LRP5G171R, can it play a role similar to the hormonal factor in its effect on the lumbar BMD?

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Abstract:

Background and aims: LRP5G171R(LRP5rs121908669) is a proven pathogenic single nucleotide polymorphism SNP for a disease associated with high bone mass HBM. There is no genotyping study for it until now. There is no idea about its relationship with other cases of BMD. There is no idea about its relationship to lumbar BMD. Hormonal factors are usually responsible for the lack of density in the spongy bone, which is the main component of vertebral bone, but what is about the responsibility of genetic factor? So,the goal is whether the genetic factor, specifically the LRP5 G171R has a role in changing the mineral density in the lumbar region. **Methods:** LRP5rs121908669 was diagnosed using PCR-RFLP and DNA sequencing in Syrian pre &post-menopuasal women. Related-Samples McNemar Change Test was used under 95% confidence level ($\alpha \leq .050$) to study distribution LRP5G171R genotypes across lumbar T-score values. The Odd Ratio test was used to identify the odd risk for lumbar T-score values when LRP5G171R genotype is absence or existed. Chi-Square Tests(□2) were used to estimate the correlation between genotypes and each of lumbar T- score under 95% confidence ($\alpha \leq .050$). **Results:** Significant chance to occurrence GG genotype, CC genotype, GC genotype in normal, osteopenia, osteoporosis lumbar T –score values are (GG 51.5%, 0.00%, 0.00%, CC 0.00%, 1%, 26.5%, GC 0.00%, 10.4%, 3%),respectively. There are significant correlation between GG genotype and normal, osteopenia lumbar T –score values, but no significant correlation with osteoporosis lumbar T –score values □2=6.302, p = . 012< .05, □2=5.919, p = . 01< .05, □2= .130, p=.719> .05, respectively. There are significant correlation between CC genotype and osteopenia lumbar T –score values, but no significant correlation with normal and osteoporosis lumbar T –score values with □2=3.846, p = . 05= .05, □2= 7.731, p = . 005< .05, □2=.204, p = . 651> .05, respectively. There are no significant correlation between GC genotype and normal, osteopenia, osteoporosis lumbar T –score values with □2= 1.658, p = . 198> .05, □2= 0.989, p = . 320> .05, □2= .363, p = . 547> .05, respectively. **Conclusions:** There is an effect of LRP5G171R on BMD in the lumbar region according to the genotype. GG is associated with normal BMD while CC GC is associated with deficient BMD

Keywords: CC genotype, lumbar T-score, GG genotype , GC genotype, LRP5G171R, LRP5rs121908669

Introduction:

Bone mass is a major determinant of the risk of osteoporotic fracture [1,2]. Twin and family studies indicate that genetic factors account for approximately 75% of the variation in peak bone mass [1,3,4]. Therefore, studying the genetic factor will be important for early intervention and prevention of osteoporosis. especially with the challenges facing dual energy X-ray absorptiometry DEXA

Bone mineral density BMD is a highly heritable trait, with a heritability estimate of 50% to 80% [5,6]. Therefore, there is a high risk to be inherted it to generations.

The LRP5 gene turned out to be an important regulator of peak bone mass in vertebrates [7]. Single nucleotide polymorphisms SNPs in the LRP5 gene may cause high or low bone mass[8-10]. Both may lead to osteoporosis and fractures[8].

LRP5rs121908669 (G171R) that causes the high bonemass phenotype is located in the aminoterminal part of the gene. Some genetic information about this SNP is explained in the table below table.1[11]

Name	Gene ID: 4041 /LRP5
Description	LDL receptor-related protein 5
Location	11:68312591-68449275
Cytogenetic region	11q13.2
SNV	G>C 511
EXON	3/23
RefSeqGene	143636 bp
Protein change	G171R
OMIM:	603506
Condition	ADO1

Table.1: Some genetic information about LRP5 G171R(LRP5rs121908669) from NCBI

Linkage analyses in people at high-risk for rare metabolic bone diseases should also yield important clues to the pathogenesis of osteoporosis[12].There is no study related to this SNP in different BMD groups. This SNP causes an increase in BMD in the cortical bone in majority[9]. But there is no idea about its effect on spongy bone. Hormonal factors are usually responsible for the lack of density in the spongy bone, which is the main component of the lumbar bone[13], but what is about the responsibility of genetic factor? So,the goal is whether the genetic factor, specifically the LRP5 G171R has a role in changing the mineral density in the lumbar region.

