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ORIGINAL PAPER

The association of $TAAAA_n$ repeat polymorphism in sex hormone-binding protein gene with polycystic ovary syndrome in Chinese population

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Abstract $TAAAA_n$ polymorphism in sex hormone-binding globulin (SHBG) gene has been found to influence the transcriptional activity of SHBG gene in vitro, and several studies have reported variable associations of this polymorphism with serum SHBG levels in women with polycystic ovary syndrome (PCOS). We have investigated the association of $TAAAA_n$ polymorphism with PCOS in 187 women with PCOS and 176 controls; in which five alleles (6–10 repeats) and 13 genotypes were found. None of the $TAAAA_n$ alleles or genotypes occurred in significant different frequency in PCOS patients compared with controls. Serum SHBG in the PCOS group was significantly lower than those in controls (33.8 ± 30.2 nmol/l vs. 65.58 ± 31.12 nmol/l; $P < 0.01$). Serum SHBG concentrations were similar for patients with PCOS whether they displayed short or long genotypes for $TAAAA_n$ (Log_{10}SHBG: 1.46 ± 0.38 and 1.58 ± 0.33, respectively). Similar results were observed for controls (Log_{10}SHBG: 1.79 ± 0.17 and 1.77 ± 0.14, respectively). In contrast, serum SHBG values were negatively associated with body mass index (BMI) and homeostasis model assessment of insulin resistance in the PCOS group ($B = -0.285$ and -0.264, respectively; $P < 0.01$). Thus, the $TAAAA_n$ polymorphism in SHBG gene was not a determinant of PCOS in this population of Chinese women, whereas, serum SHBG was significantly associated with BMI and insulin resistance in these PCOS patients.

Keywords Sex hormone-binding protein · Polycystic ovary syndrome · Polymorphism

Introduction

SHBG gene in the 17p12-p13 locus encodes a 402-amino-acid polypeptide and, after cleavage, a final 373-amino-acid signal peptide [1]. SHBG, which is produced mainly in the liver, binds androgens and estradiol in the blood, with high binding affinity for testosterone (T) and dihydrotestosterone and lower affinity for estradiol (E2), thus influencing the bioavailability of these hormones to target tissues [2, 3]. Changes in serum SHBG are influenced not only by hormonal status, e.g., androgen lowering the SHBG levels and estradiol stimulating the secretion of SHBG, but also by metabolic, nutritional, and genetic statuses [4, 5].

Polycystic ovary syndrome (PCOS) is the single most common endocrine abnormality in women at reproductive age. There is general agreement, currently, that PCOS diagnostic characteristics include hyperandrogenism, chronic anovulation, and/or polycystic ovaries [6]. Familial clustering of PCOS indicates a hereditary component is involved in the syndrome [7]. Recently, a developmental origin hypothesis has been proposed that a genetically determined exposure to androgen excess during prenatal

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life can lead to PCOS phenotype, which has been identified in animal models including rhesus fetal monkeys and sheep [8, 9]. Moreover, recent studies indicate that estrogens, even human chorionic gonadotrophin during pregnancy, have the effect of inducing ovarian cysts in some of the female pups around the time of puberty. [10–12]. No matter which sex hormone comes to effect, SHBG is the medium to regulate its bioactivity. In some hyperandrogenic patients, lower than normal amounts of SHBG in both their serum and target organs were genetically linked to a mutation in the SHBG gene coding region [13]. It is suggested that SHBG is a potential candidate gene for PCOS and it is a possible mechanism in the development of PCOS, because low levels of SHBG are commonly observed in women with PCOS and other androgen excess diseases [14–17].

