Association between osteocalcin *Hind* Ⅲ genetic polymorphism and body mass index variation****★

Investigation of 390 premenopausal women in Nanchang region

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Abstract

BACKGROUND: Body mass index (BMI) is a commonly used phenotype for obesity, which is determined by multiple genetic factors.

OBJECTIVE: To investigate whether osteocalcin (also known as bone Gla protein, BGP) *Hind* Ⅲ genetic polymorphism is associated with BMI variation.

METHODS: A total of 390 premenopausal women from a local population of Nanchang City were selected. Body weight and height were measured. All participants were genotyped at the BGP *Hind* Ⅲ locus using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

RESULTS AND CONCLUSION: The BGP genotype frequencies of HH, Hh and hh were 0.077, 0.408 and 0.515, respectively. The distribution of BGP *Hind* Ⅲ genotypes was consistent with Hardy-Weinberg equilibrium ($P > 0.05$). The BGP *Hind* Ⅲ were significantly associated with BMI (P=0.002), which could explain about 5.47% of BMI variation. On average, BMI of individuals with HH genotype was the highest [(22.81±0.73) kg/m^2], individuals with Hh genotype was intermediate [(21.50±0.53) kg/m^2], while individuals with hh genotype was the lowest [(20.23±0.63) kg/m^2]. Therefore, carriers of HH and Hh genotypes had, respectively, approximately 12.75% and 6.28% higher BMI than carriers of the hh genotype. To our best knowledge, this is the first study reporting the association of BGP *Hind* Ⅲ genetic polymorphism and BMI in healthy premenopausal women.

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INTRODUCTION

The rising tide of obesity is one of the most pressing health problems of our time[1-3]. Body mass index (BMI) is a WHO standard index for obesity, which is a complex quantitative trait determined by multiple genetic factors, with heritability ranged 20%−90%[4-8]. A number of candidate genes underlying the BMI variation were suggested[9]. However, it is far from well identified the substantial genes underlying. Osteocalcin, an osteoblast-specific protein, also called bone Gla protein (BGP), has recently been reported to be a new metabolic hormone regulating the adiposity and glucose homeostasis in experimental animal[10-12]. Osteocalcin was suggested as the only molecule made by osteoblasts that accounts for the osteoblasts-mediated regulation of glucose metabolism[13], and the results showed that osteocalcin increased adiponectin and insulin expression in adipocytes and β-cells, respectively. Furthermore, Osteocalcin-deficient mice displayed obesity[10]. Recently, several clinical studies revealed that serum osteocalcin level was associated with BMI, glucose metabolism and body fat[11, 14-17]. The BGP gene is located on 1q25−q31 and consists of four exons and three introns spanning about 2 kb. The BGP *Hind* Ⅲ polymorphism was a novel polymorphism identified at the 5' flanking promoter region due to a 1 base pair (bp) substitution from cytosine to thymine at position 298 nucleotides (nt)[18]. Since the detection of the BGP *Hind* Ⅲ polymorphism, it has been frequently used as a genetic marker in genetic study of complex disease[18-19]. To our best knowledge, up to now, no study has investigated the relationship of the BGP genetic polymorphism and BMI variation.

The observed body mass phenotype in osteocalcin null mice coupled with its potential role in glucose metabolism has encouraged us to hypothesize that BGP *Hind* Ⅲ genetic polymorphism is associated with BMI variation. In this investigation, we showed BGP *Hind* Ⅲ genetic polymorphism is associated with BMI variation in premenopausal women.

SUBJECTS AND METHODS

Design

Cross-sectional study

Time and setting

The study was performed at Department of Radioimmunology, People's Hospital of Jiangxi Province and Department of Physiology, Nanchang University, between September 2006 and December 2008.

Subjects

The study was granted by Ethical Committee of Nanchang University and People's Hospital of Jiangxi Province. In this study, 390 unrelated, pre-menopausal Han women were recruited from a local population of Nanchang City. Subjects with diseases, treatments, or conditions that would have apparent influence on healthy and causes for abnormal obesity were excluded. Good health and absence of major organ system disease were documented by clinical examination and blood

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chemistry profile. According to the *Administrative Regulations on Medical Institution* published by State Council of the People's Republic of China, the informed consent was obtained from each subject[20].

