

Significance of CTL4 Gene Polymorphisms in Susceptibility to Polycystic Ovary Syndrome of Iraqi Women

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Abstract

Background: Menstrual cycle has need to a suitable interaction between ovary and hypothalamus PCOS (Polycystic Ovary Syndrome), is a common disordering in women hormone at a certain age. Objective: to study the cytotoxicity of T-lymphocyte antigen CTLA4 and its relation with PCOS patients through investigations on 85 women. **Materials and Methods:** The tag of SNPs in CTLA4 was selected as a potential function according to the protocols issued by 1000 Genomes Project to develop a resource on human genetic variation. The study was carried out in Kamal Al-Samarrai hospital, the investigation was carried out on 85 women (45 individuals with PCOS patients), the second group was the control (40 healthy individual). Biochemical and clinical hyper-androgenism, which are, acne, hirsutism, the value of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and a history of menstrual disorders. The tagging variants rs11571316, rs62182595, rs11571315, and rs733618 in CTLA4 gene were identified and detected as well. **Results:** the differences between control (healthy women) and patients with PCOS were compared in whichever genotypes or the distribution of allelic ($p > 0.001$) for rs11571316, rs62182595, rs11571315, and rs733618. Results also show the acne, hirsutism, FSHL, LH, and the insertion of testosterone in both PCOS and control groups. **Conclusions:** there is a genetic relationship between SNP of CTAL4 and PCOS, and it confirm the involvement of CTAL4 and genotype frequencies such as rs11571316, rs62182595, rs11571315, and rs733618 this correlation may regulate the activation of the T-lymphocyte, as well as their effect on the susceptibility to schistosomiasis.

Keywords: CTL4 gene polymorphisms, susceptibility, PCOS

Introduction

PCSO (Polycystic Ovary Syndrome), is a common disordering in women hormone at a certain age, by which women are loss their productivity due to reproductive age, at this age women suffers from infrequent menstrual period (oligomenorrhea), or they suffers from increasing in the level of androgen (hormone male), these symptoms can be diagnosed using a certain technique to find out the sequence of the genes in organism, this technique called single nucleotide polymorphisms (SNPs). (Turaki, A.A. *et al.*, 2017; Petersen, R.F. *et al.*, 2007 ; Mihm, M. *et al.*, 2011; Ahn, M-J. *et al.*, 2012 ; Abreu-Sánchez, A . *et al.*, 2020).

Recently, researcher used new methods to determine and monitor ATAD (digestion, aerobic, thermophilic, and autothermallity) via evaluating PCR and in some cases PCR-DGGE and PCR, which enable the researchers to follow the dynamics of microbes with respect to temperature. Researchers also consider Acne as one of the indicators in PCSO studies (Jing Su, *et al.*, 2018; Armour, M. *et al.*, 2019; Anna, V., *et al.*, 2013 Fernández-Martínez, E.*et al.*, 2019).

Studying the types of the gene must combine with studying the allele, and the difference between them is that genotype is a certain trait of constitution to the gene while the allele is a form of gene that is variant and usually located at chromosome (Jiang, S.Y.*et al.*, 2016; Cai, L. *et al.*, 2016).

Shahmoradi and his co-workers in 2014 mentioned that body shape is determined by the genetic information which has a great effect on muscles, lung capacity as well as the women hormones which cause the infrequent in menstrual period (Zhong, J. *et al.*, 2014).

The current study aimed to find the importance of CTL4 gene polymorphisms and the effect of the gene shape in the susceptibility to polycystic ovary syndrome in the Iraqi women.

2. Materials and Methods

2.1. Patients

A total of 85 individuals were collected, 40 healthy women (control), and 45 women with PCOS (patients) were recruited from Kamal Al-Samarrai hospital from October 2019 March 2020. The analysis was achieved and carried out according to Rotterdam consensus criteria. All engaged patients had two of the following: biochemical and clinical hyper-androgenism, which are, acne, and hirsutism. The value of FSH (follicle-stimulating hormone) was ranged from 3.5 to 12.5 mIU/ML, while the LH (luteinizing hormone) was ranged from 2.4 to 12.6 mIU/M. The testosterone value was ranged from 0.025 to 0.48) ng/ml. The 40 healthy women were examined at the same gynaecology clinic during the same period. The control group had no diabetes, hypertension, and disorder. Both groups do not suffer from diseases such as cardiovascular system, abnormality in thyroid.

