Relationship Between GIP Receptor Gene Polymorphism rs10423928 and Bone Mineral Density in Postmenopausal Women in Shanghai

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Research

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Abstract

Background: GIP (glucose-dependent insulinotropic peptide) has been found to affect bone metabolism. GIPR single nucleotide polymorphism (SNP) is related to its activity, but the relationship between GIPR SNP and osteoporosis in postmenopausal women is still unclear. The Aim of this study was to investigate the association between GIPR SNP and bone mineral density (BMD) in postmenopausal women in Shanghai.

Methods: GIP SNP rs10423928 was detected in 884 postmenopausal women in Shanghai. The correlation between GIPR SNP and BMD was further assessed.

Results: There was a statistical difference between the dominant model of this site rs10423928 and the bone mineral density of the femoral neck (P = 0.035) and the Wards triangle area (P = 0.033).

Conclusions: The rs10423928 of GIPR is related to the BMD of postmenopausal women in Shanghai, China.

Background

Postmenopausal osteoporosis is related to aging and seriously affects the health of postmenopausal women. Bone mineral density (BMD) is the most commonly used indicator for the diagnosis of osteoporosis, and 60–80% of its variation is determined by genetic factors(1).

Studies have found that some commonly used drugs that regulate blood sugar, such as glucagon-like peptide-1 (GLP-1) receptor agonist, DPP-4 inhibitor (DPP-4i), can inhibit bone resorption and improve bone formation (2–7). GLP-1 is one of the substrates of DPP-4 enzyme, and other substrates of DPP-4 such as: GIP (glucose-dependent insulinotropic peptide), GLP-2 (glucagon-like peptide-1), IGF − 1 (insulin-like growth factor-1) have also been found to affect bone metabolism, or promote bone formation, or reduce bone resorption by osteoclasts (8–11).

GIP is an intestinal insulinotropic hormone that is synthesized and secreted by K cells of the duodenum and jejunum, and exerts a biological effect by binding to the corresponding GIP receptor (GIPR) on the cell surface: promotes the secretion of insulin in the first phase; improves the body's sensitivity to insulin. Our previous studies have demonstrated that there was the correlation between polymorphisms of GLP-1R gene and BMD of lumbar spine, femoral neck, and total hip in postmenopausal women of Han nationality in Shanghai, China(12). Howerver, it is unclear whether there is a correlation between polymorphisms of GIPR gene and osteoporosis. So far, few studies on GIPR gene polymorphism and osteoporosis in postmenopausal women have been performed. The purpose of our research was to explore the correlation between GIPR gene polymorphism and BMD in postmenopausal women in Shanghai.

The GIP receptor (GIPR) gene is located on chromosome 19 q13.3. GIPR is widely distributed in various tissues and organs of the human body, and is most abundantly expressed in pancreatic islet β cells,followed by islet α cells(13). In addition, it is also expressed in extra-pancreatic tissues such as osteoblasts, cardiomyocytes, vascular endothelial cells, stomach, small intestine, adipose tissue, kidney and central nervous system (14). It is speculated that gene mutations or mutations encoding GIPR may lead to abnormal expression and function of GIPR, which may be related to the risk of osteoporosis. We screened for GIPR single nucleotide polymorphism (SNP) in the GIPR locus of the CHB (Han ethnic group from Beijing) population of HapMap second stage data (http://www.hapmap.org). SNP mainly refers to DNA sequence polymorphism caused by single nucleotide variation at the genome level. SNP has now become the third-generation genetic marker. Many phenotypic

differences in human body susceptibility to drugs or diseases, etc. may be related to SNP. SNP will provide a powerful tool for the discovery of high-risk groups, the identification of disease-related genes, the design and testing of drugs, and biological basic research of science.

The 884 postmenopausal female subjects participating in the study measured lumbar spine 1–4 (L1-4), left femoral neck (Femoral neck) and total hip (Total) BMD, expressed in g/cm^2 using dual-energy X-ray absorptiometry (GE-LUNAR Prodigy USA). According to strict quality control requirements, the instrument is controlled by a special person and measured once with a standard phantom before use every day to evaluate the stability of the system. The coefficient of variation (CV) of lumbar spine, femoral neck, and total hip BMD measurements were 1.39%, 2.22%, and 0.70%, respectively.

