Association between LEPR polymorphism and susceptibility of osteoporosis in Chinese Mulao people

Guangbin Ye\textsuperscript{a,b,s}, Yandong Huang\textsuperscript{c,s}, Lianfei Yin\textsuperscript{a}, Jianchu Wang\textsuperscript{d}, Xiufeng Huang\textsuperscript{a} and Xiaoyun Bin\textsuperscript{a}

\textsuperscript{a}School of Basic Medical Sciences, Youjiang Medical University for Nationalities, Baise, China; \textsuperscript{b}Medical College of Guangxi University, Nanning, China; \textsuperscript{c}First People’s Hospital of Nanning, Nanning, China; \textsuperscript{d}Department of Hepatobiliary Surgery, Affiliated Hospital of Youjiang Medical University for Nationalities, Baise, China

ABSTRACT
To explore the association between the single nucleotide polymorphism (SNP) of leptin receptor (LEPR) gene and the susceptibility to osteoporosis (OP) among Chinese Mulao people. A total of 738 people were involved. Bone mineral density (BMD) was examined by calcaneus ultrasound attenuation measurement. Six SNPs of LEPR were detected. The genotypes, allele frequencies, linkage disequilibrium, and haplotype were analyzed. BMD decreased with age and males had higher BMD than women. The proportion of normal bone mass decreased with age, and morbidity of OP increased. Three out of six SNPs showed a difference between OP and normal group. Individuals with AA genotype of rs1137100 in OP group outnumber the normal group, AA increased the risk of OP. In rs2767485, CT increased the risk of OP. C allele may be susceptible to OP. TT genotype of rs465555 was susceptible genotype of OP, T locus may be associated with OP. Strong linkage disequilibrium was detected among rs1137100, rs1137101, and rs4655555. Four haplotypes were constructed, among which, AACGCT and GGTGTA increased the risk of OP by 3.9 and 4.2 times, respectively, whereas, GGCGTA reduced 74% of OP susceptibility. The rs1137100, rs2767485, and rs4655555 of LEPR were associated with OP in Chinese Mulao people.

Introduction
Osteoporosis (OP) is a degenerative disease with reduced bone mass, degeneration of bone micro-structure, and increased risk of bone fragility. It is easy to cause damage to bone strength and increase the risk of fracture, which seriously endangering the health and life quality of middle-aged and elderly people. OP has become an important public health problem that seriously affects human health \cite{1,2}. OP has a wide range of patients without obviously early symptoms. China is the country with the fastest ageing population and the largest elder population in the world. With the increase of life expectancy and the ageing of society, the incidence of OP is increasing year by year. The incidence of osteoporosis and osteoporotic fracture depends on bone peak and bone loss rate, which effected by factors of genetic, environmental, gender, age, nutritional status, lifestyle, physical exercise, drug use, and some other diseases, etc. \cite{3}. And the genetic factors determine the difference between 60 and 80% bone mass, 50–75% bone turnover status, and 25–35% fracture risk \cite{4}. Bone mineral density (BMD), which has high heritability, is classical diagnosis evidence for OP. Many studies have shown that single nucleotide polymorphisms (SNPs) are associated with OP or BMD \cite{5–7}.

Leptin is a secreted protein encoded by the obese (ob) gene located on human chromosome 7q32, which is mainly synthesized using white fat cells and secreted into the blood. It binds to receptors on target organs to inhibit gene transcription, thereby suppressing appetite and reducing calorie intake, improving body metabolism, and reducing fat accumulation \cite{8}. Leptin receptor (LEPR), encoded by diabetes (db) gene, which is located in human chromosome 1p32. There are six subtypes of LEPR, among which the long receptor is the main receptor for leptin signal transmission \cite{9}. Leptin is secreted into the blood by adipocytes and binds with leptin receptor. The signal transduction is mainly realized by Janus kinase, a linker with kinase structure, which regulates the transcription of a target gene using JAK-STAT pathway. Using the central and peripheral pathways, it can play the role of inhibiting appetite, increasing energy consumption, inhibiting fat synthesis, and so on. Shen et al. \cite{10} show osteonectin acts on leptin receptor + skeletal stem cells in bone marrow to promote their differentiation into osteoblasts. Leptin signalling acts on marrow stromal cells to...
enhance differentiation into osteoblasts and inhibit differentiation into adipocytes [11]. Leptin receptor can act on bone marrow stem cells, improve the proliferation of potential cells and differentiate into osteoblasts, and play an active role in bone metabolism. Di Carlo et al. [12] showed that in normal women, circulating leptin was significantly lower in women with low BMI than in normal BMI women. Li et al. [13] further showed leptin direct effects on vitamin D metabolism and osteoblast differentiation in Human Marrow Stromal Cells (hMSCs) may protect bone in obese people. Studies above have shown that leptin and leptin receptors are related to the proliferation, differentiation, and regeneration of bone cells.

