Association Between Polycystic Ovary Syndrome and Polymorphisms of *CYP11A* Gene AmongSample of Iraqi Women

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Abstract

Polycystic ovarian syndrome (PCOS) is a multifactorial disorder with significant metabolic, reproductive and psychological consequences. In order to explain the association between genetic polymorphism of CYP11A1 gene and polycystic ovary syndrome and comparing with the effect this syndromewith hormonal and lipid profile in PCOS Iraqi women.Two groups in this study was concluded. The first group involved 74 women diagnosed with PCOS and the other group involved 58 healthy women to investigate the impact of pentanucletide(AAAAT) repeat in promoter region of CYP11A1 gene between both groups. Genotyping and comparison conducted between them. The results indicated that was considerable differences ($P< 0.05$) in Prolactin and testosterone mean levels between patients and healthy groups. There was no significant results with other parameters (FSH, LH, cholesterol, triglyceride,HDL,LDL,VLDL). For genotypetwo categories were used in this study: one between genotype with age and the second between genotype with BMI for each group. The results revealed no significant ($p<0.05$) between percentage of the patients and controls regarding to age and BMI categories. Moreover, the results revealed that five different CYP11A1 (AAAAT) repeats were identified, corresponding to 3, 5, 6, 7 and 8 repeat-unit the results showed a significant difference ($p<0.05$) in pentanucleotides (AAAAT) for five and three repeats between patients and healthy women. The conclusion of the current study is that the most distributed pentanucleotide (AAAAT) repeats observed in PCOS Iraqi women was 5 repeats. Five repeats (5R) in the women can be considered as a general marker for PCOS susceptibility in Iraqi women.

Keywords: Polycystic ovarian syndrome, CYP11A1, pentanucleotide, genotype

Introduction:

Polycystic ovarian syndrome (PCOS) is a multifactorial disorder with significant metabolic, reproductive and psychological consequences (1). This significant problem for public health affecting (6-10) percent of women of reproductive age worldwide (2). The major symptoms of PCOS are now recognized as hyperandrogenism, oligo / amenorrhoea, and polycystic ovary morphology (PCOM). However, there is no agreement of doctors using the diagnostic criteria of PCOS, Rotterdam criteria or the criteria of the Androgen Excess Society (3).

Dyslipidemia is a widespread metabolic disorder in PCOS, about 70 per cent of women with PCOS have borderline or elevated lipid levels (4). Higher values of total cholesterol, LDL cholesterol, Apo B and triglycerides were found in PCOS women with a positive family history of the PCOS-related clinical abnormality (5). Another disorder correlated to this syndrome ishyperandrogenism which existent in 80-85 per cent of women diagnosed with PCOS (6). Hyperandrogenism is clinically and mechanistically estimated from elevated serum levels of androgens. The most common PCOS related endocrine disorder is an elevated androgen level. As a result, multiple genes have been identified to be correlated with PCOS in uncovering the explanation for the elevated androgen levels, such as:CYP11A, FSHR, CYP17, CYP1A1, CAPN10, INSR, SERPINE, IL-1 and SHBG.

CYP11A on 15q23-24 codes the enzyme P450 side-chain cholesterol cleavage, which catalyzes the rate limiting stage of ovarian steroidogenesis, the conversion of cholesterol to pregnenolone (7; 8). CYP11A locus is involved in PCOS development as it illustrates the relation of the development of PCOS with repetitions in 5-UTR (AAAAT) sequencing (9). Previous studies in different countries have shown a positive correlation between pentanucleotide repeats and PCOS susceptibility (10; 11; 12). Wang et al. (13) demonstrated the risk of PCOS in Chinese women, demonstrating various allele combinations that either raise or lower the risk of having PCOS among the female population. Shen et al. (14) reported a higher risk of developing PCOS with repeated*CYP11A* polymorphisms. So the aim of this study is to explain the possible association between genetic polymorphism of *CYP11A1* gene and polycystic ovary syndrome in a sample of Iraqi women and also comparing the effect of this syndromewith hormonal and lipid profile in PCOS Iraqi women.

Materials and methods

The collection of blood samples and the practical work of this study lasted from February 2019 to December 2019, for a period of eleven months.This study included seventy four infertile Iraqi women with PCOS and fifty eight healthy Iraqi women. The patients were selected from Kamal al-Samarrai Hospital, Baghdad, Iraq, and met at least two of the threefollowingcriteria depending on the (PCOS consensus workshop group sponsored by Rotterdam ESHRE / ASRMS) (15): a) Polycystic ovaries on ultrasound. b)oligovulation or Chronical anovulation. C) Clinical or biological Hyperandrogenism.

