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The association between sex hormone-binding globulin gene polymorphism with bone mineral density

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ABSTRACT

To investigate the impact of single nucleotide polymorphisms (SNPs) of SHBG gene the neighboring genes on SHBG levels, bone mineral density (BMD) and osteoporosis in Chinese males. A group of Chinese men, aged ⩾45 years were included in the analysis. BMD was measured with dual-energy X-ray absorptiometry (DXA), SHBG and total testosterone (TT) was measured using chemiluminescent immunoassay, and free testosterone (FT) was calculated. SNPs of SHBG gene and the neighboring genes were studied by means of improved multiple ligase detection reaction (iMLDR). A total of 404 men were included in our study. In the single locus analysis, significant associations were found between SHBG levels and four polymorphisms (rs11078701, rs9901675, rs9898876 and rs2541012) in age- and BMI-adjusted models. In addition, statistically significant difference was found between osteoporosis patients and control subjects in genotype distributions of rs9898876, rs2541012, rs6259 and rs3853894. In the models with or without adjustment for confounders (age, BMI, SHBG and free testosterone (FT) levels), carriers of variant genotype of rs9898876, rs2541012 and rs6259 had lower BMD and were more likely to suffer from osteoporosis, as compare to carriers of common genotype. Subjects with variant genotype of rs3853894 had higher BMD and were less likely to suffer from osteoporosis, as compared to subjects with common genotype. In the haplotypes analysis, CCGGT (constituted by rs11078701C, rs1017163C, rs9898876G, rs62059836G and rs2541012T) and haplotype CGGT (constituted by rs858521C, rs858518G, rs6259G and rs727428T) was associated with a significant risk effect for osteoporosis. Polymorphisms of SHBG or the neighboring genes were associated with SHBG levels or BMD and osteoporosis, suggesting the involvement of genetic variation of SHBG in bone health.

1. Introduction

Osteoporosis is a disease characterized by low bone mass and structural deterioration of bone tissue, leading to bone fragility and an increased susceptibility to fractures. Evidence from twins and family studies have shown that approximately 50–80% of individual variance in bone mineral density (BMD) is genetically determined [1–3].

Sex hormone-binding globulin (SHBG), a plasma glycoprotein that binds with high affinity to sex steroids and regulating their bioavailability and access to target cells, may play an important role in the pathophysiology of osteoporosis. Some cross-sectional studies and longitudinal studies among male populations have demonstrated significant associations between SHBG levels and bone health, high SHBG correlated with greater bone loss, and the presence of vertebral and peripheral fractures [4–8]. In addition, a number of clinical studies have suggested that SHBG had an independent impact on bone strength [9–12]. Our previous study [13] also showed that an increasing SHBG level was a risk factor for osteoporosis, independent from age, BMI, total testosterone (TT) and free testosterone (FT) level, which indicated that SHBG may have an independent role on bone. Apart from the metabolic and hormonal influences [14], circulating SHBG levels are also influenced by genetic variation, which may either lead to the increase in production or decrease clearance of SHBG [15–18]. Given the clinical significance of SHBG, a number of studies have

Abbreviations: SHBG, sex hormone-binding globulin; SNP, single nucleotide polymorphism; BMD, bone mineral density; DXA, dual-energy X-ray absorptiometry; TT, total testosterone; FT, free testosterone; iMLDR, improved multiple ligase detection reaction; BMI, body mass index; CV, coefficient of variation; CGAS, candidate gene association study; GWAS, genome-wide gene association study; RANK, receptor activator of nuclear factor kappa B; RANKL, receptor activator of nuclear factor kappa B ligand; PTH, parathyroid hormone; MAF, minor allele frequency; LD, linkage disequilibrium; HWE, Hardy–Weinberg equilibrium; OR, odds ratio; CI, confidence interval.

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examined the potential associations between polymorphisms of SHBG gene and serum SHBG levels that could be involved in the development of several hormone-related disorders like polycystic ovary syndrome (PCOS) [19,20], breast cancer [21,22] and prostate cancer [23,24].

Osteoporosis is a polygenic disease and BMD is affected by several genes. In the last decade, quite an amount of studies (candidate gene association studies (CGAS) or genome-wide association studies (GWAS)) have focused on identifying genetic variants which may have potential effects on bone, such as estrogen [25,26] and androgen [27,28] receptor genes and PPAR gamma gene [29,30]. However, few studies discussed the role of SHBG gene variation on bone, especially in men. And results reported were controversial [31–34]. Our current research aims to explore the association between SHBG gene polymorphisms and BMD, presence of osteoporosis and their possible influence on circulating SHBG levels among middle-aged and elderly Chinese men.

2. Materials and methods

2.1. Study design and subjects

This was a cross-sectional and candidate gene association study. The study subjects were recruited from the health checkup population of Zhongshan Hospital, Fudan University from June 2012 to May 2013. As they all live in Shanghai, their lifestyles are considered to be similar and the cohort is fairly representative of the Chinese males in big city. The purposes and procedures of the study were explained in details to the participants by researchers. Participants were asked to fill in a comprehensive questionnaire regarding personal lifestyle, risk factors for osteoporosis and personal and family disease history. Inclusion criteria: men aged $\geqslant$45 years and with willingness to participate in the study and with ability to use complete questionnaire and provided informed consent. Exclusion criteria: those who had a history or evidence of metabolic bone diseases (Paget's disease, osteomalacia, renal osteodystrophy); those who suffered tumor or bone metastases; those who had taken medications such as steroids which could affect bone metabolism; those who had used any anti-osteoporosis drugs such as bisphosphonates and calcitonin; those who had severe liver, kidney impairment; those who have been recently bedridden for more than 3 months; those who had both hips fractured or replaced. This study was approved by the Ethics Committee of Zhongshan Hospital, Fudan University, all of the study data and information were collected after participants gave informed consent.

