MITOCHONDRIAL UNCOUPLING CARRIER PROTEIN-2 DNA POLYMORPHISM (−866 G/A) AS A RISK FOR POLYCYSTIC OVARY SYNDROME IN SAUDI ARABIAN FEMALES

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Polycystic ovary syndrome (PCOS) is a common hormonal disorder characterized by gonadotropin dysregulation, hyperandrogenism, menstrual irregularity and ovarian morphology. The uncoupling protein-2 (UCP-2) gene is a member of the mitochondrial anion carrier protein (MACP) and is strongly associated with insulin resistance and obesity. An attempt is made to detect the association between PCOS and -866G/A polymorphism in UCP2 gene promoter. The study is carried on forty-seven patients with polycystic ovary syndrome and fifty–six controls. Blood samples were taken for biochemical tests and restriction fragment length polymorphism (RFLP) for detection of -866 G/A UCP2 gene polymorphisms. Significant associations between the -866A/A -866G/A of UCP2 gene promotor and PCOS are reported including a significant association between -866A/A polymorphism of UCP2 and both obesity (high BMI) and type 2 diabetes.

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinal disorder among reproductive age of women and has multiple features such as menstrual irregularity, hyperandrogenism, gonadotropin dysregulation, and ovarian morphology in the overall phenotype[1]. In addition to its reproductive features, it is associated with an increased risk of overweight or obesity, diabetes mellitus (type 2) and insulin resistance.[2]

Uncoupling protein-2 (UCP2) is a member of the mitochondrial transporter protein carrier (MTPC) and is located in the inner mitochondrial membrane.[3] It is a member of the larger family of mitochondrial anion carrier proteins (MACP).[3] It separates oxidative phosphorylation from synthesis of adenosine triphosphate (ATP) with energy release as heat (mitochondrial proton leak) and results in reduced ATP synthesis.[3] It helps the transfer of anions from inner to outer mitochondrial membrane and the return of the protons in the reverse direction from the outer to the inner mitochondrial membrane.[4] Therefore, it decreases the mitochondrial membrane potential.[4] It may have a role in thermogenesis, obesity, diabetes and atherosclerosis.[5] Now it appears that the main UCP2 function is the controlling of mitochondria reactive oxygen species. UCP-2 has three homologous protein domains.[6]

UCP2 gene is located at chromosome 11 q13.4 and expressed in many tissues, with the greatest expression in skeletal muscle,[7] and adipose tissue.[8] G/A polymorphism of the UCP2 promoter at position -866 varied in different populations.[9] G allele (wild type) is associated with reduction of mRNA expression in adipose tissue with low risk of obesity in the middle-aged humans and low risk of T2D.[10] UCP2 -866G/A polymorphism contributes to abnormal insulin hormone secretion and glucose tolerance in Italian population.[11] The same results were found in young German subjects.[12] The UCP2 is one of the responsible gene for T2D and obesity.[5] The distribution of UCP2 polymorphism in the healthy Caucasians and Iranian population is different from the Japanese population.[13]

The aim of the present study was to analyze the association between PCOS in Saudi Arabian females and -866G/A SNP of the UCP2 gene polymorphism.

Forty-seven patients with polycystic ovary syndrome were selected from outpatient's clinics of Al-Dawadmie hospital KSA from November 2015 to February 2016. The diagnosis of PCOS was made according to Rotterdam criteria (2003) which depends on present two of the three criteria needed to be fulfilled for the diagnosis of PCOS (1) clinically associated features (hirsutism or acne) and/or high serum androgen levels (2) irregular menstrual cycle (3) ultrasonographic finding of polycystic ovarian.[14] Fifty-six females without PCOS criteria were selected as control.

Complete physical, laboratory and genetic examinations were done to both groups. Local Medical Ethical Committee approved this study ethically and a written informed consent was taken from both control subjects and patients.