For further analysis BMI and age were divided into three subgroups, regarding to BMI these subgroups were (normal 18.5-24.9 k/m2 ,overweiht 25-29.9 k/m2 and obese ≥ 30 k/m2), for age (<25 years, 25-35years, >35years).A sample of venous blood (5 ml) was collected from each PCOS and healthy control women, each blood sample has been divided into two tubes: (1) EDTA tubes for Molecular analysis. (2) the blood serum to evaluate the hormones levels (FSH, LH , testosterone and prolactin) as well as to measure the lipid profile: cholesterol,triglyceride, high density lipoprotein (HDL), low-density lipoprotein(LDL),verylow-density lipoprotein(VLDL).

Molecular Analysis:

Gene selection

ReliaPrep™ Blood gDNAMiniprep System is the kit for abstracting complete genomic DNA from the blood of both groups (patients and controls) used in the present study.*CYP11A1* was the gene chosen for this study. The association between *CYP11A1* promoter pentanucleotide (AAAAT)n polymorphism and PCOS among Iraqi women was evaluated. (AAAAT)npolymorhism detection was achieved by using PCR and sequencing.

Polymerase chain reaction (PCR)

The extraction of DNA (template) from blood was carried out by the Promega extraction kit. PCR amplification (25 µl) was performed using 7µl of the template DNA, 4.9µl Nuclease free water, 12.5 µl of PCR 2X Master mix kits and 0.3 µl of each oligonucleotide primers of *CYP11A1* gene with sequence: *CYP11A1* F: GGTGAAACTGTGCCATTGCand*CYP11A1* R: GTTTGGGGGAAATGAGGGGC (16).PCR reaction was programmed with the appropriate conditions (table 1). After that, the quantity of 5µl of the PCR product and 5µl of the ladder were carefully loaded into the gel wells and operated at 90 volts for 90 minutes, following by staining with ethidium bromide stain for 20 minutes and visualized under UV light by the gel documentation system. Using gel electrophoresis on 2% (w/v) Tris acetate buffer agarose gel contained 0.1 µl/mL ethidium bromide.

Table (1): PCR Program for *CYP11A1* gene

Steps	Temperature	Time	No. of cycle
Initial denaturation	95°C	5 min	1 cycle
Denaturation	94°C	30 sec	30 cycle
Annealing	58°C	45 sec	
Extension	72°C	1 min	
Final extension	72°C	7 min	1 cycle
Holding	4°C	10 min	1 cycle

PCR products sequencing

CYPllA1 (AAAAT)n repeat-polymorphism genotyping analysis was performed by using polymerase chain reaction (PCR) and direct sequencing. In the present study all DNA specimens from cases and control were amplified by specificprimers for *CYP11A1* gene then sent for sequencing to Macrogen® - Korea. The reasons of this step were to identify the repeat-polymorphism (AAAAT)n genotyping species. The types of repeat between cases and control sequences were identified through multiple alignments by Geneious Primer 2020 software.

Statistical analysis:

Data analysis was done by utilizing SPSS for Windows, version 22 (SPSS Inc. Chicago, Illinois, United States). Shapiro–Wilk normality test was used to determine whether the studied parameters followed a gaussian distribution.

Categorical variables were expressed as proportions. Proportions were compared using the Chi-square test (χ^2).

Data were expressed as mean ± standard deviation (SD) for continuous variables. Differences between groups were analyzed by student's t test. The Tukey's Post Hoc tests for multiple comparison was applied after ANOVA tests. A *p* value less than 0.05 was considered statistically significant)(17,18).

Result and discussion

The Hormonal profile levels

Comparison of serum hormonal FSH , LH , Prolactin and testosterone was done between PCOSpatients and control groupas shown in table (2), the mean serum level of FSH in patient was (6.24±2.80)and in control group was(5.89±1.98) that shows non-significant difference ($P< 0.05$)in patient compared with control group .the present study found that the serum level of LH was not significant difference in patient group (mean 6.05±3.29)when compared with control group(5.17±2.38), the level of Prolactin in patient wasstatistically significant difference ($P< 0.05$) with mean serum(23.38±9.36) while the mean serum level (10.36±4.78) in control group, the higher concentration ofProlactin Level is considered to be reliable indicator of susceptibility for the diagnosis of polycystic ovarian syndrome, a study conducted iniraqi population by Hassan (19)indicated thatIn females with PCOS, hyperprolactinemia is more frequent than in normal ovulatory women, andfertilization is adversely affected by increased serum prolactin levels and reduces the chance of pregnancy.

The mean serum level of testosterone was slightly significant in patients group(1.41±0.55) when compared to control group (0.84±0.33) .the current study reported there was significant increase in serumprolactin andtestosterone level in women with PCOS when compared with healthy women (control).

These findings agreed with Carlos Moran et al.(20)concerning Testosterone and FSH, which reported significantly higher levels of testosterone (P <0.05) in PCOS groups than in women's control groups. No major variations between the groups were seen in the FSH values. Although the findings of the LH disagree. Another research by Kumar et al. (21)reveals significant results in patient and control group levels of LH and prolactin.

