INSR and PLIN Polymorphism in Women with Poly Cystic Ovary Syndrome (PCOS) and Its Correlation with Insulin Resistance.

Mustafa Abd- almajeed Abd-alkareem, Biology Department, College of Science, Tikrit University.
Email: Mustafa.majeed4444@gmail.com
Mobile: +9647726066925
Hadeel Abdulhadi Omear, Biology Department, College of Science, Tikrit University.
Email : hadeel.omear@tu.edu.iq
Mobile: +9647726066925
Correspondence author: Dr. Hadeel Abdulhadi Omear, Biology Department, College of Science, Tikrit University.
Email : hadeel.omear@tu.edu.iq
Mobile: +9647726066925
Received: 20/4/2020 Accepted: 26/6/2020 Published: 1st August, 2020
DOI: https://doi.org/10.32441/aajms.3.3.6

Abstract

Background: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders that affect women of childbearing age. Several genes, in addition to environmental factors, are involved in PCOS events.

Aim: To clarify the association between INSR and PLIN genes and PCOS development.

Materials and methods: SNPs in INSR and PLIN was determined using RFLP- PCR and ARMS.

Results: There was a significant increase in BMI, Triglyceride, VLDL and Sugar (29.22, 154.66, 30.01, 109.83, respectively) in the PCOS group as compared to the control group (25.19, 121.13, 25.2, 93..4). The frequency of the C allele for *INSR* in the PCOS group was 0.46, while it was 0.3 in control group.. TT genotype was associated with increased BMI, Triglyceride, HDL, VLDL and Sugar. The frequency of GG in the PCOS group was 0.3 compared to 0.17 in the control group. The increase in BMI, Triglyceride, VLDL and Sugar in PCOS

group was associated with the CC genotype of the *PLIN* gene. However, the menarche age was not significantly different between PCOS and control groups. This study indicated association of SNPs in *INSR* and *PLIN* with insulin resistance and lipid metabolism disorders in women with PCOS

Conclusion: An association of C/T polymorphism at Exon 17 of INSR with PCOS in women was observed. This indicates that the genotype CT may be a risk factor for developing PCOS. As for the PLIN gene, it was found that the group of healthy women who have the genotype CG (heterozygous asymmetric) is the genotype that is not responsible for the occurrence of the disease when compared with the group of women who have the genotype CC (homozygous symmetry).

K**eywords: Polycystic ovary syndrome, RFLP-PCR, ARMS-PCR, PCOS, INSR, PLIN.**

Introduction

Polycystic ovary syndrome is one of the most common endocrine disorders that affect women of childbearing age. It also exhibits male characteristics such as acne, hirsutism, as well as insulin resistance, obesity, type 2 diabetes and high cholesterol levels. [1]. There is growing evidence to support the syndrome as hereditary pathological condition [2,3].

Women with PCOS have metabolic disorders and the risk of central obesity, hypertension, hyperglycemia, hyperinsulinemia, low high-density lipoprotein and high serum triglycerides. Type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) are also evidence that suggest insulin resistance is a basic physiological disease associated with polycystic ovary syndrome.[4]. Type 2 diabetes was detected by insulin resistance in tissues. Insulin-dependent thus decreasing insulin secretion from beta cells in the pancreas [5,6]. The insulin receptor is a Heterotetrameric glycoprotein, a member of the Tyrosine kinase family consisting of two units α and beta linked by a disulfide bond encoded by the *INSR* gene, which is located on the short arm of chromosome 19 at region 13.2 (19q13).[2]. This gene is composed of 22 exons and more than 120 kilo base pairs [7]. The encoding area between exon (17-21) encodes for the receptor protein tyrosine kinase and is essential for the transduce of the insulin signal, automatic phosphoration of tyrosine residue [8,9].

Several types of polymorphisms have been identified within the encoded and non-coding region of the *INSR* gene for patients with PCOS syndrome. Most of these mutations are silent and the highest frequency of these single nucleotide polymorphism (SNPs) is located on the Exon17 of the *INSR* gene [10], where these mutations affect the phosphorylation of Tyrosine as well as signal transduction to insulin [11]. It has been suggested that the defect is in the beta chain. Among the exon SNPs 17 on the *INSR* gene, C / T at the His1058 site in the tyrosine kinase protein, which has an ATP binding site for insulin receptors, has been linked to the development of PCOS probably through the effects on spontaneous phosphorylation and INSR function in some women with the syndrome. [7,10,11].