This study was approved by the Ethics Committee of the Sixth People's Hospital Affiliated to Shanghai Jiaotong University, and Han women who were treated in the osteoporosis and bone disease specialty of Shanghai Sixth People's Hospital were selected. All the subjects were informed and signed the consent form.

Inclusion criteria: natural menopause lasted more than 1 year; did not receive anti-osteoporosis drugs (except calcium tablets and vitamin D); without diseases that affect bone metabolism.

We used the established postmenopausal women's database and included BMD and other clinical data to analyze the correlation between GIPR gene polymorphism and BMD of postmenopausal women in Han nationality in Shanghai. In this experiment, Shanghai Tianhao Biotechnology Co., Ltd.'s iMLDR® multiple SNP typing (15) was used to classify SNP sites.

Methods

Detection of SNPs

The selection of tag SNP is based on the International Human Genome Haplotype Program (International HapMap Project. http://www.Hapmap.org/cgi-perl/gbrowse/hapmap3_B36), and the criteria were as follows: (1) the minimum mean allele frequency (MAF) was > 0.05; (2) the coefficient of link SNP linkage disequilibrium (LD) r^2 was > 0.8; (3) GWAS (Genome-wide association study, genome-wide association analysis) SNPs that had been reported were included in this study. Finally, GIP site rs10423928 was detected in the present study.

Amplification was achieved by multiplex PCR reaction. Each measurable allele locus ligated product was obtained after two ligation reactions. The raw data files were analyzed using Gene Mapper 4.1 (Applied Biosystems, USA). A total of 884 postmenopausal women were analyzed. In this experiment, Shanghai Tianhao Biotechnology Co., Ltd.'s iMLDR® multiple SNP typing (15) was used to classify 1 SNP sites in 884 samples.

Statistical analysis

Statistical analysis was performed using SPSS version 24.0 (IBM SPSS Statistics 24, USA). The continuous variables with normal distribution are expressed as mean ± standard deviation($x \pm s$). using SPSS version 24.0 (IBM SPSS Statistics 24, USA) The continuous variables were compared with t test between two groups; the chi-square test was used to compare the categorical variables. Haploview 4.2 was used to calculate the D' value and linkage disequilibrium coefficient (r^2) of the linkage disequilibrium (LD) between SNPs, and the haplotype region

and corresponding haplotype were obtained. After adjustment for age, a linear regression model was employed to assess the relationship between GIPR SNP, and BMD in postmenopausal women. A value of $P < 0.05$ was considered statistically significant.

Results

Characteristics of subjects

A total of 907 postmenopausal women were included, but some subjects were excluded from this study because the samples were contamined, had poor quality or were not successfully typed after one failure. Finally,884 samples from postmenopausal women (mean age: 67.2 ± 10.0 years) were subjected to the detection of SNPs. In addition, subjects were divided into < 60 years group and ≥ 60 years group. The baseline characteristics of subjects included for final analysis are shown in Table 1.

