Are STK11 polymorphisms a predictor of the response to metformin in polycystic ovarian syndrome?

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Abstract. The aim of this study was to determine the frequencies of different genetic Serine threonine kinase 11 (*STK11*) variations in women with PCOS and to evaluate possible associations between the genetic polymorphisms of the STK11 gene and the response to metformin in women with polycystic ovary syndrome (PCOS). A prospective longitudinal cohort study of 57 women with PCOS was conducted. The anthropometric measurements, menstrual history, hirsutism, hair loss, acne, and biochemical parameters, in addition to gene testing for STK11 polymorphisms, were documented. Follow-up was arranged after 6 menstrual cycles whilst on oral metformin therapy, (850 mg, twice daily). Of the 120 women who were interviewed, 88 women fulfilled the inclusion criteria and 57 women completed the study. The mean age, weight, height, and BMI were 23.8 years old, 72.1 kg, 159 cm, and 28.6 kg/m^2 respectively. The frequencies of the genotypes of intron 1 of the STK11 gene were 26% CC, 44% CG, and 30% GG, and of intron 6 were 52% CC, 37% CT, and 11% TT genotype. There were statistically significant improvements following metformin therapy in menstrual frequency, blood loss, acne, ultrasound findings, and a decrease in BMI, acne, and hirsutism, but not in alopecia. Fasting insulin decreased significantly, but fasting blood sugar did not. There were no significant statistical differences in relation to the LH/FSH ratio, estradiol, FT, 17 OHP and length of menstruation. In relation to the SKT11 gene polymorphisms and metformin administration, there were variable and mostly insignificant differences in the results regarding menstrual regularity, amount of menstrual blood loss, acne, alopecia, ultrasound findings, and hirsutism score. There was a significant difference in relation to alopecia in the Intron 1 subgroups, and in relation to hirsutism score in the Intron 6 subgroups. It was concluded that polymorphisms in the STK11 gene in either Intron 1 or Intron 6 were not predictive of the response to metformin therapy at a dose of 850 mg twice daily, but may have some effect on alopecia and hirsutism.

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Introduction

Polycystic ovary syndrome (PCOS) is a well-known disorder that affects reproductive, metabolic, and cardiovascular health (1) that affects an estimated 5-10% of women (2). The reproductive effect of PCOS is characterized by oligomenorrhea or amenorrhea and chronic anovulation, hyperandrogenism, and a characteristic polycystic appearance of the ovaries (3). The etiology of PCOS is unknown. However, insulin resistance (IR) has been identified as a factor in the pathogenesis of PCOS, and it contributes to the metabolic and cardiovascular consequences of the syndrome (4).

Insulin resistance and hyperinsulinemia are associated with ovarian hyperandrogenism and a decrease in sex hormone-binding globulin (SHBG) leading to higher serum free testosterone levels (FT) (1). This hyperandrogenic state leads to the characteristic anovulation, menstrual irregularities, and hirsutism in PCOS (5). Metformin is a biguanide that is used in type 2 diabetes mellitus. A study by Dumitrescu *et al* (6) found that metformin decreases serum lipid, androgen, and plasma glucose levels as well as IR in PCOS. In contrast, Açbay and Gündoğdu (7) found that metformin does not decrease IR in PCOS, suggesting that the cellular mechanism of IR in PCOS differs from other common IR states, such as non-insulin-dependent diabetes mellitus and obesity (7). Several studies found that metformin increases the number of ovulatory cycles and improves hyperandrogenism, insulin sensitivity, menstrual regularity, metabolic disorders, and pregnancy rates in women with PCOS (6,8). It has been suggested that metformin induces a more favorable response in controlled ovulation stimulation. A meta-analysis by Lord *et al* (9) showed that metformin had a significant effect on ovulation compared to placebo, but demonstrated no effect on weight, BMI, or waist circumference.

