

## Full Length Research Paper

# Polymorphism of glucagon-like peptide-1 receptor gene (*rs1042044*) is associated with bone mineral density in Chinese Han postmenopausal women

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Previous investigations indicated that glucagon-like peptide-1 (GLP-1) played important roles in bone turnover via GLP-1 receptors (GLP1Rs) in postmenopausal state. Furthermore, polymorphisms in *GLP1R* gene were suggested to affect the function of GLP1Rs and be associated with many diseases. However, the relationships between *GLP1R* polymorphisms and osteoporosis susceptibility and bone strength remain unexplored. To address this issue, a total of 458 Chinese Han postmenopausal women were included in this study. The bone mineral density (BMD) in the lumbar spine (L<sub>2</sub>-L<sub>4</sub>) and femoral neck was measured by dual-energy X-ray absorptiometry (DEXA). A missense mutation (*rs1042044*) in *GLP1R* was genotyped using allele specific TaqMan probes. Our data showed that genetic variants of *rs1042044* were significantly associated with osteoporosis ( $P = 0.003$ ) and that the C allele of *rs1042044* was a protective factor against osteoporosis compared to the A allele with gene dosage-dependent manner (OR, 0.579; 95% CI, 0.366 to 0.916 for AC genotype and OR, 0.404; 95% CI, 0.238 to 0.688 for CC genotype). These findings indicate that polymorphisms in *GLP1R* gene may affect BMD and development of osteoporosis in Chinese Han postmenopausal women, which provide novel insight into the mechanisms of osteoporosis development and target for personal prevention and treatment of osteoporosis.

**Key words:** Glucagon-like peptide-1 receptor, single nucleotide polymorphism, osteoporosis, bone mineral density.

## INTRODUCTION

Osteoporosis is a prevalent metabolic bone disease and an important and complex health problem in postmenopausal women (Riggs and Melton, 1986; NIH,

2001). This kind of skeletal disease is characterized by low bone mineral density (BMD) and deterioration of bone tissue, and generally believed to be a multifactorial

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disorder with genetic influence accounting for up to 70% of individual variance in BMD (Jouanny et al., 1995). It was reported that, in postmenopausal state, the BMD was affected by a series of bone mass modulating factors, such as vitamin D receptor, estrogen receptor, and osteoprotegerin etc., and that polymorphisms in genes encoding these factors were significantly associated with osteoporosis risk (Horst-Sikorska et al., 2013; Gennari et al., 2005; Arko et al., 2005; Wang et al., 2013). Therefore, to define certain candidate genes responsible for regulation of bone mass and susceptibility to osteoporosis may be a beneficial approach for better understanding of the etiology and mechanisms of osteoporosis.

Recently, it was suggested that glucagon-like peptide-1 (GLP-1), a gastrointestinal hormone, plays important roles in bone modulation via its receptors (Yamada et al., 2008). The GLP-1 receptor (GLP1R) is a cognate G-protein-coupled receptor and its activation by exogenous GLP-1 analogue, exendin-4, enhances bone strength and prevents the deterioration of trabecular microarchitecture (Ma et al., 2013). These findings suggest that GLP1R is an important bone mass modulating factor. Furthermore, some single nucleotide polymorphisms (SNPs) of *GLP1R* gene were found to lead to changes of expression and function of *GLP1R* and to be associated with development of certain diseases (Sheikh et al., 2010; Beinborn et al., 2005; Sathananthan et al., 2010). Therefore, it is rational to hypothesize that *GLP1R* gene might also be a candidate gene participating in bone mass turnover and development of osteoporosis.

However, to our best knowledge, no published studies regarding this issue have been performed so far. In order to prove this hypothesis, in the present study, we investigated the relationship between a common tag-SNP (rs1042044) of *GLP1R* and BMD in Chinese Han postmenopausal women.

