Original Article
Turk J Endocrinol Metab. 2021;25:102-112

MTHFR C677T Polymorphism in Turkish Women with Polycystic Ovary Syndrome

Polikistik Over Sendromlu Türk Kadınlarında MTHFR C677T Polimorfizmi

Seher POLAT, Yasin ŞİMŞEK*

Department of Medical Genetics, Medical Faculty Erzincan University Mengücek Gazi Training and Research Hospital, Erzincan, TURKEY
*Clinic of Endocrinology, Kayseri Training and Research Hospital, Kayseri, TURKEY

Abstract

Objective: Polycystic ovary syndrome (PCOS) is one of the most common endocrine-reproductive-metabolic disorders of women at reproductive age, affecting 5-15% of the women worldwide. Although the pathogenesis of PCOS is not well defined, it is associated with an increased risk of premature coronary artery disease (CAD). Hyperhomocysteinemia (HHcy) is associated with hyperlipidemia and is an independent risk factor for CAD. The most common cause of HHcy is related to the deficiency of methylenetetrahydrofolate reductase (MTHFR). This study aimed to investigate the relationship between different genotypes of MTHFR C677T and the risk of PCOS. **Material and Methods:** Two hundred twenty voluntary premenopausal women (110 healthy controls and 110 PCOS patients) were included in the study. All the volunteers underwent a physical examination along with biochemical hormonal evaluation and genetic analysis. **Results:** The genotyping analyses and genetic model of inheritance analyses revealed that the frequencies of CC, CT, and TT genotypes in the control and PCOS group to be 51.8%, 45.5%, and 2.7% and 51.8%, 48.2%, and 0%, respectively. The frequency of C and T alleles in the control and PCOS group was determined to be 74% (C: 0.74/155) and 26% (T: 0.26/53), and 75% (C: 0.75/167) and 25% (T: 0.25/53), respectively. The "T" additive, "T" dominant, and "C" recessive models it found that the CT vs. CC (OR:1.06 CI:0.62-1.83), CC vs. TC+TT (OR: 0.99 CI: 0.58-1.72), and TC+TT vs. CC (OR: 0.99 CI: 0.58-1.70), respectively, did not show an increase in the PCOS risk. **Conclusion:** Our findings indicated that the different genotypes of MTHFR C677T were not associated with the risk of PCOS in Turkish women from Central Anatolia.

Keywords: Polycystic ovary syndrome; methylenetetrahydrofolate reductase gene; polymorphism, risk assessment

Özet

Amaç: Üreme çağındaki kadınların en yaygın endokrin-üreme-metabolik bozukluklarından biri olan polikistik over sendromu (PKOS) dünya genelinde kadınların %5-15'ini etkilemektedir. PKOS patogenezi tam olarak tanımlanmamış olmasına rağmen, PKOS'un artmış erken koroner arter hastalığı (KAH) riski ile ilişkili olduğu bilinmektedir. Hiperhomosistenemi (HHcy), KAH için bağımsız bir risk faktörü olan hiperlipidemi ile ilişkili olup HHcy'in en yaygın nedeni metilentetrahidrofolat redüktaz (MTHFR) eksikliğidir. Bu çalışma ile MTHFR C677T polimorfizmi ve PKOS riski arasındaki ilişkinin araştırılması amaçlanmıştır. **Gereç ve Yöntemler:** Çalışmaya benzer yaşta iki yüz yirmi gönüllü premenopozal kadın (110 sağlıklı kontrol ve 110 PKOS hastası) dahil edilmiştir. Tüm gönüllülere fiziksel muayene, biyokimyasal hormon değerlendirme ve genetik analizler uygulanmıştır. **Bulgular:** Genotipleme ve genetik kalıtım model analizleri ile CC, CT ve TT genotip sıklığının kontrol ve PKOS gruplarında sırasıyla %51,8, %45,5, %2,7 ve %51,8, %48,2 ve %0 olarak bulunmuştur. C ve T allellerinin sıklığı sırasıyla kontrol ve PKOS gruplarında %74 (C: 0,74/155), %26 (T: 0,26/53) ve %75 (C: 0,75/167), %25 (T: 0,25/53) olarak belirlenmiştir. "T" editif modelde CT'ye göre CC (OR: 1,06 CI: 0,62-1,83), "T" baskın modelde CC'ye göre TC+TT (OR: 0,99 CI: 0,58-1,72) ve "C" çekinik modelde TC+TT'ye göre CC (OR: 0,99 CI: 0,58-1,70) genotipine sahip olmanın PKOS riskini arttırmadığı belirlenmiştir. **Sonuç:** Mevcut çalışma ile MTHFR C677T genotiplerinin iç anadolu bölgesinde yaşayan Türk kadınlarında PKOS riski ile ilişkili olmadığı gösterilmiştir.