The molecular mechanism underlying the action of metformin seems to be related to its phosphorylation of AMPK, which inhibits glucagon-stimulated glucose production and causes an increase in glucose absorption in muscles and hepatic cells (10). Pharmacogenomics addresses how genes affect an individual's response to certain drugs. This relatively new area combines pharmacology and genomics by mapping drug response phenotypes to individual genotypes. It has the potential for more advanced screening regimes for numerous diseases, improved vaccine regimens, advanced drug discovery, quicker drug approval processes, and a decrease in the overall cost of health care (11). Further benefits of pharmacogenomics include the use of more powerful medicines, accurate methods of determining appropriate dosages, and making clinical decisions based on genetics by choosing a drug or adjusting the dosage, tailored to a person's genetic makeup (11). Serine threonine kinase 11 (*STK11*/*LKB1*), which phosphorylates AMPK, has been reported to be linked to the effect of metformin (12-14). Goldenberg and Glueck (15) in 2008, suggested an association between metformin, ovulation, and polymorphisms of the *STK11* gene in PCOS. A randomized clinical trial by Legro *et al* (16) found that the *STK11* rs8111699 polymorphism was linked to the ovulatory response to metformin and that the C allele was associated with a significantly decreased chance of ovulation in women with PCOS that are treated with metformin (16).

The aims of this novel study was to determine the frequencies of different genetic *STK11* variations in women with PCOS and to investigate the effect of *STK11* polymorphisms on the response to metformin therapy in women with PCOS.

Materials and methods

Design and setting. This study was a prospective, longitudinal cohort study of women diagnosed with PCOS. The study group was selected from women who attended the Gynecology clinics of the Jordan University of Science and Technology in the north of Jordan, between January 2016 and July 2019. Participants were fully counseled, and informed consent was obtained from all participants. Eligible women received 6 months of oral metformin therapy. The initial dose was 850 mg orally once a day. The dose was increased to a maintenance dose of 1,700 mg/day (as two doses) with meals. The study was approved by the Institutional Review Board of the Jordan University of Science and Technology.

There were 57 participants with an age range of 18-42 years, and a median age of 22 years. For Intron 1, the number of patients in groups CC, CG and GG were 15, 25, and 17, respectively. The age range for group CC was 19-42 with a median age of 23.5 years. The age range of group CG was 18-33 with a median age of 21 years, and the age range of group GG was 18-37 with a median of 21 years.

Experimental cohort. Inclusion criteria were a confirmed diagnosis of PCOS according to the Rotterdam criteria (17). This is a combination of any two of the following three criteria: i) chronic oligomenorrhea (<8 menstrual periods annually), or amenorrhea; ii) biochemical or clinical androgen excess; and iii) polycystic ovaries on ultrasonography. Enrolled women with PCOS were confirmed to be non-menopausal, between 18-45 years of age, with a minimum of 3 years' post-menarcheal, not morbidly obese (BMI >35), with normal thyroid function and serum prolactin. The exclusion criteria were chronic medical illnesses including diabetes mellitus, abnormal kidney and liver function tests, current use of oral contraceptives or use of fertility drugs within 6 months of study, and ingestion of any investigational drug within 3 months prior to the study, including metformin.

Table I. Laboratory values in the patients with PCOS (n=57).

Parameter	Mean (SD)
Fasting blood glucose, mmol/l	5.1 (0.5)
Fasting insulin, mIU/l	13.5 (7)
FSH, IU/l	5.4 (1.2)
LH, IU/l	8.0 (4.3)
LH/FSH, IU/l	1.5 (0.7)
Estradiol, pg/ml	111.1 (55.7)
Thyroid stimulating hormone, ng/dl	2.0 (0.9)
PLN, ng/dl	14.4 (5)
DHEA-S, g/dl	238.2 (109.1)
Total testosterone, ng/dl	0.3 (0.2)
17OHP, ng/dl	1.4 (0.67)

FSH, follicle stimulating hormone; LH, luteinizing hormone; PLN, prolactin; DHEA-S, dehydroepiandrosterone sulfate; 17OHP, 17-hydroxy progesterone.