Table 1
Basic information of the research object

Characteristics	Age < 60 (n = 224)	Age ≥ 60 (n = 660)	P-Value
Age (year)	54.9 ± 5.8	71.3 ± 7.4	0.00
Height (cm)	156.2 ± 5.2	152.0 ± 5.4	0.00
Weight (kg)	57.6 ± 8.4	55.2 ± 8.5	0.027
BMI(kg/cm^2)	23.6 ± 3.3	23.9 ± 3.5	0.497
Blood calcium (mmol/L)	2.34(2.27–2.40)	2.32(2.26–2.39)	0.718
Blood phosphorus (mmol/L)	1.14(1.03–1.23)	1.12(1.01–1.23)	0.700
Albumin	47.00(46.00–49.00)	46.00(44.00–48.00)	0.008
Alkaline phosphatase	69.00(56.00–80.00)	72.00(60.00–90.00)	0.004
Creatinine	54.00(49.00-60.75)	59.00(52.00–66.00)	0.00
25(OH)D(ng/mL)	20.92(16.28–26.86)	21.36(15.56–27.97)	0.87
Parathyroid hormone	40.65(32.82–53.34)	42.37(31.63–56.22)	0.128
β-Collagen specific sequence (ng/L)	403.50(223.00-5630)	366.00(216.75–551.00)	0.68
L1-4BMD(g/cm^2)	0.894(0.806–0.992)	0.859(0.773–0.968)	0.008
Neck BMD (g/cm^2)	0.758(0.708–0.848)	0.692(0.623–0.761)	0.00
TotalBMD (g/cm^2)	0.801(0.727–0.895)	0.742(0.662–0.817)	0.00
The continuous variables of the normal distribution are represented by the mean ± standard deviation (x ± s), and the data of the non-normal distribution is represented by the median and the interquartile range.			
There was no significant difference in serum, calcium and phosphorus between the two groups. Alkaline phosphatase was significantly higher in the group aged ≥ 60 years than in the control group (P = 0.004). The bone mineral density(BMD) of each group in the age group of ≥ 60 years old was lower than that of the control group (P < 0.001).			

Alleles frequency

In this study, GIPR locus rs10423928 SNP was genotyped and analyzed. SNPs were examined in which the minimum allele frequency (MAF) was greater than 0.01. The genotype distribution met the Hardy-Weinberg equilibrium, and the MAF of these SNPs was similar to the genetic variation of Beijing Han population in China (CHBS). The minimum allele frequency of the GIPR locus rs10423928 in the Han population in Beijing is 20%, and it was 20.8% in our study. The results were similar(Table 2).

Table 2
SNP site information of GLP-1R and GIPR genes

SNPs	Chr. position	SNP property	Alleles	HWE P Value	MAF in CHBS	MAF In this study
rs2268657	39020542	intron1	C/T	0.1902	0.34	0.326
rs2295006	46182304	nonsynon_exon2	G/A	0.7755	0.07	0.06
rs3765467	46182304	nonsynon_exon4	G/A	0.5521	0.23	0.255
rs6923761	39055485	nonsynon_exon5	G/A	1	0.01	0.01
rs1042044	39041502	nonsynon_exon7	C/A	1	0.47	0.46
rs2268641	39050266	intron12	C/T	0.5249	0.39	0.417
rs4714210	39055485	3'-UTR_exon13	G/A	0.1432	0.29	0.317
rs10423928	46182304	intron12	A/T	0.9107	0.20	0.208

MAF: The minimum allele frequency of the GIPR locus rs10423928 in the Han population in Beijing is 20%, our research result is 20.8%, and the results are close.

In our study there was no linkage disequilibrium relationship between GLP-1R SNPs and GIPR locus rs10423928 SNP ($0.908 < D' < 1$). (Fig. 1).

Clinical data corresponding to GIPR SNP site rs10423928 in postmenopausal women were in Table 3. The locus rs10423928 has three genotypes: T/T, A/T, and A/A. Among them, T/T genotype accounts for 556 patients (62.9%), A/T genotype accounts for 289 patients (32.7%), and A/A genotype accounts for 39 patients (4.4%).

Table 3
Clinical data corresponding to GIPR SNP site rs10423928 in postmenopausal women

rs10423928	N	Age	Height	Weight	BMI	L1-4	Femoral neck	Total hip
		(years)	(cm)	(kg)	(Kg/m^2)	BMD(g/cm^2)	BMD(g/cm^2)	BMD(g/cm^2)
T/T	556	67.1 ± 10.1	152.7 ± 5.5	55.5 ± 8.9	23.8 ± 3.6	0.877 ± 0.161	0.708 ± 0.120	0.755 ± 0.132
A/T	289	67.6 ± 9.8	153.7 ± 5.7	56.1 ± 8.2	23.7 ± 3.3	0.896 ± 0.155	0.728 ± 0.119	0.769 ± 0.130
A/A	39	65.4 ± 9.7	152.0 ± 5.8	56.5 ± 6.1	24.5 ± 3.0	0.882 ± 0.148	0.719 ± 0.124	0.773 ± 0.143

The linear regression analysis was employed to evaluate the correlation between rs10423928 and BMD. There were no relationship of rs10423928 with age, height, weight and body mass index (BMI) ($P > 0.05$). A close correlation was noted between rs10423928 and femur neck BMD (Table 4).