The evidence linking LEPR polymorphism to BMD is mixed; some studies have shown no association, whereas others have shown an association with BMD. Crabbe et al. [14] suggested the baseline BMD, longitudinal changes in hip or forearm BMD were not associated with Gln223Arg LEPR genotypes. Studies using Zhao et al. [15] also showed no correlation between LEPR polymorphism and BMD in postmenopausal women. On the other hand, LEPR polymorphism was indicated may play joint and interactive roles with TNF-α and IL-6 genes and physical inactivity in the development of OP [16]. While Lee et al. [17] found that an SNP locus c.1968G > C in LEPR was associated with femoral neck BMD, using examining postmenopausal Korean women.

LEPR expression is closely related to bone metabolism, and the mechanism of action is complex, but the effect of LEPR on bone metabolism is still unclear. The purpose of this investigation was to evaluate SNP variants of the LEPR rs1137100, rs1137101, rs1751492, rs1805094, rs2767485, rs4655555, and to determine the relationship between LEPR polymorphisms and OP of the Mulao people in Chinese.

Materials and methods

Study subjects

We recruited 738 Mulao minority people over 45 years old from China, including 422 females (Supplementary Table S1) and 316 males (Supplementary Table S2) in Guangxi Zhuang Autonomous Region of China. All of the subjects have lived there for more than three generations. The population is divided into different age groups according to every 5 years of age, individuals aged 75 and over are classified as a group. All participants gave informed written consent, and the study was approved by Medical Ethics Committee of Youjiang Medical University for Nationalities (No.2017030201).

Measurement of body parameters

Net height was measured using a standard height measuring instrument. Body composition and BMI were measured using a bioelectrical impedance analyzer (TANITA, MC-180, Japan). Achilles Express (GE, Fairfield, IA, USA) was used to perform broadband ultrasound attenuation (BUA) of the right calcaneus of subjects. Before ultrasonic BMD detection, 100 samples were chosen randomly, and their BMDs were measured both by applying the dual-energy X-ray absorptiometry (DXA) and quantitative ultrasound methods [7,18]. After comparison tests, a calibration formula was established, from which a standard module was used for the calibration to ensure the accuracy of the results from the ultrasound method. Bone mass results are represented with BUA, which was based on T-score in decibels per megahertz. Using the Chinese guideline for the Diagnosis and Treatment of Senile Osteoporosis, the standard deviation of the measured BMD was compared with the peak BMD of the same sex: T value (≥ −1.0) was normal, T value (< −2.5) < T value (−1.0) was osteopenia, and T value (< −2.5) was OP [19].

Genotyping

Blood samples were obtained from all subjects, then venous blood (3 mL) was drawn into tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). The genomic DNA was extracted using the total blood DNA extraction kit (QIAGEN Biotechnology Company). Base on articles about LEPR polymorphism associated with body composition or metabolism, and NCBI database, we screen six LEPR SNPs for multiple SNP typing (Supplementary Table S3). Primer 5 software was used to design primers, which were synthesized using GENE SKY (Shanghai Genesky Biotech Co., Ltd., Shanghai, China).

The PCR reaction and amplification were carried out described in Bin et al. [7]. Then the extension product was Sequenced on the ABI3730XL sequencer, the data was analyzed with GeneMapper 4.1 (Applied Biosystems, USA).