Data collection. Face-to-face interviews were conducted in Arabic. The demographic data included age, education, profession, income, and marital status, in addition to medical and reproductive history, use of medications, menstrual history, and hyperandrogenic symptoms (hirsutism, acne, hair loss). Pre-treatment clinical and biochemical parameters were obtained. Bilateral ovarian volumes, morphology, and the number of antral follicles, with a diameter of 2-9 mm were assessed using ultrasound during the 2nd or 3rd proliferative phase of the menstrual cycle. Ovarian morphology was defined as either PCO or not PCO. Women were asked to keep a menstrual calendar. Hirsutism was assessed according to the modified Ferriman-Gallwey criteria at 9 different body sites that included the upper lip, chin, chest, upper back, lower back, upper abdomen, lower abdomen, arm, and thigh. A score of 0 for the absence of terminal hairs to 4 for extensive terminal hair growth was assigned as appropriate. A score of ≥8 was assigned as hirsutism (18).

Sample collection and testing. Blood samples were taken between 8:00 and 9:00 a.m. after 12 h of overnight fasting; a routine practice as high protein levels may affect PCR quality. The hormonal evaluation was performed in a routine manner and included assaying of serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), FT, dehydroepiandrosterone sulfate (DHEA-S), and 17-hydroxy progesterone (17-OHP),

Table II. Clinical characteristics of PCOS before and after metformin therapy (n=57).

A, Frequency

Variable	Before, n (%)	After, n (%)	P-value
Frequency of menstruation			0.001[c]
Regular	0 (0.0)	33 (57.9)	
Irregular	57 (100.0)	24 (42.1)	
Amount of menstrual blood			0.001[c]
Low	17 (29.8)	01 (1.8)	
Normal	40 (70.2)	56 (98.2)	
Acne			0.001[c]
No	21 (36.8)	42 (73.7)	
Yes	36 (63.2)	15 (26.3)	
Alopecia			0.134
No	25 (43.9)	33 (57.9)	
Yes	32 (56.1)	24 (42.1)	
Ultrasound findings			0.001[c]
No	16 (28.1)	37 (64.9)	
Yes	41 (71.9)	20 (35.1)	
Serum progesterone, ng/dl[d]			NA
≤4	NA	1 (4.0)	
>4	NA	24 (96.0)	

B, Means

Variable	Before, mean ± SD	After, mean ± SD	P-value
Weight, kg	72.1±15.5	69.7±14.2	0.001[c]
BMI, kg/m^2	28.6±6.1	27.6±5.6	0.001[c]
Hirsutism score	16.0±6.8	13.8±5.5	0.001[c]
Fasting blood glucose	5.1±0.5	5.0±0.5	0.238
Fasting insulin	13.5±7.0	11.7±6.3	0.009[b]
LH/FSH	1.5±0.74	1.4±0.78	0.608
Estradiol	111.1±55.7	123.2±54.1	0.219
DHEA-S	238.2±109.1	262.2±139.5	0.045[a]
Total testosterone	0.3±0.21	0.3±0.20	0.218
17OHP	1.4±0.7	1.4±0.7	0.429
Length of period, days	5.6±2.2	5.8±1.4	0.333

[a]P<0.05, [b]P<0.01, [c]P<0.001. [d]Only 25 of women performed progesterone. FHS, follicle stimulating hormone; LH, luteinizing hormone; DHEA-S, dehydroepiandrosterone sulfate; 17OHP, 17-hydroxy progesterone.

thyroid-stimulating hormone (TSH), prolactin (PRL) and estradiol (E2). The metabolic assessment included fasting glucose, insulin levels, and liver and kidney function tests. On the sixth month of metformin therapy, serum progesterone (P) levels were assessed.

Women with regular cycles were tested on days 6-9 prior to the expected menstruation. The test was repeated 3-4 days later if the levels indicated anovulation. Women with irregular cycles were tested on a weekly basis. Ovulation was confirmed at a plasma progesterone of ≥4 ng/ml. A 3 ml venous blood sample was taken to study STK11 gene variation. DNA was extracted from each sample under sterile conditions using the Puregene-Qiagen DNA extraction kit (Qiagen GmbH) according to the manufacturer's instructions. The obtained DNA concentration and purity were determined using a Nanodrop Spectrophotometer.