Genes involved in type 2 diabetes are insulin resistance (PLS), a gene (rs4578621) that encodes Perilipin, one of the phosphorylated proteins that cover intracellular lipid droplets [12]. High Perilipin leads to lipolysis and increased fatty acids and Perilipin is necessary for controlling the level and transport of triglycerides in the blood, triglycerols [13]. This gene is located in humans on the long arm of chromosome 15 at site 26.1 (15q26.1) (NCBI). This site is already close to the site of obesity, diabetes and triglycerides. This protein is the target of protein kinase enzymes (PKA), no phosphorylated PLIN which can act as an obstacle to the hormone sensitive lipase (HSL) that is involved in the decomposition of triglycerides (TAGs) into intracellular lipid droplets [14,15]. In addition, phosphorylation of PLIN may stimulate the function of HSL [16]. This function may also play a role in causing obesity and fat metabolism within the body.

Several studies have shown that the level of expression of PLIN is associated with obesity. In 2003, a study conducted by San Millan et al [17] revealed that obese individuals have a lower level of PLIN compared to normal individuals and slimming. In another study, the level of PLIN was high and the concentration of mRNA in obese individuals [18], while the study conducted by Seeley and Woods [19] in 2016 showed that the *PLIN* gene is linked to obesity and related diseases. Thus this study was conducted to clarify the association between INSR and PLIN genes polymorphism and development of PCOS.

Materials and methods

Study population and sample collection

Women with PCOS were recruited from those attending Gynecology and Obstetrics Clinic in Samara General Hospital for the period from 1st July to 1st December 2018. Blood samples were collected from 70 women with polycystic ovary syndrome and 30 apparently healthy women as matched control for laboratory investigation. Their age range was 16 to 46 years.

The demographic and clinical data gathered using a predesigned questionnaire. Each blood sample was divided in to two parts, the 1st one ml was placed in EDTA test tubes and to be used later for DNA extraction. The 2nd part of the sample used for separation of serum and determination of lipid profile and blood sugar. Lipid profile and blood sugar were determined using Biolabo kits, France. The study protocol was approved by the Ethical Committee of College of Science, Tikrit University. Verbal informed consent was taken from each women before their enrolment in the study.

Molecular tests

The molecular test was performed in Molecular Biology Laboratory, College of Science, Tikrit University. DNA extracted from white blood cells preserved in EDTA tubes using the previously reported extraction method [20]. Polymorphisms are detected using RFLP- PCR and ARMS. The Exon 17 polymorphism of the *INSR* gene was detected using PCR techniques and using aprimex kit from Bioneer Korea. The primers used in the test are shown in Table 1.

Table.1. Sequence of primers used to detect*(INSR, PLIN)* polymorphisms

Gene	Sequence	Method
INSR	**F-5′CCAAGGATGCTGTGTAG ATAAG-3′** **R-5′ TCAGGAAAGCCAG CCCATGTC.-3′**	**RFLP-PCR**
PLIN	**R-wild- 5′TGGACATCTCACTGTATT GCTC-3′** **R-Mutant-5′ TGGACATCTCACTGTATTGCTG-3′** **F-common-5′ AAATGCAGGTAGCCATAAGA-3′**	**ARMS**

Following the program described previously [21], the 317bp PCR product was cut by (10 unit) *Pmll* (Biolab, England) for 4 hours and at 37 C^0. The digested DNA electrophoresis performed over the 2% agarose gel and then detected using UV-trans-illuminator. The single band 317 bp mean TT homozygous genotype. The three bands (317, 274, 43) bp mean the Heterozygous CT genotype. The two bands (274, 43) bp mean the Homozygous CC Genotype, Fig. 1.

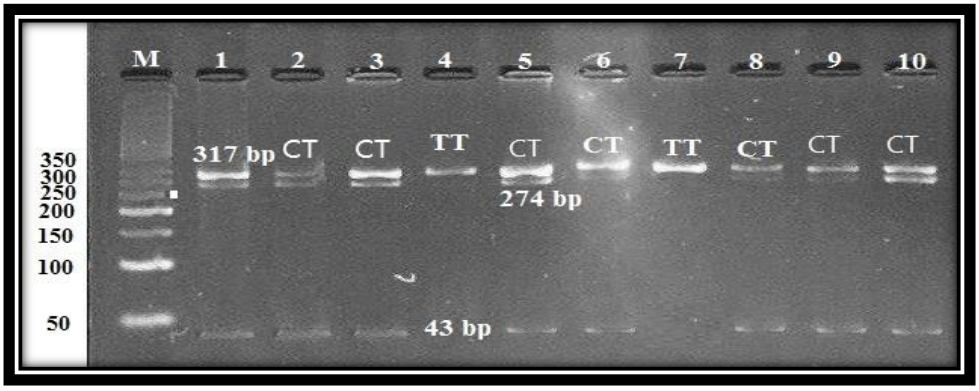

Fig.1.Restriction fragment length polymorphism analysis of the C/T polymorphism of exon 17 in the INSR gene. Agarose gel (2%) electrophoresis after *PmlI* digestion of the PCR