2.2. Measurements

2.2.1. Anthropometric measurements

Anthropometric and DXA measurements were obtained for all participants during the same visit. Height (cm) and weight (kg) was measured without shoes in light indoor clothing using a Stadiometer and body mass index was calculated as the ratio of weight (kg) to height squared (m^2).

All DXA measurements were performed by a well-trained technician using dual energy X-ray absorptiometry (Discovery A, Hologic, USA, with CV < 1%) on left hip (femoral neck, trochanter, intertrochanteric, Ward's triangle, and total hip) and lumbar spine (L1–L4, L-Total). *T* score was obtained by comparison to white males (Source: NHANES). We used the WHO diagnostic criteria for osteoporosis to classify our patients into three categories: osteoporosis (*T*-score $\leqslant -2.5$ SD), osteopenia ($-1.0 \geqslant$ *T*-score > -2.5 SD), or normal (*T*-score > -1.0 SD). The lowest *T* score at the femoral neck, intertrochanteric, total hip, or lumbar spine, was used for diagnosis.

2.2.2. Biochemical analyses

A single fasting morning venous blood sample was obtained from all subjects. Serum was separated immediately after phlebotomy and stored at -80 °C until assay at the end of the baseline study. Measurement of total testosterone (TT) and SHBG were carried out by Access Immunoassay Systems using the chemiluminescent immunoassay (Beckman Coulter, Brea, USA): total testosterone (analytical range 0.1–16 ng/ml [0.35–55.5 nmol/L]; intra-assay coefficient of variation (CV) < 3%; inter-assay CV < 5%), and SHBG (analytical range 0.33–200 nmol/l; intra-assay CV < 4.8%). Free testosterone (FT) was calculated from TT and SHBG using the Vermeulen equation [35], taking the concentration of TT, and SHBG into account and assuming a fixed albumin concentration of 43 g/l.

2.2.3. Questionnaire

A questionnaire was used to record the lifestyle and disease history of the participants, including smoking habit, alcohol consumption, etc. Participants who had smoked less than 100 cigarettes in the past five years were defined as non-smokers and the others as smokers. Participants who had consumed alcoholic beverages at least once per week for at least one year in the past five years were categorized as alcohol drinkers and the others as non-alcohol drinkers.

2.3. Selection of polymorphisms and genotyping

SNP selecting: The SNPs were selected from the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP) and the International HapMap Project database (http://hapmap.ncbi.nlm.nih.gov/) using "SHBG" as the target gene, in accordance with the following criteria: (1) minor allele frequency (MAF) of at least 5% in Chinese populations, (2) with low linkage disequilibrium (LD) using an r^2 threshold of <0.8 for each other, and the tagging SNPs are selected to constitute a minimal set of highly informative markers while minimizing redundant data. Although the MAF of rs13894 is only 3.3% in Chinese population according to HapMap data, it was genotyped in our study because it was a missense mutation (Arg126Cys). As a result, 12 SNPs of SHBG gene, which captured other 4 SNPs in the same gene, were selected. Finally we searched the literature for previous reports on SNPs of other genes influencing circulating SHBG levels [16], and included additional four SNPs (rs3853894 of ZBTB4 gene, rs9303218 of POLR2A gene, rs9901675 of CD68 gene, and rs8077824 of DNAH2 gene). Therefore, a total of 16 SNPs were chosen for analysis, including rs3853894, rs9303218, rs9901675, rs9898876, rs2541012, rs13894, rs858521, rs6259, rs727428, rs1641537, rs1017163, rs11078701, rs858518, rs59524396, rs62059836, and rs8077824.

Linkage disequilibrium: Genotype data was obtained from HapMap database and the linkage disequilibrium (LD) between SNPs in SHBG gene was examined by pairwise comparisons of r^2 using Haploview version 4.2 (Fig. 1a).

Genotyping: Blood samples were collected from patients in EDTA tubes and stored at -80 °C. The genomic DNA was extracted from peripheral blood leukocytes by salting-out method, using Relax Gene Blood DNA System (TIANGEN BIOTECH, BEIJING, China), according to the manufactures' protocol. Study subjects were genotyped for a total of 16 SNPs carried out by Shanghai Genesky Biotech Co., Ltd. (http://biotech.geneskies.com) using the improved multiple ligase detection reaction (iMLDR) assay on 3730xl genetic analyze sequencer (Applied Biosystems, Foster City, CA, USA). For each SNP, the alleles were distinguished by different fluorescent labels of allele-specific oligonucleotide probe pairs. Different SNPs were distinguished by different extended lengths at 3 end. The primers for both PCR and LDR reactions were all designed by Primer3 online software v.0.4.0 (http://primer3.wi.mit.edu).