The amplification reactions were performed in a total volume of 25 μl, consisting of 100 ng DNA template, 1 μl of each primer (10 pmol), and 12.5 μl 2X PCR MasterMix solution (i-MAX11), with the remainder accounted for by nuclease

Table III. Frequencies of the clinical characteristics of PCOS before and after therapy based on intron 1 polymorphisms (n=57).

	CC, n=15		CG, n=25		GG, n=17	
Variable	Before, n (%)	After, n (%)	Before, n (%)	After, n (%)	Before, n (%)	After, n (%)
Frequency of menstruation						
Regular	0 (0.0)	9 (60.0)	0 (0.0)	12 (48.0)	0 (0.0)	12 (70.6)
Irregular	15 (100.0)	6 (40.0)	25 (100.0)	13 (52.0)	17 (100.0)	5 (29.4)
P-value	0.0169[a]		0.0001[b]		0.0445	
Menstrual blood loss						
Low	6 (40.0)	0 (0.0)	6 (24.0)	1 (4.0)	5 (29.4)	0 (0.0)
Normal	9 (60.0)	15 (100.0)	19 (76.0)	24 (96.0)	12 (70.6)	17 (100.0)
P-value	0.017[a]		0.098		0.044	
Acne						
No	6 (40.0)	12 (80.0)	9 (36.0)	15 (60.0)	6 (35.3)	12 (70.6)
Yes	9 (60.0)	3 (20.0)	16 (64.0)	10 (40.0)	11 (64.7)	5 (29.4)
P-value	0.060		0.089		0.039[a]	
Alopecia						
No	7 (46.7)	9 (60.0)	11 (44.0)	10 (40.0)	7 (41.2)	14 (82.4)
Yes	8 (53.3)	6 (40.0)	14 (56.0)	15 (60.0)	10 (58.8)	3 (17.6)
P-value	0.464		0.774		0.032[a]	
Ultrasound findings						
No	6 (40.0)	10 (66.7)	5 (20.0)	17 (68.0)	5 (29.4)	10 (58.8)
Yes	9 (60.0)	5 (33.3)	20 (80.0)	8 (32.0)	12 (70.6)	7 (41.2)
P-value	0.143		0.0006[b]		0.0841	
Hirsutism score	17.3±6.2	14.9±5.2	15.5±6.7	13.9±5.6	15.6±7.6	12.6±6.1
P-value	0.2604		0.3642		0.2135	

[a]P<0.05, [b]P<0.001. Categorical data are presented as n (%) and continuous data are presented as mean ± SD.

free water. The thermocycling conditions were: Initial denaturation at 95˚C for 10 min, followed by 35 cycles of annealing at 60˚C for 1 min and extension at 72˚C for 1 min; with a final extension step of 72˚C for 7 min.

The primer sequences were: RS741765 forward, 5'-GCA GAAATGTAGGGTTGGG-3' and reverse, 5'-GCCCAGCCT CTGTTGTC-3'; and RS8111699 forward, 5'-GCGACACAG CGAGACTC-3' and reverse, 5'-GTGTTCCCTCCCGTT CTC-3'.

Segment rs8111699 of the *STK11* gene was amplified using the Intron Master Mix and 96-well Veriti Thermal Cycler (Applied Biosystems; Thermo Fisher Scientific, Inc.). PCR products were separated by 2% agarose electrophoresis. The gel was visualized under ultra-violet light using Gel-Doc (Bio-Rad Laboratories, Inc. Alwafi Company). Each sample was purified using bio spin columns, then sequenced separately using the Big-Dye Terminator kit (Applied Biosystems; Thermo Fisher Scientific, Inc.). Each sample was cleaned using Nucleo SEQ Columns (Macherey-Nagel GmbH), then run on the genetic analyzer 3130xl (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. DNA sequences were blasted against reference sequences to determine the variations between samples and the reference sequences.