2.2. SNP selection and genotyping analysis

The tag of SNPs in CTLA4 was selected as a potential function according to the protocols issued by 1000 Genomes Project to develop a resource on human genetic variation (<https://www.coriell.org/1/NHGRI/Collections> ; Rotterdam EA-SPCWGRevised, 2004).

The rest was carried out as depicted by Lei et al, 2005; Cai et al 2014, except the time was 6 hours for rs733618 SNP and was 7 hours for rs11571316, rs62182595 , rs11571315(Cai, L. *et al.*, 2013; Lei, C. *et al.*, 2005).

2.3. Clinical tests

All the measurements and the calculations were carried out according Li et al, 2017. The results of these tests were depicted in Table 1, and Figure 1). (Li S, *et al.*, 2017).

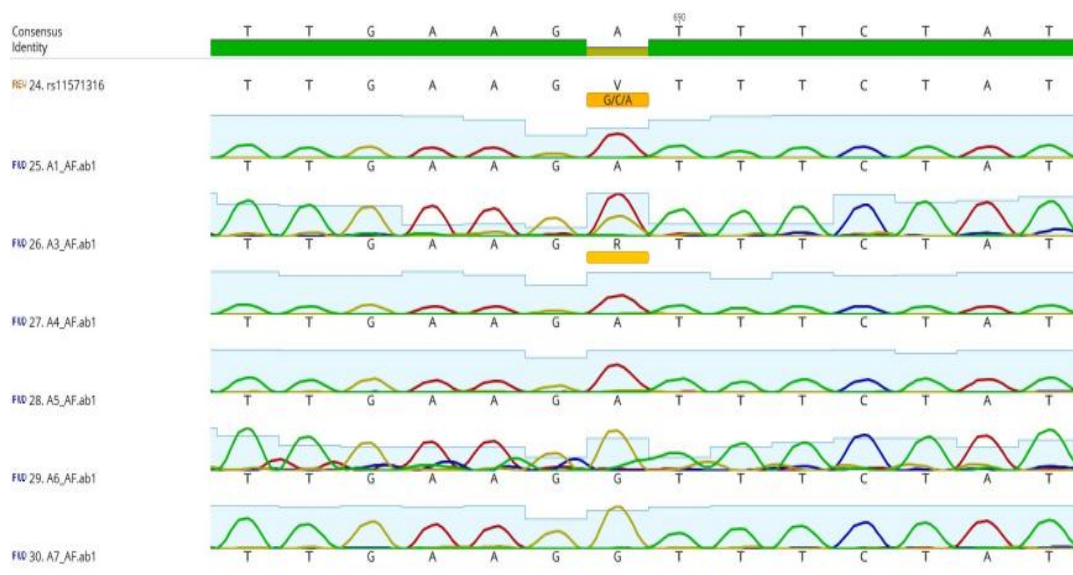


Figure 1: DNA sequence chromatogram of *CTLA4* gene SNP (rs11571316 G/C/A) showing three genotypes: AA (samples A1, A4 and A5), AG (sample A3) and GG (samples 6 and 7). In addition, the reference sequence (rs11571316) is also given.

