Polymorphism of CAG Repeat in Androgen Receptor Gene in Egyptian Women with Polycystic Ovary Syndrome

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Abstract

Background: Trinucleotide repeats CAG (n) in androgen receptor gene is thought to be central to PCOS genetic susceptibility. However, previous studies of PCOS have shown variable association of CAG (n) polymorphism with PCOS.

Objective: To assess the association of the AR gene CAG repeat length polymorphism with PCOS among Egyptian patients, and its influence on clinical and biochemical androgen traits.

Patients and Method: A cohort of 80 women with oligo-anovulatory cycles and ultrasound features of PCOS and 67 healthy women of reproductive age were investigated for CAG repeat lengths using genescan. Assay of serum total testosterone, free testosterone and DHEAS were carried out. These parameters were correlated with the CAG repeat.

Results: AR (CAG) n range were 8-25 with a median of 19 in PCOS patients and 10-29 with a median of 22 in control group. The difference in median repeat number was significant comparing PCOS with control (p<0.001). A higher frequency of short AR (CAG) n alleles (9-15) tended to be more frequent in PCOS women than controls (p=0.005). We found significant difference between the both groups as regard BMI, Hirsutism, Total testosterone, Free testosterone and DHEAS.

Conclusion: Our findings support the hypothesis that there is association of shorter CAG repeats with Egyptian PCOS patients suggesting inherited alteration in androgen sensitivity may contribute to PCOS.

Key Words: CAG repeats – Androgen receptor – Polycystic ovary syndrome – Genescan.

Introduction

The androgen receptor (AR) is a member of the family of ligand-activated transcription factors that regulate many biological processes and is encoded by a gene located at Xq11-q12q. The androgen receptor (AR) contains a polyglutamine tract of variable size in the N-terminal transactivation domain that can modulate the ability of the receptor to enhance transcriptional events in vitro [1]. This tract is encoded by a highly polymorphic CAG repeat microsatellite in exon 1 of the AR gene [2].

The AR gene contains a highly polymorphic (CAG) n repeat in exon 1 encoding aglutamine tract in the N-terminal transactivation domain of the protein, which becomes active only after AR binds to its ligand [3].

The polyglutamine tract length is inversely correlated to the transcriptional competence of the receptor, with longer tracts being associated with lower levels of AR-mediated transcription in both normal and disease states [4].

CAG repeat number normally ranges between 8 and 35 and demonstrates a stable inheritance [5].

Theoretically, an inverse relationship exists between repeat number and AR activity whereby tracts of shorter size confer greater activity than tracts of larger size. In vitro studies have indicated that ARs with shorter polyglutamine tracts have greater ability to activate reporter genes with androgen response elements [6].

Consistent with these functional studies, various metaanalyses indicated that the a smaller number of CAG repeats has been associated with hirsutism, premature pubarche, and ovarian hyperandrogenism in women [7], as well as androgen-dependent skin disorders in both men and women [8].

Polycystic Ovary Syndrome (PCOS), the leading cause of anovulatory infertility among premenopausal women, is now essentially known as androgen excess disorder. It is characterized by three key features viz., hyperandrogenism, chronic
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anovulation or infrequent ovulation and presence of numerous follicular cysts in the enlarged ovaries [9].

In view of this, a number of studies have tried to investigate the role of CAG repeats of the AR gene in PCOS yielding contrasting results. Nevertheless, some of these studies reported a trend for shorter CAG alleles to be more frequent among PCOS cases than the controls [10,11] which is consistent with the in vitro evidence of the greater receptor activity of the shorter CAG alleles. The pattern of association of CAG repeat polymorphism with the PCOS are not studied in any detail among the Egyptian Women, so we investigated the association between.

CAG repeat numbers [i.e. (CAG) n] and an increased risk of PCOS and and its influence on clinical and biochemical androgen traits.

Subjects and Methods

The study group consisted of 80 women with PCOS aged 22.5±7.2 (mean±SD) years. The subjects of this study were selected from Egyptian Women which were recruited from infertility and antenatal clinics, Elkasr Elainy Hospital, Cairo, Egypt. Due to ethnic differences in allele frequency for the human AR (hAR) (CAG) n polymorphism, women from other countries were excluded from the study.

All of these women were subject to the same infertility assessment protocol, which included reproductive history, pelvic ultrasound for ovarian morphology, blood tests for hormonal profiles, and height and weight measurements. Women with abnormal androgen levels were further assessed for adrenal hyperplasia and Cushing’s syndrome that form exclusion criteria for the diagnosis of PCOS. PCOS was based on the criteria of hyperandrogenism and anovulation [12].

