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ORIGINAL PAPER

Androgen receptor gene CAG_n trinucleotide repeats polymorphism in Chinese women with polycystic ovary syndrome

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Abstract Trinucleotide repeats CAG_n in androgen receptor gene is thought to be a potential site of genetic susceptibility to polycystic ovary syndrome (PCOS). However, previous studies of PCOS have shown variable association of CAG_n polymorphism with PCOS. In order to evaluate CAG_n polymorphism in Chinese women with PCOS, we have genotyped CAG_n repeat numbers in female Chinese subjects (148 PCOS patients and 104 control subjects). The mean CAG_n repeat lengths of PCOS patients and control subjects were similar (22.88 ± 1.76 vs. 22.85 ± 1.60; P = NS). No difference in the mean CAG_n repeat lengths of hyperandrogenic and nonhyperandrogenic subgroups of PCOS patients was found (22.86 ± 1.68 vs. 22.91 ± 1.84; P = NS). Moreover, no difference was found in the term of mean CAG_n repeat lengths in the nonhyperandrogenic subgroup and the control subjects (22.86 ± 1.68 vs. 22.85 ± 1.60; P = NS). However, mean CAG_n repeat lengths were negatively correlated with serum total cholesterol and low-density lipoprotein-cholesterol concentration in PCOS patients ($r = -0.182$, $P < 0.05$ and $r = -0.210$, $P < 0.05$, respectively), but not with total testosterone, body mass index, waist and hip circumferences. The CAG_n repeat length polymorphism may not be a major determinant of PCOS, but it may influence the lipid metabolism of PCOS patients.

Keywords Androgen receptor · Trinucleotide repeats polymorphism · Polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is the single most common endocrine abnormality in women at reproductive age [1], and is characterized by abnormal androgen production and/or activity that lead to changes in the control of follicle development and maturation [2]. Previous studies demonstrated an excess of familial clustering of PCOS, indicating a possible inherited genetic predisposition to the disorder [3–5]. Such predisposition may be mediated through an individual's hormonal activities. Androgen plays a direct role in the normal growth of ovarian follicle via androgen receptor (AR) [2], which binds androgen and subsequently stimulates the transcription of a cascade of androgen responsive genes. High serum androgen levels are associated with the inhibition of follicular development, anovulation, irregular menstruation, and the characteristic growth of small cysts in the ovary [6], and the administration of the androgen receptor antagonist

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flutamide can restore ovulation in these patients [7]. Nevertheless, there are some PCOS patients whose serum testosterone (T) concentration is normal, suggesting possible different AR gene activities. Because of these close associations, it has been proposed that AR might be one of the genetic predictors of an individual's susceptibility to PCOS [8].

The AR gene locus is at the Xq 11–12; the total genomic length is more than 90 kb, which encodes a 920 aminoacid protein. AR has three major functional domains: an androgen binding domain, a DNA binding domain, and an N-terminal domain with modulatory function. The N-terminal domain contains two polymorphic trinucleotide repeat regions, CAG_n that encodes polyglutamine and GGC_n that encodes polyglycine [9]. From the results of AR gene expression study in vitro, CAG_n repeat numbers have been identified as having an inverse relationship with the receptor transcriptional activity [10–12]. The CAG_n repeat length beyond the normal polymorphic range (e.g. >40) is strongly associated with Kennedy's syndrome, a fatal neuromuscular disorder with impaired masculinization [12, 13]. Normal polymorphic range is about 11–36 CAG_n repeats [14]; it was suggested that those with CAG_n repeats close to the upper range were associated with androgen insensitivity syndrome and male oligo-sperm infertility [15, 16], while those close to the lower CAG_n repeats range were associated with prostate cancer, benign prostate hyperplasia, and breast cancer [17–19].

Previous independent studies of PCOS have claimed the association of AR gene CAG_n polymorphism with PCOS, however, with variation. Mifsud et al. reported no difference in CAG_n repeat length for PCOS patients and control subjects. PCOS patients with lower serum total T concentration (<1.73 nmol/l) had a trend for a lower mean CAG_n repeat length than those with higher serum T; the difference was significant only when the short CAG_n repeat length was considered [20]. Recently, Jääskeläinen et al. reported CAG_n distribution between PCOS women and control subjects in Finland were similar and no correlation between the repeat length and T, body mass index (BMI), or waist and hip circumference [21]. However, Ibanez et al. claimed shorter AR gene CAG_n repeat length increased the risk for subsequent ovarian hyperandrogenism in precocious pubarche girls in Barcelona [22].