Anahtar kelimeler: Polikistik over sendromu; metilentetrahidrofolat redüktaz geni; polimorfizm, risk değerlendirmesi

Address for Correspondence: Seher POLAT, Department of Medical Genetics, Medical Faculty Erzincan University Mengücek Gazi Training and Research Hospital, Erzincan, TURKEY
Phone: +90 555 394 20 16 **E-mail:** polatdna@yahoo.com

Peer review under responsibility of Turkish Journal of Endocrinology and Metabolism.

Received: 05 Sep 2020 **Received in revised form:** 24 Nov 2020 **Accepted:** 10 Dec 2020 **Available online:** 19 Jan 2021

DOI: 10.25179/tjem.2020-78794

Introduction

Polycystic ovary syndrome (PCOS) affects 5-15% of women worldwide. It is one of the most common endocrine- reproductive-metabolic disorders and a major cause for anovulatory infertility (1-3) PCOS is not a local disease; it is a syndrome and a chronic systemic disease. Although the pathogenesis of PCOS is not well defined, in most of the cases, it is related to hyperandrogenemia, insulin resistance (IR), elevated risk of type 2 diabetes (T2DM), dyslipidemia, chronic inflammation, endometrial cancer, and an elevated risk of premature coronary artery disease (CAD) (4-6). The pathogenesis of PCOS remains unknown since it is a complex disease with a multifactorial etiology derived from the interactions between diverse genetic and environmental factors (1,7-9). In the last years, many studies were performed to observe the contribution of single nucleotide polymorphisms (SNPs) in the PCOS etiology (10-15).

The C677T (rs1801133) polymorphism located on the methylenetetrahydrofolate reductase (MTHFR) gene encodes a variant, where cytosine (C) is replaced with thymine (T) at nucleotide 677, which leads to the substitution of alanine (A) instead of valine (V) at codon 222, resulting in the reduction of enzyme activity in 30% to 70% of heterozygous and homozygous genotypes, respectively (16). In the general population, hyperhomocysteinemia (HHcy) reduces the activity of MTHFR that results from the single nucleotide polymorphism (SNP) (16). However, another factor for this can be an insufficient dietary intake of folate.

MTHFR regulates the metabolism of homocysteine (Hcy) and methionine. It converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the main form of folate in the blood. The methyl group acts as a donor for the production of methionine from Hcy. HHcy is related to hyperlipidemia (17) and is an independent risk factor for CAD (18). Laboratory findings suggested that an elevated Hcy concentration is both atherogenic and thrombogenic (19). Several studies have shown that hyperinsulinemia and insulin resistance are positively associated with HHcy (20-22). The changes in the folate metabolism may disrupt the homocysteine-methionine equilibration and maintenance of the methyl pool since folate, and homocysteine-methionine cycles work in connection (23).