Materials and Methods

The study included 150 participants who visited rheumatology clinic at Tishreen University Hospital, Lattakia, Syria, throughout the period between March 2019 and September 2021,which was interspersed

with interruptions due to the Corona pandemic. The work was approved by the Ethics Committee in Syrian Ministry of High Education, and prior written consents were obtained from all the participants.

All of the participants were interviewed using a structured questionnaire. The questionnaire included socio-demographic characteristics, work habits, physical activity, medication history, age, age of beginning and end of menstrual, pregnancy and number of children, history of family orthopedic complaint, clinical history of bone pain , measurements of height and weight, body mass index BMI (kg/m2), data of fractures, lumbar and femur Z-score, lumbar and femur T-score. All participants were women with pre-menopause or post-menopause. They were from different families. Blood phosphorous and calcium values were collected from patients' files. The controls had high or normal T-score for both femur and lumbar T-score. All patients with hypertension, diabetes, osteomalacia, surgical menopause and cancer were excluded.

Bone densitometry:

The bone mineral density (BMD; g/cm2) of the lumbar spine (L1-L4) and left femur as measured by dual energy X-ray absorptiometry (DXA) (Medix DR, France). All DXA scans were conducted by a specially trained specialist. BMD Results were converted to age- and gender-specific Z-score that matched normal Caucasians. The samples were classified into 3 groups (normal, osteopenia, and osteoporosis) according to the World Health Organization classification of T-score values.

Insilico Study:

An Insilico study on NCBI was done in 2021.It was found that there were thousands of mutations for 569 genes associated with osteoporosis. There were two proven pathogenic SNPs for osteoporosis only without any other diseases with a predictive effect on protein of 87% according to the bioinformatics application of SNP PREDICT[14,15]. Currently, there are greater numbers of genes related to osteoporosis, numbering 855 genes, and dozens of SNPs that are pathogenic proven for osteoporosis without other diseases as shown in table.2[14]

Number of genes	Number of SNPs	Clinical significance	Names of genes
569	Thousands	Coding/noncoding protein	-
483	Thousands	Coding protein	-
7	Dozens	Pathogenic for osteoporosis and other diseases	BMND7,BMND8,BMND4, CALCR,COL1A1,COL1A2,LRP5
2	3	Pathogenic only for osteoporosis	COL1A2,LRP5
The chosen SNP is LRP5rs121908669			

Table.2: results of an Insilico study(2021) to determine the pathogenetic SNPs for osteoporosis

DNA Extraction:

Blood samples were collected using EDTA anticoagulant container tubes (2.5 ml blood from each participant) in Tishreen University Hospital, Lattakia, Syria. The samples were kept at -20 c . Work had been completed in the biotechnology laboratories of the Atomic Energy Authority, Damascus, Syria, where

DNA was isolated from samples using the (QIAamp DNA Blood Mini kit , Qiagen, Germany) according to the manufacturer's procedures and was stored at -20°C. The total DNA of each sample was measured by using a spectrophotometer followed by a of quantity Ultraviolet light.

LRP5rs121908669 SNP analysis:

The studied SNP was selected using the software https://loschmidt.chemi.muni.cz/predictsnp/. The Prediction ratio for its effect on the protein was 87%. In 2021, an Insilico study was conducted on NCBI concerning the genes of osteoporosis. It was found that there are only two genes with 3 SNPs proven pathogenic for osteoporosis without other diseases. One of them is ofLRP5rs121908669.

LRP5rs121908669 polymorphism of exon 3 was amplified using a specific forward primer: (5'-TCTGTGTTAGCTGCTTCTCTT-3') and Reverse primer 5′- CCAGGACTGCGTGGGTA -3′

Primers were designed using https://www.ncbi.nlm.nih.gov/tools/primer-blast,and https://bioinfo.ut.ee/primer3-0.4.0/. The primers were manufactured using (a polygon primer designer device, in Germany). The stock concentration was 52.51 n.mol/ml for reverse primer and 63.60 n.mol/ml for forward primer. Both were diluted with dual distillation water(ddw) (10X).

The Polymerase chain reaction (PCR) was performed in a total volume of 25 µl containing 5 µl of genomic DNA, 5 µl PCR buffer, 1 µl dNTPs, 2 µl of each primer, and 1 µl of Taq DNA polymerase. PCR program included initial denaturation at 95 °C for three minutes followed by 40 cycles of 95 °C for 45 seconds, 52°C for 45 seconds, and 72 °C for 60 seconds with a final extension at 72 °C for 7 minutes. PCR reaction was conducted in a PCR T100 thermocycler (Mastercycler, Eppendorf, Germany). The amplification PCR products were run on 2% agarose gel stained with DNA Safe Stain Dye and visualized under UV light. The positive result produced bands 259 base pair (bp) (= 259 bp) which indicates the presence of the fragment which was chosen to detect this SNP.

