

**The androgen receptor gene CAG repeat length  
and PCOS**

Jääskeläinen, Jarmo M.D. <sup>a</sup>

Raimo Voutilainen, M.D. <sup>a</sup>

Seija Korhonen M.D. <sup>b</sup>

Maritta Hippeläinen, M.D. <sup>b</sup>

Seppo Heinonen, M.D. <sup>b</sup>

University of Kuopio, Finland

Department of Pediatrics <sup>a</sup>

Department of Obstetrics and Gynecology<sup>b</sup>

Reprint requests:

Jarmo Jääskeläinen, M.D. <sup>a</sup>

Department of Pediatrics

Kuopio University Hospital

70211 Kuopio

Finland

(FAX: 358-17-17; Tel: 358-17-17; e-mail:)

**Capsule**

The CAG repeat length of the androgen receptor gene was not found to be significantly associated with PCOS, which suggests that...

**Abstract**

**Objective:** To determine whether the CAG repeat length of the androgen receptor gene contributes to individual differences in the susceptibility to the development of polycystic ovary syndrome (PCOS).

**Design:** Retrospective case-control study.

**Setting:** University-based clinic.

**Patients:** 112 Caucasian women with PCOS and 112 healthy controls. Genotype frequencies were also compared with those of 574 Finnish men from a previous study.

**Intervention(s):** None

**Main outcome measures:** Androgen receptor gene CAG repeat length in PCOS and control groups.

**Results:** The mean length of the androgen receptor gene CAG repeat was similar in both groups. However, the distribution of allele frequencies was different in the PCOS group compared to that in the control group, both long and short repeat lengths being overrepresented.

**Conclusions:**

**Key words:** polycystic ovary syndrome, polymorphism, androgen receptor

## **Introduction**

By somatic cell hybridization Migeon et al. (1), found that the androgen receptor gene (AR) gene is located at chromosomal region between Xq13 and Xp11 and it is expressed nearly ubiquitously in nonreproductive and reproductive mammalian tissues, including ovaries. The androgen receptor gene is more than 90 kb long and belongs to the nuclear receptor superfamily. The protein has three major functional domains: the N-terminal domain, DNA-binding domain, and androgen-binding domain. Upon androgen binding, the receptor undergoes conformational changes, translocates into the nucleus, and stimulates transcription of androgen responsive genes by interacting with androgen response elements (2-5).

The functional significance of the androgen receptor in the male reproduction system is well studied, showing that germline androgen receptor mutations are associated with a spectrum of androgen insensitivity syndromes, ranging from minimal to complete androgen insensitivity syndrome (5, 6). However, the androgen receptor has also been linked with several other conditions including X-linked neuromuscular disorder Kennedy's syndrome(7). Studies on androgen receptor knock out (ARKO) mice revealed that female ARKO mice produced a smaller number of pups per litter probably due to defective folliculogenesis and implantation (8).

The AR CAG repeat length polymorphism in exon 1 serves not only as a polymorphism to study the putative role of AR in various conditions, it has also been shown to be inversely associated with transcriptional response to androgens *in vitro* (9). Thus, variation in AR CAG repeat length has been studied in several conditions thought to be at least partly androgen and/or androgen receptor dependent, including infertility, prostate cancer, and benign prostate hyperplasia in men and breast cancer, osteopenia, and PCOS in women (10-27). High androgen levels and insulin resistance are features of women with PCOS and Hickey et al. reported that the androgen receptor CAG polymorphism is associated with PCOS in Australian women (20).

In view of the strong evidence implicating the AR CAG repeat length polymorphism in androgen response, we have tested the hypothesis whether the length of CAG repeats is altered in a group of women with has been shown to be associated with PCOS. A control group of parous women with no signs of PCOS was also included.

## **Material and Methods**

Written approval for the study was obtained from the Ethics Committee of the Kuopio University Hospital. Informed consent was obtained from all study subjects and 112 controls.