M: 50 bp DNA ladder; Samples 1,2, 3,5,6,8,9,10: CT genotype (317-bp, 274-bp and 43-bp); Samples 4 and 7:TT genotype (317-bp)

(rs4578621) SNPs was detected by applying ARMS-PCR technique using the primers listed in Table (1) and Primexkit and following the program instructions [22]. Two reactions were used for each sample, each reaction in a separate test tube. In the first tube, the F_{common} primer was used with $R_{Wild,}$ while in the second tube, F_{Common} and R_{Mutant} were used. Electrophoresis then performed on the agarose gel and imaged in the E-graph device. If the band appears in the first tube only the genotype is CC, if a band appears in the two tubes means that the genotype is CG, but if a band appears in the second tube only, the genotype is a homozygous GG, Fig 2.

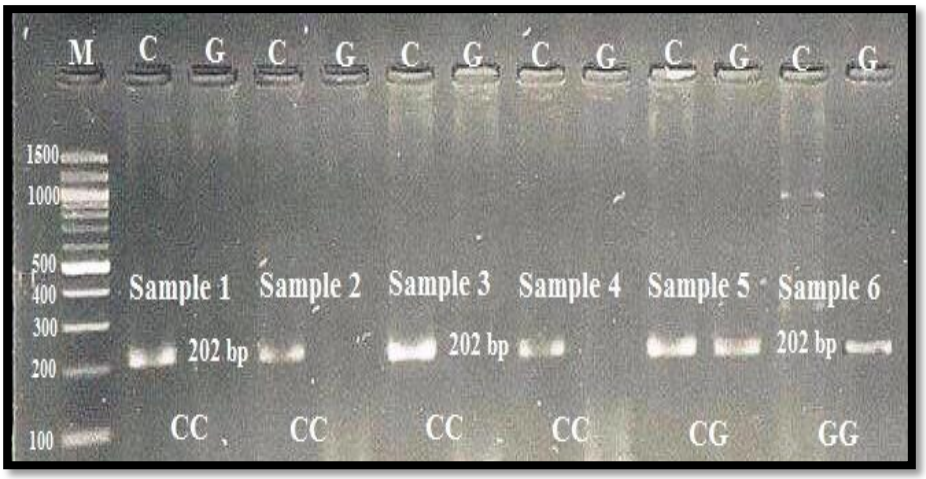

Fig. 2. Electrophoresis on 2% concentration of agarose gel using ARMS-PCR for PLIN gene with 100bp volumetric index for infected group

M: 100 bp DNA ladder; Samples 1,2, 3,4: CC genotype; Sample 5 :CG genotype; Sample 6: GG

Statistical analysis

The p and t value were calculated using SPSS version (21.0.). Alleles frequency of SNPs was calculated as described previously [23].

Results

Amenorrhoea was the common symptom (77.14%) present in women with PCOS, followed by hirsutism (72.8%), Table 2. Additionally, there was a significant differences in BMI value and mean serum concentration of Triglyceride, LDL, HDL, VLDL and Sugar in patients as compared to control group. However, there was no significant difference in the age of menarche and mean serum cholesterol between the two groups, Table 3 and Fig 1.

Detected polymorphisms in the two studied genes are shown in Table.4 and 5. For INSR gene, there was a significant differences (X^2 =8.43; P =0.015) in CC, CT, and TT genes between women with PCOS and control group. Additionally, the frequency rate of allele C was 0.46 in women with PCOS and 0.3 in control group and the difference was statistically significant (X2= 5.433; P=0.02). In

addition, the T allele frequency in patients was 0.54 and 0.70 in control group. This finding indicated that CT genotype was a risk factor for developing ovarian cyst syndrome and may suggest that T allele was protective factor for PCOS development, while C allele was a risk factor for the development of PCOS, Table 4.

Table 2. Phenotypic characteristics of women with PCOS

Phenotype	Percent
Hirsutism	**72.8**
Acne	**55.7**
Baldness	**32.8**
Infertility	**28.5**
Amenorrhea	**77.14**

Table 3. Comparison of variables in women with PCOS and control group.