In the late 1960s, the partly prevented ovulation found in the immature super-ovulated rats by an insufficient amount of folate raised questions about the effects of folic acid on ovarian function (24). HHcy caused by MTHFR deficiency may lead to impairment of the DNA methylation process (25). Incorporating a higher amount of uracil into the DNA can activate repair mechanisms, resulting in an increased risk of chromosomal breakage (26), reduced nitric oxide formation (27), reduced reactive oxygen species elimination (28), and increased proinflammatory cytokine release (29). Also, folliculogenesis and oogenesis are quite susceptible to the above-mentioned environmental changes. As shown by several experimental data, the oocyte maturation, ovulation, proliferation, and differentiation of granulosa cell and steroid biosynthesis can be affected by HHcy (24,30,31). Moreover, folate and DNA methylation both are important in Alzheimer's disease, mental health, various cancers, and neural tube defects. Additionally, Hcy may play an important role in oxidative stress as well (25,32-35).

The contribution of the MTHFR C677T polymorphism in increasing the PCOS risk was investigated in various populations with contradictory results (36-38). However, there was no study carried out on Turkish women from the Central Anatolian region. Therefore, the goals of our study were to determine whether the C677T variant was related to increase the PCOS risk among Turkish women and also to evaluate any possible relationship between the variant and the clinical and biochemical parameters.

Material and Methods

Subjects

The subjects included in this study were 220 voluntary premenopausal women (110 healthy controls and 110 PCOS patients) aged between 18 and 50 years who were examined in the endocrine clinic at Kayseri Training and Research Hospital between January 2019 and January 2020. The study was approved by the Ethics Committee of Medical School at

Erzincan University. The ethics committee decision number and date were 33216249-604.01.02 E.3294 and 15 January 2019, respectively. All the volunteers provided written informed consent and underwent a physical examination along with biochemical hormonal evaluation. A medical history form was filled, which contained information on hirsutism, menstrual cycle, reproductive and gynecological history, carbohydrate intolerance, use of medication (oral contraceptive pills (OCP)), and arterial hypertension. Ovarian and adrenal ultrasonography was not applied to all volunteers of control subjects in the study designed on a voluntary basis.

Inclusion criteria

The PCOS group included women diagnosed with PCOS according to the criteria of the Androgen Excess-PCOS Society (39).
The Control group included women who were eligible for blood donation, had a normal menstrual cycle with no excess hair growth nor any signs of hyperandrogenemia.

Exclusion criteria

Women who used OCP and/or were diagnosed with acromegaly, Cushing's syndrome, thyroid dysfunction, hyperprolactinemia, ovarian tumors, or adrenal tumors were excluded from the PCOS group.
Women who did not have a normal menstrual cycle with excess hair growth and/or any sign of hyperandrogenemia were excluded from the control group.

Diagnostic Criteria

A modified Ferriman-Gallwey score (FGS) of >8 was considered as hirsutism (40), where FGS was evaluated by the same investigator (Y.S). The intermenstrual intervals of longer than 35 days and shorter than 21 days were diagnosed as oligomenorrhea and polymenorrhea, respectively. Androgen value greater than the normal range was identified as hyperandrogenemia. Serum hormone levels were measured in the early follicular phase of the menstrual cycle after an overnight fast.

Hormone Measurements

Prolactin, thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), progesterone, luteinizing hormone (LH), estradiol (E2), dehydroepiandrosterone sulfate (DHEA-S), total testosterone (TT), and insulin levels were analyzed using the chemiluminescence immunoassay method by Roche Elecsys Cobas E601 immunoassay analyzer (Roche Diagnostics, Penzberg, Germany). Glucose was analyzed using an enzymatic method by Roche Elecsys Cobas C702 chemistry analyzer (Roche Diagnostics, Penzberg, Germany).
The reference ranges for the TT, DHEA-S, TSH, E2, LH, FSH, prolactin, progesterone, and glucose were 0.084-0.481 µg/L, 350-4070 (ng/mL), 0.27-4.2 µIU/mL, 12.4-233 pg/mL, 2.4-12.6 mIU/mL, 3.5-12.5 mIU/mL, 4.79-23.3ng/mL, 0.05-0.893 ng/mL, and 70-100 mg/dL, respectively. The homeostatic model assessment for insulin resistance (HOMA-IR) was used as insulin resistance index (41).