The genetic predisposition may play a role in PCOS. As for the AR gene CAG_n polymorphism, the strong evidence from vitro experiments has proved that it has an inverse relationship with AR transcriptional activity. And based on these studies from different population, we have performed a case–control study to find out whether CAG_n repeat length polymorphism was related to PCOS in a Chinese population.

Results

The clinical and biochemical characteristics of the women with PCOS are shown in Table 1. PCOS patients had a bigger BMI (28.38 ± 6.41 kg/m^2 vs. 20.02 ± 2.50 kg/m^2, $P < 0.05$) and were younger than the control subjects (21.54 ± 5.42 vs. 28.17 ± 3.14, $P < 0.05$).

The CAG_n repeat length ranged from 16 to 31 in PCOS patients and from 16 to 29 in control subjects, and the mean CAG_n repeat length was similar in both groups (22.88 ± 1.76 vs. 22.85 ± 1.59, $P =$ NS; Fig. 1). In the nonhyperandrogenic and hyperandrogenic subgroups ($n = 66$ and 82, respectively), the mean CAG_n repeat lengths were similar (22.86 ± 1.68 vs. 22.91 ± 1.84, $P =$ NS, respectively; Fig. 2). The clinical and biochemical characteristics were also compared in the two subgroups and age, BMI, waist circumferences, hip circumferences, waist-to-hip ratio, LH, E_2, Total T, TG, Tch, HDL-C, LDL-C in the groups were similar; only FSH concentrations were significantly different (6.01 ± 3.94 vs. 4.81 ± 2.34, $P < 0.05$). In the nonhyperandrogenic subgroup and control subjects, the mean CAG_n repeat lengths were similar (22.86 ± 1.68 vs. 22.81 ± 1.60, $P =$ NS); meanwhile, in the hyperandrogenic subgroup and the PCOS controls, the mean lengths of CAG_n were similar (22.91 ± 1.84 vs. 22.81 ± 1.60, $P =$ NS).

Further correlation analyses in the PCOS subjects found that the mean CAG_n repeat lengths correlated with Tch, short lengths of CAG_n repeats correlated with LDL-C ($r = -0.182$, $P < 0.05$, Fig. 3; and $r = -0.210$, $P < 0.05$,

Table 1 Clinical and biochemical characteristics of PCOS patients ($n = 148$)

Characteristics	Value
Age (years)	21.54 ± 5.42
BMI (kg/m^2)	28.38 ± 6.41
Oligo or Amenorrhea (%)	100
PCO (%)	77
Waist circumference (cm)	88.00 ± 13.83
Hip circumference (cm)	102.82 ± 11.66
Waist/hip	0.85 ± 0.72
FSH (mIU/ml)	5.34 ± 3.20
LH (mIU/ml)	8.60 ± 10.90
E_2 (pg/ml)	60.99 ± 69.91
Total T (ng/ml)	0.92 ± 0.33
TG (mmol/l)	1.41 ± 0.74
Tch (mmol/l)	4.50 ± 0.82
HDL-C (mmol/l)	1.32 ± 0.30
LDL-C (mmol/l)	2.59 ± 0.70

Note: FSH: follicle-stimulating hormone; LH: luteinizing hormone; E_2: estradiol; total T: total testosterone; TG: triglyceride; Tch: total cholesterol; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol

