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CAG repeat polymorphism in androgen receptor gene is not directly associated with polycystic ovary syndrome but influences serum testosterone levels

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ABSTRACT

Hyperandrogenemia has been the most consistent feature of polycystic ovary syndrome (PCOS). Androgens exert their effects through androgen receptors (ARs). The expansion of the codon CAG trinucleotide repeat polymorphism in exon 1 of the *AR* gene represents a type of genetic alteration associated with changes in the *AR* gene function. The purpose of this study was to establish a possible association of the *AR* gene CAG repeat length polymorphism with PCOS, and its influence on clinical and biochemical androgen traits. Two hundred and fourteen Croatian women with PCOS and 209 healthy control women of reproductive age were enrolled. Phenotypic hyperandrogenism, BMI and waist to hip ratio were recorded. Hormonal profiles, fasting insulin and glucose levels were measured on cycle days 3–5. Genotyping of the CAG repeat polymorphism in the *AR* gene was performed. We found no significant difference in the mean CAG repeat number between the PCOS patients and controls (22.1 ± 3.4 vs. 21.9 ± 3.2, $P = 0.286$). There was a positive correlation between the CAG repeat length and total testosterone (TT) in the PCOS group ($R = 0.225$, $P = 0.015$). A multiple linear regression model using mean CAG repeat length, BMI, age and HOMA-IR as predictors explained 8.5% (adjusted R^2) of the variability in serum TT levels. In this model the CAG repeat polymorphism was found to be a significant predictor of serum TT levels in PCOS patients ($P = 0.015$). The logistic regression analysis revealed that the CAG repeat length is not a significant predictor of hirsutism and acne status ($P = 0.921$ and $P = 0.437$, respectively). The model was adjusted for serum TT, free testosterone, androstendione and DHEAS levels as independent variables, which were also not found to be significant predictors of hirsutism ($P = 0.687$, $P = 0.194$, $P = 0.675$ and $P = 0.938$, respectively) or acne status ($P = 0.594$, $P = 0.095$, $P = 0.290$ and $P = 0.151$, respectively). In conclusion, the *AR* CAG repeat polymorphism is not a major determinant of PCOS in the Croatian population, but it is a predictor of serum TT level variability in women with PCOS.

1. Introduction

Polycystic ovary syndrome (PCOS) is the most frequent endocrine disorder in women of reproductive age with heterogenous clinical and biochemical features. Its etiology remains uncertain, however, due to a variety of predisposing genes that interact with environmental and lifestyle factors, PCOS is considered a complex genetic disorder [1]. A large number of population studies have focused on discovering genes that influence the development of PCOS using candidate gene approach [2], but their findings have been mostly irreproducible. This lack of replication might be explained by different ethnic groups studied and the local environmental influences that alter the phenotype in a particular ethnic group only.

Hyperandrogenemia and hyperinsulinemia have been the most consistent genetically determined characteristic of PCOS. Androgens exert their effects through androgen receptors (ARs), which belong to a family of ligand-activated transcription factors. Exon 1 of the *AR* gene possesses an N-terminal modulatory domain encoded by a CAG trinucleotide repeat polymorphism. The expansion of this polymorphism, with the normal range between 11 and 38 repeats, represents a type of genetic alteration associated with changes in the *AR* gene function [3]. ARs, varying in the position and size of the polyglutamine tract, were constructed *in vitro*. This model demonstrated that the transcriptional activity of the AR is inversely correlated with the number of CAG repeats [4]. The existing studies that have focused on the hypothesis that this change in transcriptional activity of the *AR* exists in PCOS patients and modulates a severity of hyperandrogenism have produced conflicting and inconsistent results [5–13]. Moreover, it is possible that a particular gene influences PCOS in one ethnic group but not in the other [1].

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The aim of the present study was to evaluate whether there is a difference in the *AR* gene CAG repeat length between PCOS patients and healthy controls in the Croatian population. Additionally, we assessed the influence of the CAG repeat number on different biochemical parameters as well as on clinical androgen traits in PCOS patients.