The lack of variations in these hormones may be due to the fact that patients in the sample received previous medical treatment.

Table (2): The hormonal profile levels of the studied groups.

Parameter	Control group(n=58)	Patients group(n=74)	*P*value
S.FSH (mIU/ml)	5.89±1.98	6.24±2.80	0.42
S.LH (mIU/ml)	5.17±2.38	6.05±3.29	0.08
S. Prolactin (ng/ml)	10.36±4.78	23.38±9.36	0.00
S.TT (ng/ml)	0.84±0.33	1.41±0.55	0.00

FSH: follicle-stimulating hormone, LH: Luteinizing hormone
TT: total testosterone

The Lipid profile levels

The present study, lipid profile comparison between patients and controls was illustrated in (Table 3). No significant differences were observed between patients and control in parameters of lipid profile, which are triglyceride, HDL, LDL, VLDL and cholesterol levels (119.40, 44.47, 114.32, 23.88 and182.60 mg\dl) in patients respectively, and (121.70, 44.08, 112.40, 24.34 and 184.08 mg\dl) levels in control women respectively.

Moreover, the lipid profile comparison (as hormones) is also subject to the same explanation. Cases getting lipid Lowery treatment could have normal limits of cholesterol, HDL, LDL, VLDL and triglyceride. In addition, Genetic and environmental variables such as diet and level of exercise are likely to contribute to these variations (22).

A study conducted by Baldani et al. (23)Showed that women with PCOS may have normal lipid profiles, the study showed that TC and total LDL-C levels did not vary between PCOS and control females. This similarity to TC and total LDL-C levels makes women with PCOS seem to have normal lipid profiles.

Table (3): Serum lipid profile of the studied groups.

Parameter	Control group(n=58)	Patients group(n=74)	*P*value
S.TC (mg/dl)	184.08±27.25	182.60±28.26	0.76
S.TG (mg/dl)	121.70±34.64	119.40±33.60	0.70
S.HDL (mg/dl)	44.08±5.00	44.47±4.86	0.65
S.LDL (mg/dl)	112.40±28.44	114.32±24.49	0.67
S.VLDL (mg/dl)	24.34±6.92	23.88±6.72	0.70

TC: total cholesterol, TG: triglycerides, HDL: high-density lipoprotein
LDL: low-density lipoprotein, VLDL: very low-density lipoprotein

Genetic studies:

Molecular analysis of *CYP 11A1* gene using PCR technique

This study used a PCR technique to detect regions of the *CYP11A1* gene. The PCR results revealed that identical bands related to the (AAAAT)n in promoter region was present. PCR amplified regions showed a molecular weight of 234bp .Figure (1).

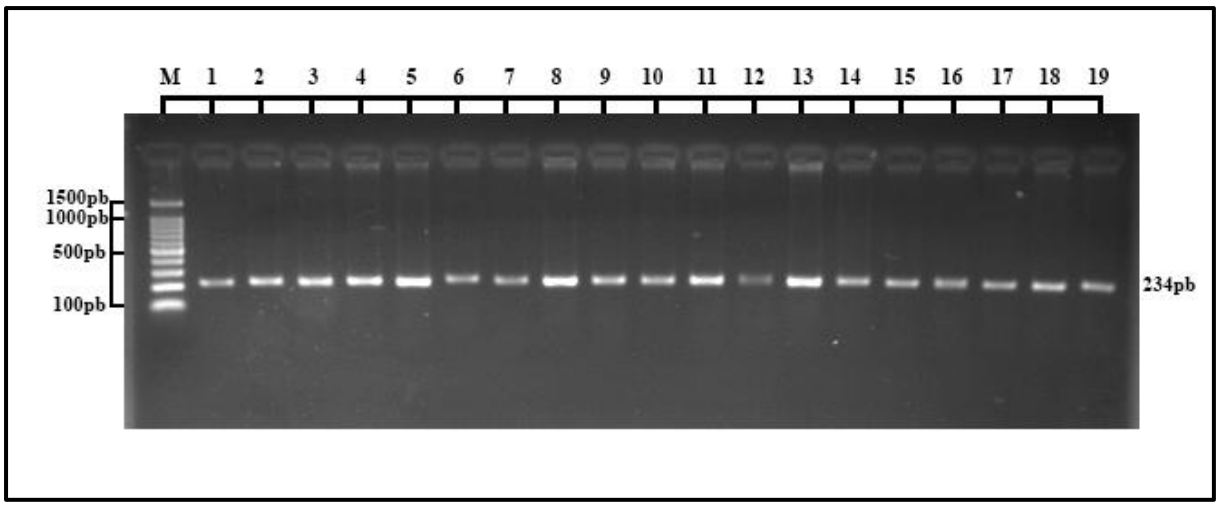

Fig.1: Agarose gel with Ethidium Bromide stain showing PCR products with *cyp11a* gene (234 bp) using specific primer for human extracted DNA. The electrophoresis was conducted at 90 volts for 90 min. Line (M) for DNA ladder (100 to 1500 bp), Line (1-19) positive results of this study samples that gave amplified products (234bp)

