Role of the CAG Repeat Polymorphism of the Androgen Receptor Gene in Polycystic Ovary Syndrome (PCOS)

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Key words
- polycystic ovary syndrome
- hyperandrogenemia
- luteinizing hormone
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- genetic polymorphism

Abstract

Background: Polycystic ovary syndrome (PCOS) is a frequent heterogenic disorder with a familial background. Androgenic effects, determining the clinical features of the syndrome, are mediated by the androgen receptor (AR), whose activity is modulated by a genetic polymorphism. We investigated the role of the CAG repeat polymorphism of the androgen receptor in PCOS.

Methods: In the infertility unit of a university clinic, 72 PCOS patients were compared with 179 ovulatory controls undergoing a standardized diagnostic work-up. The number of CAG repeats was determined by PCR, labelling with IR-800 and PAGE. X-chromosome inactivation was assessed by a methylation-sensitive assay.

Results: Compared to controls, PCOS patients displayed a shorter mean CAG repeat length, encoding for higher AR activity ($P=0.001$). CAG repeat length correlated inversely with oligomenorrhea, a central androgen dependent feature of the syndrome ($P=0.005$). In a binomial regression analysis including BMI, LH and free testosterone, CAG repeat length was identified as an independent risk factor for PCOS ($P=0.002$).

Conclusions: The CAG repeat polymorphism could constitute one of the genetic factors modulating the syndrome's phenotype, contributing to its clinical heterogeneity and associated metabolic consequences.

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Introduction

Polycystic Ovary Syndrome (PCOS) is a frequent endocrine disorder of unknown etiology affecting 6% of premenopausal women (Franks 1995, Ehrmann 2005). Although the clinical expression of the syndrome is highly variable, androgenic effects constitute the common mechanism responsible for its phenotype. Androgens transport the characteristic features of PCOS, e.g., acne, hirsutism, polyfollicular ovarian morphology and menstrual cycle disorders causing anovulation and infertility (Gilling-Smith et al. 1994; Hillier and Tetsuka 1997; Vendola et al. 1998; Mason 2000; Azziz et al. 2004; Jonard and Dewailly 2004). The association of PCOS with metabolic disturbances is mediated by insulin, enhancing synthesis and effects of androgens in these patients (Dunaif 1997; Cupisti et al. 2007). Furthermore, androgens are reported to play a pivotal role in the pathogenesis of the syndrome in utero (Abbott et al. 2005; Xita and Tsatsoulis 2006). Although family studies have demonstrated a familial clustering of the disease and a genetic basis is suspected, to date no single gene defect responsible for PCOS has been identified (Franks et al. 2001, Escobar-Morreale et al. 2005, Urbanek et al. 2005).

Androgenic effects are exerted via the androgen receptor (AR), a nuclear transcription factor and member of the steroid receptor superfamily (Chang et al. 1988). The activity of the AR is influenced by a genetic polymorphism in exon 1 of the AR gene located on the X-chromosome at Xq11–12 (Chamberlain et al. 1994). The polymorphism consists of a variable number of CAG repeats encoding for an amino-acid sequence in the receptor's transactivation domain (Chamberlain et al. 1994). In vitro experiments and in vivo studies in men showed that the number of CAG-repeats correlates inversely with the AR activity (Chamberlain et al. 1994; Zitzmann et al. 2004). Although there is a trend to a corresponding correlation in women with hyperandrogenemic conditions, the results of the studies remain

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inconsistent (Legro et al. 1994; Vottero et al. 1999; Mifsud et al. 2000; Calvo et al. 2000; Westberg et al. 2001; Hickey et al. 2002; Ibanez et al. 2003; Jaaskelainen et al. 2005; Möhlig et al. 2006; Kim et al. 2008; Xita et al. 2008; Shah et al. 2008; Liu et al. 2008; Van Nieuwerburgh et al. 2008).

Given the frequency of PCOS, its familial background and variable phenotype, a genetic polymorphism able to modulate the central pathophysiological mechanism stimulated further investigation. We conducted this study to analyze the influence of the CAG repeat polymorphism on the phenotype of women with PCOS.

Subjects and Methods

Patients

Female patients undergoing standardized diagnostics procedures in a university infertility clinic were evaluated regarding their clinical, endocrine and sonographic parameters indicating the presence of PCOS. A PCOS group was defined according to the ESHRE/ASRM consensus and compared with a control group not fulfilling the PCOS criteria (The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group 2004).