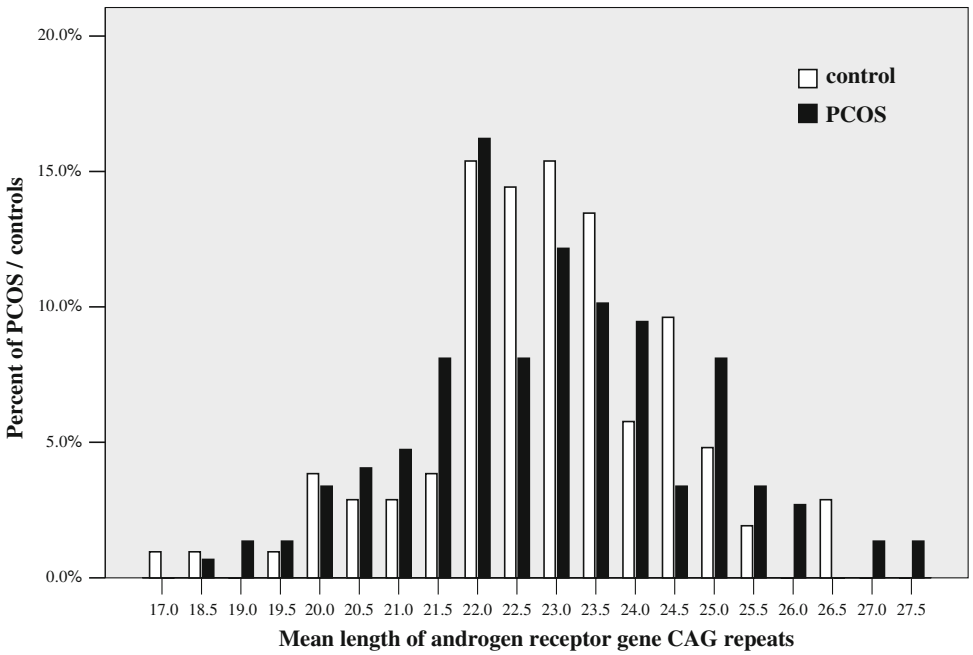

Fig. 1 Distributions of AR gene CAG_n repeat lengths in PCOS and control subjects

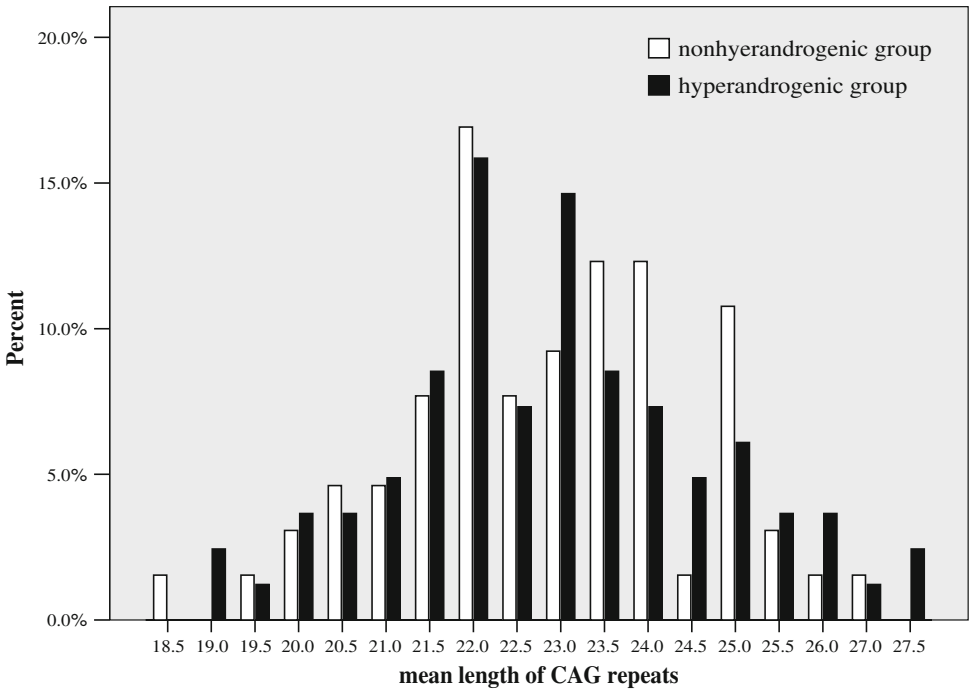

Fig. 2 Distributions of AR gene CAG_n repeat lengths in nonhyperandrogenic and hyperandrogenic PCOS patients

Fig. 4; respectively), but the CAG_n repeat lengths did not correlate with total T, BMI, hip or waist circumference (P = NS). The variables correlated with Tch consisted mean CAG_n repeat length, age, waist circumferences, TG, LDL-C ($P < 0.05$), and the variables correlated with LDL-C consisted short CAG_n repeat length, age, weight, BMI, waist circumferences, hip circumferences, waist-to-hip ratio, Tch, HDL ($P < 0.05$). However, multiple stepwise regression analyses (with Tch/LDL-C as dependent variables and the correlated variables as independent variables, respectively) revealed that the CAG_n repeat length polymorphism was not the predictor of Tch or LDL-C in PCOS. LDL-C and age were predictors of Tch ($B = 0.901$, $P < 0.01$, and $B = 0.091$, $P < 0.05$, respectively), and Tch and HDL-C were predictors of LDL-C ($B = 0.924$, $P < 0.01$, and $B = -0.276$, $P < 0.01$, respectively).

