07	5.58	7.21	-	-
P Value	0.0299		0.0860		-	

TABLE 16 Body Mass Index with the rs6426749 G/C genotype among control and the second group of patients' menopause

Parameter	Polymorphism					
	GG		GC		CC	
	control	Meno	Control	Meno	Control	Meno
Mean	34.11	29.33	32.77	-	-	29.80
SD	7.69	6.23	5.58	-	-	5.30
P Value	0.0097		-		-	

TABLE 17 Body Mass Index with the rs6426749 G/C genotype among control and the third group of patients' post menopause

Parameter	Polymorphism					
	GG		GC		CC	
	control	Post	Control	Post	Control	Post
Mean	34.11	27.60	32.77	31.28	-	29.40
SD	7.69	3.40	5.58	7.04	-	4.82
P Value	0.0973		0.9828		-	

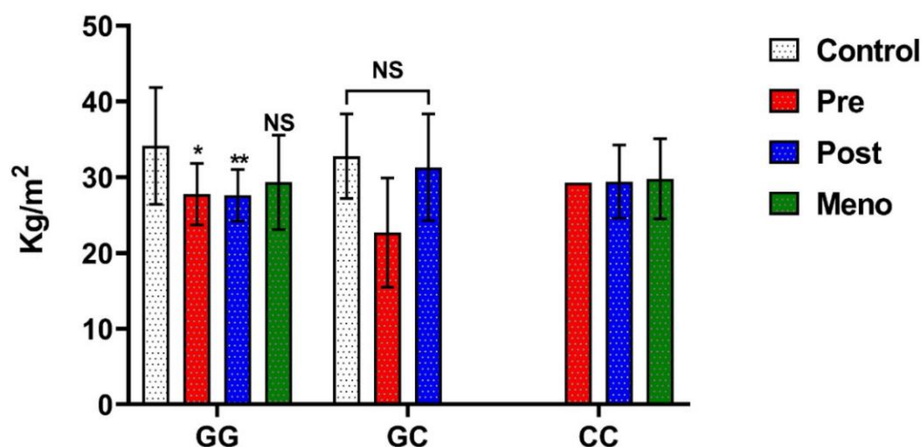

FIGURE 8 Body Mass Index and its association with the rs6426749 G/C genotype

Conclusion

The main factors that constitute risk factors for osteoporosis are BMD and BMI, with a more ratio when comparing the patients with the healthy control. These factors are related to genotypes currently studied. The genotyping and allele frequencies of ZBTB40 rs6426794 G/C for the two study groups appeared that there must have been significant gaps in genetic alleles among fracture control subjects. Compared GG was in patients 29 (58%) in control 27 (68%) This increment was with an odd ratio recorded for this relation as 1.0, the G allele showed a highly significant increment (<0.0001) of frequency in patients (0.62) than in control (0.83), and the odd ratio for this relation was (0.0013). In contrast, the GC genotype showed a frequency in control 13(32%) than in patients 4(8%) with an odd ratio equal to 0.2865 (C.I. 0.09440 to 0.9429).

The genotyping and allele frequencies of ZBTB40 rs6426794 G/C for the two study groups appeared to have momentous alterations in the genotypes among osteoporosis. Compared Bone Mineral Density and its Association with the rs6426749 G/C genotype was the bone mineral density and its association with the rs6426749 G/C (p <0.01) by making a comparison between control and the three groups of patients. The two study groups' genotyping and allele frequencies of ZBTB40 rs6426794 G/C revealed significant genotype genotypes between osteoporosis patients and controls. Compared Bone Mineral Density and its Association with the rs6426749 G/C genotype was a study in women with osteoporosis and women and its association with rs6426749 G/C. The genetic makeup of women. The results were between the control group and the three groups of patients. The differences were significant for GG and GC either CC there are no significant differences

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