Table 4
Relationship between GIPR gene SNP rs10423928 and BMD in postmenopausal women

rs10423928	age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)	L1-4 BMD(g/cm^2)	Femur neck BMD(g/cm^2)	Total hip BMD(g/cm^2)
P- Value dominant	0.753	0.176	0.527	0.982	0.331	0.035*	0.118
P-value recessive	0.271	0.449	0.702	0.377	0.331	0.737	0.427
P-value- addictive	0.898	0.403	0.504	0.725	0.639	0.059	0.111
*P ≤ 0.05, ** P ≤ 0.01							

The relationship between locus rs10423928 and BMD was further assessed in postmenopausal women. There was a correlation between locus rs10423928 and BMD at specific site. The T/T genotype of rs10423928 was positively related to femur neck BMD and wards triangle area BMD ($P < 0.05$) (Table 5).

Table 5
Linear regression analysis of GIPR gene locus rs10423928 and BMD in postmenopausal women

BMD	dominant		recessive		addictive	
	β	P value	β	P value	β	P value
L1	0.014	0.180	-0.004	0.860	0.010	0.289
L2	0.017	0.150	0.002	0.930	0.012	0.216
L3	0.021	0.123	0.000	0.988	0.015	0.194
L4	0.026	0.053	0.015	0.639	0.020	0.073
L1_2	-0.019	0.296	0.018	0.671	-0.011	0.468
L1_3	-0.023	0.271	0.039	0.426	-0.011	0.522
L1_4	-0.021	0.331	0.050	0.331	-0.009	0.639
L2_3	-0.010	0.603	0.038	0.407	-0.002	0.888
L2_4	-0.010	0.611	0.050	0.297	-0.001	0.956
L3_4	0.010	0.581	0.044	0.293	0.013	0.402
Neck	0.018	0.035*	0.007	0.737	0.013	0.059
Wards	0.019	0.033*	0.016	0.466	0.016	0.040*
Troch	0.009	0.278	0.005	0.802	0.007	0.317
Inter	0.017	0.140	0.032	0.234	0.016	0.096
Total	0.014	0.118	0.017	0.427	0.012	0.111
*P ≤ 0.05, ** P ≤ 0.01						
β: Regression coefficients						

Discussion

Genome wide association studies (GWAS) confirmed that bone mineral density is associated with multiple genetic susceptibility regions (16, 17). The correlation between vitamin D receptor gene polymorphism and osteoporosis was first revealed by Morrison et al (18) in 1994. Since then, more than one hundred gene polymorphisms related to bone metabolism regulation have been studied including sex hormones and their receptors, bone matrix component related genes, apolipoprotein E (ApoE), etc., the results have confirmed the important role of genetic factors in primary osteoporosis.

In the literature (19), the variation of GIPR gene SNP locus rs10423928 was associated with increased postprandial blood glucose and insulin. In addition, this locus was also associated with decreased body mass index (BMI), lean body mass and waist circumference (20). Therefore, we chose to study this site of GIPR gene. There is a strong linkage disequilibrium between rs10423928 and rs1800437 (located in exon 10, E354Q) (r^2 = 0.99). Related studies (21, 22) have confirmed that mutations in rs1800437 can reduce the expression of GIPR in

carriers. The same genetic variation is reflected in. Studies have found that patients with functional SNP loci rs10423928 linkage disequilibrium are associated with low BMD in early postmenopausal women (23).