Parameters	Mean ± SD		p-value
	PCOS	Control	
Menarche age	**14.39±0.01**	**14.33±0.02**	**0.37**
BMI	**29.22±0.45**	**25.19±1.04**	**0.00067**
Cholesterol	**179.67±22.32**	**190±52.07**	**0.193**
Triglyceride	**154.66±85.32**	**121.13±99.09**	**0.024**
LDL	**109.55±22.7**	**125.98±52.6**	**0.03**
HDL	**37.19±0.65**	**42.03±1.53**	**0.0007**
VLDL	**30.93±3.23**	**24.23±7.53**	**0.007**
Sugar	**109.83±20.75**	**93.4±48.1**	**0.025**

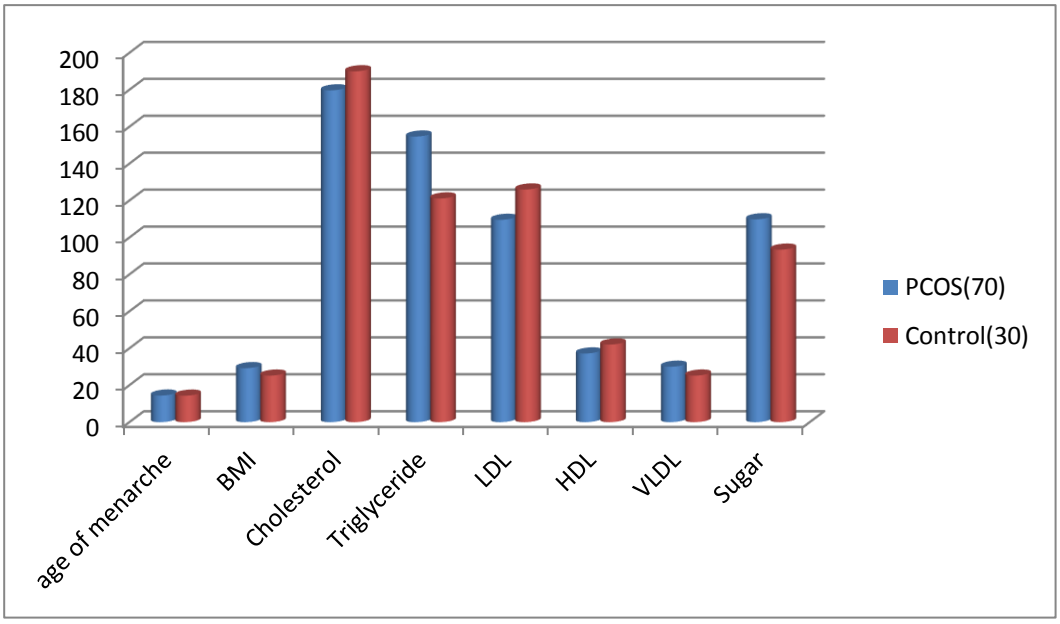

Fig. 3. Variables comparison between PCOS and control groups.

Table.4. Distribution of C/T Alleles of exon 17 of INSR in women with PCOS and control group.

Genotype	Group					
	Control			Patient		
	N	%	Freq.	N	%	Freq.
CC	2	6.6	(0.06)	8	11.4	(0.11)
CT	14	46.7	(0.47)	49	70.0	(0.70)
TT	14	40.7	(0.47)	13	18.6	(0.19)
Total	30	100%		70	100%	
X^2	Chi-Square = 8.430 ; P= 0.015					
Genotype	N (%)			N (%)		
C allele	0.30			0.46		
T allele	0.70			0.54		
X^2	Chi-Square = 5.433 ; P= 0.02					

For PLIN gene, there was a non significant differences (X^2 =2.8; P>0.05) in CC, CG, and GG genes between women with PCOS and control group. Additionally, the frequency rate of allele C was 0.50 in women with PCOS and 0.58 in control group and the difference was statistically not significant (X^2 =1.288; P>0.05). In addition, the G allele frequency in patients was 0.50 and 0.42 in control group. This finding suggest that GG genotype may be a risk factor for the development of ovarian cyst syndrome and may suggest that C allele may play as a protective factor for PCOS development, while G allele may be a risk factor for the development of PCOS. However, this finding need to be confirmed in a large scale study.

The analysis of C/G alleles of PLIN and C/T Alleles of exon 17 of INSR on the basis of age of menarche, BMI, sugar, and lipid profile strata not reveal any significant differences with the exception of BMI with INSR genotype, Table 6 and 7.

Table.5. Distribution of C/G Alleles of PLIN in women with PCOS and control group.