The following parameters were recorded: Age, body mass index (BMI), menstrual history and cycle length, presence of clinical androgenization in the form of hirsutism. After cessation of any hormonal medication for 2 months, an endocrine status was assessed in the morning (8–10 a.m.) of an early follicular phase (day 3–5) of a spontaneous or progestin induced cycle (Azziz et al. 2004, Carmina et al. 2006). Serum concentrations of estradiol, LH, FSH, testosterone, DHEAS and SHBG were measured. Basal 17-OH progesterone, prolactin and TSH were assessed to exclude other endocrine disorders causing anovulation and infertility (Azziz 2006). Non classical congenital adrenal hyperplasia was excluded by ACTH stimulation test, measuring testosterone, 17-OH progesterone and DHEAS when indicated (Carmina et al. 2006). The diameter of a dominant follicle and the presence of polycystic ovaries were evaluated by experienced reproductive gynaecologists performing a transvaginal scan on day 12–14. Transvaginal scans were re-evaluated by one of the authors (A.S.) and patients with unclear or incomplete sonographic records were not included in the study. Serum progesterone was determined on day 20–24 of a spontaneous or progestin induced cycle (Carmina et al. 2006).

After informed consent EDTA blood samples were collected from all patients and stored at −20 °C serving as a source for DNA extraction. This procedure was approved by the Ethics Committee of the Medical Faculty and the State Medical Board.

PCOS group

According to the ESHRE/ASRM-consensus, the definition of PCOS requires at least 2 of the following 3 criteria: Oligo- or anovulation, clinical or biochemical hyperandrogenism and polycystic ovaries (The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group 2004). In this study, the above criteria were defined as follows: Oligo- or anovulation was present in patients with a maximum cycle length >35 days or a progesterone <9.54 nmol/l (3 ng/ml) measured on a cycle day 20–24 and the absence of a dominant follicle >15 mm on midcycle transvaginal scan (Azziz et al. 2004; Carmina et al. 2006). Clinical hyperandrogenism was defined as hirsutism if the Ferriman Gallwey score exceeded 5 points (Ferriman and Gallwey 1961; Carmina et al. 2006). Biochemical hyperandrogenism was defined as serum concentrations of testosterone >3.05 nmol/l or of DHEAS >10.52 µmol/l. These individual reference ranges were derived from the 95th percentile of eumenorrhoeic and ovulatory patients in the control group following a procedure published before (Azziz et al. 2004). Polycystic ovaries were defined as ovaries displaying 10 or more follicles measuring 2–8 mm in diameter, arranged peripherally or scattered throughout an increased ovarian stroma (Adams et al. 1985; reviewed in Balen et al. 2003). 72 patients fulfilling the above criteria of PCOS were included in the study.

Control group

The control group consisted of 179 patients not fulfilling the Rotterdam criteria. Patients in the control group were eumenorrhoeic with cycle intervals between 21 and 35 days, ovulation was demonstrated by a rise of progesterone >12.7 nmol/l (4 ng/ml) or the presence of a dominant follicle (>15 mm) on midcycle transvaginal scan (Azziz et al. 2004).

Laboratory methods

Hormone assays

All hormone assays were performed using commercial kits as part of the clinical routine. Testosterone, DHEAS, LH and FSH were measured by ECLIA (Elecsys, Roche, Mannheim, Germany), estradiol by RIA (MAIA, Biochem Immunosystem, Guidonia Montecelio, Italy), progesterone and SHBG by FIA (DELFIA, Wallac, Erlangen, Germany), and 17-OH progesterone by RIA CT (Biosource, Nivelles, Belgium). Mean intraassay and interassay coefficients of variation were below 10 % and below 15 %, respectively. Free testosterone was calculated as published before (Mueller et al. 2006; Vermeulen 1999, www.issam/testocaculator).

CAG repeat length

Determination of CAG repeat length was performed as published before (Zitzmann et al. 2001). Briefly, DNA was extracted from peripheral lymphocytes. CAG repeat length was determined by PCR using specific primers, IRD-800 labelling and PAGE (Zitzmann et al. 2001). The number of CAG repeats was analyzed according to the bi-allelic mean, the shorter allele and the longer allele (Zitzmann et al. 2001; Hickey et al. 2002). Patients displaying 2 detected bands of CAG repeat length were considered heterozygous and subject to X-chromosome-inactivation analysis allowing the calculation of an X-weighted bi-allelic mean (Hickey et al. 2002).