2. Materials and methods

2.1. Study subjects

In the study we enrolled 214 women fulfilling the criteria for PCOS; they were 25.9 ± 5.2 years old (mean ± SD). The diagnosis of PCOS was made according to the Rotterdam consensus criteria, which are based on the presence of two out of three traits including oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries on ultrasound scanning [14,15]. Other endocrinopathies and related disorders were ruled out by measuring basal serum 17-hydroxyprogesterone (17-OHP), prolactin (PRL) and thyroid stimulated hormone (TSH) levels.

The menstrual irregularities were defined as the presence of amenorrhea or oligomenorrhea. Hyperandrogenism was assessed by the presence of hirsutism and/or acne and/or by elevated androgen levels (serum total testosterone (TT) > 2.5 nmol/l or free testosterone (FT) > 30 pmol/l). Hirsutism was defined by Ferriman–Gallwey index score >8 (FG > 8) [16]. The control group consisted of 209 healthy volunteers aged 28.7 ± 4.6 years before entering *in vitro* fertilisation (IVF) programme due to male factor infertility. They had no menstrual cycle irregularities, no clinical or biochemical hyperandrogenism, no PCO findings on ultrasound, no history of endocrinological or autoimmune disorders and no surgery to the pelvic region. The information on age at pubarche and menarche was obtained from each patient through medical interviews.

The patients discontinued medication (including hormonal contraceptives) for at least six months prior to enrolment to study.

All patients were observed in the early follicular phase of the menstrual cycle (days 3–5) or randomly in amenorrheic patients. The body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Transvaginal ultrasound (TVUS) was done to diagnose PCO according to the Rotterdam criteria [14,15]. Blood samples were drawn for the measurement of luteinizing hormone (LH), follicle stimulating hormone (FSH), total testosterone (TT), sex hormone binding globulin (SHBG), dehydroepiandrosterone sulphate (DHEAS), androstendione (A), fasting serum glucose and fasting insulin. Free testosterone (FT) was calculated from TT and SHBG as previously published [17] using a web-based calculator (http://www.issam.ch/freetesto.htm). Insulin resistance (IR) was quantified using the homeostatic model assessment of IR (HOMA-IR) ((fasting insulin (mU/l) × fasting glucose (mmol/l))/22.5). Whole blood samples were obtained for genetic analysis.

The study protocol No. 04-1116-2006 was approved by the University of Zagreb Medical School Ethics Committee. Informed written consent was obtained from all women enrolled in the study.

2.2. Hormone assays

Serum LH, FSH, SHBG, TT, DHEAS and A levels were measured. Serum LH, FSH, TSH, prolactine and TT concentrations were determined by chemiluminescent immunometric assays using LH-Vitros, FSH-Vitros, TSH-Vitros, Prolactine-Vitros and Testosterone-Vitros, respectively (Ortho Clinical Diagnostics, Johnson & Johnson, Rochester, NY, USA). Serum SHBG, DHEAS and A levels were measured using chemiluminescent immunometric assays (SHBG-Immulite, DHEAS-Immulite and Androstendione-Immulite, respectively) (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA). The concentration of 17-OH-progesterone was determined by a solid phase enzyme-linked immunosorbent assay (ELISA) based on the principle of the competitive binding (DRG-diagnostics, Marburg, Germany). The intra-assay and inter-assay coefficients of variation ranged between 1.5 and 7.9%. The plasma glucose level (Glc) was determined by the UV-photometric hexokinase method and the serum insulin (Ins) level by chemiluminescent immunometric assay using Insulin-Immulite, respectively (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA). The serum samples were obtained in the early follicular phase of the menstrual cycle or randomly in amenorrheic patients.