Genotyping Analysis

Genotyping analysis was applied to all the volunteers. The Genomic DNA isolation kit (Roche, Germany Genomic DNA) was used to isolate the DNA from leukocytes. Polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method was used to analyze the MTHFR C677T variant. The primer pairs used to amplify the C677T variant of the MTHFR gene were 5'-GCTCAAGGCAGGACAGTG-3' as the forward primer and 5'-CTGGGAAGAACT CAGCGAAC-3' as the reverse primer. DreamTaq polymerase (5U/µL, ThermoFisher, USA) was used to perform the PCR reaction. The PCR conditions were 30 cycles of 94˚C for 1 min, 63˚C for 30 s, and 72˚C for 30 s. The obtained PCR fragment of 585 bp was digested using the Taq1 restriction enzyme (ThermoFisher, USA) at 37˚C for 3 h. Agarose gel electrophoresis (2%) was used to separate the digested PCR product, which was then visualized under UV illuminator with SafeRed staining (IntronBio, Korea). In the TT homozygous mutant genotype, two fragments were obtained with a size of 359 bp, and 226 bp whereas, in the CT heterozygous mutant genotype, three fragments with a size of 585 bp, 359 bp, and 226 bp were obtained while in the CC homozygous wild genotype, only one fragment with a size of 585 bp was obtained.

Statistical Analyses

Results for the continuous variables were reported as mean±standard deviation (SD) and median (minimum-maximum) while n (%) was used for the categorical variables. The normality of variables was confirmed using the Kolmogorov-Smirnov test. The comparisons between the study groups were performed using the Student's t -test. For the variables, which were not normally distributed, the Mann-Whitney U test was used. For determining genotype frequencies of alleles between the study groups, the Chi-square test was used. Logistic regression analysis was applied to calculate the odds ratios (OR) and to test the relative risk associated with the risk allele for PCOS, confidence intervals (95% CI) were used. The variables analysis of covariance (ANCOVA) test was used to estimate the differences between the study groups with age and body mass index correction. P-values less than 0.05 were accepted as significant for all the tests. IBM SPSS 22 (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp) was used to perform all statistical analyses.

Results

Clinical and Biochemical Properties

The clinical and biochemical characteristics of the control and patients groups are presented in Table 1. Compared to the control group, the body mass index (BMI), weight, Ferriman -Gallwey score (FGS), insulin, HOMA-IR, TT, prolactin, DHEA-S, LH, and TSH were found to be significantly higher in the PCOS group, with age being significantly reduced (p=<0.05).

Genotype Distribution and Allele Frequency

To study the relationship between the MTHFR C677T variant and the PCOS risk, the genotype distribution and allele frequency were determined for each group, which is presented in Table 2.

The frequencies of CC, CT, and TT genotypes were determined as 51.8%, 45.5%, and 2.7% in the control group, and 51.8%, 48.2%, and 0% in the PCOS group. The TT genotype was not determined in the PCOS group. The C and T allele frequencies were determined as 74% (C: 0.74/164) and 26% (T: 0.26/56) in the control group and 75% (C: 0.75/167) and 25% (T: 0.25/53) in the

Table 1. Clinical and hormonal parameters in PCOS patients and the control women.

	Control	PCOS	p value
Age(year)	28 (18-49)	22 (18-40)	0.000*
Height (cm)	162.8±5.2	162.6 (±6.02)	0.808
Weight (kg)	65±11.9	71.3 (±15.9)	0.010*
BMI (kg/m2)	23.6 (17.6-45.4)	26.6 (17.1-41.2)	0.003*
FGS	2 (0-7)	10 (0-26)	0.000*
Glucose (mg/dL)	86.0 (70.3-110.0)	87.0 (58.0-107.0)	0.377
Insulin (µIU/mL)	6.7 (2-34.5)	10 (2-66.2)	0.000*
HOMA-IR	1.4 (0.36-7.92)	2.2 (0.3-14.1)	0.001*
TT (µg/L)	0.35 (0.12-0.47)	0.56 (0.09-1.51)	0.000*
Prolactin (ng/mL)	8.3 (2.3-33.62)	12.7 (4.6-67.9)	0.000*
DHEAS (ng/mL)	1781 (531.5-4048.2)	2898.0 (169.4-8799.0)	0.000*
LH (mIU/mL)	5.9 (1.8-30.0)	9.5 (2.0-86.8)	0.001*
FSH (mIU/mL)	6.76 (2.1-17.5)	6.0 (1.1-19.5)	0.027*
E2 (pg/mL)	63.5 (11.2-331.3)	57.9 (15.2-353.4)	0.924
TSH (µIU/mL)	1.5 (±0.82)	1.93 (±1.16)	0.002*
Progesterone (ng/mL)	0.36 (0.01-2.63)	0.6 (0.05-10.4)	0.005*