Statistical analysis

Data analysis was performed using the SPSS 18.0 software package. The measured data were expressed as mean ± standard deviation, and all SNP data were measured to satisfy the Hardy-Weinberg equilibrium law. The odds ratio and 95% confidence interval (95% CI) were used to measure the association between LEPR polymorphism and OP susceptibility. All statistical tests were two-sided probability tests. χ² test for comparison between two count samples; comparison between different genotypes using one-way analysis of variance (ANOVA). p < .05 was considered statistically significant. Haploview 4.2 software was used to analyze the Linkage disequilibrium (LD) of six SNPs loci of LEPR.

Results

BMD trends with age

The BUA of senior and middle-aged Mulao people showed a gradual decrease with age. The BUA value of males decreased slowly with age. We observed a significant difference between 45-year-old and over 75-year-old group (p < .05), but there was no significant difference among other age groups (p > .05). In other words, the BMD decreased evidently from the age of 75 in males. The BUA of women in the 45-year-old group is similar to that of men in the same age group, maintaining a relatively high level and gradually
decreasing with age. There were significant differences between 45-year-old and all age groups over 55-year-old (p < .05), suggesting that the BMD of Mulao women began to decrease significantly from age of 55. Compared with other age groups, BUA values of women in 70/71 and 75 groups had significant differences (p < .01), suggesting that the extent of BMD reduction of women has increased since the age of 70. Among the same age group, the BUA in male were significantly higher than those in women (p < .05) of 55~, 60~, 70~, and above 75 years groups, there was no difference in BUA between men and women in the 45~ and 50~ group (Table 1, Figure 1(A)).

**Morbidity of OP**

As shown in Table 2, the number and proportion of senior and middle-aged Mulao people with normal BMD decrease gradually with age both in males and females. The percentage of normal bone mass decreased from 82.1% in 45~ group to 38.8% in ≥75 group, while the same trend was more pronounced in females, the percentage of normal BMD declined from 92.4 to 17.65% with age in Mulao women (Table 2, Figure 1(B)). On the other hand, the number and proportion of OP increased evidently. In 45~ group, the percentage of OP was 17.9%, then it reached the peak of 61.22% in ≥75 group in male; while female, the lowest percentage of OP was 7.6% in 45~ group, and the top was 82.35% in ≥75 group. All the differences were statistically significant (Table 2, Figures 1(C,D)).

**Genotype and allelic gene frequency distribution in six SNPs of LEPR**

Six SNPs loci (rs1137100, rs1137101, rs1751492, rs1805094, rs2767485, and rs4655555) of LEPR were successful detected. Each genotype distribution was in Hardy-Weinberg
equilibrium using $\chi^2$ test ($p > 0.05$). And as shown in Table 3, Three SNPs polymorphic loci of LEPR were associated with OP susceptibility in Mulao people.

**Allele and genotype of rs1137100**

The frequency distribution of GG/GA/AA genotype in OP and normal control group had a significant difference, but no significant difference was found in the distribution of A/G alleles between OP and normal group. The genotype of rs1137100 was associated with OP susceptibility, compared with GG + GA genotype, AA genotype significantly increased the risk of OP.

**Allele and genotype of rs2767485**

There were also two genotypes of rs2767485 distributed among Mulao people, the significant difference was found both in frequency of TT and CT genotype between OP and normal group, and the frequency of C/T alleles. Compared with TT genotype, CT genotype significantly increased the risk of OP, the C allele may be susceptible to OP.

**Allele and genotype of rs4655555**

A significant difference was found in frequency distribution of AA/TA/TT genotype in OP group and normal control group, and also significant difference was detected in the distribution of A/T alleles between OP and normal group. TT was the susceptible genotype of OP relative to AA/TA, and the T locus may be associated with OP susceptibility (Table 3).

**Correlation between BMD and LEPR polymorphism**

The three SNPs (rs1137100, rs2767485, rs4655555) of LEPR, which distribute distinct differences between the normal and OP groups, had relative with BMD. BMD in AA genotype of rs1137100 was lower than that in GG or GA genotype. BMD in CT genotype was lower than that in TT genotype of rs2767485. In rs4655555, the BMD of TT genotype was lower than that of AA or TA genotype (Table 4).

