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ARTICLE

Polymorphism of CAG and GGN repeats of androgen receptor gene in women with polycystic ovary syndrome

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Abstract One characteristic of polycystic ovary syndrome (PCOS) is hyperandrogenism, which may be related to the activity of androgen receptor (AR). This study was designed to investigate the polymorphism of CAG and GGN repeats in the AR gene in women with PCOS. The frequency distributions of CAG and GGN repeat alleles, as well as their X-inactivation patterns, were compared between 76 age-matched normal women (control group) and 80 women with PCOS (PCOS group). The expression of *AR* mRNA in the ovarian tissues of seven patients with PCOS and five normal women was also tested using real-time quantitative PCR. It was found that PCOS patients had significantly higher frequency of longer GGN biallelic mean (29.8%) and X-weighted biallelic mean (33.3%) than controls (6.1% and 3.2%, respectively, $P = 0.002$, $P = 0.003$). The odds ratio of the long GGN repeat length ($n > 16$) before and after X-chromosome inactivation (XCI) in the PCOS group was significantly higher than in controls ($P = 0.0001$, $P = 0.005$). AR-GGN repeat mRNA expression was higher in the ovarian tissue of controls compared with PCOS patients ($P = 0.022$). In conclusion, the data suggest that the GGN repeat polymorphism in the *AR* gene is associated with PCOS.

KEYWORDS: androgen receptor, endocrinology, hyperandrogenism, polycystic ovary syndrome, polymorphism

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Introduction

Polycystic ovary syndrome (PCOS), a common anovulatory condition, affects 6–8% of premenopausal women (Gluszak et al., 2012). The 2003 Rotterdam consensus workshop concluded that PCOS was a syndrome with two out of three criteria: (i) oligo- or anovulation; (ii) clinical and/or biochemical signs of hyperandrogenism; and (iii) polycystic ovaries (PCO) of ultrasound imaging (with the exclusion of other aetiologies) (Rotterdam, 2004). Hyperandrogenism often presents abnormal biochemical parameters (elevated serum concentrations of testosterone, free testosterone, androstenedione, dehydroepiandrosterone sulphate) (Gluszak et al., 2012), and/or clinical signs (acne, hirsutism, seborrhea, androgenic alopecia, virilization) (Das et al., 2013). The mechanism of hyperandrogenism is still unclear, although the ovarian origin of androgen is well studied (Schweighofer et al., 2012). Actually, there are significant differences in androgen concentration and androgen action among patients with PCOS. Clinical signs may not be apparent in some PCOS patients with biochemical hyperandrogenism, while clinical signs could be very apparent in other patients without biochemical hyperandrogenism. The so-called functional androgen excess in patients with PCOS was estimated to range from 4% to 14% (Sanchon et al., 2012). One of the mechanisms leading to this difference could be molecular mediation of androgen receptor (AR).

AR activity is physiologically modulated by its variably sized polyglutamine and polyglycine tracts in the N-terminal transactivation domain. The tracts are encoded by a highly polymorphic CAG and GGN repeat sequence in exon 1 of the *AR* gene located on the X chromosome (Bennett et al., 2010). The CAG repeat varies in length from 8 to 35 repeats, while GGN repeat, a complex repeat represented by $(GGT)_3GGG(GGT)_2(GGC)_n$, varies in length from 10 to 30 repeats (Faber et al., 1989). The in-vivo study showed that the shorter CAG repeat could be related to the increased transcription of androgen-responsive target genes (Sankar and Hampson, 2012). However, the effect of GGN polymorphism on AR activity remains unclear, as there were discrepancies in previous studies (Jaaskelainen, 2012). One in-vitro study indicated that in response to both testosterone and 5α-dihydrotestosterone, those *AR* with GGN10, GGN27 and GGN24 in the N-terminal showed significantly lower AR activities than the AR with GGN23 (Lundin et al., 2007). However, there were little epidemiological investigations on the association between GGN repeat length and female infertility (Panda et al., 2011). According to classic genetics, one X chromosome becomes inactive in every female cell. X-chromosome inactivation (XCI) causes transcriptional inactivation in two X chromosomes through a series of events, such as DNA methylation, which could induce some diseases due to inappropriate regulation (Lee and Bartolomei, 2013). Other than the microsatellite CAG and GGN repeat analysis, some studies investigated the XCI patterns in some cases (Rajender et al., 2013). However, CAG and GGN repeat polymorphisms in the Asian population with PCOS have not been studied in detail, and the XCI pattern of GGN repeat number has not been investigated.

The issues regarding the familial nature of PCOS and its potential genetic relevance, both autosomal and X-linked patterns, are yet to be defined in patients with PCOS. It is much more difficult to elucidate the aetiopathogenesis of PCOS because of its heterogeneity of clinical presentation and variable progression, such as the individual difference of androgen excess. It is true that the pathophysiological mechanism of PCOS involves the combined actions of genetic, environmental and epigenetic factors. The number variation of CAG/GGN repeat in the *AR* gene is correlated with the transcription of androgen-responsive genes, which is associated with susceptibility to many human diseases (Brokken et al., 2013; Peng et al., 2014). To the best of our knowledge, no studies have been published on the effect of *AR* gene polymorphism on the aetiopathogenesis of PCOS, especially androgen excess. This study was designed to investigate CAG and GGN repeat polymorphisms in the *AR* gene, the XCI pattern and AR expression in Chinese women with PCOS.

Materials and methods

Study population

This study recruited 156 Chinese Han women aged 21–34 years old comprising 80 PCOS cases (the PCOS group) and 76 age-matched healthy women (the control group), at the study centre from 2012 to 2015. All women provided their informed consent. The diagnosis of PCOS was based on the revised criteria of the European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine in 2003 (two out of three criteria): (i) oligo- and/or anovulation; (ii) clinical and/or biochemical signs of hyperandrogenism; and (iii) polycystic ovaries; and exclusion of other aetiologies (e.g. congenital adrenal hyperplasia, androgen-secreting tumours and Cushing syndrome). Body mass index (BMI), androgen parameters, total testosterone (TT), sex hormone-binding globulin (SHBG), hirsutism (defined as a modified Ferriman–Gallwey score) and acne description were recorded. Blood (5 ml) was collected at 08:00 h.

Ovarian tissues from five women with normal menstrual cycles (women undergoing sex reassignment surgery) and seven PCOS cases (during surgical treatment) were collected after receiving signed consents. All volunteers had stopped hormonal treatment for at least 3 months.

This study was approved by the ethical review board of the First Affiliated Hospital of Nanjing Medical University on 16 April 2012 (reference number: 2012-SR-048).

Clinical and biochemical measurements

Whole blood was sampled on day 2–3 of the menstrual cycle or during the period of amenorrhoea in PCOS patients. Basal sex hormone concentrations were measured in all PCOS and control subjects; TT and SHBG were evaluated using radioimmunoassay kits (North Institute of Biological Technology, Beijing, China) using an automatic clinical chemistry analyser (Olympus AU5400). Free androgen index (FAI = TT/SHBG $\times$ 100%) was used to evaluate free testosterone concentrations.