Genotype distribution of *CYP 11A1* gene

The types of repeat between cases and control sequences were identified through multiple alignments by Geneious Primer software. The results revealed that five different *CYP11A* (AAAAT) repeats were identified, corresponding to 3, 5, 6, 7 and 8 repeat-unit. The distribution of these repeats are (29.30, 62.10, 1.7, 3.4 and 3.4) % respectively in control, as compared with (14.9, 79.7, 4.1, 0.0 and 1.4) % respectively in PCOS patients as shown in table (4).

Table (4): The genotype distribution of *CYP11A1* gene

Samples 180			
PCOS Patients (100)		Control (80)	
Possible to Analyze	74	Possible to Analyze	58
5 Repeats (%)	59 (79.7%)	5 Repeats (%)	36 (62%)
3 Repeats (%)	11 (14.8%)	3 Repeats (%)	17 (29.3%)
6 Repeats (%)	3 (4%)	7 Repeats (%)	2 (3.4%)
8 Repeats (%)	1 (1.3%)	8 Repeats (%)	2 (3.4%)
		6 Repeats (%)	1 (1.7%)

*** In compare to Reference cyp11A gene (NG_007973.1) with (AAAAT) Repeats located between (4589–4628)**

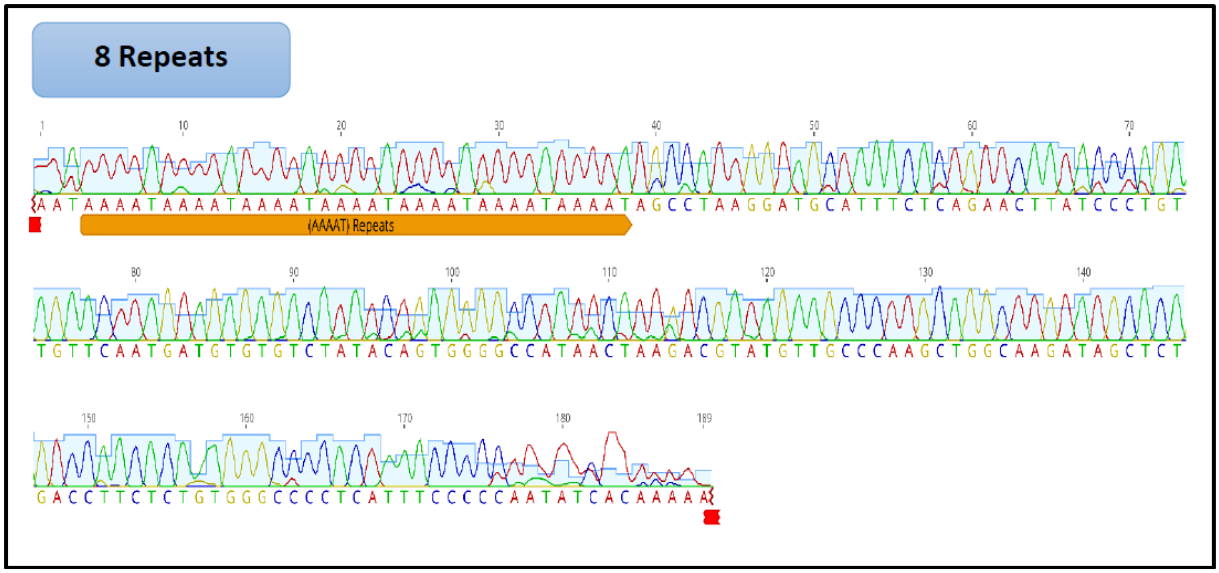

Figure (2):illustrates 8 repeats of pentanucleotide (AAAAT) in promoter region of *CYP11A1* gene

