The relationship between CAG repeat length polymorphism and infertility in Southern Chinese Han women

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ABSTRACT. Objective: To research the relationship between polymorphism of trinucleotide (CAG)n repeat alleles of the Exon 1 of androgen receptor gene and women with polycystic ovary syndrome (PCOS), or with endometriosis. Materials and methods: One hundred and forty-one control women and 74 women with PCOS and with endometriosis were recruited. The (CAG)n repeat alleles were genotyped with 3100 genetic analyser. The repeat number and frequency distributions of (CAG)n alleles were compared and analyzed statistically. Results: The results showed that mean repeat number of the (CAG)n was significantly lower in women with PCOS than in controls (p<0.001). The mean repeat number of the (CAG)n was significantly different between infertile women with endometriosis (p<0.05). However, the differences between infertile women with PCOS and fertile women with PCOS were not significant (p>0.05). Conclusions: These data indicated that (CAG)n repeat polymorphism have some influence, but have not a straight relationship in infertile women with PCOS and with endometriosis in this research population.

INTRODUCTION

Infertility and sterility are important subjects in clinical medicine; according to the World Health Organization (WHO) reports, 10-15% of reproductive-aged couples are infertile (1). Infertility and sterility are diseases caused by complex, multiple factors; in particular, the causes of female infertility are very complex. Recently, research on the genetic mechanism of disease, in particular, the relationship between the androgen receptor (AR) gene on the X chromosome and infertility has become a hot topic. However, researches worldwide have mainly focused on the relationship between this gene and male infertility (2-5). Studies regarding the correlation between this gene and certain diseases causing female infertility are rarely reported.

The human AR gene located at Xq11-12 contains a polymorphic trinucleotide short tandem repeat allele (CAG)n locus in the coding region of Exon 1 which codes polyglutamine residues. Studies have found that the repeat number of (CAG)n in the healthy population is 9-36 (6). It is reported that the allele repeat number of (CAG)n is negatively correlated with AR function (7, 8). An abnormally repeat number of (CAG)n could lead to androgen insensitivity syndrome, male infertility, breast cancer in males and females, prostate cancer, and other diseases (9-11). Brys et al. reported that mutation of the female breast cancer gene BRCA1 is associated with repeat number of AR-(CAG)n (12). Hickey et al. reported that the AR CAG repeat polymorphism and X chromosome inactivation in Australian Caucasian women with infertility related to polycystic ovary syndrome (PCOS) (13). However, the research results obtained by Ibáñez were inconsistent with those obtained by Hickey et al. (14). These research results all indicated that the AR gene had an immense impact on human reproductive function. Therefore, the following questions arise: how does this gene influence females who possess 1 active X and 1 inactive X chromosome? What are the polymorphisms of this gene in infertile Han Chinese females? In this study, in order to clarify the relationship between the repeat number of (CAG)n locus and some diseases that cause female infertility and to explore the possible genetic basis underlying these diseases, we analyzed allele repeat polymorphisms of AR-(CAG)n in southern Han Chinese women with normal androgen levels, including PCOS and endometriosis patients.

MATERIALS AND METHODS

Study groups

A total of 74 female patients aged between 22 and 43 yr were recruited from Reproductive Centers, the First and Second Hospitals Affiliated to Sun Yat-sen University. No abnormality was found in the chromosome test and in androgen levels of these patients [total testosterone (TT): 0.014-3.4 mmol/l]; further, they did not have mycoplasma and chlamydia infections. There were 41 infertile women and 9 fertile women patients with PCOS or polycystic ovary symptom, 24 with endometriosis. The PCOS due to oligo-anovulation and polycystic ovaries were diagnosed by ultrasonography and polycystic ovarian morphology according to the Rotterdam Consensus Conference criteria (15). The endometriosis due to menstrual disorder and infertility was diagnosed with laparoscopic visualization, followed by histopathologic assessment of putative lesions. The stage of endometriosis was not assigned. None of the patients diagnosed with PCOS and endometriosis received endocrine therapy before inclusion in the study. We recruited 141 age-matched healthy fertile wom-
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en which test paternity from the center of medicine expertise of Sun Yat-sen University to serve as controls. All control participants completed a questionnaire including history of disease and menstrual symptoms. They had no history of endocrinologic or other clinic disorders. Informed consent was obtained from the subjects, and the study was approved by the Ethics Committee of our University.