The length of the $TAAAA_n$ polymorphism at the promoter region of SHBG appears to influence its transcriptional activity: a pentanucleotide polymorphism appears to influence its transcriptional activity, and a six-TAAAA allele has been associated with decreased transcriptional activity in hepatoblastoma cells in vitro [18]. However, the results of efforts to determine the relationship between this polymorphism and circulating SHBG levels in at least three separate studies of PCOS and hirsutism have been inconsistent [14, 15, 19, 20]. In the study of Xita, women with PCOS had a significantly greater frequency of $TAAAA_n$ alleles in SHBG (>8 repeats) than their healthy counterparts who had shorter repeats (<8 repeats). Furthermore, in their PCOS group, carriers of the longer $TAAAA_n$ genotypes had lower SHBG levels than those with shorter $TAAAA_n$ genotypes [14]. However, in Ferk's study, none of the SHBG alleles or genotypes was present at a significantly higher rate in PCOS patients compared with controls; Even so, serum SHBG values were strongly influenced by the polymorphism in both the PCOS (55.3%) and the control (33.1%) groups [19]. In a recent report of Xita, long $TAAAA_n$ alleles (>8 repeats) were observed to occur at a greater frequency in women with PCOS than in healthy women. Patients with PCOS that display a combined long $TAAAA_n$ repeat in the SHBG gene together with short CAG_n alleles in the androgen receptor (AR) gene had significantly lower serum SHBG levels and higher serum androgens than individuals with other genotype combinations [20]. Furthermore, SHBG levels were significantly higher in hirsute women who were homozygous for six-TAAAA repeats than in the nine-repeat homozygous patients, and the levels lay between the two for eight-repeat homozygous patients [15].

On the basis of these studies from different populations, we have performed a case–control study to investigate the association of $TAAAA_n$ repeat polymorphism and PCOS in a population of Chinese women.

Materials and methods

Studied population

All participants in this study were non-related Han Chinese living in Shanghai.

One hundred and eighty-seven were patients recruited (June 2004 to December 2007) from the Specialized Outpatient Clinic for Obesity in Ruijin Hospital. They were diagnosed PCOS for the first time and did not have any long term hormonal therapy for artificial menstrual cycle or metformin therapy for the three preceding months before the study. PCOS was based on the criteria of Rotterdam Revised 2003 (2 out of 3) diagnosis: oligomenorrhea or amenorrhea for at least 6 months; clinical and/or biochemical signs of hyperandrogenism; polycystic ovaries (presence of 12 or more follicles in each ovary measuring 2–9 mm in diameter, and/or increased ovarian volume (>10 ml) and exclusion of congenital adrenal hyperplasia, Cushing's syndrome, androgen-secreting tumor, hyperprolactinemia, and thyroid dysfunction [21, 22]. There were 176 controls divided into two subgroups: (1) 146 females (1st control group) of proven fertility, with normal menses and ovarian morphology and no history of subfertility treatment, were recruited from the Department of Gynecology and Obstetrics in Ruijin Hospital at the time of their delivery; (2) thirty healthy females (2nd control group), with normal menses (in self and family), ovarian morphology and sex hormone concentrations and without hirsutism, were recruited during non-gestational period. The study was approved by the Ethics Committee of the Ruijin Hospital, Shanghai JiaoTong University School of Medicine, and informed consent was obtained from all participants.

Clinical and biochemical measurement

For the patients and 2nd control groups, the weight, height, waist and hip circumferences were recorded; ultrasonography was performed in the early follicular phase (3rd–5th) of a spontaneous or progestin-induced menstrual cycle, and overnight fasting serum samples were collected. Laboratory examinations were operated as follows: Chemiluminescent Microparticle Immunoassay for the quantitive determination of serum luteotrophic hormone, follicle-stimulating hormone, prolactin, and total testosterone, using the kit from Abbott Laboratories (Abbott Park, IL, USA). Serum SHBG concentrations were measured using the immunoradiometric assay kit (DSL-7400 ACTIVE ®) from Diagnostic Systems Laboratories (Webster, Texas, USA), with the interassay coefficient of variation of 11.5%. Serum total cholesterol and triglycerides were measured by the enzymatic method, whereas

high-density lipoprotein cholesterol was measured with a specific precipitation method (Beckman LX-20, Brea, CA, USA), and low-density lipoprotein cholesterol was calculated using the Friedewald formula [23]. The serum glucose concentration was determined immediately after blood centrifugation by a hexose-kinase method (Beckman CX-7 Biochemical Autoanalyser, Brea, CA, USA). Serum insulin concentration was measured by radioimmunoassay (Sangon Company, Shanghai, China).