Experimentals

Mitochondrial DNA extraction

Mitochondrial DNA was purified from whole blood samples with the QIAamp® DNA Blood Mini Kit (Holliston, MA, USA). DNA was eluted in 150 µl elution buffer and examined on 1% agarose gel and stored at – 20°C for analysis.

PCR and restriction fragment length polymorphism (PCR-RFLP)

DNA fragment corresponding to the UCP2 G (−866) A polymorphism (rs659366) was amplified by 5'-CAC GCT GCT TCT GCC AGG AC-3' as forward primer and 5'-AGG CGT CAG GAG ATG GAC CG-3' as reverse primer.[15]

PCR products were digested by MluI restriction enzyme (NEB, Ipswich, MA, USA) and separated on 2% agarose gel electrophoresis.[16] The −866A/A genotype was marked by a single 363 bp fragment due to loss of MluI site (Figures. 1 and 2), while the wild-type (−866) G/G genotype was digested into 295 and 68 bp fragments (Fig. 2). (−866) A/G genotype was digested into 363, 295 and 68 bp fragments. 68 bp band does not present in (-866G/G), and (-866A/G) in the Figure 2.

Biochemical determinations

Blood samples from the PCOS patients and controls were analyzed using biochemical assays, including FSH (Human FSH EIA Test Kit Catalog No: 40-052-115017 GENWAY BIOTECH IN), LH (Ultra Sensitive Luteinizing Hormone (LH) LumELISA Catalog No. 40-101-325027 GENWAY BIOTECH IN), TSH (Thyroid Stimulating Hormone (TSH) Human ELISA Kit (Abcam), PRL (PRL human ELISA KIT KA0217 Catalog No (Abnova), DHEA-S (DHEA sulfate (DHEA-S) ELISA Kit (ab108669) (Abcam) and E2 (Prostaglandin E2 High Sensitivity ELISA Kit (ab133055) Abcam).

Anthropometry assessment

Anthropometric evaluation was performed for all the patients in both groups. Body weight, height and waist circumference were measured.[17] Waist circumference was measured at the level of the umbilicus with the standing position, the face directed forward, shoulders relaxed; and normal breathing by using non-stretchable plastic tape to the nearest 0.1 cm. Body mass index (BMI) was calculated as body weight divided by height squared (kg m^{-2}).

Statistical analysis

Allele's frequency and genotypes were computed using the Arlequin software (version 3.1) and SNP stats (http://bioinfo.iconcologia.net/SN Pstats). Data were presented by means ± SD and percentages. The compiled data were computerized and analysed by SPSS V 12. The following tests of significance were used: t-test between means we used to analyse mean difference, t-test between percentage to analyse percent difference and chi–square. A level of $p < 0.05$ was considered significant.

Results

A total of 47 polycystic ovary syndrome patients and 56 control subjects were included in this study. General and clinical characteristics of all the subjects enrolled in this study are shown in Table 1. Patients with significantly high serum TSH, LH, testosterone and DHEA-S in PCO than control subjects were observed.

Comparing -866G/A polymorphism of UCP2 gene and alleles in PCOS Patients and controls were tabulated (Tables 2 and 3). The -866 A/A and A/G UCP2 gene polymorphisms and -866 A allele were significantly higher in PCOS than control group. Relations of -866G/A polymorphism of UCP2 gene and both $HbA1_c$ (Table 4) and BMI (Table 5) were shown that -866 A/A UCP2 gene polymorphism was significantly higher in both uncontrolled DM (Table 4) and obesity (Table 5).