Our study found that there is a positive correlation between the dominant model T / T of rs10423928 and BMD of the femoral neck and Wards triangle area. That is to say, if the GIPR gene rs10423928 is homozygous T / T in postmenopausal women, it will enhance BMD of the femoral neck and Wards triangle area.

Regarding the effect of GIP on bone, a study (8) found that in mice with GIPR deficiency (GIPR KO), the cortical bone becomes thinner, the number of endosteal osteoclasts increases, and the bone mineral density decreases, which indicates the bone strength and quality variety. 14 ovariectomized 12-week-old BALB / c female mice were randomly divided into 2 groups, respectively treated with GIP analog N-AcGIP (25 nmoles / kg / day bw) and normal saline, and 10 BALB // c Female rats were injected with normal saline as a control group. Micro-CT tomography and 3D reconstruction techniques found that the number of bone trabeculae increased after GIP treatment in ovariectomized mice. TRACP staining and bone microstructure indicators suggested that the number of osteoclasts decreased and bone resorption decreased (9). (N-AcGIP: It is a GIP analogue. After acetylation of the 1st tyrosine at the amino terminus, it can enhance the resistance to DPP-4 enzyme without being destroyed and inactivated by the enzyme.). Daily injection of GIP into Sprague-Dawley rats with ovary removed proved that GIP can inhibit the acceleration of bone turnover caused by estrogen loss (24). Studies have shown that transgenic mice overexpressing GIP have higher BMD and bone mineral content (BMC) compared to the control group. In addition, their serum GIP and total osteocalcin levels are increased (25).Gene knockout mice lacking GIPR disturb the cortical microstructure of bones, leading to a decrease in bone "quality", strength, and fat content (8). These observations indicate that there is a complex shared molecular mechanism between osteoporosis and diabetes, and incretin GIP can improve bone metabolism.

At present, it is found that GIP affects bone directly and indirectly (10). Direct route: GIP combined with GIPR on osteoblasts can increase intracellular Ca^{2+} and cAMP concentrations, thereby promoting bone alkaline phosphatase activity and type I collagen mRNA expression (14, 26), promoting bone matrix Mineralization.In addition, the addition of GIP to the cultured osteoblast precursors can promote their differentiation, increase their proliferation and show anti-apoptotic activity in pluripotent mesenchymal stem cells in the bone marrow (27). Higher levels of transforming growth factor-β (TGF-β) are known to stimulate osteoblasts, and GIP can promote the secretion of TGF-β and improve bone metabolism. For osteoclasts, GIPR expressed in osteoclasts can downregulate bone resorption, inhibit osteoclast resorption activity, and reduce the expression of osteoclast differentiation markers (such as TRACP enzyme), as well as cathepsins K and the expression of G-CSF (granulocyte colony stimulating factor) receptor (28).

Indirect route: 1. It is known that the combination of insulin and insulin receptor on the surface of osteoblast membrane will promote bone formation. GIP acts on pancreatic β cells, enhances glucose-induced insulin secretion (19), and promotes insulin secretion.2. GIP receptors (GIPR) are also distributed on fat cells, which produce inflammatory cytokines (such as leptin, lipase, adiponectin, amylin etc.), GIP acts on fat cells and regulates secretion of leptin and adiponectin etc. LP can promote the differentiation of bone marrow mesenchymal stem cells into osteoblasts and inhibit their differentiation into osteoclasts and adipocytes, thereby maintaining the balance of bone metabolism, maintaining bone mass and bone quality. 3. GIP can regulate endothelial cell production of vascular endothelin-1 and nitric oxide, both of which have effects on bone turnover (29) Therefore, GIP indirectly plays an important role in the process of bone conversion (30).

So far, only one study at home and abroad has investigated the correlation between GIPR and BMD. It is reported that patients with functional SNP loci rs10423928 linkage disequilibrium are associated with low BMD in early postmenopausal women (23). Some studies have found that GIP polymorphism is not related to serum GIP and young women; but it is inversely related to the following components in the elderly: bone strength, including density, mineral content and microstructure (31).Another study reported that postmenopausal women's serum GIP levels increased (32) and were regulated by estrogen replacement therapy (33).