Table 1: PCR-DGGE primers to the detected SNP

SNP Tm	Temp.	50 -30 Primers	Fragment length
rs231775	51 °C	CCTGAAAGGTTTTGCTCTA AGAAGACAGGGATGAAGAG	198 bp
rs733618	59 °C	CTAAGAGCATCCGCTTGCAC TTGGTGTGATGCACAGAAGC	186 bp

Statistical analysis

Baseline data of patients and control were given as either mean \pm standard deviation (SD) or number and percentage frequencies. Significant differences between means were assessed by Student t-test, while between frequencies by Pearson's Chi-squared test. Direct gene counting method was used to calculate allele frequencies of *CTLA4* gene SNPs, and departure from Hardy-Weinberg equilibrium which measured by Pearson's Chi-squared test. SNPs, and PCOS association was expressed in OR (Odd Ratio), CI (Confidence Interval), and when p value ≤ 0.05 it was considered as significant changes. The statistical package SPSS version 25.0 was used to carry out these analyses.

Results and Discussion

Figure 1 and table 1 indicates the DNA sequence of CTLA4 gene and the PCR-DGGE primers for each detected SNP respectively, these investigations were widely used by Turaki et al in 2017(Turaki, A.A. *et al.*,2017).

The baseline data that was collected for polycystic ovary syndrome for control and patients was listed in table 2.

Table 2: Baseline data of PCOS patients and control

Data*		PCOS cases (N = 45)	Control (N = 40)	p-value
Age (year)		29.6 ± 5.2	30.0 ± 6.8	0.178
BMI (kg/m ²)		27.3 ± 3.8	25.2 ± 3.0	0.005
Acne	Yes	29 (64.4%)	8 (20.0)	< 0.001
	No	16 (35.6%)	32 (80)	
Hirsutism	Yes	33 (73.3%)	3 (7.5)	< 0.001
	No	12 (26.7%)	37(92.5)	
Irregular cycle	Yes	31 (68.9%)	0 (0.0)	< 0.001
	No	14 (31.1%)	40 (100.0)	
FSH (mIU/ml)		3.2 ± 1.7	8.4 ± 2.2	< 0.001
LH ((mIU/ml))		17.9 ± 5.6	8.7 ± 2.3	< 0.001
Testosterone (ng/ml)		1.8 ± 2.1	0.3 ± 0.1	< 0.001

Table 2 indicates that patients suffers from polycystic ovary syndrome may have suffers from acne (64% patients, 20% control), same thing with hirsutism (73.3% for patients and 7.5% in control). As it is clear from table 2, the percent of PCSO patients that suffers from irregular cycle are 68.9% while the percent in control was 0.0%. In PSCO patients, the decreases and increases in the levels of FSH and LH respectively, as well as the high level of testosterone this will affect the insertion of estrogens and thyroid hormones. The elevated levels of testosterone usually pointed to hyperandrogenism which eventually cause many side effects such as hirsutism (increasing in the coarse hair growth).(Li, S. *et al.*, 2017; Shahmoradi, S. *et al.*, 2014; Shi, J . *et al.*, 2020; Laisk, T. *et al.*, 2018; Li, T.*et al.*, 2015; Wetterslev, J. *et al.*, 2017; Becker M, *et al.*, 2017).

Allele frequencies for rs11571316 SNP of *CTLA4* gene in PSCO patients were depicted in table 3.

Table 3: Allele and genotype frequencies of rs11571316 SNP of *CTLA4* gene in polycystic ovary syndrome patients and control

rs11571316 SNP Allele / Genotype	PCOS cases (N = 45)		Control (N = 40)		OR	95% CI	p-value
	N	%	N	%			
G	34	37.8	61	76.2	Reference		
A	56	62.2	19	23.8	5.29	2.72 - 10.28	< 0.001
GG	8	17.8	25	62.5	Reference		
GA	18	40.0	11	27.5	5.11	1.71 - 15.27	0.003
AA	19	42.2	4	10.0	14.84	3.89 - 56.71	< 0.001
HWE p-value	0.362		0.128				

rs11571316 is a SNP of the *CTLA4* gene can influence the susceptibility of PSCO patients and cause schistosomiasis infection, while the existence of the 'G' allele can considerably cause the increases in the cytotoxicity of T-lymphocyte-associated protein 4 (*CTLA4*) gene, also patients with PSCO, usually shows decreases in GA genotype due to schistosomiasis with an interesting difference ($p=0.003$). The AA shows a significant difference between control and patients with ($p<0.001$), these results matched with the finding of Jing et al, 2018(Jing, Su. *et al.*, 2018).