**Linkage disequilibrium (LD) analyses**

To further confirm the association between LEPR polymorphism and OP, we used Haplovie 4.2 free software to analyze LD for the six SNP loci of LEPR. As shown in Supplementary Tables S4, S5, the LD coefficients of $D'$ > 0.8 and $r^2 > 0.3$ were observed between rs1137100 and rs1137101, between rs1137100 and rs4655555. These results suggested that there was strong linkage disequilibrium among the loci rs1137100, rs1137101, and rs4655555 (Supplementary Figure S1).

**Haplotype frequency distribution of LEPR polymorphisms**

Using SHEsis software to construct haplotype of six polymorphisms of LEPR, it is found that there are four haplotype
Table 4. Comparisons of BMD in three SNPs loci of LEPR among normal bone mass and OP groups.

| Loci       | Genotype | Normal    | OP         | Total    |
|------------|----------|-----------|------------|----------|
| rs1137100  | GG       | 116.47 ± 12.99 | 94.39 ± 8.72 | 109.71 ± 15.62 |
|            | GA       | 121.50 ± 12.92  | 95.73 ± 10.18  | 108.55 ± 13.77  |
|            | AA       | 106.67 ± 6.43   | 93.28 ± 4.31   | 97.30 ± 7.96    |
|            | p-Value  | .315         | .700        | .034      |
| rs2767485  | TT       | 115.93 ± 12.63  | 94.78 ± 8.74  | 109.65 ± 15.11  |
|            | CT       | 112.36 ± 9.03   | 93.81 ± 10.35  | 102.37 ± 14.21  |
|            | p-Value  | .931         | .040        | 2.058      |
| rs1137100  | AA       | 116.47 ± 12.99  | 94.39 ± 8.72  | 109.71 ± 15.62  |
|            | CT       | 112.36 ± 9.03   | 93.81 ± 10.35  | 102.37 ± 14.21  |
|            | p-Value  | .315         | .700        | .034      |

Table 5. Correlation analysis of haploid type constructed by LEPR polymorphism site and OP.

| Haplotypes | Case (freq) | Control (freq) | Chi² | p     | OR (95% CI) |
|------------|-------------|----------------|------|-------|-------------|
| AAGCGTT    | 12 (0.046)  | 7 (0.012)      | 9.326 | .0022 | 3.902 (1.529–9.957) |
| AGCGTT     | 19 (0.070)  | 20 (0.034)     | 5.486 | .019  | 2.145 (1.118–4.117) |
| GGCGTA     | 156 (0.583) | 445 (0.770)    | 49.207 | .252 (0.168–0.376) |
| GGTGTA     | 41 (0.151)  | 24 (0.034)     | 3.433 | .019  | 3.402 (1.118–10.362) |

combinations among Mulao minority population (all those frequency <.03 were ignored in analysis). Among them, there were two haplotypes of OP risk, which were higher in the case group than in the control group: Haploid type AAGCGCT increased the risk of OP by 3.9 times, and haploid type GGTGTA increased the risk of OP by 4.2 times. The distribution of haploid GGCGTA in the control group was higher than that in the case group, and it was a protective haploid type, which could reduce 74% of OP susceptibility (Table 5).

Discussion

Age-related changing trends of bone mass

The calcaneal BMD of Mulao population is similar to that of similar age groups in previous studies. For example, in 10 regions of China, the variation range of calcaneal BMD of men over 50 years old is BUA (114.3–109.8) dB/MHz, and that of women over 50 years old is BUA (109.8–98.4) dB/MHz [20]. It was also observed that calcaneal BMD decreased with age, among the male groups of Mulao minority, the degree of BMD reduction in males was slow. The BMD in group over 75 years old was significantly different from the 45–75 group, while no difference was found in other age groups (Table 1), suggesting that the BMD of Mulao males began to decrease significantly after 75 years old, the occurrence of BMD reduction was late.