The results are presented as mean±standard deviation or median (minimum-maximum). * Shows a significant difference. BMI: Body mass index; HOMA-IR: Homeostatic model assessment of insulin resistance; DHEA-S: Dehydroepiandrosterone sulfate; TT: Total testosterone; TSH: Thyroid-stimulating hormone; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; E2: Estradiol; PCOS: Polycystic ovary syndrome.

Table 2. Frequencies of C677T genotypes and alleles in PCOS patients compared with the control women.

MTHFR C677T	Control n (%)	PCOS n (%)	p value
Genotype			
CC	57 (51.8)	57 (51.8)	0.194
CT	50 (45.5)	53 (48.2)	
TT	3 (2.7)	0 (0)	
Allele			
C	0.74 (164)	0.75 (167)	0.198
T	0.26 (56)	0.25 (53)	

PCOS: Polycystic ovary syndrome.

PCOS groups. Both the genotype distribution and allele frequencies were not significant between the study groups (p=<0.05).

Genetic Model of Inheritance Analyses

C677T variant was further analyzed using genotyping test models "dominant/recessive/additive" to have a better understanding of the genotype-phenotype association between the genes and PCOS (Table 3). Since the "TT" genotype was not determined in the PCOS group, "C" additive, "C" dominant, and "T" recessive models could not be applied.

In the "T" additive, "T" dominant, and "C" recessive models, it was found that CT vs. CC (OR: 1.06 95% CI: 0.62-1.83), CC vs. TC+TT (OR: 0.99 95% CI: 0.58-1.72), and TC+TT vs. CC (OR: 0.99, 95% CI: 0.58-1.70), respectively, did not increase the PCOS risk.

Clinical and Hormonal Parameters in Each of the C677T Genotypes

The genotypes of C677T were compared with the hormonal and clinical data of the PCOS and control group. The data of the analysis is presented in Table 4. The "TT" genotype was determined in only three volunteers; therefore, it could not be included in the statistical analysis (due to the numbers being less than five).

Our results found no significant difference between the C677T genotypes and the clinical and hormonal data from each genotype (p=<0.05). Similarly, even when the volunteers were not separated based on groups and analyzed only based on genotype, no significant differences were found between the C677T genotypes and the clinical and hormonal data of the volunteers (Table 5).

Table 3. Genetic test model analyses of C677T genotypes.

MTHFR C677T		OR (95% CI)	p value
"T" Additive model	CC	Ref.	0.972
	TC	1.06(0.62-1.83)	
	TT	ND	
"T" Dominant Model	CC	Ref.	0.988
	TC+TT	0.99 (0.58-1.70)	
"C" Recessive Model	TC+TT	Ref.	0.988
	CC	0.99 (0.58-1.72)	

ND: Not determined.

Discussion

PCOS is not a local disease; it is a syndrome and chronic systemic disease with a highly strong inheritance of up to 70% (9). Moreover, it has a multifactorial and polygenic etiology. Multifactorial diseases are probably related to the effects of multiple genes (often the genes are larger in quantity but smaller in effect) in combination along with lifestyle and environmental factors.