Table 1. General and clinical characteristics of PCOS and control groups

Parameters	Control subjects (mean ± SD)	PCOS patients (mean ± SD)
Age (years)	34.6 ±12.3	37.7 ±13.9
Mean BMI	22.54 ± 1.4	26.31±2.6*
Waist/hip ratio	0.82±0.06	0.99 ±0.032
E2 levels (ng mL^{-1})	32.7 ±14.7	22.9±19.4
Prolactin levels (ng mL^{-1})	16.4±4.8	16.8 ±6.7
Testosterone (ng mL^{-1})	0.32 ±0.12	0.59 ±0.18*
DHEA-S level (µg mL^{-1})	129.5±24.2	200±34.3*
TSH levels (µIU mL^{-1})	1.44 ±0.6	1.39 ±0.7
FSH levels (µIU mL^{-1})	5.1 ±3.9	6 ±3.3
LH levels (µIU mL^{-1})	4.3±2.1	9 ±1.4*
LH/FSH	0.8 ±0.5	1.5 ±0.4*

*Significant change ($p< 0.05$) between PCOS subjects and controls

Table 2. Distribution of -866 G/A polymorphism in PCOS patients and controls

Parameters	Control subjects (*n*=56)	PCOS Patients (*n*=47)
-866 AA(*n*,%)	16 (28.5%)	15 (32%)*
-866 AG(*n*,%)	10 (18%)	22(47%)*
-866 GG(*n*,%)	30 (53.5%)*	10 (21%)

*Significant change ($p<0.05$) between PCOS subjects and controls

Table 3. Distribution of -866 G/A alleles in PCOS patients and controls

Parameters	Control subjects (*n*=112)	PCOS patients (*n*=94)
-866 A (*n*,%)	42 (37.5 %)	52 (55 %)*
-866 G (*n*,%)	70 (62.5 %)*	42 (45 %)

*Significant change ($p<0.05$) between PCOS Subjects and controls.

Table 4. Relation between HbA1c and -866 (G/A) genotypes

Parameters	HbA1c≤ 7% *n*=71	HbA1c>7% *n*=32
-866 AA (*n*, %)	18 (25.4 %)	13 (40.6 %)*
-866 AG (*n*, %)	22 (31 %)	10(31.3 %)
-866 GG *n* (%)	31 (43.7%)	9 (28.1%)

*Significant change ($p<0.05$) between PCOS subjects and controls.

Table 5. relation between BMI and -866 (G/A) genotypes

Parameters	BMI ≤ 25 n (65)	BMI > 25 n (38)
-866 AA (*n*, %)	15(23.1%)	16 (42.1%)*
-866 AG (*n*, (%)	20 (30.8%)	12 (31.6%)
-866 GG (*n*, %)	30 (46.2%)*	10 (26.3%)

*Significant change ($p < 0.05$) between PCOS Subjects and controls.

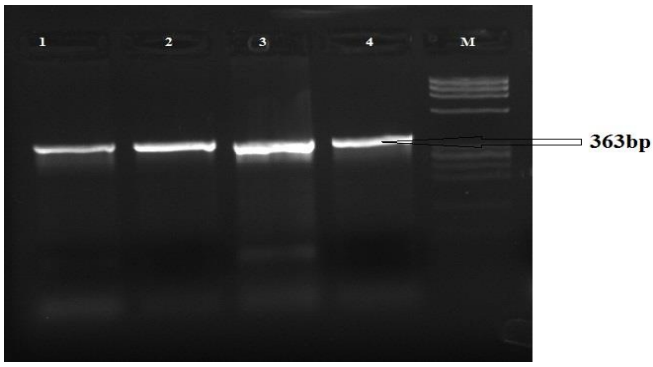

Figure 1. PCR amplification for −866A/A polymorphism UCP2 gene: Lanes 1, 2, 3 and 4 PCR product with length 360 base pair. Lane M ϕx 147 marker.