The OP% in Mulao males was about 5% or lower before the age of 70, the OP% was 13% in the 70–75 group and 18.37% in the over 75 year old group (Table 2). These results showed that the prevalence of OP increased gradually after 70 years old, and the BMD decreased after 75 years old, suggesting that the important population of OP prevention should focus on the propaganda and prevention of the population under 70 years old.

The prevalence of OP in Mulao women was not high. Before the age of 65, the prevalence of OP was under 5%, and it began to exceed 20% after the age of 65. However, the reduction of BMD in the senior and middle-aged Mulao females was relatively large, the BMD decreased rapidly since the age of 55, especially after 70 years old. It suggested that Mulao women after the age of 65 were affected by the dual effects of oestrogen reduction and ageing. At this age, most women have menopause, oestrogen secretion is suddenly reduced, bone resorption is greater than bone formation, they will experience the rapid bone loss phase, and will lose the most bone strength over the menopause transition [21]. It is speculated that the decrease of BMD in Mulao women around 55 years old is mainly influenced by the decrease of oestrogen. After 70 years old, the decrease of BMD is more obviously affected by both low oestrogen and ageing.

Distribution of LEPR SNPs in Mulao OP and normal bone mass individuals

Variation at the LEPR locus has been shown to influence the BMI [22], and LEPR is also associated with the people’s obesity index [23,24], or metabolic syndrome [25], speculated that the LEPR mediates the relationship between obesity and BMD. Lee et al. showed that polymorphism of c.1968G > C at an SNP locus in LEPR correlates with femoral neck BMD in postmenopausal Korean women [17]. In view of the correlation between LEPR and proliferation, differentiation and regeneration of bone cells [26,27]. In this study, we speculated that LEPR polymorphism and its haplotypes may be related to the occurrence of OP. We screened six SNPs of LEPR, analyzed the relationship between gene polymorphism and BMD of Mulao minority people in China, and speculated the relationship between gene polymorphism and OP risk.

Rs1137100 is the conversion of A > G at the nucleotide position of LEPR so that the 326th amino acid is converted from lysine to arginine, and also rs1137100 is the most extensively studied SNP site of LEPR. However, most of the current research on the polymorphism of rs1137100 is about metabolic-related diseases, such as diabetes [28], overweight [29], suggesting that LEPR mediates the relationship between obesity and BMD. Lee et al. showed that polymorphism of c.1968G > C at an SNP locus in LEPR correlates with femoral neck BMD in postmenopausal Korean women [17]. In view of the correlation between LEPR and proliferation, differentiation and regeneration of bone cells [26,27]. In this study, we speculated that LEPR polymorphism and its haplotypes may be related to the occurrence of OP. We screened six SNPs of LEPR, analyzed the relationship between gene polymorphism and BMD of Mulao minority people in China, and speculated the relationship between gene polymorphism and OP risk.

Rs1137100 is the conversion of A > G at the nucleotide position of LEPR so that the 326th amino acid is converted from lysine to arginine, and also rs1137100 is the most extensively studied SNP site of LEPR. However, most of the current research on the polymorphism of rs1137100 is about metabolic-related diseases, such as diabetes [28], overweight [29], suggesting that LEPR mediates the relationship between obesity and BMD. Lee et al. showed that polymorphism of c.1968G > C at an SNP locus in LEPR correlates with femoral neck BMD in postmenopausal Korean women [17]. In view of the correlation between LEPR and proliferation, differentiation and regeneration of bone cells [26,27]. In this study, we speculated that LEPR polymorphism and its haplotypes may be related to the occurrence of OP. We screened six SNPs of LEPR, analyzed the relationship between gene polymorphism and BMD of Mulao minority people in China, and speculated the relationship between gene polymorphism and OP risk.

Rs1137100 is the conversion of A > G at the nucleotide position of LEPR so that the 326th amino acid is converted from lysine to arginine, and also rs1137100 is the most extensively studied SNP site of LEPR. However, most of the current research on the polymorphism of rs1137100 is about metabolic-related diseases, such as diabetes [28], overweight [29], suggesting that LEPR mediates the relationship between obesity and BMD. Lee et al. showed that polymorphism of c.1968G > C at an SNP locus in LEPR correlates with femoral neck BMD in postmenopausal Korean women [17]. In view of the correlation between LEPR and proliferation, differentiation and regeneration of bone cells [26,27]. In this study, we speculated that LEPR polymorphism and its haplotypes may be related to the occurrence of OP. We screened six SNPs of LEPR, analyzed the relationship between gene polymorphism and BMD of Mulao minority people in China, and speculated the relationship between gene polymorphism and OP risk.
detect any significant differences in BMD levels by the genotypes.