Genotype	Groups					
	Control			Patient		
	N	%	Freq.	N	%	Freq.
CC	10	33.3	(0.33)	25	35.7	(0.36)
CG	15	50.0	(0.50)	24	34.3	(0.34)
GG	5	16.7	(0.17)	21	30.0	(0.30)
Total	30	100%		70	100%	
X^2	Chi-Square = 2.800; P=0.247					
Genotype	N (%)			N (%)		
C allele	0.58			0.5		
G allele	0.42			0.5		
X^2	Chi-Square = 1.288; P=0.256					

Table.6. Correlation of *INSR* Genotypes with some biochemical parameters, BMI and Age of menarche.

Genotype parameters		Number	Mean ± SD	P value
Age of Menarche	CC	8	14.37±.51	0.917
	CT	49	14.40±.81	
	TT	13	14.30±.75	
	Total	70	14.38±.76	
BMI	CC	8	24.29±4.75	0.004
	CT	49	28.86±6.33	
	TT	13	33.58±5.77	
	Total	70	29.21±6.51	
Cholesterol	CC	8	171.62±24.30	0.607
	CT	49	182.40±37.23	
	TT	13	174.30±33.04	
	Total	70	179.67±35.10	
Triglyceride	CC	8	139.25±85.75	0.163
	CT	49	146.48±69.70	
	TT	13	194.92±125.02	
	Total	70	154.65±84.99	
HDL	CC	8	34.87±4.29	0.539
	CT	49	37.42±6.48	
	TT	13	37.69±6.34	
	Total	70	37.18±6.22	
VLDL	CC	8	27.85±17.15	0.618
	CT	49	29.30±13.94	
	TT	13	33.98±24.29	
	Total	70	30.00±16.48	
LDL	CC	8	112.27±19.01	0.469
	CT	49	112.16±37.45	
	TT	13	98.01±43.89	
	Total	70	109.54±37.12	
Sugar	CC	8	88.37±15.43	.637
	CT	49	105.32±51.35	
	TT	13	104.61±40.63	
	Total	70	103.25±46.634	

Table.7. Correlation of *PLIN* Genotypes, BMI, biochemical, and Age of Menarche parameters.

Genotype parameters		N	Mean ± SD	P value
Age of Menarche	CC	25	14.36±.81	
	CG	24	14.37±.82	
	GG	21	14.42±.67	
	Total	70	14.38±.76	0.953
BMI	CC	25	29.48±7.79	
	CG	24	30.89±5.36	
	GG	21	27.24±5.53	
	Total	70	29.29±6.46	0.165
Cholesterol	CC	25	178.2±35.42	
	CG	24	172.25±25.47	
	GG	21	190.61±42.45	
	Total	70	179.90±35.09	0.208
Triglyceride	CC	25	175.96±104.30	
	CG	24	138.16±76.79	
	GG	21	150.71±65.60	
	Total	70	155.42±85.20	0.290
HDL	CC	25	35.40±5.20	
	CG	24	37.91±7.24	
	GG	21	38.0476±6.24	
	Total	70	37.05±6.30	0.264
VLDL	CC	25	32.60±20.09	
	CG	24	27.6333±15.35	
	GG	21	30.1429±13.12	
	Total	70	30.16±16.53	0.581
LDL	CC	25	106.22±39.23	
	CG	24	104.28±29.40	
	GG	21	118.91±41.93	
	Total	70	109.36±37.06	0.369
Sugar	CC	25	119.28±66.73	
	CG	24	90.16±26.07	
	GG	21	100.66±28.90	
	Total	70	103.71±46.60	0.085

Discussion

PCOS is one of the most widely studied endocrine diseases in women. Many patients with PCOS are more likely to develop T2DM and have symptoms such as glucose intolerance and insulin resistance [24]. Lipid metabolism disorders, central obesity, hypertension and high triglyceride level [25] were associated with PCOS. The current study shows a significant difference in BMI between the PCOS and the control group. It was also observed that there was a significant difference in triglycerides, VLDL, HDL, LDL and blood sugar levels in PCOS group compared with the control group. No difference was observed in the mean age of menarche. Cholesterol, LDL and HDL were significantly lower in the PCOS group as compared to the controls. Fat metabolism is one of the most common disorders in PCOS patients with an incidence of more than 70% [26]. It was suggested that genes related to insulin action and lipid metabolism with a particular focus on the *INSR* and *PLIN* gene may act as a cause of PCOS. In other words women with certain gene focus are more susceptible for the development of PCOS than those without such genes.

The *INSR* receptor gene is composed of 22 exons located on a chromosome 19 extending over 120 kbp. Several single nucleotide polymorphisms of the *INSR* gene have been detected, the most important being the expression region exon 17 [27]. This region encodes the tyrosine kinase protein for insulin receptors and is therefore associated with severe insulin resistance and hyperinsulinism and is therefore highly associated with PCOS [28,29]. The present study findings reveals an association between a single nucleotide polymorphism in exon 17 of the *INSR* gene and PCOS development.