Statistical analysis. For statistical analysis, collected data were double entered on data sheets and analyzed using SPSS version 22 (IBM Corp). Descriptive statistical analysis was performed using frequencies, or means and standard deviations (SD) to describe categorical and numeric data as appropriate. The Kolmogorov-Smirnov test was used to examine all numeric variables for normal distribution. The Pearson's χ^2 and Fisher's exact tests of independence were used to assess if there was a significant, dependent relationship between categorical variables. One-way ANOVA was used to examine differences in the means of hirsutism scores in intron 1 and 6 of the *STK11* gene. A paired-samples t-test was used to evaluate the difference between the means of normally distributed variables before and after metformin therapy. The medians were compared when variables exhibited a significantly skewed distribution using Wilcoxon Signed Rank tests were used for paired samples. P<0.05 was considered to indicate a statistically significant difference.

Results

For this study, 120 women were interviewed, 88 women fulfilled the inclusion criteria, and of these, 57 completed the study and another 31 women were excluded due to non-compliance or

Table IV. Frequencies of clinical characteristics of PCOS before and after therapy based on intron 6 polymorphisms (n=57).

	CC, n=30		CT, n=21		TT, n=6	
Variable	Before, n (%)	After, n (%)	Before, n (%)	After, n (%)	Before, n (%)	After, n (%)
Frequency of menstruation						
Regular	0 (0.0)	17 (56.7)	0 (0.0)	11 (52.4)	0 (0.0)	5 (83.3)
Irregular	30 (100.0)	13 (43.3)	21 (100.0)	10 (47.6)	6 (100.0)	1 (16.7)
P-value	<0.0001[b]		0.0005[b]		1.000	
Menstrual blood loss						
Low	11 (36.7)	01 (3.3)	3 (14.3)	0 (0.0)	3 (50.0)	0 (0.0)
Normal	19 (63.3)	29 (96.7)	18 (85.7)	21 (100.0)	3 (50.0)	6 (100.0)
P-value	0.0025[a]		0.2317		0.1818	
Acne						
No	12 (40.0)	20 (66.7)	9 (42.9)	15 (71.4)	0 (0.0)	4 (66.7)
Yes	18 (60.0)	10 (33.3)	12 (57.1)	6 (28.6)	6 (100.0)	2 (33.3)
P-value	0.0384		0.0614		0.0606	
Alopecia						
No	13 (43.3)	17 (56.7)	11 (52.4)	10 (47.6)	1 (16.7)	6 (100.0)
Yes	17 (56.7)	13 (43.3)	10 (47.6)	11 (52.4)	5 (83.3)	0 (0.0)
P-value	0.3017		0.7576		0.0152[a]	
Ultrasound findings						
No	11 (36.7)	21 (70.0)	3 (14.3)	12 (57.1)	2 (33.3)	4 (66.7)
Yes	19 (63.3)	9 (30.0)	18 (85.6)	9 (42.9)	4 (66.7)	2 (33.3)
P-value	0.0097[a]		0.0038[a]		0.5671	
Hirsutism score	17.7±6.7	15.1±5.6	12.1±4.3	11.0±3.8	21.2±8.2	18.0±7.2
P-value	0.1083		0.3850		0.4890	

[a]P<0.01, [b]P<0.001. Categorical data are presented as n (%) and continuous data are presented as mean ± SD.

loss to follow-up. The mean (SD) of age, weight, height, and BMI of the 57 participants were 23.8 (5.7) years, 72.1 (15.1) kg, 159 (5.2) cm, and 28.6 (6.1) kg/m^2 respectively. The average monthly family income was 1,000 JD ($1,400), 70% were single and 28% were university graduates.