About 50% of postprandial insulin secretion is the result of GIP. In type 2 diabetes patients, the insulinotropic effect of GIP has been reduced, and studies have shown that impaired GIP response is associated with insulin resistance (34). Assuming that patients with type 2 diabetes may express lesser amounts of GIPR or defective GIPR (35), then GIPR deficiency may be the pathophysiological mechanism of type 2 diabetes (36). Various intestinal hormones such as GIP and leptin can regulate bone turnover, which we call the "gut-bone axis" system, and there is a close relationship between bone turnover and intestinal hormones (37). Discussing the genetic polymorphism of intestinal hormones and their receptors can further clarify their important role in osteoporosis.

Conclusion

Our research shows that there is a positive correlation between the dominant model T / T of the polymorphism GIPR gene rs10423928 in serum and BMD of the femoral neck and Wards triangle in postmenopausal women of Han nationality in Shanghai, China. GIPR gene polymorphism SNP sites can guide the evaluation of the body's metabolic diseases. Regarding the correlation between the rest of the GIPR gene polymorphism and BMD, we will further study and confirm. This study laid a certain research foundation for clarifying the effect of GIPR on osteoporosis and diabetic osteoporosis.

Abbreviations

GIP glucose-dependent insulinotropic peptide

GIPR glucose-dependent insulinotropic peptide receptor

SNP Single-nucleotidepolymorphisms

BMD Bone mineral density

DPP-4 Dipeptidyl peptidase-4

GLP-2 glucagon-like peptide-1

IGF -1 insulin-like growth factor-1

MAF mean allele frequency

LD linkage disequilibrium

GWAS Genome-wide association study

CHBS Beijing Han population in China

BMI body mass index

TRACP tartrate resistant acid phosphatase

G-CSF granulocyte colony stimulating factor

TGF-β transforming growth factor-β

Declarations

Ethics approval and consent to participate

ethical approval was granted by Ethics Committee of Shanghai Sixth People's Hospital.

Consent for publication

Both written and verbal informed consent were also sought from all the participants during the data collection exercise.

Availability of data and material

All data generated or analysed during this study are included in this published article [and its supplementary information files]

Competing interests

The other authors have no conflicts of interest to declare.

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Author's contribution

LZ and BF designed the study, LZ and JH collected the data, analyzed the data, interpreted the results and prepared the submission for publication. XS, DP and JH collected samples and data, performed data analysis and interpreted the results.

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Contributions

(I) Conception and design: L Zhang, B Feng; (II) Administrative support: B Feng; (III) Provision of study materials or patients: J He; (IV) Collection and assembly of data: J He, L Zhang ,X Sun; (V) Data analysis and interpretation: X Sun, W He, Dongyue Pang, Jingjing Hu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