Rs2767485 is the transformation of T→C at the intron site of LEPR gene, which has been reported may affect energy balance during weight loss among Brazilians obese [31], and rs2767485 is also associated with the occurrence of Adolescent Idiopathic Scoliosis (AIS) [32]. However, there is no literature reported about rs2767485 related to osteoporosis, bone metabolism, or BMD. This study found that the SNP locus rs2767485 only showed two genotypes of TT and CT in the Mulao people. The distribution of wild-type TT genotype in normal population (70.28%) is more than that in OP patients (29.72%), while the distribution of CT genotypes of mutant heterozygotes in the normal population (40.74%) was significantly lower than that in OP patients (59.26%), and no mutation homozygotes were detected, allele C may be a susceptibility site for OP. The BMD of CT genotype was significantly lower than that of TT genotype ($p < .05$). It suggested that CT genotype at rs2767485 increased the risk of OP compared with TT genotype ($p = .001$), and people with C allele might be more susceptible to OP ($p = .002$).

Rs4655555 is the transformation of T→A at the intron site of LEPR gene. A genome-wide association study revealed that rs4655555 remained associated with plasma soluble leptin receptor (sOB-R) levels in 1504 women of European ancestry [30]. Whereas, a genetic association study found that rs4655555 had no significant association with AIS in the Chinese Han population [31]. This study found that the distribution of AA genotype in the normal group (72.28%) was higher than that in the OP group (29.72%), while the distribution of TT genotype in the OP group (57.89%) was higher than that in the normal group (42.11%). Allele T may be the susceptibility site of OP, and BMD of TT genotype was also significantly lower than that of AA genotype ($p = .019$), suggesting that TT genotype at rs4655555 increased the risk of OP compared with AA genotype ($p = .005$), and people with T allele may be more susceptible to OP ($p = .002$).

Compared with genetic susceptibility factors, the existence of each polymorphic locus does not only play an isolated role in the occurrence and development of the disease but also has a certain internal relationship and effect between them. LD analysis is a way to test this genetic association. In this study, LD index $D'$ and $r^2$ were comprehensively analyzed, and it was found that among the six SNP sites of LEPR gene, there was strong linkage disequilibrium among the loci rs1137100, rs1137101, and rs4655555, and among the three SNPs detected related to BMD in the Mulao people, rs1137100 and rs4655555 have a strong linkage disequilibrium relationship ($D'=0.815, r^2=0.511$), suggesting that rs1137100 and rs4655555 have a positive effect on bone density. The effect of rs2767485 may be chained, but this study has not confirmed whether the combined effect of these two sites has an effect on the function of LEPR gene, while the effect of rs2767485 on bone density is independent.

Studies on haplotype are more likely to reveal the association between multiple SNPs and disease susceptibility. We constructed haplotypes on the polymorphic sites of LEPR gene and analyzed the association with OP. In our study, a total of 25 haplotypes were constructed from six SNP sites, and the distribution of four haplotypes in the case group and the control group was significantly different. Among them, there were three haplotypes (AACGCT, GGTCGA, GGTCGA) in the case group is higher than the control group, individuals with AACGCT, GGTCGA, or GGTCGA haplotypes. Haplotype AACGCT increased the risk of OP by 290%, and haplotype GGTCGA increased the risk of OP by 322%. The distribution of haploid GGCGTA in the control group was higher than that in the case group, and it was a protective haploid type, which could reduce 74% of OP susceptibility. Therefore, haploid AACGCT and GGTCGA are potential genetic susceptibility factors, and haplotype GGCGTA may be a protective factor of BMD. However, the mechanism of LEPR and haplotype in relation to OP is still worth further study.