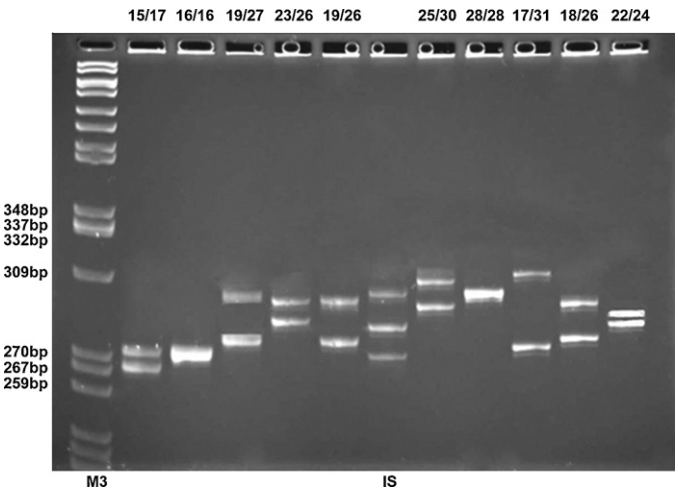

Fig. 1. Identification of *AR* CAG repeat length genotype from gel electrophoresis of PCR products. Individual *AR* genotypes are marked as number of CAG repeats for both *AR* alleles; numbers are separated by a slash. Accuracy of determining number of CAG repeats are estimated on ±2 repeats (±6 bp). M3 – marker designed for precise size determination of unknown DNA fragments (Elchrom Scientific, Cham, Switzerland); IS – internal standard for CAG repeats.

2.3. Genotype analysis

Genomic DNA was isolated from whole blood using FlexiGene Kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's protocol. Genotyping for the *AR* (CAG)*n* polymorphism was performed by PCR using High-Fidelity Platinum DNA Polymerase (Invitrogen, Paisley, Scotland, UK), forward primer 5′-TCC AGA ATC TGT TCC AGA GCG TGC-3′ and reverse primer 5′-GCT GTG AAG GTT GCT GTT CCT CAT-3′ [18], and followed by electrophoresis on Spreadex Mini gels (Elchrom Scientific, Cham, Switzerland). The number of CAG repeats was calculated from the size of the PCR products in relation to the M3 marker (Elchrom Scientific, Cham, Switzerland) and our own internal standard. The latter was prepared from three different PCR fragments, 264 bp, 276 bp, and 333 bp in size, that were, after direct sequencing, identified as having 14, 18, and 37 CAG repeats, respectively. The gel was documented with ChemiGenious Bio Imaging System (Syngene, Cambridge, UK) (Fig. 1) and analyzed with GeneTool analysis software (Syngene, Cambridge, UK) which allows accurate measurement of PCR fragment length. Genotype analysis was conducted at the Department of Obstetrics and Gynaecology, Division of Medical Genetics, University Medical Centre Ljubljana.

2.4. Statistical analysis

We used the independent Student's *t*-test to compare the values of the means between cases and controls. Differences in categorical

characteristics between cases and controls were assessed using Chi-square test.

For genotype analysis, the biallelic mean of the CAG repeat from the two alleles in each subject was calculated. The biallelic mean was considered in two ways. Firstly, it was analyzed as a continuous variable. The median number of allelic CAG repeats was calculated from the CAG repeat number of each allele observed in both groups. Subjects with extreme heterozygous genotype combinations, having one shorter and one longer allele than the median were excluded from subsequent analyses. Secondly, we created a binary nominal variable with the two states being less than or equal to or greater than the median CAG repeat number observed in both groups.

The association analysis of the biallelic means of the CAG repeat and the PCOS was carried out using logistic regression, adjusting for age and BMI as independent variables. The Pearson's correlation coefficient was used for assessing linear relations. To explore the effect of the CAG repeat length on testosterone concentration, a multiple linear regression model was constructed with testosterone as a dependent variable, and age, BMI, HOMA-IR and biallelic average repeat length as independent variables. Logistic regression model was conducted to explore the association of the CAG repeat length with clinical androgen traits in PCOS patients, adjusting for serum androgen levels as independent variables.

All statistical analyses were done using the SPSS for Windows (version 15.0; SPSS Inc., Chicago, IL, USA). A P-value <0.05 was considered statistically significant.