The mean hirsutism score was 16.0±6.8 and the mean length of menstrual periods was 5.6±2.2 days. The means of laboratory values are presented in Table I. Comparing the frequencies of clinical characteristics before and after metformin therapy revealed statistically significant differences in the frequency of menstruation (P=0.001), amount of menstrual blood (P=0.001), acne (P=0.001), ultrasound findings (P=0.001), but not in alopecia (P=0.134; Table II). Post metformin therapy, there was a statistically significant decrease in body weight (P=0.001), BMI (P=0.001), hirsutism score (P=0.001), and DHEA-S (P=0.045). Fasting insulin did significantly decrease after therapy (P=0.009), but fasting blood sugar did not (P=0.238). There was no significant statistical difference in relation to LH/FSH ratio (P=0.608), E2 (P=0.219), TT (P=0.218), 17 OHP (P=0.429) and length of menstruation (P=0.333; Table II). Of the 25 women who underwent luteal progesterone tests, the level was >4 ng/ml in 24 women. Out of 17 women that were trying to conceive, 7 became pregnant during the study period.

The frequency distribution of the genotypes of intron 1 of the STK11 gene was 26% CC, 44% CG, and 30% GG. The frequency distribution of the genotypes of intron 6 of the STK11 gene was 52% CC, 37% CT, and 11% TT. As for the SKT11 gene polymorphism, the results before and after therapy in intron 1 in the CC, CG, and GG subgroups in relation to menstrual regularity, amount of menstrual blood loss, acne, alopecia, ultrasound findings, and the hirsutism score were consistently variable and mostly insignificant (Table III). Regarding intron 6 polymorphism, the results before and after therapy in the CC, CT, and TT subgroups in relation to menstrual regularity, amount of menstrual blood loss, acne, alopecia, ultrasound findings, and the hirsutism score were consistently variable and mostly insignificant too (Table IV).

At the start of the study, 32 patients in groups CC, CG, and GG of intron 1 (8/15, 14/25, and 10/17 respectively, complained of alopecia. Following metformin therapy, 24 patients reported significant improvements (P=0.024), particularly in the GG subgroup (3/17) compared to the CC (6/15) and CG (15/25) groups, respectively (Table V). In addition, there were significant differences in relation to intron 6 and the hirsutism score; from 17.7 to 15.1 for CC, 12.1 to 11.0 for CT, and 21.2 to 18.0 for TT (P=0.006, Table VI).

The number of women with ovulatory levels of progesterone among the subgroups was 9 with CC, 7 with CG, and 8 with

Table V. Frequencies of clinical characteristics of PCOS before and after therapy based on intron 1 polymorphisms (n=57).

	Before, n (%)			After, n (%)		
Variable	CC, n=15	CG, n=25	GG, n=17	CC, n=15	CG, n=25	GG, n=17
Age, years						
≤35	13 (86.7)	25 (100.0)	15 (88.2)	NA	NA	NA
>35	2 (13.3)	0 (0.0)	2 (11.8)	NA	NA	NA
P-value	0.184			NA		
BMI, kg/m^2						
≤24.99	6 (40.0)	9 (36.0)	5 (29.4)	06 (40.0)	9 (36.0)	5 (29.4)
>24.99	9 (60.0)	16 (64.0)	12 (70.6)	09 (60.0)	16 (64.0)	12 (70.6)
P-value	0.815			0.815		
Frequency of menstruation						
Regular	0 (0.0)	0 (0.0)	0 (0.0)	09 (60.0)	12 (48.0)	12 (70.6)
Irregular	15 (100.0)	25 (100.0)	17 (100.0)	06 (40.0)	13 (52.0)	5 (29.4)
P-value	0.927			0.340		
Menstrual blood loss						
Low	6 (40.0)	6 (24.0)	5 (29.4)	0 (0.0)	1 (4.0)	0 (0.0)
Normal	9 (60.0)	19 (76.0)	12 (70.6)	15 (100.0)	24 (96.0)	17 (100.0)
P-value	0.563			0.521		
Acne						
No	6 (40.0)	9 (36.0)	6 (35.3)	12 (80)	15 (60.0)	12 (70.6)
Yes	9 (60.0)	16 (64.0)	11 (64.7)	3 (20)	10 (40.0)	5 (29.4)
P-value	0.956			0.409		
Alopecia						
No	7 (46.7)	11 (44.0)	7 (41.2)	9 (60)	10 (40.0)	14 (82.4)
Yes	8 (53.3)	14 (56.0)	10 (58.8)	6 (40)	15 (60.0)	3 (17.6)
P-value	0.952			0.024		
Ultrasound findings						
No	6 (40.0)	5 (20.0)	5 (29.4)	10 (66.7)	17 (68.0)	10 (58.8)
Yes	9 (60.0)	20 (80.0)	12 (70.6)	5 (33.3)	8 (32.0)	7 (41.2)
P-value	0.391			0.818		
Progesterone, ng/dl						
≤4	NA	NA	NA	0 (0.0)	0 (0.0)	1 (12.5)
>4	NA	NA	NA	9 (100.0)	7 (100.0)	8 (87.5)
P-value	NA			0.396		
Pregnancies						
No	NA	NA	NA	3 (60)	3 (60)	4 (57.1)
Yes	NA	NA	NA	2 (40)	2 (40)	3 (42.9}
P-value	NA			NA		
Hirsutism score	17.3±6.2	15.5±6.7	15.6±7.6	14.9±5.2	13.9±5.6	12.6±6.1
P value	0.708			0.552		