## MATERIALS AND METHODS

### Subjects

A case-control study was performed with a total of 458 Chinese Han postmenopausal women, including 223 randomly selected primary postmenopausal osteoporosis patients (age from 48 to 79), who were enrolled from Kunming General Hospital of Chengdu Military Command between 2009 and 2013, and 235 health controls (age from 49 to 82). Menopausal status was defined as the date of last menses followed by 12 months of no menses. Individuals with current hepatic disease, renal disease, diabetes mellitus, surgical removal of both ovaries, and/or taking any drugs known to affect bone metabolism were excluded. The study protocol was designed in compliance with the principles of the Helsinki Accord, and was reviewed and approved by the local Ethical Committees. Informed consent statement was obtained from all participants after full explanation of the procedure.

### Measurement of BMD

BMD was measured at the lumbar spine (L<sub>2</sub>-L<sub>4</sub>) and femoral neck

by dual-energy X-ray absorptiometry (DEXA) (Lunar Expert 1313, Lunar, Madison, WI). The value of BMD was automatically calculated from bone mineral content (g) and bone area (cm<sup>2</sup>) and expressed in g/cm<sup>2</sup>.

### Genotyping assay

Peripheral venous blood samples (3 ml) were collected into tubes containing ethylenediaminetetraacetic acid and stored at -80°C for analysis. Genomic DNA extraction was performed using the QIAGEN DNA MicroKit® (Valencia, CA) according to the manufacturer's instructions. The genotyping of the non-synonymous tag-SNP (rs1042044) was performed according to previous report (Sheikh et al. 2010) by TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA). Briefly, TaqMan PCR was performed in a 10 µL system containing 1× ABI TaqMan Genotyping Master Mix, TaqMan 40× probe mix, and 25 ng of DNA on an ABI StepOne™ Real-Time PCR System (Applied Biosystems) and analyzed using SDS v3.0 software (Applied Biosystems). The PCR conditions were: DNA denaturing at 95°C for 10 min, 50 cycles of replication at 95°C for 15 s, and then annealing and extension at 60.0°C for 1 min followed with final extension for 3 min. The primers used were as follows: forward 5'CCTAGCCAGAGATGTGAGCT3' and reverse 5' CAGCAGTGC GTTCCAAGTAC 3' (chr6:39041252+39041636).

### Statistical analysis

Data were analysed with SPSS 18.0 for Windows (PASW Statistics, SPSS Inc., Chicago, IL). The genotypic frequency for rs1042044 was tested for departure from Hardy-Weinberg Equilibrium (HWE). Chi-square ( $\chi^2$ ) test was used to determine the differences of genotypic and allelic distributions between osteoporosis case and control group, and also the odds ratios (OR) with 95% confidential intervals (CIs) of osteoporosis risk. Student *t* test was used to determine the differences in anthropometric characteristics between case and control group. Differences in anthropometric characteristics according to genotypes were tested by one-way analysis of variance. Differences in BMD according to genotypes were analyzed by using analysis of covariance adjusting for potential confounders, that is, age, body mass index (BMI) and years since menopause.

## RESULTS

### Population characteristics

Descriptive statistics of both the case and control groups are shown in Table 1. There were no significant differences regarding the age ( $P = 0.979$ ), BMI ( $P = 0.662$ ), and years since menopause ( $P = 0.834$ ) between the 2 populations. Meanwhile, the BMD values in osteoporosis case group were significantly lower compared to health controls at both lumbar spine and femoral neck ( $P < 0.001$  for both positions).

### Distributions of genotype and allele frequencies of rs1042044 between osteoporosis patients and health controls

The distributions of genotype and allele frequencies of

**Table 1.** Characteristics of osteoporotic and control postmenopausal women.

Parameter	Case (n = 223)	Control (n = 235)	P <sup>a</sup>
Age (years)	59.9 ± 7.2	59.9 ± 7.3	0.979
BMI (kg/m <sup>2</sup> )	23.4 ± 2.8	23.3 ± 2.8	0.662
Years since menopause (years)	10.1 ± 6.9	10.0 ± 7.0	0.834
BMD (g/cm <sup>2</sup> )			
Lumbar spine	0.785 ± 0.039	0.973 ± 0.073	<0.001
Femoral neck	0.653 ± 0.071	0.825 ± 0.058	<0.001