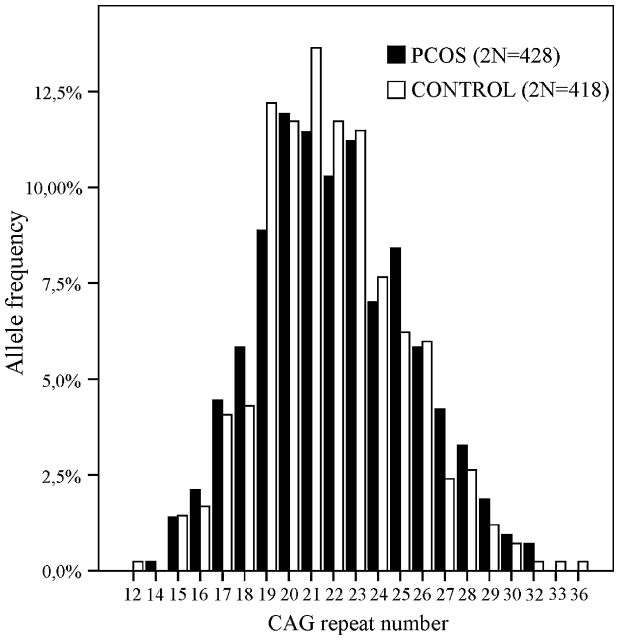

Fig. 2. Distribution of CAG repeat alleles in PCOS patients and controls.

3. Results

3.1. Clinical and biochemical characteristics of PCOS patients and controls

The clinical and hormonal parameters in PCOS patients and control women are presented in Table 1. As expected, LH, mean serum TT, FT and HOMA-IR were significantly higher in the PCOS group, whereas the mean serum SHBG and FSH levels were significantly lower than in the control group. The PCOS patients had higher BMI, and a significantly higher frequency of hirsutism than controls; the age at pubarche and menarche did not differ between the groups.

3.2. Association of CAG repeat polymorphism with PCOS

The distribution of the number of allelic CAG repeats for the PCOS patients and controls is presented in Fig. 2.

The difference in the mean CAG repeat number between the PCOS patients (22.1 ± 3.4; $2n$ = 426 alleles) and controls (21.9 ± 3.2; $2n$ = 418 alleles) was not significant (P = 0.286). The frequency distribution of the PCOS and control women bearing repeat values below and above the median number of allelic CAG repeats is presented in Table 2, separately for the total alleles ($2n$), biallelic mean

Table 1
Mean ± SD of clinical and hormonal parameters in the PCOS and control patients.

	PCOS (n = 214)	Control (n = 209)	P-value[a]
Age (years)	25.9 ± 5.2	28.7 ± 4.6	<0.001
BMI (kg/m^2)	23.1 ± 3.9	22.2 ± 3.1	0.013
BMI < 25 kg/m^2	79.4%	90.9%	0.001
WHR	0.78 ± 0.1[b]	0.79 ± 0.1[c]	ns
FSH (IU/l)	4.1 ± 2.0	5.1 ± 1.4	<0.001
LH (IU/l)	9.1 ± 5.9	3.1 ± 1.2	<0.001
TT (nmol/l)	2.5 ± 0.9	1.2 ± 0.4	<0.001
FT (pmol/l)	45.8 ± 27.2	13.5 ± 5.3	<0.001
Androstendione (nmol/l)	11.5 ± 5.2	7.6 ± 2.5	<0.001
DHEAS (μmol/l)	6.8 ± 2.9	5.0 ± 1.9	<0.001
SHBG (nmol/l)	41.0 ± 19.5	64.0 ± 20.4	<0.001
HOMA-IR	2.2 ± 2.6	1.3 ± 0.5	<0.001
Pubarche (years)	11.1 ± 1.6	11.4 ± 1.5	ns
Menarche (years)	12.7 ± 1.6	12.8 ± 1.5	ns
Menstrual cycle			
Normal	9.9%	100%	<0.001
Oligomenorrhea	67%	0%	<0.001
Amenorrhea	24.2%	0%	<0.001
Hirsutism	76.9%	11.1%	< 0.001
Acne	51.6%	18.2%	<0.001
CAG biallelic mean	22.1 ± 2.6	21.9 ± 2.5	ns

Abbreviations: BMI, body mass index; WHR, waist/hip ratio; FSH, follicle stimulating hormone; LH, luteinizing hormone; TT, total testosterone; FT, free testosterone; DHEAS, dehydroepiandrosterone sulphate; SHBG, sex hormone binding globulin; HOMA-IR, homeostatic model assessment of insulin resistance; ns, not significant.