The frequencies of the CC, CT and TT genotypes were significantly different between PCOS patients and the control group. Interestingly, the TT genotype was higher in control women than in patients while the CC genotype was higher in women with PCOS. This finding indicated that TT genotype was a protective gene while CC genotype was a risk factor for the development of PCOS. Moreover, obesity was associated with the genotype (TT) compared to the other genotype, the mean of BMI in women with TT type was

higher and a significantly different in their frequency from that in women with CT and CC genotype [30].

The polymorphism of 1085 C / T in the encoded segment of the tyrosine kinase on the *INSR* gene indicated that women with the syndrome may be overweight or thin [10,29]. This study proved that women with PCOS were overweight and had higher BMI values than control group. This finding suggest that there was a clear effect of C / T polymorphism in exon 17 of the *INSR* gene on physiological and biochemical variables in women with PCOS.

Women with genotype TT had higher mean level of glucose as compared to those with other genotypes. In other words there is insulin resistance predominance. In cases where mutations occur in exons 17-21, the region that encodes tyrosine kinase for insulin receptors is highly resistant to insulin and hyperinsulinemia [31]. Excessive phosphorylation of serine residues from *INSR* and signal transfer molecules is causing insulin resistance. This molecular imbalance reduces the activity of tyrosine kinase in INSR, thereby reducing the signal transmission pathway [31,32]. Studies of *INSR* function in some women with PCOS have found that changes in auto-phosphorylation that may be secondary to tyrosine kinase polymorphisms selectively affect metabolic pathways rather than dilution pathways in conventional insulin-targeted tissues and ovaries [33]. Insulin stimulates obesity by affecting the brain causing hunger. Recent evidence suggests that the brain processes information from fatty tissue signals such as insulin, which are spread in proportion to body fat mass, and integrates this input with nutrient signals such as fatty acids [34,35]. In response, the brain sends signals to control nutritional behavior and metabolism of essential substances in ways that promote balance in both energy stores and fuel metabolism, the liver works by making fat by converting extra calories into fat, and on fat cells in the muscle belly to be filled with fat by reducing mitochondrial function [36,37].

In the *PLIN* gene (rs4578621), the results showed that the frequency of homozygous mutant patterns (GG) was higher in women

with the polycystic ovary syndrome (0.3) compared to the control group (0.17). The frequency of alleles C in patients was (0.5) while it was (0.58) in the control group, while alleles G was with frequency of (0.5) and (0.42) in patients and control groups respectively. From the equilibrium of the society and the stability of the allele frequency, the mutant allele frequency was higher in patients than in the control group [23]. This variation in genes and alleles may explain the prevalence and incidence rates differences between different social groups.

Women with PCOS who show INSR polymorphism were with significant lower BMI mean value in those with CC genotype as compared to those with CT and TT genotypes. In addition, serum cholesterol, triglycerides, VLDL, HDL and blood sugar were lower in CC genotype as compared to CT and TT genotypes. However, the differences not reach a significant levels and thus warranted the need for conduction of large scale study to confirm or exclude this finding.

The present study data showed that women with polycystic ovary syndrome carrying C alleles had a higher value of BMI than other alleles in women with PLIN polymorphism. Additionally, genotypes (CG and CC) showed a higher mean values of BMI than the genotype (GG). Blood glucose mean serum value was higher in women with CC genotypes as compared to those with CG and GG genotypes. PLIN protein is an important phosphorylated proteins in the process of controlling the level and transport of triglycerides [14]. This may play a role in the events of obesity and obstruction of fat metabolism within the body as previous studies have shown that individuals who are obese and related diseases they have a lower level of PLIN protein [16,19]. At the same time, the age of a menarche showed no difference between the three genotypes, while HDL and LDL cholesterol concentration was low in CC genotypes compared to other genotypes.

This study is the first research on the relationship of the PLIN gene in Iraqi women with PCOS. Global previous studies had reported fewer links between the PLIN gene and PCOS. This is explained the current study finding concerning the genetic variation in the PLIN gene. It was not associated with the risk of PCOS. However, the

present study sample size is small and this warranted the need for performance in a large scale study.

In conclusion, an association of C/T polymorphism at Exon 17 of INSR with PCOS in women was observed. This indicates that the genotype CT may be a risk factor for developing PCOS. For the PLIN gene, it was found that the group of healthy women who have the genotype CG (heterozygous asymmetric) is the genotype that is not responsible for the occurrence of the disease when compared with the group of women who have the genotype CC (homozygous symmetry).

