# Morphological Traits According to BMI of LRP5rs121908669 Genotypes Carriers

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**Abstract:**

**Background & Aim:** Descriptive diagnostics is a part of the clinical diagnosis. Not reliable but it is an important prompt. Therefore, it is necessary to know the phenotypes of the genotyped carrier for the LRP5G171R. This study is the first of its kind worldwide. **Methods:** This cross-sectional study was performed on 150 pre& post -menopausal women with a mean age of 40 years. Body mass index (BMI) was calculated. To determine the genotypes of LRP5rs121908669, PCR-RFLP assay was performed and confirmed by DNA sequencing. Likehood test, Chi-Square test, The binary logistic regression test, Odd Ratio test were used as statistical studies to determine the relationship between BMI and genotypes of LRP5G171R. **Results:** Likehood test confirms the relationship between BMI and LRP5G171R ($\varkappa^2$ =15.658, p=0.048).The results indicate that there are positive correlations between obese and extremely obese BMI and GG($\varkappa^2$ =23.707, 14.013 p=0.000, 0.00), successively. There is a negative correlation between normal BMI and GG ($\varkappa^2$ =19.675, p=0.000). There is a negative correlation between extremely obese BMI and CC($\varkappa^2$ =12.466, p=0.000). There is a negative correlation between obese BMI and GC($\varkappa^2$ =19.997, p=0.000).**Discussion:** No previous studies have identified morphological traits in terms of BMI, but in general there are studies confirming the relationship of the LRP5 gene has a significant role in metabolism and adipocyte biology. This is consistent with the results of this study.**Conclusion:** Finally, obesity and extremely obesity are positive things. The carriers of the mutanted LRP5G171R(GC,CC) will be protected from obesity, unlike the carriers of normal genotype (GG) will suffer from obesity. It can be added to NCBI as an associated SNP to obesity.

**Keywords:** LRP5G171R, Obesity, postmenopausal women, BMI, LRP5rs121908669

**Introduction:**

Lipoprotein receptor-related protein 5 (LRP5),consisting of 23 exons and spacing 136.6 kb, is mapped to chromosome 11q13.4 in humans and is part of the low density lipoprotein receptor family of cell surface receptors[1,2]. LRP5 plays an important part in the WNT signaling pathway,[3–6] may regulate bone,[7, 8] and is important for glucose and cholesterol metabolism[9]. The discovery of LRP5 as an important actor in bone metabolism resulted in a major interest in the role of *LRP5* as a susceptibility gene in the regulation of BMD and/or fracture risk in the general population. This led to several recent reports on association studies between *LRP5* single-nucleotide polymorphisms (SNPs) and different bone phenotypes [10 ,11]. several studies have shown a positive association between body mass index (BMI( and bone mineral density (BMD), and low body weight and substantial sudden weight loss induce bone loss [12].

LRP5, a member of the low-density lipoprotein receptor family, is also important for glucose and cholesterol metabolism[13,14]. Several studies have found that LRP5 polymorphisms are associated with complex diseases or traits that are related to obesity[15, 16]. However, only one study has reported the relationship between LRP5 polymorphisms and obesity in Caucasian nuclear families[17]. Therefore, the LRP5 gene could be a pleiotropic genetic fac-tor influencing both osteoporosis and obesity phenotypes. However, a clear relationship between LRP5's single nucleotide polymorphisms (SNPs) and peak BMD and obesity[18].

Although a genetic determination of obesity has been established, with estimates of heritability .0.50 for the variation in body mass index (BMI) [19–23]the underlying susceptibility genes remain largely unknown. LRP5 polymorphisms have been found important to complex diseases or traits that are related to obesity[1, 8, 9 ,24–27]. Morphological features in each disease may guide the final clinical diagnosis, so it is important to study them in all diseases[28].

The morphological traits of carriers of all mutations should be studied, and it should fall under a heading that could be called morphological genetics.

The direct relationship between LRP5G171R and obesity has never been studied. This mutation is related to bone diseases. Could it also be related to other metabolic diseases and obesity? This would be useful as part of the morphological genomics that serves the final clinical diagnosis.

**Materials and Methods**

-Study population and Phenotype measurement:

The study included 150 participants who visited rheumatology clinic at Tishreen University Hospital, Lattakia, Syria, between March 2019 and September 2021, interspersed with interruptions due to the Corona pandemic. The work was approved by the Ethics Committee in Syrian Ministry of High Education and written informed consent was obtained from all the participants. BMI was calculated as body weight (kg) divided by the square of height (m). Weight was measured in light indoor clothing, using a calibrated balance beam scale, and height was measured using a calibrated stadiometer. We divided the participants into 5 groups according to the classification of the World Health Organization Obesity Assessment. All participants were women with per-menopause or post-menopause. Theywere from different families. All patients with hypertension, diabetes, osteomalacia, surgical menopause and cancer were excluded.