[a] Student's t-test was used for continuous and Chi-square test for categorical variables.

[b] n = 91.

[c] n = 98.

Table 2
Distribution (%) of PCOS cases and controls according to the qualitative categories of CAG repeats.

Repeat size	Total number of CAG repeat alleles[a]		CAG repeat biallelic mean[b]		CAG repeat biallelic mean with excluded extreme heterozygous genotypes[c]	
	PCOS ($2n = 428$)	Control ($2n = 418$)	PCOS ($n = 214$)	Control ($n = 209$)	PCOS ($n = 116$)	Control ($n = 124$)
≤22	56.5	61.0	55.6	58.9	61.2	68.5
>22	43.5	39.0	44.4	41.1	38.8	31.5

[a] $\chi^2 = 1.74$, $P = 0.187$.
[b] $\chi^2 = 0.00$, $P = 0.99$.
[c] $\chi^2 = 1.42$, $P = 0.233$.

and biallelic mean with excluded extreme heterozygous combination.

The distribution of the CAG repeats was not significantly different between cases and controls, even though the PCOS population tended to have a longer CAG repeat than controls. The logistic regression was performed on the entire cohort of cases and controls to assess the association of CAG repeat length with the PCOS status, but the result was non-significant ($P = 0.379$).

3.3. Association of the CAG repeat polymorphism and androgen traits in PCOS

We found no significant differences in biochemical parameters between the PCOS group with short and long alleles (Table 3) except for the level of the TT which was higher in the CAG repeat >22 allele group ($P = 0.019$).

Pearson's correlation analysis revealed a positive correlation between the CAG repeat length and the TT level in the PCOS patients ($R = 0.225$, $P = 0.015$). Among PCOS patients, BMI was not found to be in significant correlation with the TT ($R = 0.028$, $P = 0.345$). A positive correlation was observed between the CAG repeat length and the TT concentration either unadjusted or after adjustment for age, BMI and HOMA-IR (Table 4). A model using the mean CAG repeat length, BMI, age and HOMA-IR as predictors explained 8.5% (adjusted R^2) of the variability in the serum TT levels ($P = 0.015$) (Table 4).

To explore the association of the CAG repeat length with clinical androgen traits (hirsutism or acne), a logistic regression model was constructed with hirsutism or acne status (no/yes) as dependent variable, CAG repeat length status (short/long) as independent variable, and androgens (total and free testosterone, androstendione and DHEAS) as covariates, but the result was non-significant ($P = 0.921$ for hirsutism; $P = 0.437$ for acne). The serum levels of total testosterone, free testosterone, androstendione and DHEAS were also not found to have a significant effect on hirsutism ($P = 0.687$, $P = 0.194$, $P = 0.675$ and $P = 0.938$, respectively) or acne status ($P = 0.594$, $P = 0.095$, $P = 0.290$ and $P = 0.151$, respectively).

Table 3
Mean ± SD of biochemical and clinical parameters in PCOS patients with short and long CAG repeat alleles.