Restriction enzymes for RFLP were chosen from https://nc2.neb.com/NEBcutter2/. The Restriction Fragment Length Polymorphisms (RFLP) of the LRP5 gene was carried out by PCR product gestion for 16h at 37 °C with 0.8 µl Bfi1 (MBI Fermentas, Vilnius, Lithuania). Then, 15 µl of the digested PCR products were added to 3 (6X) loading dye and loaded on 3.5% agarose gel, and run at 80V for 60 minutes. PCR products for L 5rs121908669 were then visualized using the gel documentation system BIO-RAD (Gel-DocSy1-L8-M5). The lengths of the digested product were 192pb*67bp; 259pb; 259bp*192*67bp for the normal genotype GG, Hhomozygous genotype CC, and heterozygous genotype GC, respectively. The ladder is 20pb. There was no positive or negative control sample.

All were confirmed by direct sequencing using SeqStudio Genetic Analyzer (Applied Biosystems, USA). The cycle-sequencing reaction was performed in a 10 µl volume containing 1 µl of the ready reaction of the terminator, 5 p.mol of either the forward or reverse primer, and 10 ng of purified PCR product (ExoSAP-IT

kit; Amersham BioSciences, Piscataway, NJ, USA). The thermal cycle protocol was 95˚C for 4 minutes followed by 30 cycles at 96˚C for 10 seconds, 50˚C for 5 seconds, and 60˚C for 4 minutes (ABI GeneAmp PCR System 9700, Applied Biosystems). Centri-Sep columns (Princeton Separations, Adelphia, NJ, USA) were used for the effective and reliable removal of excess dye terminators (DyeEx 2.0, Qiagen, Germany) from completed DNA sequencing reactions. Data were compared and aligned with different sequences using the NCBI BLAST Assembled Genomes tool(http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical study:

Statistical analysis was performed using SPSS computer software version 20 (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp; 2011). Related-Samples McNemar Change Test was used to examine the correlation in the distribution between the presence of LRP5G171R genotypes and BMD of lumbar region under 95% confidence level ($\alpha \leq .050$), and to study a null hypothesis concerning mild OI of distribution across osteopenia and osteoporosis cases. Chi-Square Test was used to estimate the correlation between LRP5G171R genotypes and BMD of lumbar region which are of under 95% confidence ($\alpha \leq .050$). the Odd Ratio test was used to identify the odd risk for lumbar T-score values when LRP5G171R genotype is absence or existed. The results are as showen in table4

Results:

All data about age, age of beginning and end of menstrual, pregnancy and number of children, history of family orthopedic complaint, bone complaint, measurements of height and weight, body mass index BMI (kg/m2), data of fractures, classification of cases according to WHO*, are contained in the table below table.3. All participants had normal blood concentrations of calcium and phosphorous.

Variable	Case
Total number	150
Age	60(40, 80)
Age of beginning of menstrual	14(11, 17)
Age of end of menstrual	50.5(46, 55)
Weight	69.5(40,99)
Height	165(150,180)
BMI	29.69(17.99, 41.4)
Data on fractures(YES/NO)	85/65
History of family orthopedic complaint(YES/NO)	56/94
Clinical history of bone complaint(YES/NO)	139/11
L2-L4(lumbar) Z-score	(-4.1, 3.1)
L2-L4 (lumbar)T-score	(-5.6, 1.2)
Femur Z-score	(-1.9, 1.1)
Femur T-score	(-2.2, 1.1)

Normal(T-score ≥1) *	74
Osteopenia (-2.5) < T-score <(-1)	48
Osteoporosis T-score ≤ (-2.5)	28
Total	150

* World Health Organization Definition of Osteoporosis by T-score values

Table.3: Clinical, laboratory, demographic and radiological information for participants

LRP5rs121908669 detection and genotyping were determined using PCR-RFLP figure.1 and DNA sequencing figure.2. It was found that there were 97(64.66%) GG genotype, 20 (13.3%) homozygous genotype CC, 33 (22%) heterozygous genotype GC .