**Table 2.** Frequency distribution of *GLP1R* rs1042044 polymorphisms and their associations with the risk of osteoporosis in postmenopausal women.

rs1042044	Case	Control	OR (95% CI)	P <sup>a</sup>
	n (%)	n (%)		
Genotype				
AA	69 (30.9)	44 (18.7)	1.000 [Ref]	
AC	109 (48.9)	120 (51.1)	0.579 (0.366 - 0.916)	<b>0.003</b>
CC	45 (20.2)	71 (30.2)	0.404 (0.238 - 0.688)	
Allele				
A	247 (55.4)	208 (44.3)	1.000 [Ref]	
C	199 (44.6)	262 (55.7)	0.640 (0.493 - 0.830)	<b>0.001</b>

OR odd ratio, CI confidence interval, Ref reference category. <sup>a</sup> P values were calculated from two-sided chi-square tests for genotype or allele distribution.

*GLP1R* rs1042044 in the two groups are listed in Table 2. The  $\chi^2$  test revealed that the genotypic frequencies of both groups did not deviate from HWE ( $\chi^2 = 0.027$ ,  $P = 0.870$  for case group; and  $\chi^2 = 0.287$ ,  $P = 0.592$  for control group). In the following association analysis, we found that the genotypic frequencies in osteoporosis case group were significantly different from those of healthy control group ( $P = 0.003$ ). Furthermore, osteoporosis was much less frequently observed in the AC (OR, 0.579; 95% CI, 0.366 to 0.916) and CC (OR, 0.404; 95% CI, 0.238 to 0.688) genotypes than in the AA genotype, which exhibited an obvious gene dosage effect. Accordingly, data about the distribution of allele frequency between the two groups also showed that the C allele was significantly frequent in control group and may be a protective factor against osteoporosis ( $P = 0.001$ ; OR, 0.640; 95% CI, 0.493 to 0.830).

#### Association of *GLP1R* rs1042044 genetic polymorphisms with BMD

We then combined the two groups and analyzed the differences of anthropometric characteristics according to genotypes. As shown in Table 3, no significant differences in age ( $P = 0.204$ ), BMI ( $P = 0.181$ ), or years since menopause ( $P = 0.117$ ) according to genotypes of rs1042044 were observed in the present study. However,

the rs1042044 polymorphism was found to be still significantly associated with BMD at both lumbar spine and femoral neck ( $P < 0.001$ ) even after adjustment for the possible confounding factors (age, BMI, and years since menopause). Interestingly, these data also exhibited a gene dosage effect that the number of the C allele in the genotypes was positively correlated with the BMD values (Table 3).

#### DISCUSSION

GLP-1, which induces a variety of physiological effects by activating GLP1R, is mainly produced by L-cells primarily localized in the ileal/colonic mucosa and is hence considered as an important gastrointestinal hormones (Drucker, 1998; Kieffer and Habener, 1999). However, the role of *GLP1R* gene in the pathology of osteoporosis is unclear and need to be investigated. It was recently reported that the entero-bone endocrine axis was proposed as a mediator of bone turnover (Isales and Hamrick, 2008). Therefore, the association between *GLP1R* and BMD and osteoporosis seems to be quite plausible with regard that growing evidence indicated that osteoporosis is a multifactorial and polygenic disease (Zhao et al., 2005; Lee et al., 2014). In the present study, we assessed the effects of rs1042044 polymorphism in *GLP1R* gene on BMD values and osteoporosis risk in

**Table 3.** The characteristics of *GLP1R* rs1042044 polymorphisms in the total group of subjects in the total group of postmenopausal women.