The participants were divided into 5 groups of BMI and studied the relationship of the effect of each group on the emergence of a mutant(GC,CC) genotype instead of a normal (GG)genotype. Each genotype was studied on the five groups of BMI.

-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.1[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 | | | |

Table1: 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). Likehood test was used to know the existence of an important relationship between BMI and LRP5G171R .The binary logistic regression test was used to study the predictive effect of BMI on LRP5G171R genotypes($p = .000<.05$). Chi-Square Test was used to estimate the correlation between LRP5G171R genotypes and BMI under 95% confidence ($\alpha \leq .050$). Odd Ratio test was used to identify the odd risk for BMI values when LRP5G171R genotype is absence or existed.

**Results and discussion:**

It is the first study of its kind in the world. There is no previous study linking the body mass index to the genotypes of LRP5G171R.The results of the statistical study are showen in the table below, table2.

| Genotypes | N. | BMI groups | B/ OR | CI / P | Exp(B)/ OR | Chi-Square | P |
|---|---|---|---|---|---|---|---|
| GG genotype | 1 | <18.5 | .915 | 0.173-4.832 | .915 | 0.011 | 0.917 |
| | 2 | [ 18.5-24.9] | .179 | 0.68- .471 | .179 | 19.675 | 0.000 |
| | 3 | [ 25- 29.9] | .707 | 0.452- 1.107 | .707 | 2.507 | 0.113 |
| | 4 | [30- 34.9] | 5.491 | .2.499-12.064 | 5.491 | 23.707 | 0.000 |
| | 5 | ≥35 | 16.472 | 2.145-126.510 | 16.472 | 14.013 | 0.000 |
| CC genotype | 1 | <18.5 | -8.244 | .118 | .000 | 0.060 | 0.806 |
| | 2 | [ 18.5-24.9] | -8.374 | .064 | .000 | 6.868 | 0.009 |
| | 3 | [ 25- 29.9] | -4.712 | 0.016 | .009 | 0.307 | 0.579 |
| | 4 | [30- 34.9] | -3.280 | .082 | .038 | 1.952 | 0.162 |
| | 5 | ≥35 | .154 | .049- .484 | .154 | 12.466 | 0.000 |
| GC genotype | 1 | <18.5 | -2.271 | .455 | .103 | 0.104 | 0.748 |
| | 2 | [ 18.5-24.9] | -2.802 | .300 | .061 | 8.808 | 0.003 |
| | 3 | [ 25- 29.9] | -1.288 | .421 | .276 | 1.883 | 0.170 |
| | 4 | [30- 34.9] | .650 | .673 | 1.915 | 19.997 | 0.000 |
| | 5 | ≥35 | .423 | .127- 1.411 | .423 | 2.023 | 0.155 |
| Likehood Ratio Test ($\varkappa^2$ =15.658, p=0.048) | | | | | | | |

Table2: Results of the statistical relationships that study the relationship between BMI and genotypes of LRP5G171R

According to the Likelihood Ratio Test, there is a significant correlation for BMI ($\varkappa^2$ = 15.658, p = .048 <.05) on genotyping of LRP5rs121908669.

For BMI<18.5(under weight) ,It has no significantly impact on tendency toward CC genotype (p = .118 > .05). The odds ratio for the studied group indicates that every unit decrease in BMI is associated with a 97.37% increasing in chance odds to having CC genotype more than GG genotype.

According to the tendency type, there is a negative correlation between the studied group and CC genotype observing (B = -8.244). The studied group has no significantly impact on tendency toward GC genotype (p = .455 > .05). The odds ratio for the studied group indicates that every unit decrease in BMI is associated with a 89.68% increasing in chance odds to having GC genotype more than GG genotype. According to the tendency type, there is a negative correlation between the studied group and GC genotype observing (B = -2.271). There are no significant correlation between GG, CC, GC and BMI<18.5(Chi-Square =0 .011, 0.060, 0.104, p = 0.917> 0.05, 0.806>0.05, 0.748> .05), respectively.