Methods

Measurement

Body weight was measured to the nearest 0.01 kg using a digital scale, height was measured to the nearest 0.1 cm using a wall-mounted stadiometer, and BMI was calculated as weight (kg) divided by height squared (m^2).

Genotyping

Genomic DNA was isolated using the phenol-chloroform extraction method[21]. A 253 bp fragment containing the *Hind* Ⅲ polymorphism the BGP gene was amplified by polymerase chain reaction (PCR) using previously described primers and amplification conditions[22]. The PCR products were digested with *Hind* Ⅲ restriction endonucleases (Promega Corp., Madison, WI, USA) and separated by 1.5% agarose gel electrophoresis with ethidium bromide staining. The genotypes for all subjects were represented as HH, Hh, and hh by restriction fragment length polymorphism procedures (RFLP). Uppercase letters represent absence and lowercase letters represent presence of restriction sites.

Main outcome measures

BGP *Hind* Ⅲ genotype and the BMI values of all the subjects.

Statistical analysis

All statistical analysis was conducted using SAS version 6.12 (SAS Institute, Cary, NC, USA). Hardy-Weinberg equilibrium (HWE) at the BGP *Hind* Ⅲ marker locus was examined by the 2 test. The BMI values were verified for normal distribution by Shapiro-Wilks test. Bartlett's test was performed to test the homogeneity of variances in BMI within each of the three BGP *Hind* Ⅲ genotypes. Since age generally affected BMI variation significantly[23], linear regression analyses were performed to adjust for age as covariate. One-way analysis of variance (ANOVA) was used to evaluate the association between BGP *Hind* Ⅲ genetic polymorphism and BMI adjusted for age. A P value < 0.05 was considered statistically significant.

RESULTS

Summarization basic characteristics of the study subjects (Table 1).

The BGP genotype frequencies of HH, Hh and hh were 0.077, 0.408 and 0.515, respectively. The distribution of BGP *Hind* Ⅲ genotypes in our subjects was consistent with the HWE (P > 0.05).

Table 1 Basic characteristics of the subjects (n=390)

	Age (yr)	Height (cm)	Weight (kg)	Body mass index (kg/m^2)
$\bar{x}\pm s$	31.19±5.90	159.90±5.60	55.19±8.05	21.58±2.95
Minimum	21.3	142.0	39.00	15.24
Maximum	42.7	172.5	87.00	30.96

Body mass index is the unadjusted raw data

BGP *Hind* Ⅲ genotype and the BMI values of all the subjects

Bartlett's test for homogeneity of variances of BMI for the three BGP *Hind* Ⅲ genotypes was not significant (P=0.203). Thus the analyses were not significant violation of the assumptions of ANOVA. All the results of one-way ANOVA analysis were summarized (Table 2). Statistical analysis showed that the BGP *Hind* Ⅲ were significantly associated with BMI (P = 0.002), which could explain about 5.47% of BMI variation. On average, BMI of individuals with HH genotype was the highest [(22.81±0.73) kg/m^2], individuals with Hh genotype was intermediate [(21.50±0.53) kg/m^2], while individuals with hh genotype was the lowest [(20.23±0.63) kg/m^2]. Therefore, carriers of HH and Hh genotypes had, respectively, approximately 12.75% and 6.28% higher BMI than carriers of the hh genotype.

Table 2 Association analysis for the osteocalcin *Hind* Ⅲ gene and body mass index ($\bar{x}\pm s$)

Marker	Genotypes	n	Body mass index (kg/m^2)	P value
Osteocalcin *Hind* Ⅲ	HH	30	22.81±0.73	0.002
	Hh	159	21.50±0.53	
	hh	201	20.23±0.63	

The body mass index values are adjusted for age

DISCUSSION

Some evidence has shown that BGP may play an important role in obesity[10-12, 14, 16-17]. However, so far, the association between the BGP polymorphism and BMI has not been reported. In a population of healthy and premenopausal Han original women, we firstly indicated BGP *Hind* Ⅲ were significantly associated with BMI.