The control group consisted of 67 healthy Egyptian women with normal menstrual cycles (28-30 days) and no signs of hyperandrogenism.

Five mls of venous blood was collected from each one and divided into 2 samples. The first one is 2ml venous blood was left to clot in plain polypropylene tube at 25 °C for 30 minutes, then the separated serum was used for Hormone analysis (total testosterone, free testosterone and DHEAS) by electrochemiluminescence assays (automatic elecsys 2010, Germany).

The other blood sample is 3ml blood on EDTA was used for DNA extraction and CAG-repeats analysis by automated ABI 310 Genetic Analyzer.

CAG repeats:

DNA was isolated from blood samples using the QIA amp RNA blood mini kits catalog No. 52304 (Qiagen, Gmbh, Hilden). Testing condition was done according to the manufacturer’s recommended protocol.

The CAG repeat was genotyped using a PCR-based assay. Genomic DNA was amplified by PCR using fluorescently labeled primers that flank the CAG repeat. Amplification was performed in a reaction volume of 20 µL containing 0.1 µg genomic DNA, 8 pmol fluorescently labeled forward primer (5'-TCC AGA ATC TGT TCC AGA GCG TGC3'), 8 pmol unlabeled reverse primer (5'-GCT GTG AAG G- TT GCT GTT CCT CAT-3'), 0.1mmol/l dNPTs, and 1U Taq polymerase. Amplification was performed as follows: Initial denaturation/enzyme activation at 94 °C for 7 minutes; followed by 30 cycles for 45 seconds at 94 °C, 45 seconds at 55 °C, and 45 seconds at 72 °C, followed by a final extension of 72 °C for 10 minutes. Amplification was performed in an automated thermal cycler 9700 (PE, Applied Biosystems, USA).

Two microliters of PCR products were mixed with 24 µL formamide and 1 µL of the internal size standard TAMRA 500. The mixture was denatured at 95 °C for 3min and placed on ice until analysis. Electrophoretic analysis was performed using POP4 gel in ABI 310 Genetic Analyzer (ABI Prism 310; Applied Biosystems, Perkin Elmer, USA). The amplified products were analyzed by Gene-scan software (Applied Biosystems). Accurate sizing of alleles was estimated by comparison with the internal standard TAMRA 500.

Statistical analysis:

The data collected were statistically analyzed using statistical package for social sciences (SPSS/version 16) software. (Inc., Chicago, USA). Unpaired t-tests were used to compare clinical characteristics between women with and without PCOS.

The primary genotypic unit used in the association analyses was the biallelic mean (the mean of the CAG repeat number from the two alleles in each subject). Biallelic mean was considered in two ways. First, it was analyzed as a continuous quantitative variable. Second, it was treated as a binary qualitative variable, with the two states being either less than or greater than or equal to the median repeat number observed in the control group. Significance was taken as \(p<0.05\). Correlations among variables were done by spearman’s correlation coefficient. Qualitative data are pre-
sented as number and percent. Quantitative data are presented as Mean±Standard deviation or median (minimum-maximum) where appropriate.

**Results**

This study was conducted on 80 women with PCOS and 67 healthy Egyptian women.

The clinical characteristics of the women with PCOS were as follows:

Of 80 patients 43 (38.4%) had irregular periods, 69 (61.6%) had Oligomenorrhea, 32 (40%) had hirsutism, 15 (18.75%) had acne, 69 (61.6%), and only 2 (2%) had alopecia areata. 80% of cases were defined polycystic ovaries as the presence of at least eight peripheral cysts less than 10mm in diameter, with increased ovarian stroma on ultrasound, occurring bilaterally.

Hyperandrogenism was found in 71.25% of cases. 58.75% of our studied group were obese (BMI >27kg/m²), and 68.75% were infertile.

Table (1) presents Comparison of hormonal and AR-CAG repeat length between 80 PCOS cases and 67 control women. We found significant difference between the both groups as regard BMI, Hirsutism, Total testosterone, Free testosterone and DHEAS.

The range of CAG repeats was 8-25, with a median of 19 in the women with PCOS, while in control women CAG repeat numbers ranged from 10 to 29, with a median of 22. The difference in median repeat number was significant comparing PCOS with control ($p<0.001$).

The allele distribution pattern was different in the PCOS group compared to control group. 45/80 (56.25%) of PCO womens carrying the very short ($\leq 15$) AR CAG repeats, while in control group 18/67 (30%) only carrying the very short ($\leq 15$) AR CAG repeats ($p=0.005$).