	CAG repeat length		
	≤22 ($n = 71$)	>22 ($n = 45$)	P-value[a]
Age (years)	26.9 ± 5.9	25.8 ± 5.9	ns
BMI (kg/m^2)	23.4 ± 4.2	22.4 ± 3.2	ns
WHR	0.76 ± 0.1[b]	0.82 ± 0.1[c]	ns
FSH (IU/l)	3.8 ± 1.5	4.5 ± 2.9	ns
LH (IU/l)	8.9 ± 6.1	9.3 ± 6.8	ns
TT (nmol/l)	2.2 ± 0.9	2.6 ± 1.0	0.019
FT (pmol/l)	40.1 ± 21.9	45.0 ± 23.3	ns
Androstendione (nmol/l)	12.3 ± 6.0	10.2 ± 4.1	ns
DHEAS (μmol/l)	7.0 ± 2.8	7.6 ± 2.4	ns
SHBG (nmol/l)	41.3 ± 19.7	43.9 ± 17.2	ns
HOMA-IR	2.0 ± 2.1	2.4 ± 2.9	ns
Pubarche (years)	11.5 ± 1.7	10.7 ± 1.5	ns
Menarche (years)	13.0 ± 1.6	12.5 ± 1.8	ns
Menstrual cycle			
Normal	10.8%	7.7%	ns
Oligomenorrhea	67.6%	53.8%	ns
Amenorrhea	21.6%	38.5%	ns
Hirsutism	70.3%	69.2%	ns
Acne	45.9%	61.5%	ns

Abbreviations: BMI, body mass index; WHR, waist/hip ratio; FSH, follicle stimulating hormone; LH, luteinizing hormone; TT, total testosterone; FT, free testosterone; DHEAS, dehydroepiandrosterone sulphate; SHBG, sex hormone binding globulin; HOMA-IR, homeostatic model assessment of insulin resistance; ns, not significant.
[a] Student's t-test was used for continuous and Chi-square test for categorical variables.
[b] $n = 37$.
[c] $n = 19$.

Table 4
Multiple linear regression models with the TT as the dependent variable.

Variable	Standardized β coefficient	P-value
Unadjusted model ($R^2 = 0.048$)		
CAG repeat length (biallelic mean)	0.220	0.019
Adjusted model ($R^2 = 0.076$)		
CAG repeat length (biallelic mean)	0.215	0.022
Age	−0.137	0.140
BMI	0.091	0.327
Adjusted model ($R^2 = 0.085$)		
CAG repeat length (biallelic mean)	0.231	0.015
Age	−0.124	0.186
BMI	0.114	0.232
HOMA-IR	−0.099	0.301

Abbreviations: BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance.

4. Discussion

In the present study we evaluated the difference in the *AR* gene CAG repeat length between PCOS patients and healthy controls. To our knowledge this is the first study to examine the association of the *AR* CAG repeat length and the PCOS in the Croatian population. The PCOS patients tended to have a higher frequency of longer *AR* CAG repeats than controls, but allele distribution was not significantly different between the groups studied, indicating that the *AR* gene is not the major determinant of PCOS (Table 2). This finding is concurrent with the observation of some previous studies [5,7,8,11,19].

While there is a significant variation in the number of the CAG repeats in different populations, different studies used different approaches in defining the CAG repeat groups. Most of them used biallelic means to analyze the results, with short and long alleles defined according to the median in their population. In women, the *AR* gene is under the phenomenon of the X-inactivation by methylation of HpaII and HhaI sites [20]. The X inactivation patterns are neither perfectly random (50:50), nor perfectly non-random (100:0) which may result in differences in the active AR protein [21]. The preferential expression of shorter CAG repeats of the *AR* gene would contribute to the increased androgen sensitivity.

Additionally, we assessed the influence of the CAG repeat number on different biochemical parameters as well as on clinical androgen traits in the PCOS patients. In our cohort of patients, the androgens were significantly higher in the PCOS group compared to controls and the same was true for the androgen traits – hirsutism and acne (Table 1). The analysis of the differences between biochemical and clinical parameters of the PCOS patients with short and long alleles revealed significant difference in serum TT levels ($P = 0.019$). The Pearson's correlation analysis showed a linear association between the CAG repeats and serum TT levels ($R = 0.225$, $P = 0.015$). This positive correlation was observed after the adjustment for age, BMI and HOMA-IR using a multiple linear regression model. The same analysis in the control group revealed no significant difference in any biochemical parameter. BMI alters circulating androgen levels by decreased levels of the SHBG or through decreased insulin sensitivity, whereas insulin directly stimulates thecal cell androgen production [22]. There is a normal age-related decline in androgen levels, which was confirmed in the study by Spencer et al. on 260 healthy eumenorrheic women [23]. This is the reason why BMI, insulin sensitivity and age should be considered when establishing the presence of hyperandrogenemia in women.