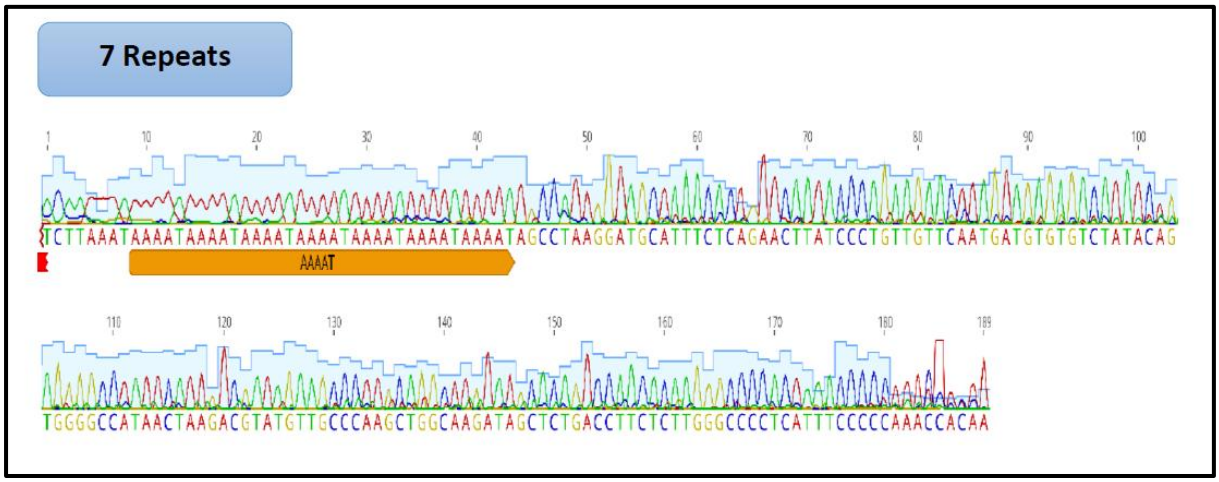

Figure (3):illustrates 7 repeats of pentanucleotide (AAAAT) in promoter region of *CYP11A1* gene

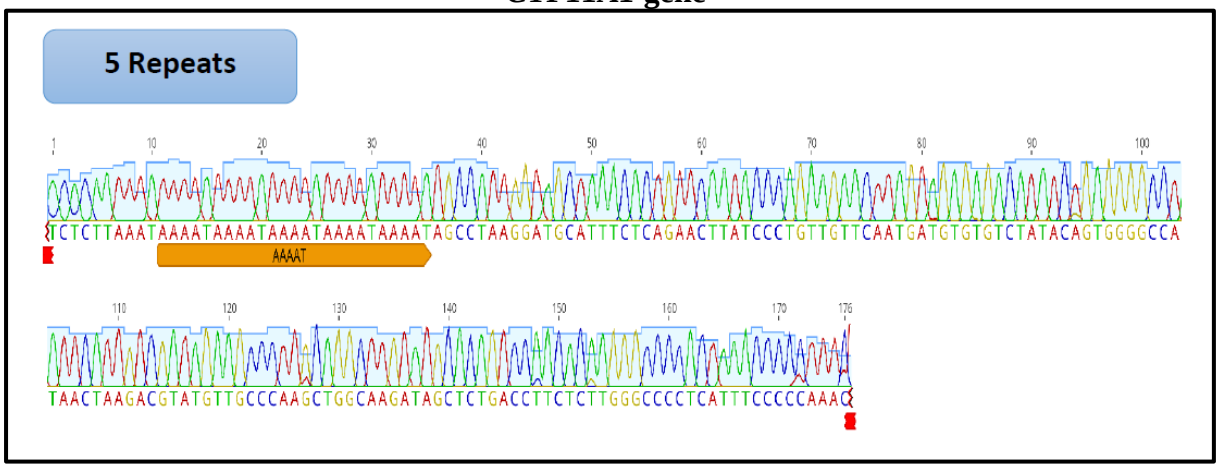

Figure (4):illustrates 5 repeats of pentanucleotide (AAAAT) in promoter region of *CYP11A1* gene

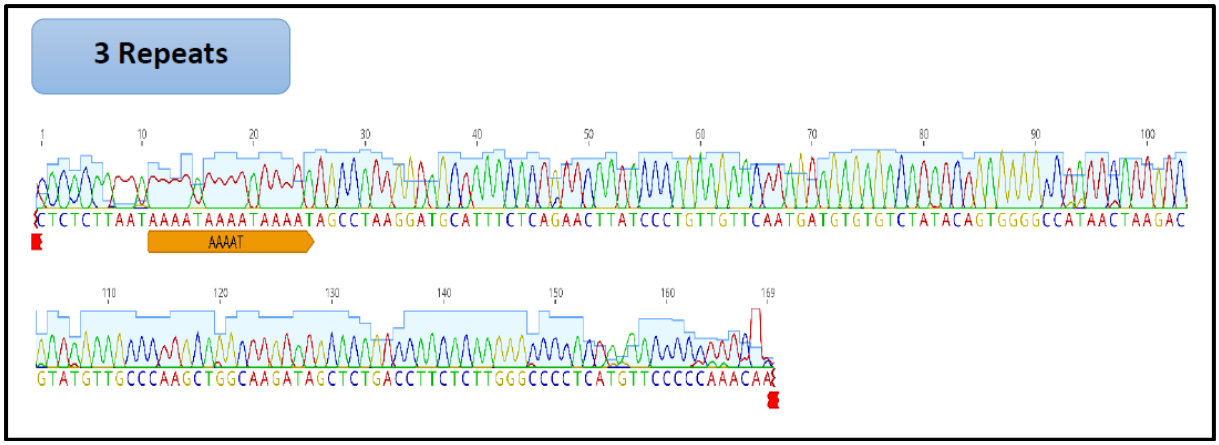

Figure (5):illustrates 3 repeats of pentanucleotide (AAAAT) in promoter region of *CYP11A1* gene