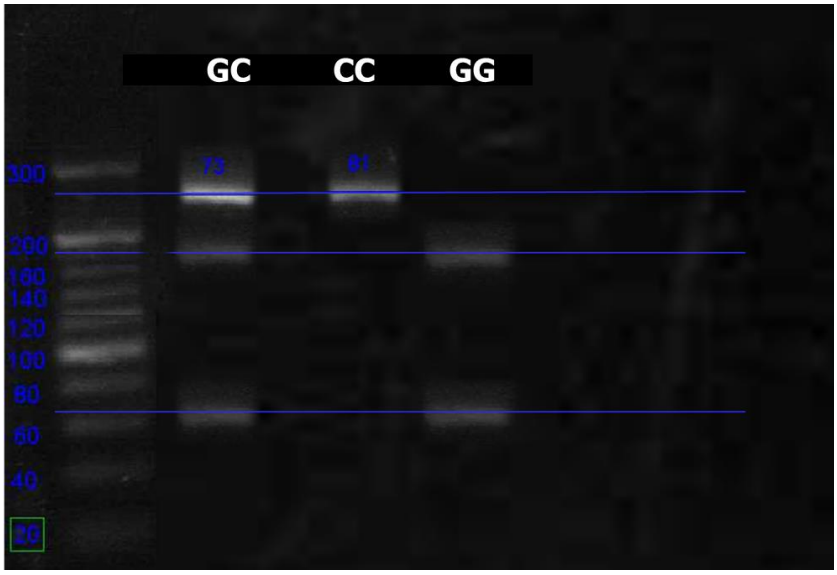

Figure.1: Agarose gel electrophoresis of PCR products for the Bfi1 polymorphism: the far left lane, 20bp DNA ladder, The rest of lanes,GG genotye, CC genotype, GC genotype.

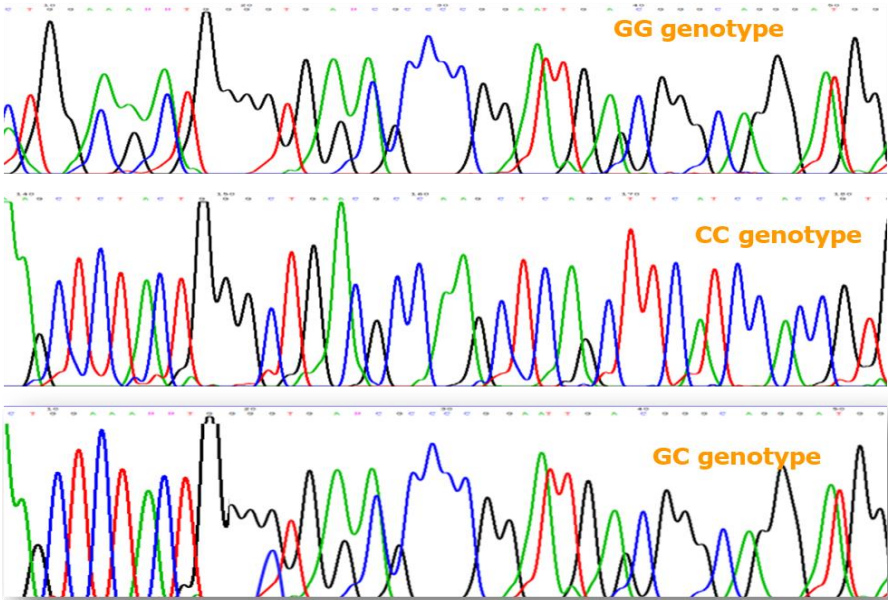

Figure.2: GG(GGGGT),CC(GGGCT),GC(GGG(G/C)T) genotypes of LRP5G171R by DNA sequencing analysis

The results of statistical studies of the relationship between bone mineral density in the lumbar region and the genotypes of LRP5G171R are showen in the table below

genotypes	N.	BMD	McNemar%	OR	CI	Chi-Square	P
GG	1	normal	51.5	1.4000	1.092- 1.794	6.302	0.012
	2	osteopenia	0	0.495	0.268- 0.913	5.919	0.015
	3	osteoporosis	0	0.813	0.263- 2.516	0.130	0.719
CC	1	normal	0	0.712	0.546- 0.928	3.846	0.05
	2	osteopenia	1	3.462	0.910- 13.165	7.731	0.005
	3	osteoporosis	26.5	0.846	0.202 – 3.540	0.204	0.651
GC	1	normal	0	0.822	0.625- 1.081	1.658	0.198
	2	osteopenia	10.4	1.375	0.714- 2.648	0.989	0.320
	3	osteoporosis	3	1.551	0.362- 6.655	0.363	0.547

Table4: Results of Related-Samples McNemar Change Test, Risk estimate tests, Chi-Square Tests to evaluate the relationship of genotypes to lumbar T-score values

Discussion:

It is the first study of its kind in the world to link the genotypes of LRP5rs121908669 (G171R) with lumbar T-score values. There is only one study related to this SNP.