Ovarian hyperandrogenism is considered the fundamental abnormality of PCOS. Ovarian theca cells express androgen receptors. According to the hypothesis and findings of *in vitro* study it is expected that shorter CAG repeat alleles result in amplified androgen receptor activity [4]. Regulation of AR function and identity of target genes for the receptor are not well understood [24]. Amplified transcriptional activity of AR promotes intra-ovarian androgenic microenvironment which presumably stimulates early follicular growth and contributes to mechanisms of follicular arrest found in PCOS. Increased number of small antral follicles is positively correlated to the serum testosterone and androstendione levels in PCOS patients [25]. Insulin and insulin-like growth factor 1 (IGF-1) are capable of enhancing androgen response to LH as well as independently stimulate androgen production [26]. Furthermore the androgens have been also shown to induce expression of the genes for IGF-1 and IGF-1 receptor [27] establishing a vicious cycle that promotes androgen production.

Only a few studies have found an association between the CAG repeats and serum testosterone levels in PCOS patients [6,8,12,19]. Ibanez et al. [12] found that post-menarcheal girls with premature pubarche and shorter alleles (<20 CAG repeats) had higher serum androgen levels and disposed with androgen traits. Westberg et al. [28] examined 270 premenopausal Swedish women and found those with fewer CAG repeats to display higher levels of serum androgens than women with longer repeats. Our findings of a positive linear relationship between the CAG repeat alleles and testosterone levels are consistent with the findings of Hickey et al. [6] and Kim et al. [8] in Australian and Korean PCOS women, respectively. Hickey et al. [6] found preferential expression of longer CAG alleles in the PCOS women (greater than 22 repeats) that were also positively correlated with serum testosterone. The mechanisms in the background of these findings remain to be elucidated. Mifsud et al. [19] found a trend of lower CAG repeat numbers in Chinese PCOS patients with lower testosterone levels, but bivariate correlation analysis revealed that this relationship was not linear.

Since the CAG repeat length is a predictor of the variability in TT only in the PCOS population, we believe that it contributes, at least to some extent, to the etiology of PCOS which still remains to be elucidated. Even though we have found a significant positive correlation between the CAG repeat length and TT, it is only a modest correlation ($R = 0.225$, $P = 0.015$), and the adjusted model explains only 8.5% of the variability in serum testosterone, therefore other factors influencing the serum TT level are probably involved as well. Recent advances in molecular biology have identified many coregulators (coactivators and corepressors) of the AR which influence the androgen action via cross-talk with the AR; this has revealed potentially multiple mechanisms through which the transcription activity may be modulated [24,29].

Although it is expected that due to a higher receptor activity the women with shorter alleles will have a higher expression of clinical androgen traits of acne and hirsutism, this has not been confirmed in our study. Also, levels of the TT, FT, androstendione or DHEAS were not found to be significant predictors of hirsutism or acne status. This is consistent with the findings of some studies which have found no differences in the number of the CAG repeat length between the hirsute patients and normal controls [10,30,31]. Vottero et al. [31] recorded the shortest alleles only in the hirsute patients, but this was not confirmed by other groups [10,30].

5. Conclusion

In summary, the *AR* CAG repeat polymorphism is not a major determinant of PCOS in the Croatian population, but it is a predictor of serum TT level variability in Croatian women with PCOS. However, since the adjusted linear regression model explains only 8.5% of the variability of serum TT, the major limitation of our study is that we analyzed the CAG repeat length as one of the factors influencing the transcriptional activity of the *AR*. Further studies are needed to reveal other signaling pathways influencing the androgen action, especially the role of numerous newly discovered AR coregulators.

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