DNA amplification and genotype analyses

DNA was extracted from the peripheral blood leukocytes of subjects with PCOS and control subjects using the standard phenol–chloroform method, quantified by spectrophotometry, and stored at −20°C [24]. Genomic DNA (50 ng) was used for PCR amplification with the fluorescent-labeled primers, which flank the region of SHBG gene promoter $TAAAA_n$ repeats. The sense primer used for this work was—AGACAGGAGGATCGCTTGAA and the antisense primer was—CGCCTCTCAGTTATCTTCCCT. PCR was performed in a volume of 50 µl containing 1.5 mM $MgCl_2$, 10 mM dNTP, 50 ng genomic DNA, 2 pmol of each primer and 2.5 U Taq DNA polymerase (Shenergy Biocolor, Shanghai, China). Amplification was performed with preheating at 95°C for 5 min, followed by denaturation at 94°C for 30 s, annealing at 59°C for 30 s, and extension at 72°C for 45 s for 30 cycles. PCR production fragments labeled with D3/D4 fluorescent dye were analyzed in a Beckman-Coulter CEQ 8800 sequencer (Beckman Coulter, Fullerton, CA, USA). Data collection and analysis were performed with Fragment Analysis Module (Beckman Coulter, Fullerton, CA, USA).

Data analysis

Data were analyzed with the SPSS statistical package in Windows version 11.0, (SPSS, Chicago, IL, USA). Chi-square test was used to assess the Hardy–Weinberg equilibrium principle. All variables were checked for normal distribution by the Kolmogorov–Smirnov one-sample test. Serum SHBG was calculated in logarithm to adjust to normal distribution. Chi-square test was performed to compare between patients and two controls groups the frequencies in SHBG alleles and genotypes as well as the frequencies in short and long genotypes. Student's *t*-test was used to compare mean values of body mass index (BMI), age, $\log_{10}$SHBG, $\log_{10}$ total T, LH, FSH, and homeostasis model assessment of insulin resistance (HOMA-IR) between the patient and 2nd control groups. The serum SHBG levels for women with short and long $TAAAA_n$ genotypes in the PCOS group were compared using the Student's *t*-test. A similar analysis was performed for the serum SHBG levels for women with short and long $TAAAA_n$ genotypes in the 2nd control group. The correlation analysis was performed using Pearson's correlation coefficient. Multiple stepwise regression analysis with serum SHBG as a dependent variable was performed. In all the tests, the level of significance was designated as $P \leq 0.05$.

Results

The clinical and biochemical characteristics of the patients and the 2nd control group are shown in Table 1. Serum SHBG levels of the patients were significantly lower than those of the controls (33.8 ± 30.2 nmol/l vs. 65.58 ± 31.12 nmol/l, respectively, $P < 0.01$), while BMI and serum total T, LH, HOMA-IR were significantly higher than those of the controls ($P < 0.01$).

In this study, five-$TAAAA_n$ alleles ranging from 6 to 10 repeats were found; and 13 genotypes were observed: 6/6, 6/7, 6/8, 6/9, 6/10, 7/7, 7/8, 7/9, 7/10, 8/8, 8/9, 9/9, and 9/10, but genotype 6/10 was found only in the control groups (Figs. 1 and 2). The alleles in PCOS and the two control groups were in Hardy–Weinberg equilibrium ($P = 0.53$ and $P = 0.56$, respectively). The most common alleles were 9, 6, and 7-$TAAAA_n$ repeats, whereas the most common genotypes were 6/9, 7/9, and 9/9 (Figs. 1 and 2), and these were found in both the patient and two control groups; whereas the frequencies of short and long genotypes between the two groups were similar (short genotypes: TAAAA6/6, TAAAA6/7, TAAAA7/7: 18.7% vs. 25%; long genotypes: TAAAA9/9, TAAAA9/10: 17.1% vs. 18.2%, respectively). Figures 1 and 2 show between the two groups the similar frequencies of alleles

Table 1 Clinical and biochemical characteristics in PCOS and 2nd control group (mean ± SD)