X-chromosome-inactivation assay

Because the AR gene is located on the X-chromosome of which one is inactivated in women, in case of heterozygous CAG repeat alleles the individual X-chromosome-inactivation status is of interest (Lyon 1992; Vottero et al. 1999). X-chromosome inactivation correlates with DNA-methylation at the HpaII site near the CAG repeats of exon 1 of the AR gene (Allen et al. 1992). X-chromosome inactivation was assessed as previously described using a methylation sensitive restriction enzyme for pre-digestion (Hickey et al. 2002; Zitzmann et al. 2004). The enzyme specifically cuts the unmethylated, hence active DNA, leaving all methylated, inactive DNA undigested and available for amplification. To calculate the degree of X-inactivation the signals were analyzed densitometrically and quantification was assessed as described (Naumova et al. 1996; Iitsuka et al. 2001; Zitzmann et al. 2004).

Statistical analysis

Clinical data and CAG repeat lengths were compared between groups by a Wilcoxon W-test. Comparisons displayed in **Table 1** were conducted as an explorative analysis and *P*-values were not adjusted. The distribution of categorized CAG repeat lengths was analyzed with a Chi^2-test and calculation of a Somer's d coefficient. The correlation of CAG repeat length with cycle length was evaluated using the Spearman Rho coefficient. A one-sided t-test was used to test for random X-chromosome inactivation. In a backwards stepwise binomial regression analysis continuous parameters independently predicting PCOS were identified. Predictors that did not contribute to the model were excluded (exclusion threshold $P > 0.04$). A $P < 0.05$ was considered statistically significant. All tests were generated using the statistical software package SPSS (Chicago, IL; release 13.0).

Results

Characterization of patients

Clinical and endocrine patient and control data are displayed in **Table 1**. As expected, patients in the PCOS group showed longer cycle intervals, elevated total testosterone, free testosterone and DHEAS serum concentrations, a higher BMI, a higher LH level and a correspondingly elevated LH/FSH ratio. Patients of the PCOS group were 2.5 years younger than controls ($P < 0.001$). Among PCOS patients, the symptoms of the syndrome were distributed as follows: 61 patients (84.8%) displayed PCO ovaries, 44 patients (61.2%) suffered from oligo-/anovulation, 38 patients (52.8%) had evidence of biochemical hyperandrogenism and 17 patients (23.6%) were hirsute.

CAG repeat length compared between groups

The length of CAG repeat alleles in all patients followed a normal distribution pattern with a bi-allelic mean of 21.83 alleles, ranging from a minimum of 17 alleles to a maximum of 29 alleles (**Table 1**). The shortest CAG repeat length observed was 12, the longest 31. Comparing both groups, the bi-allelic mean of PCOS patients was significantly smaller compared with controls (21.43 vs. 21.99, $P = 0.031$). Analyzing the longer of 2 given CAG repeat alleles, this difference between groups was confirmed, the PCOS patients showing a significantly shorter CAG repeat length compared with controls (22.69 vs. 23.75, $P = 0.001$, **Table 1** and **Fig. 1**). In contrast, for the shorter of 2 CAG repeat alleles, no significant difference between groups was observed. Because the difference of the bi-allelic mean was caused by the longer CAG repeat allele, further investigations in this study are focused on those alleles.