***The Sequence Analysis performed by using Geneious Prime 2020 Software.**

The number and the percentage of genotype in PCOS women and control group are shown in table (5), there was a significant difference ($p<0.05$) between percentage of the patients and controls of three repeats (3R) and five repeats (5R).The percentage of (3R) wassignificant higher in controls group than in patients(29.30,14.90)% respectively, while the percentage of (5R) wassignificant higher in patients group than in controls (79.70 ,62.10)% respectively. There was no significant results with other repeats (6R, 7R, 8R).

CYP11A locus is involved in PCOS development as it illustrates the relation of the development of PCOS with repetitions in 5-UTR (AAAAT) sequencing (9). Previous studies in different countries have shown a positive correlation between pentanucleotide repeats and PCOS susceptibility (10; 11; 12). Wang et al. (13) demonstrated the risk of PCOS in Chinese women, demonstrating various allele combinations that either raise or lower the risk of having PCOS among the female population. The correlation of higher risk of developing PCOS and repeated *CYP11A* polymorphisms has been confirmed by Shen et al. (14).

This study showed that *CYP11A* (AAAAT) microsatellite contained 5 repeats in Iraqi women as the most common repeats, In addition, the distribution of the five repeats of *CYP11A* gene is different from that of the other ethnic populations. The common alleles are 6 and 8 repeat units in PCOS Chinese women, 4 repeat units in Caucasian from Spain and Greece and 9 repeat unit in American white women (16). This could be due to ethnic or racial variations between people of different countries and races.

Table (5): Number and percentages of (AAAAT) repeats in patients and control groups.

Number of (AAAAT) repeats	Control group (n=58)	Patients group (n=74)	χ^2	*P*value
3	17(29.30%)	11(14.90%)	4.06	0.04
5	36(62.10%)	59(79.70%)	5.02	0.02
6	1(1.70%)	3(4.10%)	0.60	0.43
7	2(3.40%)	0(0.00%)	2.59	0.10
8	2(3.40%)	1(1.40%)	0.64	0.42

Correlation between genotypes and age groups

The number and percentages (AAAAT) repeats in the control and patient subgroups, based on the age categories shown in Table (6) and Table (7) respectively.There was no significant difference ($p<0.05$) between percentage of the patients and controls regarding to age categories.

These findings show that there is no age impact in different (AAAAT) repeats of PCOS patients comparing with control women in the Iraqi population.As we mentioned earlier,the common alleles in Chinese women were 6 and 8-repeat units, 4-repeat units in Caucasian from Spain and Greece and 9-repeat unit in American white PCOS-women. Ethnicity was not included in the diagnostic criteria for polycystic ovary syndrome (PCOS).It is widely recognized that ethnic variations are likely to lead to the different manifestations ofPCOS (24).There is, therefore, a need for comparative studies throughout different ethnic groups to ascertain if the epidemiological variations observed represent a true ethnic difference in the PCOS phenotype and if there is an Iraqi phenotype for PCOS.

Table (6): Number and percentages of (AAAAT) repeats in control sub-groups depending on age categories.

Number of (AAAAT) repeats	n	Age categories			χ^2	*P*value
		<25 y (n=4)	25-35y (n=30)	>35y (n=24)		
3	17	2(11.80%)	9(52.90%)	6(35.30%)	1.04	0.59
5	36	2(5.60%)	18(50.00%)	16(44.40%)	0.51	0.77
6	1	0(0.00%)	1(100.00%)	0(0.00%)	0.95	0.62
7	2	0(0.00%)	2(100.00%)	0(0.00%)	1.93	0.38
8	2	0(0.00%)	0(0.00%)	2(100.00%)	2.93	0.23

Table (7): Number and percentages of (AAAAT) repeats in patient's sub-groups depending on age categories.

Number of (AAAAT) repeats	n	Age categories			χ^2	*P*value
		<25 y (n=33)	25-35y (n=30)	>35y (n=11)		
3	11	2(18.20%)	6(54.50%)	3(27.30%)	3.98	0.13
5	59	28(47.50%)	23(39.00%)	8(13.60%)	1.04	0.59
6	3	2(66.70%)	1(33.30%)	0(0.00%)	0.84	0.65
8	1	1(100.00%)	0(0.00%)	0(0.00%)	1.25	0.53

Correlation between genotypes and BMI groups

Number and percentages of (AAAAT) repeats in control and patient sub-groups based on BMI were demonstrated in Table (8) and (9) respectively .There was no significant difference ($p<0.05$) between percentage of the patients and controls regarding to BMI.