	PCOS	Controls	*P*
Number	187	30	–
Age (years)	21.72 ± 5.7	22.5 ± 3.1	NS
BMI (kg/m^2)	28.3 ± 6.4	20.48 ± 1.18	<0.05
SHBG (nmol/l)	33.8 ± 30.2	65.58 ± 31.12	<0.05
Total T (µg/ml)	0.91 ± 0.38	0.35 ± 0.21	<0.05
HOMA-IR	4.1 ± 3.5	1.10 ± 0.6	<0.05
LH (mIU/ml)	7.57 ± 5.61	3.48 ± 1.34	<0.05
FSH (mIU/ml)	5.28 ± 2.95	6.11 ± 3.14	NS

BMI: body mass index

FAI: free androgen index

HOMA: homeostasis model assessment

IR: insulin resistant

NS: non-significant

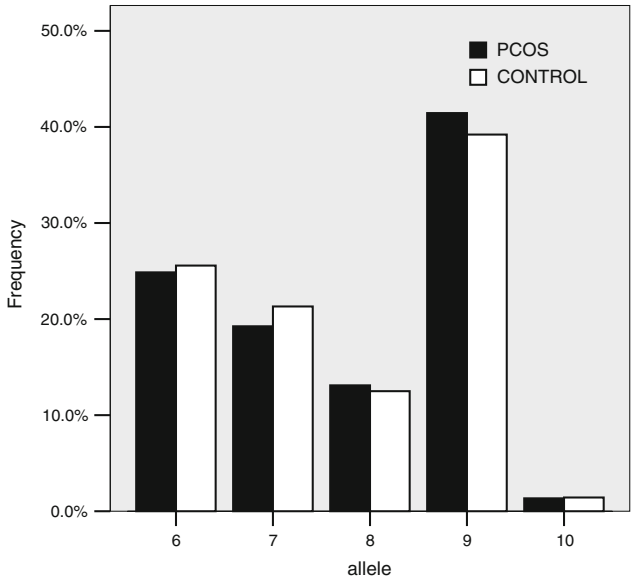

Fig. 1 Distribution of $TAAAA_n$ alleles of SHBG gene in PCOS and two control groups ($n = 187$ and 176, respectively). The allele frequencies were not statistically different

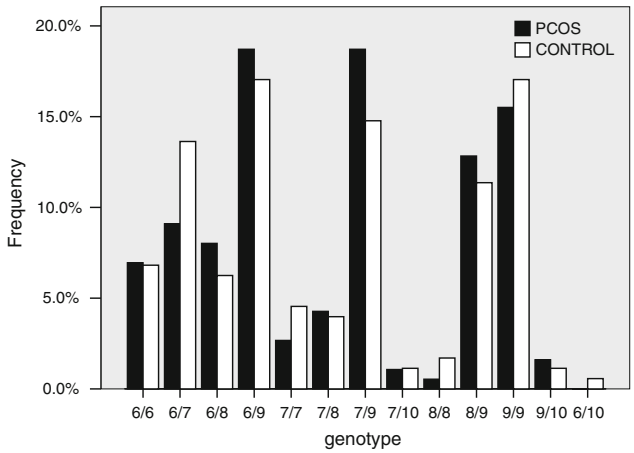

Fig. 2 Distribution of $TAAAA_n$ genotypes of SHBG gene in PCOS group and two control groups ($n = 187$ and 176, respectively). The short (6/6, 6/7, 7/7) and the long (9/9, 9/10) genotypes were not statistically different in the PCOS group and the two control groups

and the genotypes. Serum SHBG concentrations between the short and long genotypes in the PCOS group ($n = 35$ and 32, respectively) and between the two in 2nd control group were similar (Table 2). To explore the possible influence of the recruitment of overweight/obese PCOS patients on the polymorphism of $TAAAA_n$, we also analyzed the allele frequencies in overweight/obese PCOS patients and the lean PCOS patients, and found the similar distributions (Fig. 3). Correlation analysis revealed that serum SHBG levels correlated with BMI, serum glucose, and insulin concentrations in PCOS group. Multiple stepwise regression analysis found that serum SHBG was

Table 2 Serum SHBG[a] comparison between women with the short and the long genotypes in PCOS group and in 2nd control group (mean ± SD)