References

1. Ding T, Hardiman PJ, Petersen I, Wang FF, Qu F, Baio G. The prevalence of polycystic ovary syndrome in reproductive-aged women of different ethnicity: a systematic review and meta-analysis. Oncotarget. 2017;8(56):96351–58.
2. Tehrani RF, Behboudi-Gandevani S. Polycystic ovary syndrome . INTECH Open Science, Chapter (4). Cotemporary Gynecologic Practice: 81. 2015.
3. Bozdag G, Mumusoglu S, Zengin D, Karabulut E, Yildiz BO. The prevalence and phenotypic features of polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod.2016;31:2841–55.
4. Santoro N, Eisenberg E, Trussell JC, Craig LB, Gracia C, Huang H, et al. Fertility-related quality of life from two RCT cohorts with infertility: unexplained infertility and polycystic ovary syndrome. Hum Reprod 2016;31: 2268–79.
5. Anwar S, Shikalgar N. Prevention of type 2 diabetes mellitus in polycystic ovary syndrome: A review. Diabetes and Metabolic Syndrome: Clin Res Rev 2017;11:913- 917.
6. Hallberg SJ, Mckenzine AL, Willamms PT, Bhanpuri NH, Peters NL, Campbell WW, et al. Effectiveness and Safety of a Novel Care Model for the Management of Type 2 Diabetes at 1 Year : An Open- Lael, Non-Randomized, Controlled Study.Diabetes Ther 2018;9:583-612.
7. Zhu JL, Luo XM, QianYQ, Jin FH, Uang HF. A novel SNP at exon 17 of INSR is associated with decreased insulin sensitivity in Chinese women with PCOS. Molecular Human Reproduction 2006; . 12:151–155.
8. Ward CW, Lawrence MC, Streltsov VA, Adams TE, McKeon NM. The insulin and EGF receptor structures: new insights into

ligand–induced receptor activation. Trends in Biochemical Science. 2007; 32(3):129– 137.
9. Li M, Youngren JF, Dunaif A, Goldfine ID, Maddux BA, Zhang BB, et al. Decreased insulin receptor (IR) autophosphorylation in fibroblasts from patients with PCOS: effects of serine kinase inhibitors and IR activators. J Clin Endocrinol Metab 2002; 87: 4088-4093.
10. San Millán JL, Cortón M, Villuendas G, Sancho J, Peral B, Escobar-Morreale HF. Association of polycystic ovary syndrome with genomic variants related to insulin resistance, type 2 diabetes mellitus, and obesity. J Clin Endocrinol Metab 2004; 89: 2640-2646.
11. Diamanti-Kandarakis E, Papavassiliou AG. Molecular mechanisms of insulin resistance in polycystic ovary syndrome. Trends Mol Med 2006; 12: 324-332.
12. Sohn JH, Lee YK, Han JS, Jeon YG, Kim JI, Choe SS, Kim SJ, Yoo HJ, Kim JB. Perilipin 1 (Plin1) deficiency promotes inflammatory responses in lean adipose tissue through lipid dysregulation. J Biol Chem. 2018;293(36):13974-13988..
13. Tucci S, Futterweit W, Concepcion ES, Greenberg DA, Villanueva R, Davies TF, et al. Evidence for association of polycystic ovary syndrome in caucasian women with a marker at the insulin receptor gene locus. J Clin Endocrinol Metab 2001; 86: 446-449.
14. Bialesova L, Kulyté ***A***, Petrus P, Sinha I, Laurencikiene J, Zhao C, et al. Epigenetic Regulation of PLIN 1 in Obese Women and its Relation to Lipolysis. Sci. Rep.2017; **7**: 10-152.
15. Lee EJ, Yoo KJ, Kim SJ, Lee SH, Choi DH, Cha KU, et al. Relationship between the C/T single nucleotide polymorphysim in Exon 17 of the Insulin Receptor Gene and Polycystic Ovary Syndrome. J Biol Sci .2005; 5: 832-836.
16. Spritzer PM. Polycystic ovary syndrome: reviewing diagnosis and management of metabolic disturbances. Arq Bras Endocrinol Metabol 2014;58(2):182–187.
17. San Millán JL, Cortón M, Villuendas G, Sancho J, Peral B, Escobar-Morreale HF. Association of polycystic ovary syndrome with genomic variants related to insulin resistance, type 2 diabetes mellitus, and obesity. J Clin Endocrinol Metab 2004; 89: 2640-2646.