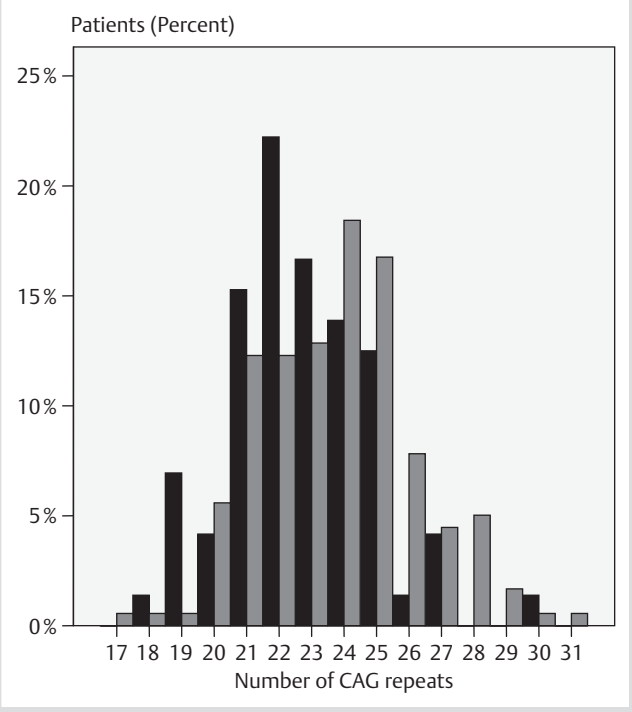

Fig. 1 Short CAG repeat length is associated with PCOS. PCOS patients are shown in black, controls are shown in grey. The figure displays the longer of 2 given CAG alleles.

Table 1 Clinical characteristics and CAG repeat length of the study population.

	All subjects n = 251		PCOS n = 72		Controls n = 179		Wilcoxon W-Test
	Mean (Median) ± SD	Range	Mean (Median) ± SD	Range	Mean (Median) ± SD	Range	*P*
Characteristics							
age (yrs)	31.84 (31.91) ± 3.90	(19.75–41.73)	30.17 (29.75) ± 3.69	(21.58–40.03)	32.64 (33.04) ± 3.76	(19.75–41.73)	<0.001
height (cm)	168.92 (170.0) ± 6.90	(141–189)	169.38 (170.0) ± 7.37	(141–185)	168.79 (169.0) ± 6.70	(150–189)	0.544
weight (kg)	67.69 (65.0) ± 12.20	(47–110)	71.65 (68.50) ± 14.37	(50–110)	66.02 (65.0) ± 10.84	(47–110)	0.001
BMI	23.74 (22.80) ± 4.07	(15.49–36.72)	24.97 (24.22) ± 4.67	(15.49–36.44)	23.18 (22.41) ± 3.65	(16.26–36.72)	0.001
LH (U/l)	4.97 (4.40) ± 2.71	(0.9–22.8)	6.08 (4.85) ± 4.12	(0.9–22.8)	4.52 (4.30) ± 1.70	(0.9–12.9)	<0.001
FSH (U/l)	6.94 (6.70) ± 2.08	(1.2–15.7)	6.23 (5.95) ± 1.76	(2.4–12.9)	7.24 (7.10) ± 2.14	(1.2–15.7)	<0.001
LH/FSH	0.78 (0.66) ± 0.53	(0.11–4.05)	1.05 (0.78) ± 0.80	(0.11–4.050)	0.67 (0.63) ± 0.32	(0.15–2.60)	<0.001
T (nmol/l)	1.73 (1.40) ± 1.24	(0.10–11.80)	2.62 (2.50) ± 1.74	(0.40–11.80)	1.38 (1.30) ± 0.73	(0.10–3.40)	<0.001
free T (nmol/l)	0.023 (0.017) ± 0.022	(0.001–0.145)	0.039 (0.034) ± 0.030	(0.002–0.145)	0.0164 (0.014) ± 0.011	(0.001–0.065)	<0.001
DHEAS (µmol/l)	5.93 (5.80) ± 2.65	(0.40–14.90)	6.69 (6.30) ± 3.33	(0.40–14.90)	5.63 (5.45) ± 2.25	(0.50–14.10)	0.004
SHBG (nmol/l)	71.13 (64.80) ± 36.64	(0.6–200.0)	61.93 (56.90) ± 38.52	(7.1–200.00)	75.10 (69.90) ± 35.29	(0.60–198.10)	0.011
cycle length (d)	43.94 (29.00) ± 58.14	(21–365)	80.76 (40.00) ± 99.72	(27–365)	29.06 (28.00) ± 3.35	(21–35)	<0.001
CAG length							
shorter allele	20.21 (20.20) ± 2.11	(12–26)	20.14 (20.00) ± 2.15	(12–26)	20.23 (20.00) ± 2.10	(15–26)	0.760
longer allele	23.45 (23.00) ± 2.38	(17–31)	22.69 (22.50) ± 2.18	(18–30)	23.75 (24.00) ± 2.41	(17–31)	0.001
Bi-allelic mean	21.83 (22.00) ± 1.84	(17–29)	21.43 (21.50) ± 1.87	(17–27)	21.99 (22.00) ± 2.07	(17–29)	0.031
X-Bi-allelic mean	21.94 (21.93) ± 2.02	(16.57–30.76)	21.48 (21.49) ± 1.87	(17.58–26.50)	22.11 (22.00) ± 2.07	(16.57–30.76)	0.027

Table 2 Distribution of categorized CAG repeat length.