Our report indicating the association between the BGP *Hind* Ⅲ genotypes and BMI variation was consistent with previous evidence suggesting a potential role of BGP in determining BMI. First, the BGP *Hind* Ⅲ RFLP was caused by 1 bp substitution from C to T at the 5' flanking promoter region of the BGP gene, which was the important promoter region containing osteocalcin box[24], responsible for glucocorticoid repression[25] and osteoblastspecific regulatory elements[18, 26-27],

and was likely to be functional. Although the molecular mechanisms that underlie the association between the BGP *Hind* Ⅲ gene and BMI remain unclear, we consider that the *Hind* Ⅲ or related linked polymorphisms might alter BGP protein function and might be associated with BMI. Second, the BGP gene was located on 1q25-q31 in human genome (http: www.ncbi.clm.nih.gov/OMIM). This is coincides with the previous quantitative trait locus (QTL) analysis suggesting human genome 1q and 1q25-q41 harbor an important QTL for BMI and weight, respectively[28-29]. Our observation confirm to a certain that the BGP gene is an important gene determining BMI in human genome 1 q. Therefore, it is worth while to focus on the BGP gene for further study to search for gene underlying BMI variation in the genomic region 1q.

It should be pointed out that BMI is a complex trait determined by multiple genetic and environmental factors. Other genetic and environmental factors, such as VDR gene[30], physical activity, diet habits and smoking history[31-32], were not considered in this study. More important genetic and environmental factors should be taken into account in further studies. Second, the sample size in this study is relatively small, the association found here is required to confirm in larger samples in different ethnic origins. In addition, our regular population association study is not family based, which may be plagued by the problem of population admixture[33-34]. The transmission-disequilibrium test (TDT)[35], genomic control (GC) and structured association (SA)[36] is robust approaches immune to population admixture. Thus avoid false positive/negative results due to population admixture[37], the analyses of TDT, GC and SA are needed to confirm the association reported here.

In conclusion, we showed the association of BGP gene and BMI in Chinese. Although the exact mechanism underlying the associations we describe here remains to be elucidated, as does the relative importance of other genetic and environmental variables, our observations should be considered as preliminary.

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骨钙素基因 *Hind* Ⅲ多态性与体质量指数变异的相关联性：南昌地区绝经前女性 390 例调查*****

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摘要

背景： 体质量指数(BMI)是研究肥胖的常用表型，它是由多基因决定的复杂性状。

目的： 验证骨钙素基因 *Hind* Ⅲ多态性是否与体质量指数的变异相关联。

方法： 征集南昌当地绝经前女性 390 例，并测量身高和体质量。用聚合酶链式反应-限制性片段长度多态性(PCR-RFLP)法对所有个体的骨钙素基因 *Hind* Ⅲ位点进行基因分型。

结果与结论： 骨钙素 HH，Hh 和 hh 基因型频率分别为 0.077，0.408 和 0.515。骨钙素基因型分布符合哈的温格尔平衡($P > 0.05$)。骨钙素基因 *Hind* Ⅲ与体质量指数存在显著的关联($P=0.002$)，它可以解释大约 5.47%体质量指数的变异。HH 基因型个体的体质量指数最高[(22.81±0.73)kg/m^2]，Hh 基因型个体的体质量指数居中[(21.50±0.53) kg/m^2]，而 hh 基因型个体的体质量指数最低[(20.23±0.63) kg/m^2]。因此，HH 和 Hh 基因型个体的体质量指数比 hh 基因型个体的体质量指数分别高大约 12.75%和 6.28%。文章首次在健康绝经前女性中报道骨钙素基因 *Hind* Ⅲ多态性与体质量指数的变异相关联。

关键词： 骨钙素基因；*Hind* Ⅲ；多态性；体质量指数；关联
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来自本文课题的更多信息--

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利益冲突：无利益冲突。

课题的创新点：通过 PubMed 数据库搜索关键词：BGP BMI 有相关的文章 14 篇，但是至今尚没有一篇是关于 BGP 和 BMI 的遗传关联研究，因此本文是首篇报道 BGP *Hind* Ⅲ基因多态性与 BMI 的关联性。

课题评估的“金标准”：没有公认的“金标准”。

设计或课题的偏倚与不足：不足之处在于肥胖具有许多因素，包括经济状态和其他相关遗传背景，设计中没有考虑这方面的因素。

提供临床借鉴的价值：文章应用 PCR 方法，对 390 例绝经前妇女 osteocalcin 基因多态性和体质量指数的关系进行了分析，得出 *Hind* Ⅲ基因的多态性与体质量指数相关的结论。对于探索人类肥胖的遗传背景具有一定的价值。实验方法简单可靠。