18. Musso C, Cochran E, Moran SA, Skarulis MC, Oral EA, Taylor S, et al. Clinical course of genetic diseases of the insulin receptor (type A and Rabson-Mendenhall syndromes): a 30-year prospective. Medicine (Baltimore) .2004; 83: 209-222.
19. Seeley RJ, Woods SC. Monitoring of stored and available fuel by the CNS: Implications for obesity. Nat Rev Neurosci .2003; 4: 901-909.
20. Nicolaiou K, Isoldi KK, Ramer NJ, Sarcona A. The Role of Perilipins in the Development of Obesity and Obesity-Related Diseases. Topics Clin Nutr 2016;31(3): 248 -256 .
21. Bartlett J, Stirling D. PCR protocols: Method in Molecular Biology. 2nd ed. Springer, 2003..
22. Mutib MT, Hamdan FB, Al-Salihi AR. INSR gene variation is associated with decreased insulin sensitivity in Iraqi women with PCOS. Iran J Reproduct Med;2014;12(7): 499–506.
23. Noorzehi SR, Galavi N, Ranjbar HR, Lotfian M. Association of Perilipin and insulin receptor substrate-1 genes polymorphism with lipid profiles, central obesity , and type2 diabetes in a sample of an Iranian population. Iran Red. Crescent Med J 2017;19(6): 1-8.
24. Snustad DP, Simmons MJ. Genetics. 6th ed. John Wiley & Sons, Inc., 2012.
25. Bagheri M, Abdi-Rad I, Hosseini-Jazani N, Zarrin R, Nanbakhsh F, Mohammadzaie N. An association study between INSR/NsiI (rs2059806) and INSR/PmlI (rs1799817) SNPs in women with polycystic ovary syndrome from west Azerbaijan province. Iran J Reprod Infer 2015;16: 109–112.
26. Daghestani MH. RS1799817 in INSR associates with susceptibility to polycystic ovary syndrome. J Med Biochem 2019;38: 1–11.
27. Panz VR, Ruff P, Joffe BI, Kedda MA, Seftel HC. SSCP analysis of the tyrosine kinase domain of the insulin receptor gene: polymorphisms detected in South African black and white subjects. Hum Genet 1996; 97: 438-440.
28. AI-Jefout M, Alnawaiseh N, Al-Qtaitat A. Insulin resistance and obesity among infertile women with different polycystic ovary syndrome phenotypes. Scientific Reports 2017;.7: 39-53.
29. Takasawa K, Tsuji-Hosokawa A, Takihima S, Wada Y, Nagasaki K, Dateki S, et al. Clinical characteristics of adolescent cases with

Type A insulin resistance syndrome caused by heterozygous mutations in the β-subunit of the insulin receptor (INSR) gene. J Diabetes 2019;11(1):46–54.
30. Lee EJ, Yoo KJ, Kim SJ, Lee SH, Choi DH, Cha KU, et al. Relationship between the C/T single nucleotide polymorphysim in Exon 17 of the Insulin Receptor Gene and Polycystic Ovary Syndrom. J Biol Sci. 2005;5: 832-836.
31. Chang AY, Lalia AZ, Jenkins GD, Dutta T, Carter RE, Singh RJ, et al. Combining a non targeted and targeted metabolomics approach to identify metabolic pathways significantly altered in polycystic ovary syndrome. Metabolism 2017;71:52–63.
32. Bremer AA, Miller WL. The serine phosphorylation hypothesis of polycystic ovary syndrome: a unifying mechanism for hyper androgenemia and insulin resistance. Fertil Steril, 2008;89(5):1039–1048.
33. Tsuji-Hosokawa A, Takasawa K, Nomura R, Miyakawa Y, Numakura C, Hijikata A, et al.. Molecular mechanisms of insulin resistance in 2 cases of primary insulin receptor defect-associated diseases. Pediatr Diabetes. 2017;18(9): 17–24.
34. Hubbard SR. Insulin receptor: both a prototypical and atypical receptor tyrosine kinase. Cold Spring Harb Perspect Bio. 2013;15 (3):46-89.
35. Pocai A, Lam TK, Obici S, Gutierrez-Juarez R, Muse ED, Arduini A, et al. Restoration of hypothalamic lipid sensing normalizes energy and glucose homeostasis in overfed rats. J Clin Invest 2006; 116: 1081-1091.
36. Murray RK, Granner DK, Mayes PA, Rodwell VW. Overview of Metabolism. In: Mayes P A and Bender D A (ed). Harper's Illustrated Biochemistry. 26th Ed. New York, Lange Medical Books/McGraw-Hill; 2003: 125-128.
37. Hosoe J, Kadowaki H, Miya F, Aizu K, Kawamura T, Miyata I, et al. Structural basis and genotype–phenotype correlations of INSR mutations causing severe insulin resistance. Diabetes. 2017;66: 2713–2723.