Categorized CAG repeat length	All subjects n = 251	PCOS n = 72	Controls n = 179
short alleles (< 22 CAG)	21.9 %	27.8 %	19.6 %
medium alleles (22–25 CAG)	61.8 %	65.3 %	60.3 %
long alleles (> 25 CAG)	16.3 %	6.9 %	20.1 %
all alleles	100.0 %	100.0 %	100.0 %

Chi-square Pearson $P = 0.027$; Somers-d $P = 0.009$, the longer CAG repeat allele is analyzed

Table 3 CAG repeat length and extent of oligomenorrhea as assessed by length of cycle interval (days).

	Correlation	P*
shorter allele	none	0.750
longer allele	negative	0.005
bi-allelic mean	negative	0.026
x-weighted bi-allelic mean	negative	0.066

*Non parametrical test: Spearman's correlation

Table 4 Short CAG repeat length as an independent risk factor for PCOS.

Risk factor	P*
elevated free testosterone	< 0.001
short CAG repeat length	< 0.001
elevated LH	0.011

*Backwards stepwise binomial regression analysis, the longer CAG repeat allele is analyzed

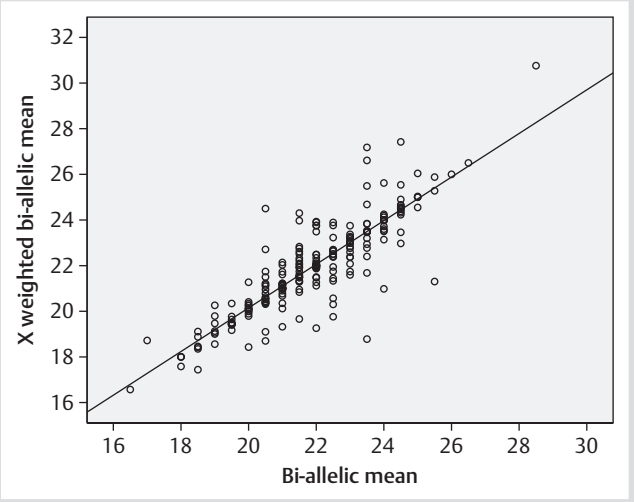

Fig. 2 Pattern of X-chromosome inactivation. Correlation between bi-allelic mean and X-weighted bi-allelic mean of CAG repeat length in all subjects.

CAG repeat length is associated with PCOS

When evaluating the longer of 2 CAG repeat alleles according to 3 categories representing short (< 22), medium (22–25) and long (> 25) CAG repeat alleles, short alleles, encoding for more active AR, were significantly more frequent in the PCOS group ($P = 0.027$, Chi-square Pearson and $P = 0.009$ Somers-d). Correspondingly, long alleles, protecting from PCOS by encoding for a weaker AR, were more frequent in the control group (**Table 2**).

CAG repeat length correlates with cycle length

In PCOS ovaries follicular development is impaired by androgenic effects resulting in prolonged cycle intervals and accordingly oligomenorrhoea is regarded as a key feature of the syndrome. We investigated cycle intervals as expressed in days to test for an influence of the genetic polymorphism on a continuous phenotypic parameter of PCOS. In a non-parametrical test the length of cycle intervals correlated inversely with CAG repeat length of the longer CAG repeat allele ($P = 0.005$) and the bi-allelic mean ($P = 0.026$), see **Table 3**. There was no correlation with other phenotypic traits of PCOS, such as the presence of hirsutism, androgen levels or polycystic ovaries (data not shown).

CAG repeat length as an independent risk factor for PCOS

We then investigated the role of CAG repeat length in context with established risk factors for PCOS. To this end, we included BMI, serum LH, free serum testosterone and CAG repeat length of the longer allele in a backwards stepwise binomial regression analysis and identified the CAG repeat polymorphism as an independent risk factor for PCOS ($P \leq 0.001$), **Table 4**.