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Figures

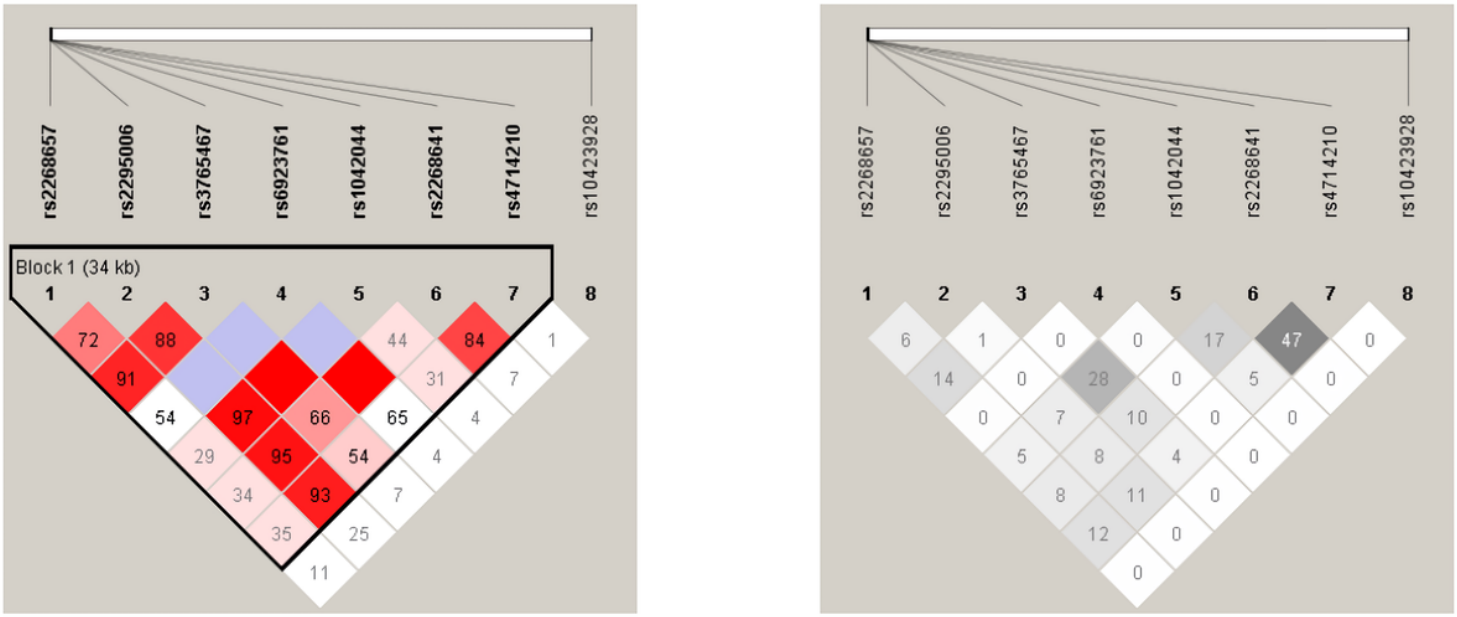
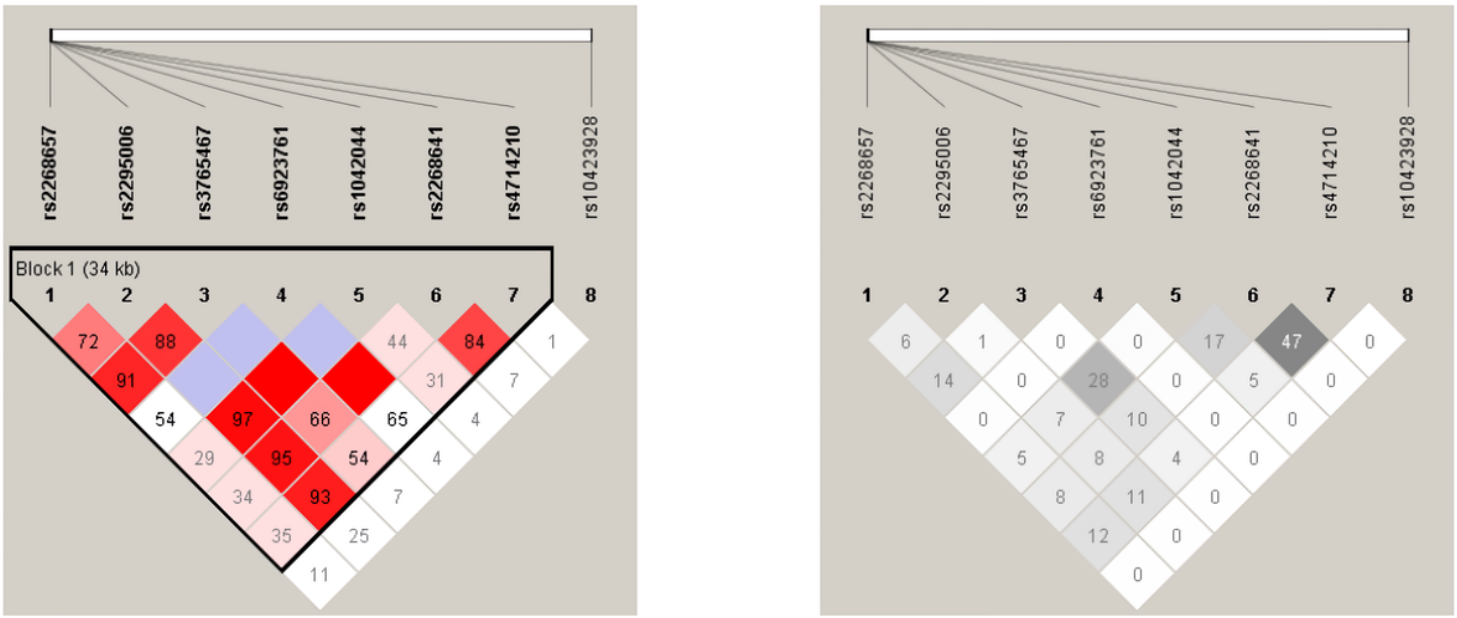