To understand the etiology and genetic basis of PCOS, case-control studies were carried out, which were related to the genes involved in different mechanisms, including adrenal and ovarian steroidogenesis (STAR, CYP11B1, CYP21A2, CYP19A1, CYP17A1, and VDR), the function of steroid hormones (AR, SHBG), function and regulation of gonadotropin (FSHR, LHR, GnRHR), function and secretion of insulin (INS, INSR, IRS-1, IRS-2, INSL3, and CAPN10), energy home-

Table 4. Clinical and hormonal parameters in PCOS patients and the control women having C677T genotypes.

MTHFR 677	Control			PCOS		
Genotypes	CC	CT	TT	CC	CT	TT
Age (year)	27 (18-49)	28 (18-49)	30 (28-32)	22 (18-40)	22 (18-37)	na
Height (cm)	161.5±5.3	162±5.2	167±1.4	161.5±5.5	161.7±6.6	na
Weight (kg)	64.9±12.3	63.4±11.8	57.5±0.7	71.7±15.4	68.8±16.3)	na
BMI (kg/m^2)	24 (17.6-45.4)	23.5 (18.8-34.3)	20.6 (20.2-21.1)	27 (17.8-41.2)	26.3 (17.1-39.1)	na
FGS	2 (0-7)	3 (0-8)	3 (0-6)	10 (0-22)	11 (0-26)	na
Glucose (mg/dL)	86 (70.3-110)	85 (73-110)	78 (78-78)	89 (58-107)	83 (59-106)	na
Insulin (µIU/mL)	6.9±3.9 5.7 (2-20.7)	9.2±6.7 8.1 (2-34.5)	3.2±1.7 3.2 (2-4.5)	13.6±11.2 11.6 (2-66.2)	10.4±9.1 8.59 (2-51.9)	na
HOMA-IR	1.4 (0.36-4.3)	1.6 (0.36-7.92)	0.86 (0.86-0.86)	2.4 (0.41-14.1)	1.75 (0.3-11.5)	na
TT (µg/L)	0.34 (0.12-0.47)	0.36 (0.19-0.70)	0.36 (0.34-0.38)	0.58 (0.18-1.27)	0.5 (0.09-1.51)	na
Prolactin (ng/mL)	7.3 (2.3-33.6)	8.8 (3.5-26.8)	8.2 (7.9-8.5)	13.7 (4.7-67.9)	10.7 (4.6-30.2)	na
DHEAS (ng/mL)	1825.8 (585.9-4048.2)	1635 (531.5-3952.4)	1567.4 (1207.0-1927.7)	2900.3 (1001.0-8799.0)	2889.3 (169.4-8050.0)	na
LH (mIU/mL)	4.9 (2.1-30.0)	6.9 (1.8-22)	10.5 (10.5-10.5)	10 (2.6-86.8)	8.1 (2.1-32.5)	na
FSH (mIU/mL)	6.7 (2.1-17.5)	6.8 (3.46-12.3)	7.8 (7.8-7.8)	6 (1.6-19.5)	6.1 (1.1-15.1)	na
E2 (pg/mL)	69.1 (19.6-331.3)	60.6 (11.2-244.5)	55.5 (55.5-55.5)	56.8 (15.2-300.1)	58.4 (18.7-353.4)	na
TSH (µIU/mL)	1.4 (±0.7)	1.4 (±0.9)	2.4±1.7	1.7±1.03	2.1±1.3	na
Progesterone (ng/mL)	0.35 (0.01-2.63)	0.39 (0.08-0.57)	0.34 (0.34-0.34)	0.58 (0.09-8.91)	0.63 (0.05-10.45)	na

The results are presented as mean±standard deviation or median (minimum-maximum). BMI: Body mass index; FGS: Ferriman-Gallwey score; HOMA-IR: Homeostatic model assessment of insulin resistance; DHEA-S: Dehydroepiandrosterone sulfate; TT: Total testosterone; TSH: Thyroid-stimulating hormone; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; E2: Estradiol; PCOS: polycystic ovary syndrome. NA: not applicable.

ostasis (FTO, PPARG), chronic inflammation (TNF-α, TNF-β1, IL6, IFN-γ, and IL10), oxidative stress (SOD1, SOD2, GPx, and CAT), and cardiovascular risk factors (PAI-1 and MTHFR) (9-15,42). However, incompatible results stemmed from these studies and also the populations from which the study groups were formed (9,37,43-46). A lack of globally accepted diagnostic criteria of PCOS and incomplete knowledge of its pathophysiology may be the additional reasons for the inconsistency.