The significant chance of occurrence of GG genotype in normal, osteopenia, osteoporosis lumbar T –score values are 51.5%, 0.00%, 0.00%,respectively. So, the null hypothesis is rejected and the alternative hypothesis is retained which indicates that the distributions of GG genotype across osteoorosis and osteopenia, respectively, are significantly variant with a confidence level of 95% or more ($p \leq .05$). The null hypothesis is retained which indicates that the distributions of GG genotype across normal lumbar T –score values are equally likely with a confidence level of 95% or more ($p \leq .05$),.table4

There are significant correlation between normal, osteopenia lumbar T –score values and GG genotype (Chi-Square = 6.302, p = . 012< .05), (Chi-Square = 5.919, p = . 015< .05), respectively. There is no significant correlation between osteoporosis lumbar T –score values and GG genotype (Chi-Square = .130, p = . 719> .05).table4. The Odds presence of normal, osteopenia, osteoporosis lumbar T –score values are *1.400 times greater, * .495 times less, * .813 times less, respectively when GG genotype exists compared with their odd without GG genotype exists,table4. So, GG genotype is a factor that reduces the risk of osteopenia and osteoporosis and increases the likelihood of obtaining a normal BMD . This corresponds to Van Wesenbeeck E et al. 's study2003 [7].

The significant chance of occurrence of CC genotype in normal, osteopenia, osteoporosis lumbarT –score values are 0.00%, 0.01%, 26.5%, respectively. So, the null hypothesis is rejected and the alternative hypothesis is retained which indicates that the distributions of different values across normal, osteopenia, respectively and CC genotype are significantly variant with a confidence level of 95% or more ($p \leq .05$). The null hypothesis is retained which indicates that the distributions of CC genotype across osteoporosis lumbar T–score values are equally likely with a confidence level of 95% or more ($p \leq .05$), table4. There are significant correlation between normal and osteopenia lumbar T –score values and CC genotype (Chi-Square = 3.846, p = . 050≤ .05, Chi-Square = 4.881, p = . 027< .05), respectively. There is no significant correlation between osteoporosis lumbar T –score values and CC genotype (Chi-Square = .052, p = . 820> .05), table4. The Odds presence of normal, osteopenia, osteoporosis lumbar T –score values are *.712 times less, *3.462 times greater, *.846 times less, respectively when CC genotype exists compared with their odd without CC genotype exists, table4 .So, CC genotype is a factor that increases the risk of osteopenia and osteoporosis in lumbar position and reduces the likelihood of obtaining a normal BMD. This contradicts Van Wesenbeeck E et al. 's study2003 [7].

The significant chance of occurrence GCgenotype in normal, osteopenia, osteoporosis lumbar T –score values are 0.00%, 10.4%, 0.03%,respectively. So,the null hypothesis is rejected and the alternative hypothesis is retained which indicates that the distributions of GC genotype across normal, osteoporosisT-score, respectively are significantly variant with a confidence level of 95% or more ($p \leq .05$). The null hypothesis is retained which indicates that the distributions of GC genotype across and osteopenia lumbar T –score values, are equally likely with a confidence level of 95% or more ($p \leq .05$),table4. There are no significant correlation between normal, osteopenia, or osteoporosis lumbar T –score values, respectively, and GC genotype (Chi-Square = 1.658, p = . 198> .05, Chi-Square = .989, p = . 320> .05, Chi-Square = .363, p = . 547> .05,respectively,table4. The Odds for the presence of normal, osteopenia, and osteoporosis lumbar T –score values are *.822 times less, *1.375 times greater, *1.551 times greater, respectively when GC genotype exists compared with their odd without GC genotype exists,table4. So,GC genotype is a factor that increases the risk of osteopenia in lumbar position and has no effect on osteoporosis and normal BMD. This contradicts Van Wesenbeeck E et al. 's study2003 [7].

The limitations of this study are the small size of the sample, no positive control for RFLP, and the necessity to sequence all hot spots for the LRP5 gene.