Information was collected retrospectively in regard to 112 non-diabetic women with PCOS at endocrinology/infertility clinics in the region of Kuopio University Hospitals, Finland, and in regard to 112 non-hirsute, fertile control women with regular cycles and normal ovaries who delivered at Kuopio

University Hospital between January 1999 and December 1999. In the study group, the indications for referral were menstrual cycle disturbances, infertility and symptoms of hyperandrogenism. In this study the diagnosis of PCOS was based on observation of anovulation and polycystic ovaries in ultrasonography and exclusion of other reasons for anovulation and hyperandrogenism such as hypothyreosis, hyperprolactinemia, hypercortisolism and late onset of congenital adrenal hyperplasia. In addition, the subjects included had at least one of the following clinical or biochemical disturbances: hirsutism, infertility, laboratory testing revealing androgen excess (serum total testosterone concentration > 2.5 nmol/l or free plasma testosterone > 40 pmol/l, evaluated by means of assay of SHBG and total testosterone, and an elevated LH/FSH ratio (>2). Hirsutism was defined as the presence of excessive body hair in an androgen-dependent pattern, with a modified Ferriman-Gallwey score of 8 or more(28).

DNA was extracted from peripheral blood lymphocytes using a standard phenol-chloroform extraction method. The polymorphic 5'-terminal poly-CAG repeat region was amplified with PCR. Each reaction contained 0.5  $\mu$ M forward (CY5-5'TCCAGAATCTGTTCCAGAGCGTGC-3') and reverse (5'-GCTGTGAAGGTTGCTGTTCCCTCAT-3') oligonucleotide primers (TAG, Copenhagen, Denmark), 0.25 mM dNTPs (Amersham Pharmacia Biotech, Uppsala, Sweden), 1 U DyNAzyme<sup>TM</sup> II Recombinant DNA polymerase (Finnzymes, Espoo, Finland), and standard 1x reaction buffer containing 1.5 mM MgCl<sub>2</sub>. The total volume was 20  $\mu$ l and each reaction was covered with 30  $\mu$ l of mineral oil. The samples were cycled (x30; 94 C 60 sec for denaturation, 64 C 45 sec for annealing, 72 C 45 sec for extension) using Hybaid PCR Express thermal cycler (Ashford, Middlesex, UK).

One  $\mu\text{l}$  of the PCR product was mixed with 1  $\mu\text{l}$  of 250 and 300 bp ALFexpress sizers, respectively, and 3  $\mu\text{l}$  loading dye (Amersham Pharmacia Biotech). The mixed samples were heat denaturated at 96 C for 5 min, put immediately on ice, and run on a denaturing gel (Reprogel High Resolution, Amersham Pharmacia Biotech) by automated fluorescence detection (ALFexpress DNA Analysis System, Amersham Pharmacia Biotech). The data were analyzed using the ALFwin Fragment Analyzer 1.00 software package. Ten percent of the samples were analyzed twice to confirm the integrity of the results. The sizes of the products ranged from 258 bp (12 CAG repeats) to 309 bp (29 CAG repeats).

#### Statistical analyses

The AR CAG repeat length was compared between the PCOS group and the two control groups with  $t$  test. The CAG distribution in the three groups was studied with  $\chi^2$  test, in all tests the  $p$  level of significance was set to 0.05.

#### Results

The clinical characteristics of the women with PCOS are shown in Table 1. The 112 women with PCOS were genotyped for the GAC repeat polymorphism of the androgen receptor gene. Androgen receptor CAG repeats ranged from 16-29 in control women and 13-29 in PCOS women (Table 2, Figure 1). Mean CAG lengths were similar in PCOS and control groups ( $21.5 \pm 2.2$  vs.  $21.5 \pm 2.1$ ;  $p$  0.8; Figure 2). Furthermore, the mean CAG repeat length of the PCOS group did not differ from the mean CAG repeat length of a previously reported Finnish control population ( $21.5 \pm 2.9$ ;  $p$  0.9)(28). The mean shorter allele CAG length was  $19.9 \pm$

2.6 in PCOS group and  $19.8 \pm 2.3$  in the control group ( $p = 0.8$ ; Figure 3).

Though the mean CAG repeat lengths were similar in PCOS and control groups, there were differences in the allele distribution (Table 3; Figure 1). There were five women carrying alleles with CAG repeats less than 16, all in the PCOS group. When the CAG repeat lengths were divided into three groups ( $\leq 18$ , 19-24, and  $\geq 25$ ), there were significant differences between the PCOS group and the previously studied control group. However, when the PCOS group was compared to the control group of this study, the difference remained statistically insignificant.