	Short-genotype carrier	Long-genotype carrier	*P*
PCOS group	1.46 ± 0.38	1.58 ± 0.33	NS
Control group	1.79 ± 0.17	1.77 ± 0.14	NS

[a] Log_{10} SHBG

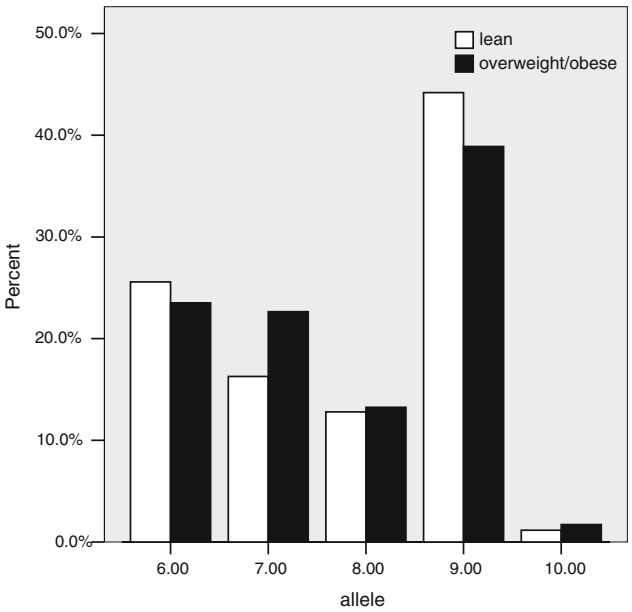

Fig. 3 The allele distribution in the overweight/obese PCOS group and the lean PCOS group. The allele frequencies were not statistically different

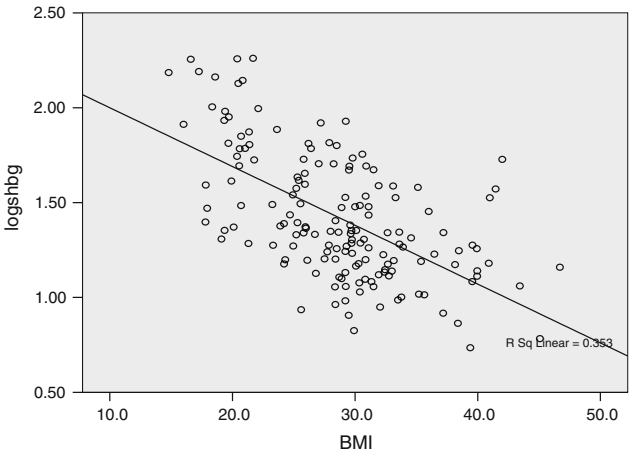

Fig. 4 Negative association of serum SHBG (Log value) and BMI in PCOS group

significantly and negatively associated with BMI and HOMA-IR in PCOS group ($B = -0.285$, $P = 0.004$ and $B = -0.264$, $P = 0.005$; respectively, Figs. 4 and 5); but these associations were not found in the 2nd control group.

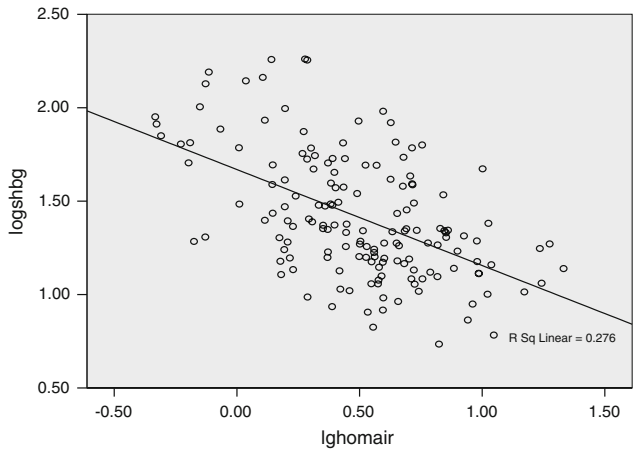

Fig. 5 Negative association of serum SHBG (Log SHBG) and HOMA-IR (Log value) in PCOS group

Discussion

This study found that $TAAAA_n$ repeat polymorphism in the SHBG gene was not a determinant of PCOS, because the frequencies of alleles as well as genotypes between PCOS patients and controls were similar; and it is the same with serum SHBG which were similar in all the groups with either short or long genotypes. Meanwhile, we found that serum SHBG was negatively associated with BMI and HOMA-IR in PCOS group. Together, these results suggest that serum SHBG concentrations may be influenced by other factors, e.g., BMI, insulin resistance, but not $TAAAA_n$ polymorphism.