Genotype	AA	AC	CC	P <sup>a</sup>
n (%)	113 (24.7)	229 (50.0)	116 (25.3)	-
Age (years)	59.2 ± 7.1	59.7 ± 7.0	60.9 ± 7.8	0.204 <sup>a</sup>
BMI (kg/m <sup>2</sup> )	23.8 ± 2.8	23.2 ± 2.7	23.3 ± 2.9	0.181 <sup>a</sup>
Years since menopause (years)	9.2 ± 6.8	9.9 ± 6.6	11.1 ± 7.5	0.117 <sup>a</sup>
BMD (g/cm <sup>2</sup> )				
Lumbar spine	0.838 ± 0.099	0.887 ± 0.110	0.912 ± 0.112	<0.001 <sup>b</sup>
Femoral neck	0.700 ± 0.108	0.741 ± 0.106	0.780 ± 0.098	<0.001 <sup>b</sup>

BMI body mass index, BMD Bone mineral density. <sup>a</sup> P values were calculated by one-way analysis of variance (ANOVA). <sup>b</sup> P values were calculated with analysis of covariance adjusted for age, body mass index and years since menopause.

Chinese Han postmenopausal women. Our findings revealed for the first time that genetic variants of rs1042044 in *GLP1R* were significantly associated with osteoporosis and that the C allele of rs1042044 was a protective factor against osteoporosis, which gene dosage-dependently increased BMD values.

The rs1042044 of *GLP1R* is a tag-SNP located in exon 7 of the *GLP1R* gene. This SNP is a nonsynonymous variant resulting in a cytosine (C) to adenine (A) change at the sequence level and phenylalanine (Phe) to leucine (Leu) at position 260 of the GLP1R protein (Phe260Leu). Furthermore, the minor allele frequency of this SNP is much greater than 5% in the Chinese Han population according to Data from HapMap (<http://www.hapmap.org>). Therefore, we chose this SNP in this study trying to assess its association with osteoporosis. According to our data, the minor allele (A) frequencies of rs1042044 in total participants was 0.497, which was close to the allele frequency data of Chinese Han population from HapMap (<http://www.hapmap.org>), indicating that the participants consist of a representative population sample for this case-control study.

The functionality of rs1042044 has previously been studied by Fortin et al. (2010) who demonstrate, using a cAMP reporter gene assay, that this polymorphism did not alternate the pharmacological properties of GLP1R (Fortin et al., 2010). However, given the location of the amino acid substitution, it is possible that other aspects of the receptor function may be affected (desensitization or internalization), which need to be further clarified. Since the nature of the present study is exploratory, we did not preformed further mechanism investigations, which is an intrinsic limitation of this study. However, our findings are in accordance with previous investigation which also indicated that variants of this SNP altered the protein function of GLP1R (Sheikh et al., 2010). Some characters of rs1042044 may explain the mechanisms. The location of this SNP is within the second intracellular loop of the mature translated GLP1R protein, whose variation may possibly affect the interaction between GLP1R and G-

proteins and therefore alter downstream GLP1R signaling (Bavec et al., 2003). It is also possible that this tag-SNP variant may capture other nearby functional SNPs that influence either ligand affinity or downstream signaling of GLP1R (Sheikh et al., 2010; Bavec et al., 2003; Al-Sabah and Donnelly, 2003; Lopez de Maturana and Donnelly, 2002; Lopez de Maturana et al., 2004; Xiao et al., 2000). Further research is needed to elucidate this issue in the future. Another limitation of our study is the relatively small sample size (223 cases and 235 controls). Therefore, larger sample size as well as meta-analysis is warranted to validate the results of our work. Meanwhile, the race of the studied participants is Han Chinese. Whether our present conclusions can be appropriately drawn in other human races or not still needs to be clarified.

In summary, our present study finds novel evidence that variants of rs1042044 in *GLP1R* gene is associated with BMD values and osteoporosis risk in Chinese Han postmenopausal women, which provides new insight into the mechanisms of osteoporosis development and target for personal prevention and treatment of osteoporosis.

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## Conflict of interest

The authors have not declared any conflict of interest.

**Abbreviations:** BMI, Body mass index; BMD, bone mineral density; CI, confidence interval; GLP-1, glucagon-

like peptide-1; **GLP1R**, glucagon-like peptide-1 receptor; **HWE**, Hardy–Weinberg Equilibrium; **ORs**, odds ratios; **SNP**, single nucleotide polymorphism.

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