Categorical data are presented as n (%) and continuous data are presented as mean ± SD.

GG in the intron 1 genotype subgroup, and 14 with CC, 6 with CT, and 4 with TT in the intron 6 genotype subgroup. Of the 17 women trying to conceive, 7 got pregnant during the study period. Their distribution among the subgroups was CC 2/5, CG 2/5, and GG 3/7 in the intron 1 sub-groups, and CC 3/7, CT 3/8, and TT 1/2 pregnancies in the intron 6 genotype subgroup.

Discussion

PCOS is one of the most common endocrine disorders in women of reproductive age, with no known definitive therapy. Metformin, a well-tolerated insulin sensitizer with minimal side effects, proved useful in correcting IR, hyperandrogenism,

Table VI. Frequencies of clinical characteristics of PCOS before and after therapy based on intron 6 polymorphisms (n=57).

	Before, n (%)			After, n (%)		
Variable	CC, n=30	CT, n=21	TT, n=6	CC, n=30	CT, n=21	TT, n=6
Age in years						
≤35	27 (90.0)	20 (95.2)	6 (100.0)	NA	NA	NA
>35	3 (10.0)	1 (4.8)	0 (0.0)	NA	NA	NA
P-value	NA			NA		
BMI, kg/m^2						
≤24.99	12 (40)	5 (23.8)	3 (50)	11 (36.7)	6 (28.6)	3 (50.0)
>24.99	18 (60)	16 (76.2)	3 (50)	19 (63.3)	15 (71.4)	3 (50.0)
P-value	0.354			0.603		
Frequency of menstruation						
Regular	0 (0.0)	0 (0.0)	0 (0.0)	17 (56.7)	11 (53.4)	5 (83.3)
Irregular	30 (100.0)	21 (100.0)	6 (100.0)	13 (43.3)	10 (47.6)	1 (16.7)
P-value	0.407			0.392		
Menstrual blood loss						
Low	11 (36.7)	3 (14.3)	3 (50.0)	1 (3.3)	0 (0.0)	0 (0.0)
Normal	19 (63.3)	18 (85.7)	3 (50.0)	29 (96.7)	21 (100.0)	6 (100.0)
P-value	0.119			0.633		
Acne						
No	12 (40.0)	9 (42.9)	0 (0.0)	20 (66.7)	15 (71.4)	4 (66.7)
Yes	18 (60.0)	12 (57.1)	6 (100.0)	10 (33.3)	6 (28.6)	2 (33.3)
P-value	0.138			0.933		
Alopecia						
No	13 (43.3)	11 (52.4)	1 (16.7)	17 (56.7)	10 (47.6)	6 (100.0)
Yes	17 (56.7)	10 (47.6)	5 (83.3)	13 (43.3)	11 (53.4)	0 (0.0)
P-value	0.298			0.071		
Ultrasound findings						
No	11 (36.7)	3 (14.3)	2 (33.6)	21 (70)	12 (57.1)	4 (66.7)
Yes	19 (63.3)	18 (85.7)	4 (66.7)	9 (30)	9 (42.9)	2 (33.3)
P-value	0.206			0.636		
Progesterone						
≤4	NA	NA	NA	0 (0.0)	1 (14.3)	0 (0.0)
>4	NA	NA	NA	14 (100.0)	6 (85.7)	4 (100.0)
P-value	NA			0.262		
Pregnancies						
No	NA	NA	NA	4 (57.1)	5 (62.5)	1 (50.0)
Yes	NA	NA	NA	3 (42.9)	3 (37.5)	1 (50.5)
P-value	NA			NA		
Hirsutism score	17.7±6.7	12.1±4.3	21.2±8.2	15.1±5.6	11.0±3.8	18.0±7.2
P-value	0.001[b]			0.006[a]		