For BMI [ 18.5- 24.9](normal), The studied group no significantly impact on tendency toward CC genotype (p = .064 > .05). The odds ratio for the studied group indicates that every unit decrease in BMI is associated with a 99.97% increasing in chance odds to having CC genotype more than GG genotype. According to the tendency type, there is negative correlation between the studied group and CC genotype observing (B = -8.374). The studied group has no significantly impact on tendency toward GC genotype (p = .300 > .05). The odds ratio for the studied group indicates that every unit decrease in BMI is associated with a 93.93% increasing in chance odds to having GC genotype more than GG genotype. According to the tendency type, there is negative correlation between the studied group and GC genotype observing (B = -2.802). There is no significant correlation between GG and BMI[18.5-24.9] (Chi-Square = 19.675, p = 0.000< 0.05). There are significant correlation between CC,GC and BMI[18.5-24.9] (Chi-Square = 6.868, 8.808, p = 0.009< .05, 0.003< .05).

For BMI [ 25- 29.9]( over weight), The studied group has significantly impact on tendency toward CC genotype (p = 0.016 <0.05). The odds ratio for the studied group indicates that every unit decrease in BMI is associated with a 96.23% increasing in chance odds to having CCgenotype more than GG genotype. According to the tendency type, there is negative correlation between the studied group and CC genotype observing (B = -4.712). The studied group has no significantly impact on tendency toward GC genotype (p = .421 > .05). The odds ratio for the studied group indicates that

every unit decrease in BMI is associated with a 72.41% increasing in chance odds to having GC genotype more than GG genotype. According to the tendency type, there is negative correlation between the studied group and GC genotype observing (B = -1.288). There are no significant correlation between GG,CC,GC and BMI[25-29.9] (Chi-Square = 2.507, .307, 1.883, p = .113> .05, .579> .05, .170> .05 ).

For BMI [30- 34.9](obese), The studied group has no significantly impact on tendency toward CC genotype (p = .082 > .05). The odds ratio for the studied group indicates that every unit decrease in age is associated with a 96% increasing in chance odds to having CC genotype more than GG genotype. According to the tendency type, there is negative correlation between the studied group and CC genotype observing (B = -3.280). The studied group no significantly impact on tendency toward GC genotype (p = .673 > .05). The odds ratio for the studied group indicates that every unit increase in BMI is associated with a 1.915 times increasing in chance odds to having GC genotype more than GG genotype. According to the tendency type, there is positive correlation between the studied group and GC genotype observing (B = .650). There are significant correlation between GG,GC and BMI[30-34.9] (Chi-Square = 23.707, 19.997, p = .000< .05, .000< .05 ). There is no significant correlation between CC and BMI[30-34.9] (Chi-Square = 1.952, p = .162> .05).



For BMI [≥35](extremely obese), the relative odd risk possibility for being at BMI≥35 group and not having GG genotype is 16.472 times greater than the relative odd risk for being at the other BMI groups and not having GG genotype. The relative odd risk possibility for being at BMI≥35 group and not having CC genotype is 84.6 % less than the relative odd risk for being at the other BMI groups and not having CC genotype. The relative odd risk possibility for being at BMI≥35 group and not having GC genotype is 57.7% less than the relative odd risk for being at the other BMI groups and not having GC genotype. There is significant correlation between GG and BMI≥35 (Chi-Square =14.013, p = .000< .05)**.** There are no significant correlation between CC,GC and BMI≥35 (Chi-Square = 12.466, 2.023 p = .000< .05, .155> .05).

The final results that there is a positive correlation between obese and extremely obese BMI and GG, a negative correlation between normal BMI and GG. There is a negative correlation between obese and extremely obese BMI and CC. There is a negative correlation between obese BMI and GC and a negative correlation between normal BMI and GC.

These results consistent with the results of studies confirming the significant role of LRP5 gene in metabolism and adipocyte biology[18,29,30] But these results contradict a Chinese study confirming that there is no relationship between LRP5 and BMI[18]. But there is no study that demonstrates the relationship of LRP5G171R with BMI to compare the results of this study with it.

**Conclusion:** People are classified according to BMI as (under weight, normal, overweight, obese, and over obese). The GG and GC genotypes appear less in normal BMI carriers. Over weight is a protective factor against the appearance of CC. Obesity increases the onset of normal GG and decreases the appearance of GC. Over obesity increases the appearance of GG and reduces the appearance of CC. In other words, one of the morphological characteristics of the carrier of the GG genotype is obesity and over obesity, and the carrier cannot be a normal BMI. The carrier of the GC genotype does not have specific characteristics but cannot be normal or obese. The carrier of the CC genotype cannot be overweight or over obese.

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**Declaration:** I confirm that this work is a part of an approved PhD thesis which was approved by university board's decision No.1698 of 05/02/2019, and this work is an original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

**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.

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