The comparison between control and PSCO for Allele and genotype frequencies of rs62182595 SNP shown in table 4

Table 4: Allele and genotype frequencies of rs62182595 SNP of *CTLA4* gene in polycystic ovary syndrome patients and control

rs62182595 SNP Allele / Genotype	PCOS cases (N = 45)		Control (N = 40)		OR	95% CI	p-value
	N	%	N	%			
G	74	82.2	74	92.5	Reference		
A	16	17.8	6	7.5	2.67	0.99 - 7.15	0.066
GG	29	64.4	34	85.0	Reference		
GA	16	35.6	6	15.0	3.13	1.08 – 9.03	0.035
AA	ND		ND				
HWE p-value	0.146		0.608				

ND: Not detected.

From table 4, it was clear that there was no significance differences in A and GA with p-value 0.066, and 0.035 respectively.

Table 5, demonstrate the frequencies of rs11571315 SNP of *CTLA4* gene in PSCO patients and compare it with control.

Table 5: Frequencies of rs11571315 SNP of *CTLA4* gene in polycystic ovary syndrome patients and control

rs11571315 SNP Allele / Genotype	PCOS cases (N = 45)		Control (N = 40)		OR	95% CI	p-value
	N	%	N	%			
<i>C</i>	61	67.8	80.0	100	Reference		
<i>T</i>	29	32.2	0	0.0	77.23	4.71 - 1266.76	< 0.001
<i>CC</i>	20	44.4	40	100.0	Reference		
<i>CT</i>	21	46.7	ND		71.08	4.26 - 1184.90	< 0.001
<i>TT</i>	4	8.9	ND		8.78	0.47 - 162.50	0.119
HWE p-value	0.646		1.000				

ND: Not detected.

The p-value in table 5 for T, and CT are 0.001 for both of them which indicates a noticeable difference between patients and control, while for TT the p-value is 0.119 which reflect a negligible relationship between it and the PCOS (Becker, M. *et al.*, 2017).

In table 6 it is clear the noticeable association between C, TC, by which the p-value for each of them is <0.001, while the CC does not shows any correlation with PCOS.

Table 6: Allele and genotype frequencies of rs733618 SNP of *CTLA4* gene in polycystic ovary syndrome patients and control

rs733618 SNP Allele / Genotype	PCOS cases (N = 45)		Control (N = 40)		OR	95% CI	p-value
	N	%	N	%			
<i>T</i>	64	71.1	75	93.8	Reference		
<i>C</i>	26	28.9	5	6.2	6.09	2.23 -- 16.69	< 0.001
<i>TT</i>	20	44.4	35	87.5	Reference		
<i>TC</i>	24	53.3	5	12.5	8.00	2.69 - 23.83	< 0.001
<i>CC</i>	1	2.2	ND		2.73	0.11 - 66.33	1.000
HWE p-value	0.046		0.6733				

Conclusions

1. Findings indicate a genetic relationship between SNP of *CTLA4* and PCOS.
2. Findings in this research confirm the involvement of *CTLA4* and genotype frequencies such as rs11571316, rs62182595, rs11571315, and rs733618 this correlation may regulate the activation of the T-lymphocyte as well as their effect on the susceptibility to schistosomiasis infection in addition to that it may cause increases in the expression of

the cytotoxicity of the T-lymphocyte-associated protein 4 (CTLA4) gene, and increasing in the body mass.

3. The current research is novel analysis regarding the involvement of PCOS with 4 functional SNP tag in CTLA4 gene
4. Findings show that PCOS patients suffer from Acne, Hirsutism, FSHL, LH, and abnormality in insertion of testosterone and other symptoms.

Ethical statement

The current study was approved by Research Ethical Committee, College of Science, Mustansiriyah University, Baghdad, Iraq.

Source of Funding

Self fund.

Conflict of Interest

No conflict of interest

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