Previous studies on the polymorphism of $TAAAA_n$ repeat in SHBG gene in PCOS and other diseases revealed different frequencies of alleles and genotypes in different populations. In a Greek population, the most common allele were 6, 8, and 9-$TAAAA_n$ repeats [11]; which were the same for a Slovene population [19] and a French population [15], with 6/8, 6/9, 8/9 and 6/8, 8/8, 8/9 as the most common genotypes, respectively for the last two populations; in contrast, our study found that 6, 7, and 9-$TAAAA_n$ repeats alleles and the 6/9, 7/9, and 9/9 genotypes are the most common in our population of Chinese women. Our results agree with Ferk's study [19] in which the frequencies of alleles as well as genotypes presented similarly in PCOS and control groups. We speculate that this variation of alleles and genotypes in different populations may be due to the ethnic background and genetic heterogeneity. Further, the variable results obtained with regard to previous analyses of the influence of the genotypes of $TAAAA_n$ in the SHBG gene on serum SHBG may be partly explained by genetic heterogeneity [14, 15, 19].

Long alleles or genotypes may have influenced the interpretation of the data. In the study of Xita, the long-alleles were defined as the alleles longer than 8 while the short-alleles were those shorter than 8, and the short/long genotypes were defined using the same criteria [14]. In a recent research in Turks, the authors classified the alleles in another way: 6/6, 6/x, x/x (x referred to the alleles longer than 6) [25]. The present study defined the short and long genotypes in line with the Hogeveen's study [18], considering their result of six-TAAAA allele being associated with decreased transcriptional activity more significantly than other alleles in an in vitro assay. This definition would represent the gap between the short and long genotypes more powerfully. Although we found serum SHBG in the subjects with the short and long genotypes were similar between the two genotype carriers in both the PCOS and 2nd control groups, it is possible that the sample number was not enough to display the association, for the number of study subjects with the short and long genotype were 35 and 32 respectively in PCOS group; 7 and 4 respectively in the 2nd control group. However, at least the lower serum SHBG levels in PCOS group could not be explained by the polymorphism of $TAAAA_n$ because there was no difference in the frequencies of the short or long alleles as well as the short and long genotypes in the PCOS and control groups. In this respect, we infer that other influences, genetic and/or environmental, may be considered to play an important role in the regulation of serum SHBG [26].

According to other studies, serum SHBG would take part in the mechanism of PCOS [14, 15]. However, it is not clear how SHBG was regulated by metabolic and other statuses. Hyperinsulinemia is known to suppress SHBG synthesis in the liver [27], and SHBG is an integrated marker of insulin resistance, which may be of use to identify insulin-resistant individuals for targeted treatment with insulin-sensitizing agents [28]. Some previous studies reported that serum SHBG levels are also significantly and negatively influenced by BMI, but this influence was mostly restricted to non-obese patients [15]. Furthermore, we found that BMI and HOMA-IR were possible regulators, and the association of serum SHBG levels and BMI is not limited to non-obese patients. In this study, most of the PCOS patients were overweight (13.6%) or obese (39%), but only a small proportion of the PCOS subjects were overweight or obese in previous studies. In this sense, our PCOS patients consisted of a wide range of BMI, facilitating the demonstration of the association of the serum SHBG and BMI. However, in the control group, these associations were not found, may partly be because of the centered distribution of BMI, as well as HOMA-IR (Table 1).

In conclusion, our study found that the $TAAAA_n$ polymorphism of SHBG gene is not a determinant of PCOS or the predictor of serum SHBG levels in our Chinese population, whereas BMI and HOMA-IR are associated with serum SHBG.

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