No difference in X-chromosome inactivation pattern between PCOS and controls

Because mere determination of CAG repeat length cannot give information about the methylation status of the X-chromosome and hence the activity of assessed CAG repeat alleles, in heterozygous patients information about the methylation status is of interest, especially when X-chromosome inactivation does not occur at random but preferably involves the longer or the shorter of 2 given CAG repeat alleles (skewed X-chromosome inactivation) (Vottero et al. 1999). To account for X-chromosome inactivation status, we calculated the X-weighted bi-allelic mean of the CAG repeat alleles (Hickey et al. 2002; Zitzmann et al. 2004). The difference from the bi-allelic mean was calculated, negative values indicating a preferential expression of the shorter CAG repeat allele and positive values indicating a preferential expression of the longer CAG repeat allele. In the whole study population there was no trend to a skewed X-chromosome inactivation, neither in favour of the shorter nor of the longer CAG repeat allele. Analyzing the PCOS group and the control group separately, no skewed X-chromosome inactivation in either of the groups was detected. However, in some individuals a preferential X-chromosome inactivation was present preferring either the longer or the shorter allele (**Fig. 2**). In those subjects the assessment of X-chromosome inactivation status might generate genotype-phenotype correlations more precise than the mere number of CAG repeats alone. However, for the whole group, the X-weighted bi-allelic mean yielded correlations of comparable precision to the bi-allelic mean (**Table 1**).

Discussion

This study demonstrates an association of the CAG repeat polymorphism of the AR with the phenotype of PCOS. Considering the longer of 2 given alleles, mean CAG repeat allele length was significantly smaller in the PCOS group compared to controls. Furthermore, short CAG repeats were more frequent in PCOS possibly enhancing androgenic effects in these patients, while longer CAG repeat allele lengths were more frequent in the control group, presumably exerting a protective effect.

The CAG repeat polymorphism as a modulator of AR activity has been recently investigated in PCOS because androgens constitute the central element in the etiology of the disorder and transport its clinical phenotype. In line with our findings, some reports support the concept of a more active AR in PCOS, observing shorter CAG repeat lengths compared with controls (Xita et al. 2008; Shah et al. 2008). However, findings remain inconsistent, and other studies do not report associations of CAG repeat lengths with the condition of PCOS (Mifsud et al. 2000; Jaaskelainen et al. 2005; Kim et al. 2008; Ferk et al. 2008; Liu et al. 2008; van Niewerburgh et al. 2008). Notably, when subgroups of PCOS were analyzed according to androgen levels in 2 of these studies, PCOS patients with distinctly elevated androgens displayed increased CAG lengths, representing less active receptors (Mifsud et al. 2000, Kim et al. 2008). Congruently in another study, PCOS patients with a short bi-allelic mean (<21 CAG repeats) displayed lower serum androgens albeit a high proportion of hirsutism, an overt symptom of female hyperandrogenism (Van Nieuwerburgh et al. 2008). In line with these observations, not all PCOS patients of our study displayed symptoms of androgenization in form of hirsutism or overt hyperandrogenemia (Cupisti et al. 2011; Dewailly et al. 2006; Barber et al. 2007). Probably because patients were recruited in a reproductive medicine unit, the symptoms most frequent were related to infertility as reflected by the high incidence of polycystic ovaries and impaired ovulation (Cussons et al. 2005). Accordingly, the findings of our study suggest that the subtile influence of the CAG repeat polymorphism could play a role predominantly in PCOS populations not presenting as the fully developed syndrome (Dewailly et al. 2006; Barber et al. 2007). Summarized in a unifying hypothesis, these findings are support for a modulating role of the genetic AR polymorphism in PCOS: more active receptors (short CAG repeats) can cause a hyperandrogenic phenotype in absence of markedly elevated androgens while patients with a decreased receptor's activity (long CAG repeats) will develop the disorder preferably in the presence of distinctly elevated androgens.