[a]P≤0.01, [b]P≤0.001. Categorical data are presented as n (%) and continuous data are presented as mean ± SD.

and ovulation, and is relatively safe for the fetus; it is classed as a category B medication which is where animal studies have failed to demonstrate a risk to the fetus, in the absence of adequate and well-controlled studies in pregnant women (19).

Metformin has been used to treat several reproductive and metabolic abnormalities associated with PCOS; however, further studies are required to substantiate its use as current guidelines only specifically support the use of metformin for glucose intolerance in PCOS (20,21). However, prospective studies with adequate follow-up times following metformin therapy are required to better elucidate the role of metformin in treating reproductive and metabolic abnormalities, a research gap that the present study addresses (21). This study of the effect of metformin in 57 women with PCOS showed statistically

significant improvement in cycle regularity, amount of blood loss, acne, and ovarian ultrasound morphology. This is consistent with the findings of other studies (22,23). In agreement with the findings of other studies, metformin therapy in this study demonstrated a significant decrease in hirsutism in women with PCOS (24,25). As suggested by Palomba *et al* (26), the results of this study suggest that it would be reasonable to use Metformin as a first-line therapy in women, with relative contraindication to the long term use of the combined oral contraceptive pill, who wish to have regular menstruation, where menstruation is simply a withdrawal bleed. This may be more pertinent in communities where young and unmarried women are reluctant to use hormonal contraceptives for menstrual cycle regulation.

Unfortunately, women with oligomenorrhea failed to comply with weekly progesterone tests, whereas, women with normal cycles had a 96% ovulatory progesterone level, with 41% positive β-hCG in women trying to get pregnant. These results are consistent with the results of another report (9).

The study of intron 1 and intron 6 revealed that their subgroups responded to metformin differently in relation to various parameters. The intron 1 subgroup's response to metformin revealed no statistically significant difference in all parameters except for alopecia. A study of the same parameters in intron 6 showed no significant difference in response in all sub-groups in response to metformin therapy except for hirsutism. The ovulatory response was similar, and pregnancies were reported in all subgroups. Previous studies have shown that polymorphisms in STK11 are associated with the response to metformin in type 2 diabetes and PCOS (27,28). Although we did not reproduce these findings across all characteristics studied, the results did show that the polymorphisms identified a subset of women whose alopecia improved following metformin therapy.

One of the limitations of this study is the small sample size. Secondly, the levels of free testosterone and SHBG levels were not included to evaluate the androgenic response to therapy (29).

In conclusion, metformin therapy did improve period regularity, ovarian appearance, acne, hirsutism, and ovulatory response. SKT11 polymorphism of both intron 1 and intron 6 may have effects on alopecia and hirsutism, respectively.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

JH conceived the study. JH, SH, HA, and ZA designed the study. NA analyzed the data. JH, SH and ZA performed the experiments. JH and NA collected the data. JH, SH, ZA, HA, and NA wrote the manuscript. ZA edited the manuscript. All authors confirm the authenticity of the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The study design and consent forms for all procedures were approved by the Ethics Committee for Human Participants at the Jordan University of Science and Technology (Irbid, Jordan) and complied with the guidelines described in the Declaration of Helsinki.

Patient consent for publication

Written informed consent was obtained from all the patients for publication of their data.

Competing interests

The authors declare that they have no competing interests.

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