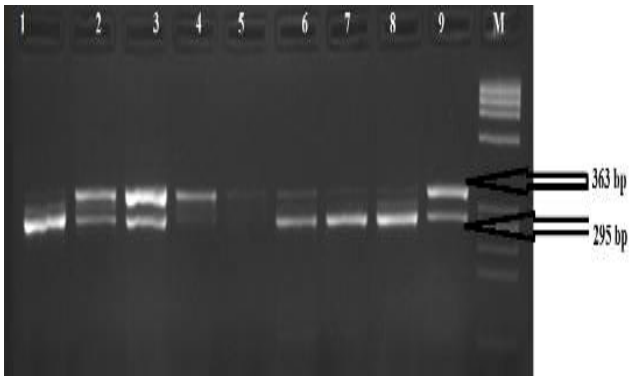

Figure 2. Restriction enzyme analysis for −866G/G and −866G/A polymorphisms of UCP2 gene PCR: Lanes 1, 6, 7 and 8 with 295 bp (-866G/G), lanes 4 and 5 with 363 bp (-866A/A) and lane 2, 3, and 9 with 363bp and 295 bp (-866A/G). Lane M ϕx 147 marker.

Discussion

Polycystic ovary syndrome (PCOS) is a common cause of female infertility during reproduction period associated with production of surplus of androgens causing irregular ovulation, or even a lack of ovulation[18]. Androgens are responsible for body changes in male like muscle mass and hair growth. In women, androgens are necessary to make estrogen.[18] In the current research, PCOS women have significantly high androgen levels. The excess androgen in women with PCOS can cause acne and excessive hair growth.[18]

Excess androgen production in case of PCOS also leads to irregular or absent ovulation[19]. In the current research, PCOS women have significantly high serum LH/FSH (more than 1). Because of its anovulatory effect, PCOS women may have difficulty in getting pregnant.[19]

Many PCOS women are resistant to insulin action. This means that they need larger than normal amounts of insulin to maintain normal blood sugar levels.[2] These women are at high risk for diabetes. In the current study, PCOS women have significantly high serum $HbA1_c$. High insulin levels caused by insulin resistance can lead to excessive production of androgen[3]. Beside the reproductive disorders in PCOS there are an association with an increased risk of obesity[7] and T2D.[5] In our study, PCOS women have significantly high BMI.

Several genetic studies of PCOS were caarried out and it wwas found that many genetic region were associated with it such as CAPN10 (calpain 10),[20] CYP11A (cytochrome P450, family 11, sub family A),[21] the insulin gene VNTR (variable number of tandem repeats),[22] and D19S884 (di nucleotide repeat marker mapping to chromo some 19p13.2).[23] In the present study, UCPs has a key role in the regulation of human energy production by uncoupling respiration from oxidative phosphorylation, and thus converting stored energy to free heat.[24] UCP-2 -866G/A SNP was identified in the promoter region.[25-27]. This polymorphism was reported in fat metabolism, diabetes and obesity,[28&29] UCP-2 -866A/A gene allele was frequently reported not only with increased adipose mRNA expression and obesity[30-32] but also, with type 2 diabetes (T2D) secondary to reduction in insulin potency.[30] UCP-2 -866G/A gene alleles were reported to have a low risk of coronary artery disease,[33] very low energy levels in the peripheral nerve function,[34] high risk of metabolic syndrome, higher waist-to-hip ratio[35] and higher serum oxidative stress markers.[36] UCP-2 -866G/G gene allele was shown to be associated with a reduced mRNA expression in adipose tissue, low BMI,[37] decrease risk of obesity[28], high insulin sensitivity and a reduced risk of T2D.[26] These genotype form were reported to have low blood triglyceride levels[37] and higher levels of LDL-cholesterol.[34] UCP-2 -866A/A and -866G/A genotype is assumed that the UCP2 gene is associated with obesity and type 2 diabetes, it is also associated with obesity in females with PCOS.

In conclusion, there are a significant association between the -866A/A and -866G/A gene polymorphisms in the UCP2 gene promoter and PCOS in Saudi Arabian females. The -866A/A polymorphism of UCP2 is associated with (high BMI) obesity and T2D in the same study group. Further investigations for studying -866G/A polymorphism of the UCP2 gene promotor and PCOS patients on a large scale or in different ethnic groups is required.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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