**Conclusions**

This study found that BMD of the senior and middle-aged Mulao ethnic group in China decreases with age. The minority of OP in Mulao people was similar to the average of China. The decrease of BMD of men (significantly decreased after 75) was more later and slowly than that of women (significantly decreased after 55) in Mulao ethnic group. Advocating a healthy lifestyle, active intervention and early diagnosis and treatment are of great significance for the prevention and treatment of osteoporosis. The polymorphism of rs1137100, rs2767485, and rs4655555 in LEPR was associated with BMD in Mulao people, and AA, CT, TT GG type were the susceptible genotypes of OP. Four haplotypes were constructed, among which, AACGCT and GGTCGA could increase the risk of OP, whereas GGCGTA might reduce OP susceptibility.

**Disclosure statement**

The authors declare that they have no competing interests.

**Funding**

This work was supported by the National Natural Science Foundation of China [32060208 and 82060441], the Guangxi Natural Science Foundation [2017JJA10377 and 2019JJA140524], and the Scientific Research and Technology Development Project of Baise City [Baike20192501].

**ORCID**

Xiaoyun Bin http://orcid.org/0000-0001-5122-7414

**Data availability statement**

The analyzed data sets generated during the study are available from the corresponding author on a reasonable request.

**References**

[1] Kanis JA, McCloskey EV, Johansson H, et al. A reference standard for the description of osteoporosis. Bone. 2008;42(3):467–475.
[2] Cosman F, de Beur SJ, LeBoff MS, et al. Clinician's guide to prevention and treatment of osteoporosis. Osteoporos Int. 2014;25(10):2359–2381.

[3] Boudin E, Fijalkowski I, Hendrickx G, et al. Genetic control of bone mass. Mol Cell Endocrinol. 2016;432:3–13.

[4] Sigurdsson G, Halldorsson BV, Styrkarsdottir U, et al. Impact of genetics on low bone mass in adults. J Bone Miner Res. 2008;23(10):1584–1590.

[5] Li Y, Zhou J, Wu Y, et al. Association of osteoporosis with genetic variants of circadian genes in Chinese geriatrics. Osteoporos Int. 2016;27(4):1485–1492.

[6] Zhou X, Qiu YH, He P, et al. Why SNP rs227584 is associated with human BMD and fracture risk? A molecular and cellular study in bone cells. J Cell Mol Med. 2019;23(2):898–907.

[7] Bin X, Lin C, Huang X, et al. FGF-2 gene polymorphism in osteoporosis among Guangxi’s Zhuang Chinese. JIMUS. 2017;18(7):1358.

[8] Zhang Y, Chua S Jr. Leptin function and regulation. Compr Physiol. 2017;8(1):351–369.

[9] Dam J. Traffic and signalisation of the leptin receptor. Biol Aujourd'hui. 2018;212(1–2):35–43.

[10] Shen B, Vardy K, Hughes P, et al. Integrin alpha11 is an osteolec- tin receptor and is required for the maintenance of adult skeletal bone mass. Elife. 2019;8:e42274.

[11] Jiang H, Chen Y, Chen G, et al. Leptin accelerates the pathogenesis of heterotopic ossification in rat tendons tissues via mTORC1 signaling. J Cell Physiol. 2018;233(2):1017–1028.

[12] Di Carlo C, Tommaselli GA, De Filippo E, et al. Menstrual status and serum leptin levels in anorectic and in menstruating women with low body mass indexes. Fertil Steril. 2002;78(2):376–382.

[13] Li J, Gao Y, Yu T, et al. Obesity and leptin influence vitamin D metabolism and action in human marrow stromal cells. J Steroid Biochem Mol Biol. 2020;198:105564.

[14] Crabbe P, Goemaere S, Zmierczak H, et al. Are serum leptin and resistin polymorphisms with obesity parameters in Hammam Sousse Sahlioul Study. J Clin Lab Anal. 2017;31(6):e22148.

[15] Zhao HY, Liu JM, Ning G, et al. Study of the impact of candidate genes on bone mineral density in postmenopausal women. Chin J Obst Gynecol. 2005;40:803–807.