## **Discussion**

The aim of the present study was to investigate whether variants in the AR gene were associated with PCOS. The familial tendency towards PCOS and the hyperandrogenity observed in PCOS lead us to investigate the role of the CAG repeat polymorphism in the gene encoding androgen receptor in women with PCOS (29). Based on *a priori* information we hypothesized that the short repeat length would increase the risk of PCOS, whereas the long repeat length would be protective against the disease. The main finding of this study was that androgen receptor CAG repeat length is not a major factor in the etiology of PCOS. The mean repeat length was similar in the PCOS group compared to both control groups. However, the allele distribution pattern was different. The women carrying the very shortest ( $\leq 15$ ) AR CAG repeats all belonged to the PCOS group. Furthermore, there were less PCOS women carrying the medium length alleles than in the group of

standard population. Our result is partly in concordance with two previous studies on PCOS women. In all these three studies, including the present study, the mean AR CAG length is similar in PCOS women and healthy controls. The role of AR CAG repeat length becomes more evident only when a more detailed analysis is carried out. Mifsud et al. (22) found that PCOS women with serum T lower than their normal laboratory mean value had a significantly lower mean AR CAG repeat length in their shorter allele than those with higher serum T concentrations. However, even so, the difference remained non significant when both alleles were considered. Hickey et al. found in Australian population that PCOS women carry more alleles with AR CAG repeats  $\geq 23$  (20). This difference became even more significant when biallelic means were adjusted for preferential expression via X-inactivation analysis.

Furthermore, in their study, longer allele AR CAG repeat length correlated with serum T.

Perhaps, the most striking evidence on the role of AR CAG repeat length as a mechanism of ovarian hyperandrogenism comes from a study on Spanish girls (30). Ibáñez et al. followed girls with premature pubarche (PP) and found that the AR CAG repeat length is shorter in these girls compared to healthy controls. They also showed that those developing ovarian hyperandrogenism post menarche, had shorter mean repeat length than those with normal ovarian function.

In a population based Swedish cohort of 270 women (31), serum T concentration was higher in women with fewer AR CAG repeats and the authors speculated that higher transcriptional activity of the receptor could lead to an increased ovarian androgen production, thus AR could have a stimulatory role for androgen production in females, opposite to that found in males (21). The familial nature of PCOS clearly indicates a significant genetic component, and this genetic component probably comprises multiple gene variants each contributing a

small effect (29). The study of PCOS has been hampered by a lack of a male phenotype and therefore genetic studies have mainly focused on candidate genes. The androgen receptor gene has previously been implicated ... (9)

This study shows that there are differences across populations and in affected individuals due to the multifaceted nature of PCOS.

Power studies...



**Table 1. Clinical characteristics of the women with PCOS**

	N=112	%
Ovulatory disorders	112	100
- oligoamenorrhea <sup>a</sup>	69	61.6
-irregular periods <sup>b</sup>	43	38.4
Ultrasound: PCO	112	100
Hyperandrogenism	65	58.0
-hirsutism <sup>c</sup>	42	37.5
-acne	20	17.8
-alopecia areata	4	3.6
-total testosterone > 2.5 nmol/l <sup>d</sup>	45	40.2
Infertility	70	62.5
Obesity (BMI > 27 kg/m <sup>2</sup> )	67	59.8

PCO = polycystic ovary; BMI = body mass index.

<sup>a</sup> Ferriman-Gallwey score of > 8.

<sup>b</sup> Or free plasma testosterone > 40 pmol/l, evaluated using SHBG

**Table 2. Androgen receptor gene CAG repeat length divided into three categories and percentages of PCOS women and controls in each category.**

<b>Population</b>	<b>n≤18</b>	<b>n= 19-24</b>	<b>n≥25</b>	<b>p (<math>\chi^2</math>-test) (PCOD vs controls)</b>
PCO n=112	12,9%	66,5%	20,5%	
Controls (this study) n=112	14,7%	71,4%	13,8%	0.168
Controls (32) n=574	9,6%	77,9%	12,5%	0.003

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