Conclusion:

As final result, GG is an independent protective factor against low BMD, i.e. GG is a prognostic factor for the absence of low BMD. Whereas, GC genotypes have not any real influence on BMD. CC genotype is a

non- independent influencing factor on BMD., LRP5G171R can be added as a risk factor. LRP5G171R can be both a diagnostic and a therapeutic goal for osteoporosis.

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Ethical approval statement: The work was approved by the Ethics Committee in Syrian Ministry of Higher Education and written informed consent was obtained from all the participants according to the Declaration of Helsinki.

Informed Consent Statement:"Informed consent was obtained from all subjects involved in the study**.** Written informed consent has been obtained from the patient(s) to publish this paper.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author (Eiman M. Shahrour), upon reasonable request. All relevant material is included in this publication

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Conflict of Interests

Eiman shahrour , Bassel AL-Halabi , Amir N Dabboul, Walid Al-achkar, Abd Alrazak Hassan, Atieh Khamis, and Haissam Yazigi declare that they have no conflict of interest.

References:

1. Boyden LM, Mao J, Belsky J, Mitzner L, Farhi A, Mitnick MA, Wu D, Insogna K, Lifton RP (2002) High bone density due to a mutation in LDL-receptor-related protein 5. N Engl J Med 346: 1513–1521.

2. Heaney RP, Abrams S, Dawson-Hughes B, et al. Peak bone mass. Osteoporos Int 2000;11:985-1009.

3. Worth HM, Wollin DG. Hyperostosis coticalis generalisata congenita. J Can Assoc Radiol 1966;17:67-74.

4. Ades LC, Morris LL, Burns R, Haan EA.(1994) Neurological involvement in Worth type endosteal hyperostosis: report of a family. Am J Med Genet 51:46-50.

5. Shan CH, Mahim J, Shalini J, Zeynep A, Philippe CA, Robert KL, Carrie NI, Hongzheng D, Donna M, Eric B, Richard G, Eric O, James L, Jennifer P, and Brendan L (2019) Genetic Burden Contributing to Extremely Low or High Bone Mineral Density in a Senior Male Population From the Osteoporotic Fractures in Men Study (MrOS). JBMR Plus (WOA) DOI: 10.1002/jbm4.10335.

6. Boudin E, Fijalkowski I, Hendrickx G, Van Hul W.(2016) Genetic control of bone mass. Mol Cell Endocrinol. 432:3–13.

7. Van Wesenbeeck E, Cleiren E, Gram J, Beals R, Benichou O, Scopelliti D, Key L, Renton T, Bartles C, Gong Y, Warman M, Vernejoul M, Bollerslev J, Van Hul W (2003) Six novel missense mutations in the LDL receptor-related protein 5 (LRP5) gene in different conditions with and increased bone density. Am J Hum Genet 72:763–771.

8. Mark J., Kimberley H., Roel N. and Wim HU.(2004) LRP5 and Wnt Signaling: A Union Made for Bone J Bone Miner Res. Vol.19 DOI: 10.1359/JBMR.040816.

9. Little RD, Carulli JP, Del Mastro RG, et al.(2002) A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant highbone- mass trait. Am J Hum Genet 70:11-9.

10. Whyte MP (1999) Sclerosing bone disorders. In: Favus M-J (ed) Primer on the metabolic bone diseases and disorders of mineral metabolism, 4th ed. Lippincott Williams and Wilkins, Philadelphia, pp 367–383

11. https://www.ncbi.nlm.nih.gov/

12. Joseph M. Zmuda, et al.(1999) Recent Progress in Understanding the Genetic Susceptibility to Osteoporosis. Genetic Epidemiology 16:356–367

13. Abdul Razak, H.; Kaser, A. ; (2014)Rheumatology locomotor system diseases. Tishreen university publications.

14. Kato, M., Patel, M.S., Levasseur, R., Lobov, I., Chang, B.H., Glass, D.A., Hartmann, C., Li, L., Hwang, T.H., Brayton, C.F. et al. (2002) Cbfa1-independent decrease in osteoblast proliferation, osteopenia, and

persistent embryonic eye vascularization in mice deficient in Lrp5, a Wnt coreceptor. J. Cell. Biol., 157, 303–314.

15. Wu, W., Glinka, A., Delius, H. and Niehrs, C. (2000) Mutual antagonism between dickkopf1 and dickkopf2 regulates Wnt/b-catenin signalling. Curr. Biol., 10, 1611–1614.