**Determination of trinucleotide repeat lengths**

Genomic DNA was extracted from the peripheral blood of each subject using a DNA IQ™ kit (DNAIQ™ protocol, Promega Corporation, Madison, WI, USA) according to the manufacturer’s instruction. The primers for amplifying (CAG)n repeat fragments were as follows: sense, 5’-GCT GTG AAG TTG CTG TTC TCT AT-3’ and antisense, 5’-TCC AGA ATC TGT TCC AGA GCG TGC-3’, as described previously (4). They were synthesized by Shanghai Sangon Biological Engineering Technology & Service Co., Ltd (Shanghai, China). The sense primer was labeled with 6-carboxyfluorescein (6-FAM) at the 5’ end. A 20-μl volume of PCR reaction mixture contained DNA templates (10-100 ng), 0.8 U Taq polymerase (New England Biolabs, Inc. Beverly, MA, USA), 0.2 mmol/l dNTP (Roche, Grenzacherstrasse, Switzerland), 10 pmol primers, 1.5 mmol/l MgCl2, and 1× PCR buffer (ABI, Foster City, USA). The PCR parameters were as follows: pre-denaturing at 95 C for 5 min; 35 cycles at 95 C for 1 min, 60 C for 1 min, and 72 C for 1 min; and final extension at 72 C for 5 min, then stored at 4 C. PCR products were separated by electrophoresis in a 2% agarose gel. Then, 2.0 μl PCR products were mixed with 9 μl formamide and 1.0 μl ILS-600 (Promega Corporation, Madison, WI, USA) as an internal lane standard, heated at 95 C for 3 min, snap-cooled on ice for 3 min according to the PowerPlex Y system protocol (Promega Corporation, Madison, WI, USA), and loaded on the 3100 Genetic Analyzer (PE Applied Biosystems, CA). GeneScan analysis was performed using Gene Mapper 3.1 v software. PCR products (135, 259, and 283 bp) were sequenced by Invitrogen Co. (Shanghai, China), resulting in (CAG)n repeat number of this 3 PCR products. The (CAG)n repeat numbers of other products were calculated based on those of the 3 (CAG)n alleles.

**Statistical analysis**

All values were expressed as mean±SD. A statistics analyzing software package SPSS 13.0 (SPSS Incorporated, Chicago, USA) was used for all analyses. A value of p<0.05 was considered statistically significant. Chi-square distribution ($\chi^2$) test was performed for each group according to numbers of alleles repeats. All experimental groups and the control group were classified according to the allele repeat number of (CAG)n locus, with reference to Hickey et al. and Lavery et al. and other methods (4, 13). Alleles with different repeat number were analyzed using the $\chi^2$ test in order to identify differences in their distribution between each experimental group and the control group. The patient groups and the normal control group were divided into 3 groups as follows: those with average allele repeat number between 21 and 24 (medial cut point), ≤20 (shorter length cut point), and ≥25 (longer length cut point).

**RESULTS**

**Statistical results regarding allele repeat number of (CAG)n**

The statistical results are shown in Table 1. By using t-test analysis, significant difference for the allele repeat number of (CAG)n, was found between the patients with the PCOS and normal control groups (21.41 vs 24.19, p<0.0001). Furthermore, the differences between the value in the endometriosis (22.83 vs 24.19, p=0.035) and the value in the control group were significant. However, the differences between the value in fertile women patients with PCOS groups (21.89 vs 21.30, p=0.555) and the value in the infertile patients with PCOS were not found. The comparison of the results of mean value of (CAG)n repeat length in different groups are shown in Figure 1.

**Allele (CAG)n frequency distribution**

The allele frequency of (CAG)n locus was calculated in each group. In the normal control group, allele 24 had

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**Table 1 - Androgen receptor exon 1 (CAG)n, repeat allele distribution profiles in control and infertile women groups.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean repeat number</th>
<th>Median</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (no.=141)</td>
<td>24.19</td>
<td>24</td>
<td>4.18</td>
<td>11-39</td>
</tr>
<tr>
<td>PCOS (no.=50)</td>
<td>21.41</td>
<td>21</td>
<td>3.78</td>
<td>10-31</td>
</tr>
<tr>
<td>Endometriosis (no.=24)</td>
<td>22.84</td>
<td>23</td>
<td>3.56</td>
<td>12-29</td>
</tr>
<tr>
<td>Infertility with PCOS (no.=41)</td>
<td>21.30</td>
<td>21</td>
<td>3.83</td>
<td>11-31</td>
</tr>
<tr>
<td>Fertility with PCOS (no.=9)</td>
<td>21.89</td>
<td>22</td>
<td>3.58</td>
<td>14-28</td>
</tr>
</tbody>
</table>

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**Fig. 1 - Comparison of the mean values of (CAG)n, repeat length in the different groups.**