In humans, the MTHFR gene is located on chromosome 1 and consists of 12 exons. It has three transcripts with the sizes of 2.2 kb, 7.5 kb, and 9.5 kb (47). This gene has fourteen nucleotide polymorphisms associated with the enzymatic deficiency (48), out of which, C677T and A1298C (rs1801131) are the most commonly reported variants that can reduce the MTHFR activity by various degrees. As opposed to the C677T variant, although the A1298C reduces the MTHFR activity, it is not related to HHcy or a lowered plasma folate concentration, neither in the homozygous nor heterozygous state (33). PCOS women are more susceptible to CAD, and HHcy is an independent risk factor for CAD. Insufficient activity of 5,10-MTHFR is one of the most common causes for a high amount of plasma levels of Hcy (16,49). The frequency of the variant differs significantly based on the population from different geographic regions (50,51). Almost 17% of the subjects with cardiovascular disease (CVD) (52) and 28% of the subjects with HHcy and premature vascular disease (53) were found to have C677T polymorphism.

The latest meta-analysis was performed with 21 articles published between the years 1995 and 2020, where the authors found that the T allele was related to an increased risk of PCOS compared to the C allele. The result indicated that C677T polymorphism was related to an elevated risk of PCOS in both homozygous and heterozygous mutant genotypes. Ethnicity dependent subgroup analyses showed that the

Table 5. Clinical and hormonal parameters of each C677T genotypes in the study group.

	Genotypes		
Genotypes	**CC**	**CT**	**TT**
Age (year)	23 (18-49)	24 (18-49)	30 (28-32)
Height (cm)	161.5±5.4	161.9±5.9	167±1.4
Weight (kg)	68.4±14.4	66.4±17.7	57.5±0.7
BMI (kg/m^2)	25.6 (17.6-45.4)	24 (17.1-39.1)	20.6 (20.2-21.1)
FGS	4 (0-22)	5 (0-26)	3 (0-6)
Glucose (mg/dL)	87 (58-110)	83 (59-110)	78 (78-78)
Insulin (µIU/mL)	7.9 (2-66.2)	8.5 (2-51.9)	3.2 (2-4.5)
HOMA-IR	1.67 (0.36-14.1)	1.7 (0.3-11.5)	0.86 (0.86-0.86)
TT (µg/L)	0.42 (0.12-1.27)	0.41 (0.09-1.51)	0.36 (0.34-0.38)
Prolactin (ng/mL)	11.28 (2.3-67.9)	10.3 (3.5-30.2)	8.2 (7.9-8.5)
DHEAS (ng/mL)	2253.5 (585.9-8799)	2151.0 (169.4-8050.0)	1567.4 (1207.0-1927.7)
LH (mIU/mL)	7.5 (2.1-86.8)	7.8 (1.8-32.5)	10.5 (10.5-10.5)
FSH (mIU/mL)	6.3 (1.6-19.5)	6.5 (1.1-15.1)	7.8 (7.8-7.8)
E2 (pg/mL)	59.1 (15.2-331.3)	58.4 (11.2-353.4)	55.5 (55.5-55.5)
TSH (µIU/mL)	1.5±0.89	1.7±1.1	2.4±1.7
Progesterone (ng/mL)	0.45 (0.05-8.91)	0.5 (0.05-10.45)	0.34 (0.34-0.34)

The results are presented as mean±standard deviation or median (minimum-maximum). BMI: Body mass index; FGS: Ferriman-Gallwey score; HOMA-IR: Homeostatic model assessment of insulin resistance; DHEA-S: Dehydroepiandrosterone sulfate; TT: Total testosterone; TSH: Thyroid-stimulating hormone; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; E2: Estradiol; PCOS: polycystic ovary syndrome. "TT" genotype was determined in only three volunteers (less than 5), so it is not included in the statistical analysis.