While subgroup analysis according to androgen levels in our PCOS group did not reveal differences in CAG repeat lengths (data not shown), we report novel evidence that the AR's genetically determined activity was continuously correlated with increased cycle length, a central androgen dependent feature of PCOS. Because it has been shown that enhanced recruitment, prolonged development and premature atresia of follicles are androgen dependent processes contributing to the clinical sequelae of oligomenorrhea and amenorrhea which are regarded as a key feature of the syndrome (Hillier and Tetsuka 1997; Vendola et al. 1998; Franks 1995; Ehrmann 2005; The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group 2004), our findings support the hypothesis of the AR's genetic variation modulating the phenotype of PCOS. Although absolute CAG repeat differences of 1.06 between groups appear limited considering absolute numbers, they are comparable to other reports (Mifsud et al. 2000; Ibanez et al. 2003; Kim et al. 2008). Furthermore, our study demonstrates that the genetic polymorphism influenced the PCOS phenotype by functioning as an independent risk factor, comparable to established markers of PCOS e.g., elevated LH, increased BMI and elevated free testosterone. Recently, a novel concept of "androgenicity" was proposed in male individuals, considering both androgen concentrations and AR activity responsible for androgenic effects (Zitzmann et al. 2004; Canale et al. 2005). The data of this study suggest the validity of this concept for androgen dependent disorders also in female individuals.

It is not easy to explain the partially inconsistent results in studies on the CAG repeat polymorphism and female hyperandrogenism. Because large and well characterized groups of genetic homogeneity are required when assessing phenotypic effects of genetic polymorphisms (Edwards et al. 1992), different ethnic backgrounds could have influenced findings in a Caucasian population of an immigration country (Hickey et al. 2002) and in a heterogenic population of Chinese and Indians in Singapore (Mifsud et al. 2000). Ethnic differences had to be accounted for by separate analysis in another study, finding a weaker genotype-phenotype association in white individuals compared with Afro-Americans (Shah et al. 2008). Furthermore, subjects of control groups tend to be assessed differently or recruited in a different population, not subject to the specific biochemical and sonographic evaluations applied in the respective PCOS group (Mifsud et al. 2000; Hickey et al. 2002; Jaaskelainen et al. 2005; Kim et al. 2008). The consistent genotype-phenotype correlation observed in our study might therefore find its explanation in the detailed characterization of genetically homogeneous PCOS patients and controls, both recruited in an identical clinical setting and assessed in a standardized pattern.

Epigenetic X-chromosome-inactivation by methylation adjacent to the CAG repeat allele site can further modify the genotype-phenotype correlation of the AR polymorphism in female hyperandrogenism (Allen et al. 1992). In hirsute patients, preferential X-chromosome inactivation of the shorter allele augmented androgenic effects (Vottero et al. 1999) and different patterns of X-chromosome inactivation accounted for a varying clinical phenotype in sister pairs with PCOS, hinting on an epigenetic etiology of female hyperandrogenism (Hickey et al. 2006). On the contrary, no difference in X-inactivation pattern was detected in another study on hirsute patients (Calvo et al. 2000) and X-inactivation was not a major determinant in a recent study on PCOS, although a discrete preferential expression of the shorter CAG allele was observed (Shah et al. 2008). In our PCOS population as a whole, preferential X-chromosome inactivation did not play a significant role, but was present in individual subjects. Because X-chromosome inactivation can vary in different tissues assessment of inactivation patterns in target tissues such as the ovaries or the skin could help to further elucidate the role of epigenetic processes in different conditions of female hyperandrogenism.

Since data collection for this study included patients recruited before the Rotterdam PCOS consensus, we applied a definition of polycystic ovarian morphology widely used before, which was not changed throughout the study to preserve consistency (Adams et al. 1985; Polson et al. 1988; reviewed in Balen et al. 2003). This definition requires 10 small follicles instead of the Rotterdam criterion of 12 small follicles (The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group 2004), the latter treshold identifiying PCOS with a higher sensitivity and a lower specificity (Jonard and Dewailly 2004). In this context, a possible bias on the selection of some PCOS individuals in our study cannot be completely excluded.

We conclude that the CAG repeat polymorphism of the AR contributes to the development of a PCOS phenotype, modulating the clinical appearance of the disorder. Our findings are further support for the pivotal role of androgens in PCOS with the genetically determined activity of the AR contributing to central features of the condition: The CAG repeat polymorphism could

constitute a factor in the familial background, convey part of the distinct phenotypic variability and transport metabolic consequences of the syndrome.

Conflict of Interest: None.

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