[16] Lin CC, Li TC, Liu CS, et al. Associations of TNF-α and IL-6 polymorphisms with osteoporosis through joint effects and interactions with LEPR gene in Taiwan: Taichung community health study for elders (TCHS-E). Mol Biol Rep. 2016;43(10):1179–1191.

[17] Lee HJ, Kim H, Ku SY, et al. Association between polymorphisms in leptin, leptin receptor, and β-adrenergic receptor genes and bone mineral density in postmenopausal Korean women. Menopause. 2014;21(1):67–73.

[18] Chen X, Kong C, Yu H, et al. Association between osteosarcopenic obesity and hypertension among four minority populations in China: a cross-sectional study. BMJ Open. 2019;9(7):e026818.

[19] Microsurgery Department of the Orthopedics Branch of the Chinese Medical Doctor Association, Group from the Osteonecrosis and Bone Defect Branch of the Chinese Association of Reparative and Reconstructive Surgery, Microsurgery and Reconstructive Surgery Group of the Orthopedics Branch of the Chinese Medical Association. Chinese guideline for the diagnosis and treatment of osteonecrosis of the femoral head in adults. Orthop Surg. 2017;9:3–12.

[20] Qiao YJ, Li X, Wu M, et al. Levels of calcaneus bone mineral density in adults from 10 regions of China. Chin J Epidemiol. 2018;39:422–427.

[21] Karlamangla AS, Burnett-Bowie SM, Crandall CJ. Bone health during the menopause transition and beyond. Obstet Gynecol Clin North Am. 2018;45(4):695–708.

[22] Couto Alves A, Díaz Silva NMG, Karhuinen V, et al. GWAS on longitudinal growth traits reveals different genetic factors influencing infant, child, and adult BMI. Sci Adv. 2019;5(9):eaaw3095.

[23] Farias DR, Franco-Sena AB, Rebelo F, et al. Polymorphisms of leptin (G2548A) and leptin receptor (Q223R and K109R) genes and blood pressure during pregnancy and the postpartum period: a cohort. Am J Hypertens. 2017;30(2):130–140.

[24] Nesrine Z, Haithem H, Imen B, et al. Leptin and leptin receptor polymorphisms, plasma leptin levels and obesity in Tunisian volunteers. Int J Exp Path. 2018;99(3):121–130.

[25] Zayani N, Omezzine A, Boumaïza I, et al. Association of ADIPOQ, leptin, LEPR, and resistin polymorphisms with obesity parameters in Hammam Sousse Sahlioul. J Clin Lab Anal. 2017;31(6):e22148.

[26] Bao D, Ma Y, Zhang X, et al. Preliminary characterization of a leptin receptor knockout rat created by CRISPR/Cas9 system. Sci Rep. 2015;5:15942.

[27] Rodeheffer MS, Horowitz MC. Fat decisions: leptin regulates bone mass versus fat in the marrow. Cell Stem Cell. 2016;18(6):684–686.

[28] Cao X, Huo P, Li W, et al. Interactions among moderate/severe periodontitis, ADIPOQ-rs1501299, and LEPR-rs1137100 polymorphisms on the risk of type 2 diabetes in a Chinese population. Arch Oral Biol. 2019;103:26–32.

[29] Dos Santos Rocha A, de Cássia Ribeiro-Silva R, Nunes de Oliveira Costa G, et al. Food consumption as a modifier of the association between LEPR gene variants and excess body weight in children and adolescents: a study of the SCAALA cohort. Nutrients. 2018;10(8):1117.

[30] Sun Q, Cornelis MC, Kraft P, et al. Genome-wide association study identifies polymorphisms in LEPR as determinants of plasma soluble leptin receptor levels. Hum Mol Genet. 2010;19(9):1846–1855.

[31] Corgosinho FC, Almeida SS, Tock L, et al. LEPR polymorphism may affect energy balance during weight loss among Brazilians obese adolescents. Neuropeptides. 2017;66:18–24.

[32] Liu Z, Wang F, Xu LL, et al. Polymorphism of rs2767485 in leptin receptor gene is associated with the occurrence of adolescent idiopathic scoliosis. Spine. 2015;40(20):1593–1598.