Furthermore, there were less PCOS women 25/80 (31.25%) carrying the longest length alleles (>20) than in the control group 40/67 (59.7%). In the entire cohort, biallelic mean of repeats was associated with PCOS.

**Discussion**

Hyperandrogenism is a key feature of the PCOS syndrome and might contribute to IR, which is often observed in PCOS women [13-15]. At the molecular level, testosterone effects are mediated through activation of the AR. The ability of the receptor to enhance transcription of testosterone-regulated genes was shown in vitro to depend on a highly polymorphic CAG repeat microsatellite in exon 1 [16]. Therefore, it appears reasonable to consider the CAG length polymorphism, when investigating the PCOS.

Thus, we hypothesized that the shorter alleles would be more frequent and preferentially more active among the PCOS women than the controls. To the best of our knowledge, There is no studies
of its kind on Egyptian women to examine the association of AR polymorphism with PCOS.

Our study revealed the range of CAG repeats was 8-25, with a median of 19 in the women with PCOS, while in control women CAG repeat numbers ranged from 10 to 29, with a median of 22. We found a significant association between shorter repeat length and the presence of PCOS.

A smaller biallelic mean was associated with increased frequency of PCOS. Our results are consistent with some recent studies where Xita et al., [11] and Shah et al., [10] found shorter alleles to be more frequent among the PCOS cases than controls.

However Dasgupta et al., [17] study revealed a range of 8-31 CAG repeats in the AR gene of South Indian PCOS women and the controls. And neither the distribution of biallelic mean values nor the mean repeat sizes were found significantly different between the PCOS cases and controls. Similarly, another study found no overall association of CAG repeats with PCOS but observed higher mean CAG repeats in a subgroup with higher testosterone levels [18].

Alternatively, in the study by Hickey et al., [19] paradoxically a longer CAG repeat biallelic mean was associated with an increased risk of PCOS in Australian Caucasian women, a finding that appears to be contrary to functional data. These contradictory results can be attributed to the different ethnic background of studied population, in which there is a significant variation in the number of AR gene CAG repeats in different populations and this may account for the inconsistent findings of association studies in different populations.

In our cohort study, there was correlation between hirsutism, total testosterone, free testosterone, DHEAS and the CAG length, which is in accordance with a recently published study [20]. Some prior studies found an association between CAG repeats and testosterone levels [21-23]. Other investigators found that the CAG repeat length of the AR influenced the relationship between free testosterone and insulin resistance [measured by homeostatic model assessment (HOMA-IR)], such that at lower CAG lengths, free testosterone and HOMA-IR were positively correlated [24]. Another study found that shorter CAG repeats were associated with increased androgen levels only in the presence of longer alleles of the SHBG (TAAAA) n variant, suggesting that simultaneous lesions in both androgen availability and sensitivity could result in hyperandrogenism [11]. Presumably alterations in androgen sensitivity could lead to altered androgen secretion, similar to altered insulin secretion when insulin resistance is present.

However other study revealed no significant association between testosterone levels and CAG repeat length [17]. The allele distribution pattern was different in PCOS group compared to control group in Egyptian women. 45/80 (56.25%) of PCO women carrying the very shortest (<15) AR CAG repeats. Furthermore, there were less PCOS women 25/80 (31.25%) carrying the longest length alleles (>20) than in the control group 40/67 (59.7%). Our result in concordance with Jaaskelainen et al., [25] study in which all subjects with CAG repeat length 15 or less had PCOS, suggesting some influence of the shorter CAG repeat in the pathogenesis of PCOS.

In this study, significant association between CAG repeat length and BMI. This also was reported by some recent studies as Dasgupta et al., [17] who found significant heterogeneity in the CAG biallelic mean between the obese and lean PCOS cases. The lean PCOS women, who have a significantly higher frequency of the middle range CAG repeats, would possibly be conferred with a moderate receptor activity as compared to the obese PCOS cases. This is can explain the differential receptor activity among them, which may also probably explain the manifestation of hyperandrogenic features in such cases.

The difference of our results results can be explained by the difference in sample size, heterogeneous group of patients, and genetic background of patients.

As our study is the first of its kind from Egypt, although many studies about this contradictory subject were carried on different ethnic populations. Egyptian are known to be of mixed ethnic origin (Middle Eastern, African and European), so Egyptian studies are expected to add to the data available for different ethnic backgrounds [26]. And therefore further studies are required among other ethnic groups, encompassing the geographic heterogeneity of Egypt, including Delta regions, Upper Egypt and Nubia before reaching any conclusions on the precise role of androgen receptor CAG repeat polymorphism in the manifestation of this immensely heterogeneous PCOS phenotype in the populations of this region.

References