A case-and-control association study conducted by Wang et al. (13) Showed that polymorphism with 6 repeats is the most widespread allel of this polymorphism in the population of Han Chinese PCOS-women, although this allel is weakly associated with BMI in patients with PCOS.The differences in findings in the Iraqi population compared to other

ethnic studies warranted more analysis to decide whether CYP11A1 pentanucleotide polymorphism is a racial and ethnic variant.

Table (8): Number and percentages of (AAAAT) repeats in control sub-groups depending on BMI.

Number of (AAAAT) repeats	n	BMI			χ^2	*P*value
		Normal (n=20)	Overweight (n=25)	Obese (n=13)		
3	17	5(29.40%)	10(58.80%)	2(11.80%)	2.77	0.25
5	36	14(38.90%)	12(33.30%)	10(27.80%)	3.85	0.14
6	1	0(0.00%)	1(100.00%)	0(0.00%)	1.34	0.51
7	2	0(0.00%)	1(50.00%)	1(50.00%)	1.44	0.48
8	2	1(50.00%)	1(50.00%)	0(0.00%)	0.63	0.72

Table (9): Number and percentages of (AAAAT) repeats in patient's sub-groups depending on BMI.

Number of (AAAAT) repeats	n	BMI			χ^2	*P*value
		Normal (n=16)	Overweight (n=20)	Obese (n=38)		
3	11	3(27.30%)	3(27.30%)	5(45.50%)	0.27	0.87
5	59	10(16.90%)	16(27.10%)	33(55.90%)	4.12	0.12
6	3	2(66.70%)	1(33.30%)	0(0.00%)	4.58	0.10
8	1	1(100.00%)	0(0.00%)	0(0.00%)	3.67	0.15

Correlation between genotypes with hormones and lipids

Three repeats (3R) genotype for control and patients groups

The relationship between 3R genotype and parameter study in control and patient for hormonal and lipid profile showed in Table (10) and (11) respectively. The statistically significant differences were FSH, Prolactin and Testosterone level with P value of (0.002, 0.01and 0.005) mean levels respectively. While there was no significant differences with other parameters.

Our analysis of the (3R) polymorphisms in CYP11A1 promoter regions shows a significant association with FSH, Prolactin and T levels. An association with PCOS also is seen. The study shows that these polymorphisms are predisposing factors for increased levels of Prolactin and T and lower FSH levels in PCOS women relative to healthy groups.

Table (10): The hormonal profile of (AAAAT) repeat 3 in control and patients groups.

Parameter	Number of (AAAAT) repeats= 3		*P*value
	Control group (n=17)	Patients group (n=11)	
S.FSH (mIU/ml)	7.28±3.09	4.39±0.53	0.002
S.LH (mIU/ml)	4.64±1.39	5.00±1.63	0.79
S. Prolactin (ng/ml)	10.51±4.32	14.29±2.46	0.01
S.TT (ng/ml)	0.83±0.25	1.40±0.51	0.005

Table (11): The lipid profile of (AAAAT) repeat 3 in control and patients groups.

Parameter	Number of (AAAAT) repeats= 3		*P*value
	Control group (n=17)	Patients group (n=11)	
S.TC (mg/dl)	180.05±25.20	181.90±30.90	0.86
S.TG (mg/dl)	128.35±37.70	132.45±32.83	0.77
S.HDL (mg/dl)	41.88±4.91	44.09±4.25	0.23
S.LDL (mg/dl)	123.87±21.43	113.01±24.47	0.22
S.VLDL (mg/dl)	25.67±7.54	26.49±6.56	0.77

Five repeats (5R) genotype for control and patients groups

The relationship between 5R genotype and parameter study in control and patient for hormonal and lipid profile showed in Table (12) and (13) respectively. The statistically significant differences were FSH, Prolactin and Testosterone levels with P value of (0.006, 0.00 and 0.00) mean levels respectively. While there was no significant differences with other parameters.

When the frequency of different genotypes was compared between groups, 5 repeats were extremely high in patients with FSH, Prolactin and Testosterone levels.

The findings of the present study are shown the most distributed repeats observed in PCOS and controls groupswas 5 repeats (79.7 % vs.62.1 %). five repeats (5R) in the women can be considered as a general marker for PCOS susceptibility in Iraqi women.

We suggest that the generation of significant variations in the number of repeats in various populations may be due to different extrinsic and intrinsic influences. Hence,knowing the common repeat number related to various diseases in different populations couldhelp to understand the gene-environment interaction. Moreover,in order to realize the importance of this marker, cell-based studies with different repeat numbers in relation to expression should be performed to translate the information produced and to understand the susceptibility of different populations to hormone-related diseases.

Table (12): The hormonal profile of (AAAAT) repeat 5 in control and patients groups.