Discussion

The results of present study suggested that the AR gene CAG_n repeat length polymorphism may not play a major

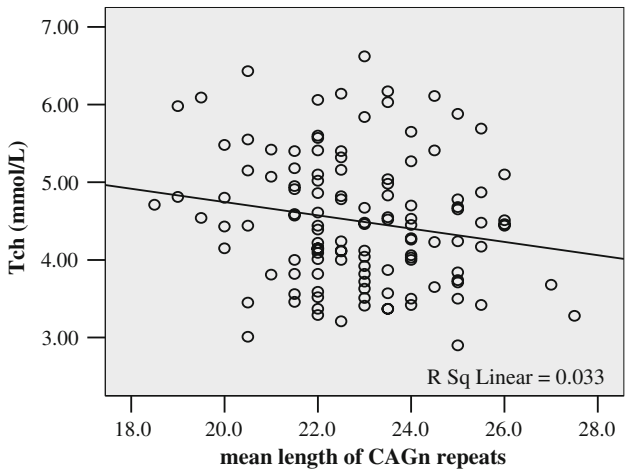

Fig. 3 Negative relationship between mean lengths of CAG_n repeats and Tch

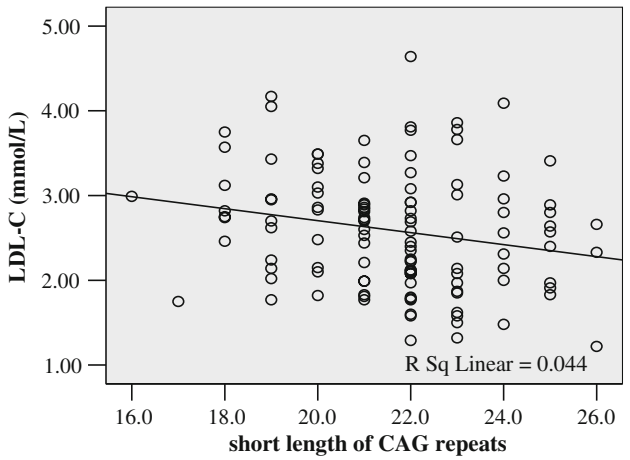

Fig. 4 Negative relationship between the short lengths of CAG_n repeats and LDL-C

role in the development of PCOS, since no difference in the term of mean CAG_n repeat length was found in the PCOS patients and the control subjects, in the nonhyperandrogenic and hyperandrogenic subgroups, especially in the nonhyperandrogenic subgroup and control subjects. The results were corresponding with two former studies. Mifsud et al. reported that the CAG_n repeat lengths in PCOS patients and control subjects were similar in a Chinese population (Singapore), and no correlation of serum total T with the mean CAG_n repeat length was found. PCOS patients with lower serum total T concentration only had a significant short CAG_n repeat length than those with higher serum T. The mean CAG_n repeat lengths in their report were almost the same with ours [20]. In their research, the cut-off point for the low- and high-T subgroups of PCOS was 1.73 nmol/l, which was the mean value of total T of the female population in Singapore. While 0.8 ng/ml in our study came from a former investigation on local normal female and PCOS patients by the Shanghai Institute of Endocrine and Metabolic Disease (data unpublished) and integrated with the reference range of the kits for the total T measurement in the Abbott Laboratories. In a recent research of PCOS patients of Finnish population, the mean CAG_n repeat length between PCOS patients and PCOS controls were similar. No correlation of mean CAG_n repeat length with total T was found and the hyperandrogenic PCOS patients did not differ from the patients with normal circulating testosterone concentrations in respect to the mean CAG_n repeat length [21]. However, there are some differences in aspects of sensitivity, exactness, and operation in different measurements for T [23]. We confirmed that the mean CAG_n repeat lengths were not associated with total T in present study; whether the CAG_n repeat lengths associated with other T measurements like free T, bio-T needs further experiments. Recently, free T was reported to correlate with the mean CAG_n repeat lengths in the PCOS patients [24].

In addition, we found that the CAG_n repeat length and the lipid profiles for Tch and LDL-C had negative correlations in PCOS patients; however, mean CAG_n repeat polymorphism was not the predictor of lipid profiles after multiple stepwise regression analyses. We infer that the correlations may not occur by chance, since previous researches reported that the androgen-AR complex decreases the catabolic removal of LDL by attenuating estrogen receptor (ER)-mediated induction of low-density lipoprotein receptor activity [25]. Potential mechanisms for the interaction of AR and ER could be that AR directly interacts with ER, thus blocking ER activation, or a cofactor is being shared by AR and ER, whereby dominance of AR prohibits the activation of ER-induced genes [26]. We infer that the correlations in our data may represent some clue about the possible relationship between the CAG_n repeat polymorphisms and the lipid metabolism in the PCOS condition.

One limit of the current research is that we did not perform an X chromosome inactivation analysis. Hickey et al. found that the long allele was expressed preferentially in their patients with PCOS and was correlated with higher total T serum concentrations. Their results were the opposite of their initial hypothesis and contradicted other reports regarding X chromosome inactivation analysis which indicated that the short allele was expressed preferentially in other populations of women [27, 28]. However, inactivity analysis in peripheral blood lymphocytes does not necessarily reflect X chromosome inactivation status in specific target tissues like the ovary, and further work would be required to validate the use of peripheral blood lymphocytes for understanding inactivation status in a hormonally regulated tissue.