Figure 1

The linkage disequilibrium diagram of GLP-1R SNPs and GIPR



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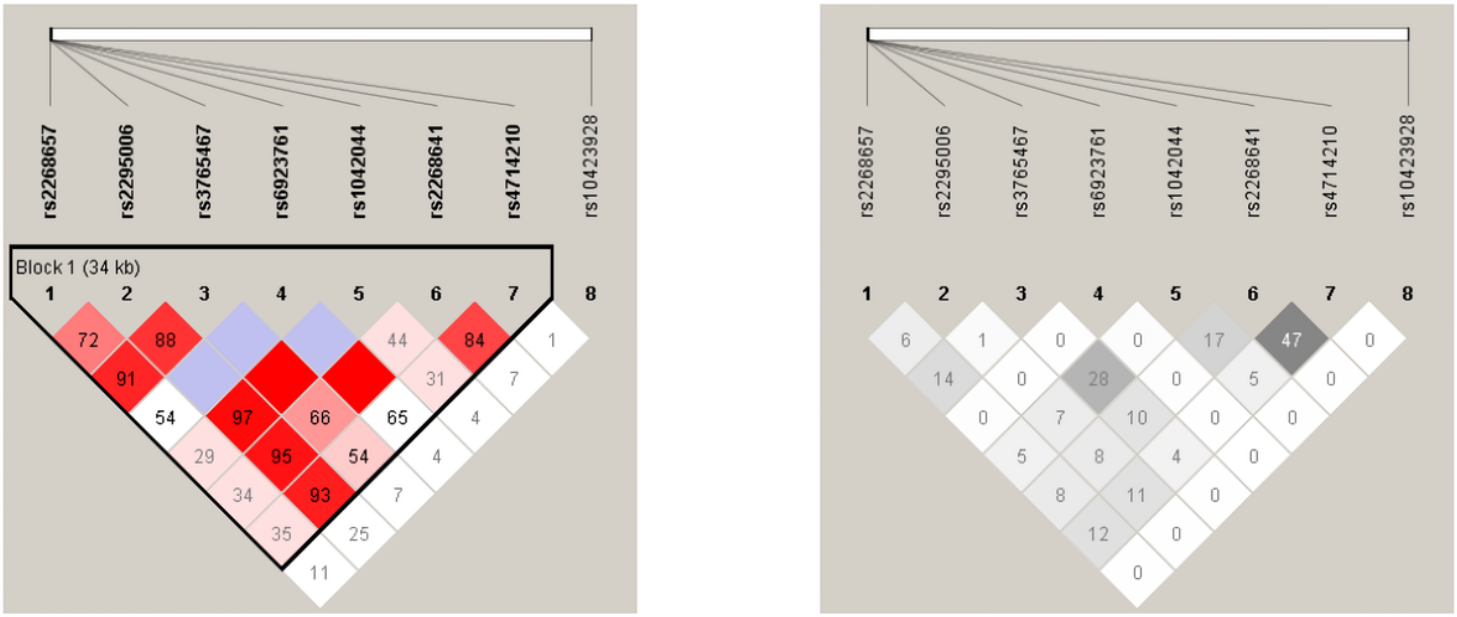

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