Parameter	Number of (AAAAT) repeats= 5		*P*value
	Control group (n=36)	Patients group (n=59)	
S.FSH (mIU/ml)	5.22±2.10	6.69±2.96	0.006
S.LH (mIU/ml)	5.54±2.66	7.03±3.00	0.24
S. Prolactin (ng/ml)	10.63±3.10	25.13±9.35	0.00
S.TT (ng/ml)	0.89±0.35	1.44±0.62	0.00

Table (13): The lipid profile of (AAAAT) repeat 5 in control and patients groups.

Parameter	Number of (AAAAT) repeats= 5		*P*value
	Control group (n=36)	Patients group (n=59)	
S.TC (mg/dl)	185.41±28.64	181.89±27.86	0.55
S.TG (mg/dl)	118.58±32.31	116.66±32.33	0.77
S.HDL (mg/dl)	45.00±4.86	44.45±4.91	0.60
S.LDL (mg/dl)	107.65±31.05	114.04±23.96	0.26
S.VLDL (mg/dl)	23.71±6.46	23.33±6.46	0.77

Six repeats (6R) genotype for control and patients groups

The relationship between 6R genotype and parameter study in control and patient for hormonal and lipid profile showed in Table (14) and (15) respectively. There was no significant differences with all parameters.

A large variation was shown In the distribution of (AAAAT)n repeat in different ethnic groups, including ours.Studies in the Caucasian and Asian populations identified an association of 4 and 6 repeat alleles corresponding to that of PCOS (12), another study (10) found an over-representation of 9 repeat alleles in PCOS patients.Our results showed very low distribution of 6 repeats in iraqi women and there was no association between PCOS and control groups regarding to hormones and lipid profile.

Table (14):The hormonal profile of (AAAAT) repeat 6 in control and patients groups.

Parameter	Number of (AAAAT) repeats= 6		*P*value
	Control group (n=1)	Patients group (n=3)	
S.FSH (mIU/ml)	5.70	4.33±1.72	0.56
S.LH (mIU/ml)	7.50	4.00±1.02	0.08
S. Prolactin (ng/ml)	12.00	24.86±4.50	0.13
S.TT (ng/ml)	0.90	1.02±0.30	0.75

Table (15): The lipid profile of (AAAAT) repeat 6 in control and patients groups.

Parameter	Number of (AAAAT) repeats= 6		*P*value
	Control group (n=1)	Patients group (n=3)	
S.TC (mg/dl)	189.00	185.00±30.51	0.92
S.TG (mg/dl)	116.00	132.66±61.97	0.83
S.HDL (mg/dl)	47.00	45.66±8.32	0.90
S.LDL (mg/dl)	96.80	109.73±34.50	0.77
S.VLDL (mg/dl)	23.20	26.53±3.10	0.45

Eight repeats (8R) genotype for control and patients groups

The relationship between 8R genotype and parameter study in control and patient for hormonal and lipid profile showed in Table (16) and (17) respectively. The statistically

significant difference was Prolactin level with P value of 0.02 mean levels. There was no significant differences with other parameters.

A study conducted by Reddy et al. (12) in South India recorded that fifteen different alleles ranging from 2–16 repeats were found in the study group and 8 repeat were the most common alleles observed in the controls.The existence of > 8 repeat alleles was widespread in patients.These findings are not in agreement with our research as well as with other studies mentioned above, the same reason also applies that race, gene-gene and gene-environmental interactions may play a key role in these variations in this multifactorial disease.

Table (16): The hormonal profile of (AAAAT) repeat 8 in control and patients groups.

Parameter	Number of (AAAAT) repeats= 8		*P*value
	Control group (n=2)	Patients group (n=1)	
S.FSH (mIU/ml)	4.90±1.27	6.60	0.47
S.LH (mIU/ml)	4.70±1.69	6.00	0.51
S. Prolactin (ng/ml)	10.20±3.39	24.00	0.02
S.TT (ng/ml)	0.46±0.23	0.60	0.71

Table (17): The lipid profile of (AAAAT) repeat 8 in control and patients groups.

Parameter	Number of (AAAAT) repeats= 8		*P*value
	Control group (n=2)	Patients group (n=1)	
S.TC (mg/dl)	171.50±33.23	190.00	0.61
S.TG (mg/dl)	130.00±34.85	98.00	0.51
S.HDL (mg/dl)	48.00±5.65	46.00	0.82
S.LDL (mg/dl)	116.80±18.95	114.40	0.89
S.VLDL (mg/dl)	26.00±2.82	19.60	0.31

Conclusion:

The findings of the present study are shown the most distributed pentanucleotide (AAAAT) repeats observed in PCOS groupwas 5 repeats. Five repeats (5R) in the women can be considered as a general marker for PCOS susceptibility in Iraqi women.Hormones included in this study (Prolactin, Testosterone) were significantly impacted in PCOS Iraqi women comparing with healthy women while (FSH, LH) eventhough it was higher in patients but didn't reach significant limits. There was no impact of this syndrome in lipid profile between the groups.

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