Obesity is associated with abnormal lipid metabolism, and both obesity and abnormal lipid metabolism are common clinical characteristics in at least some cohorts of patients with PCOS [29, 30]. Although PCOS patients of this study were recruited from the outpatient obesity clinic and most of them (72.5%) were obese or overweight, no significant clinical and biochemical differences were found except for the BMI, waist and hip circumferences between the lean and overweight/obesity. The mean values of their lipid profiles were in the normal range. It suggests that obesity was not an independent variable with regard to lipid metabolism in these individuals at the time we collected the serum samples. Further studies to follow up these PCOS patients for the clinical and biochemical changes including hormones, lipid profiles were in schedule.

In a Chinese population, we confirmed that AR gene CAG_n polymorphism may not be a major determinant of PCOS, while it may play a possible role in lipid metabolism in PCOS condition.

Materials and methods

Studied population

All PCOS patients and control subjects included in this study were unrelated Han Chinese living in Shanghai. One hundred and forty-eight PCOS patients were recruited (2004 June to 2007 August) from the specialized outpatient clinic for obesity in Ruijin Hospital. The women did not have any hormonal therapy for the past 3 months before the study and all were diagnosed at the first time. 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). Congenital adrenal hyperplasia, Cushing's syndrome, androgen-secreting tumor, hyperprolactinemia, and thyroid dysfunction were excluded [31, 32]. One hundred and four control subjects of proven fertility, with normal menstrual cycles and ovary morphology, and without the history of subfertility treatment, were recruited from the Department of Gynecology and Obstetrics in Ruijin Hospital when they normally delivered a baby. 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

The weight, height, waist and hip circumferences of the PCOS patients were recorded. For the PCOS patients, ultrasonography was performed in the menstrual 3rd to 5th day. Fasting serum samples were collected. Laboratory examinations were operated as follows: Chemiluminescent Microparticle Immunoassay was use to determine serum luteotrophic hormone, follicle-stimulating hormone, prolactin, and estradiol; total T and the kits were from Abbott Laboratories (Abbott Park, IL, USA). Serum Tch and triglycerides (TG) were measured by the enzymatic method, while high-density lipoprotein-cholesterol (HDL-C) was measured with a specific precipitation method (Beckman LX-20, Brea, CA, USA), and LDL-C was calculated using the Friedewald formula.

DNA amplification and genotype analyses

DNA was extracted from the peripheral blood leukocytes of PCOS patients and control subjects in the standard phenol-chloroform method and quantified by spectrophotometry [33], then stored at –20°C. Genomic DNA (50 ng) was used for PCR amplification with the fluorescent-labeled primers, which flank the hAR CAG_n repeats region, with the sense primer as -TCCAGAATCTGTTCCAGAGCGTGC and the antisense primer as -GCTGTGAAGGTTGCTGTTCCTC AT, as according to the Ghadessy's study [20]. The PCR was performed in a volume of 50 μl containing 1.5 mM $MgCl_2$, 10 mM dNTP, 50 ng genomic DNA, 2 μmol 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 using CEQ™ 8800 Genetic Analysis System (Beckman Coulter, Inc., Fullerton, CA, USA) according to the manufacturer's instruction.

Data analysis

Data were analyzed within the SPSS for Windows version 11.0, statistical package (SPSS, Chicago, IL, USA). All variables were checked for normal distribution by the Kolmogorov–Smirnov one-sample test. Total T was calculated in logarithm to adjust to normal distribution. The data were displayed by mean ± SD. PCOS group were divided in to nonhyperandrogenic and hyperandrogenic subgroups by serum total T concentration 0.8 ng/ml: over 0.8 ng/ml indicated hyperandrogenism. As two X linked AR were present in the female, mean lengths of CAG_n repeat were adopted to represent the genotype of CAG_n repeats.

The mean CAG_n repeat lengths were compared by the two-sample independent *t*-test. The clinical and biochemical characteristics of the nonhyperandrogenic and hyperandrogenic subgroups were compared by the

two-sample independent *t*-test, as well. The correlation analysis was performed using Pearson's correlation coefficient. In order to explore the influence of CAG_n repeat polymorphisms on lipid profiles in women with PCOS, two stepwise multiple regression models were constructed, with the variables correlated with LDL-C as independent variables in the regression of LDL-C and the variables correlated with Tch as independent variables in the regression of Tch. In all tests the *P* level of significance was set to 0.05.

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