C677T mutant in the Middle Eastern population showed greater PCOS risk (TT+CT vs. CC: OR: 2.66, 95% CI: 1.54-4.58; CT vs. CC+TT: OR: 2.64, 95% CI: 1.27-5.49; TT vs. CC: OR: 2.21, 95% CI: 1.16-4.21; T vs. C: OR: 1.82, 95% CI: 1.39-2.37). However, such risk was not found in the Asian or Caucasian population (34). The analysis also showed that ethnicity played a significant role in the etiology of PCOS in terms of genetics. The author concluded that further studies need to be performed to explore the contribution of MTHFR C677T polymorphism toward the PCOS risk in the Turkish population since only one study was performed on the Turkish population till then. In our study, we did not find any correlation between PCOS and C677T genotype (CT vs. CC OR: 1.06 95% CI: 0.62-1.83, CC vs. TC+TT OR: 0.99 95% CI: 0.58-1.72, TC+TT vs. CC OR: 0.99, 95% CI: 0.58-170). On the contrary to our findings, Karadeniz et al. found that women with PCOS had four times the higher CT genotype, statistically higher frequency of the T allele, and a statistically higher level of Hcy compared to the healthy control although the Hcy level was not correlated with the C677T genotype. Therefore, the authors concluded that the MTHFR genotypes did not affect the plasma Hcy levels in patients with PCOS in Turkey (54). Van der Put et al. found that the C677T genotypes were associated with reduced folate and higher Hcy levels (33). The previous meta-analysis by Zhu et al. performed using 21 articles, available from the inception till 17 June 2019, found that the T allele in MTHFR C677T polymorphism could be a genetic risk factor for PCOS, especially in the Asian population (TT+CT vs. CC OR: 1.47 95% CI: 1.12-1.94, TT vs. CT+CC OR: 1.50 95% CI: 1.25-1.81). However, the evidence did not support the same association in the Caucasians (TT+CT vs. CC OR: 1.30 95% CI: 0.78-2.61, TT vs. CT+CC OR: 1.01 95% CI: 0.68-1.50) (37). Another meta-analysis by Wang et al. performed using 16 studies published before December 2016 found that the T allele was not a risk factor for PCOS (OR: 1.08; 95% CI: 0.96-1.21). Upon ethnicity-based analysis, an increased risk was found in the PCOS

in the Asian population because of the T allele (OR: 1.31; 95% CI: 1.09-1.58), but such results were not found in the Middle Eastern population (OR:1.26; 95% CI: 0.96-1.67). The authors claimed that the T allele had a protective effect against PCOS in the Caucasian population (OR: 0.82; 95% CI: 0.68-0.99). Additionally, they concluded that MTHFR C677T polymorphism might be providing diverging effects on the PCOS etiology depending on the ethnicity. HHcy can not only be seen in the case of genetic defects, but it can also be due to nutritional deficiencies (insufficient dietary intake of folate), which can be the reason for the discordant results besides ethnicity.

PCOS women from different ethnicity were presented with different clinical appearances of the syndrome. Therefore, differences in ethnicity seem to have an important effect on the disease etiology. Different genetic components may contribute differently to the disease phenotype. For instance, Caucasian patients with PCOS were unlikely to have diabetes when compared to East Asian patients (55), or the European and Maori women were more prone to hirsutism compared to the other ethnic groups (56).

No correlation was observed between the MTHFR C677T genotype and the PCOS risk in our study. Moreover, even in the current literature, there were discordant results. Clinical studies showed that HHcy levels in PCOS patients returned to normal levels upon folic acid supplementation (21,57). Therefore, in the PCOS treatment, folic acid supplementation may be considered to lower the Hcy levels, considering the possibility of HHcy in PCOS patients. The interactions between gene-environment, gene-gene, and even different alleles of the same gene may modulate PCOS risk. Hence, the investigation of polymorphisms on other genes involved in the folate metabolism may be of great importance to understand the role of folate metabolism in PCOS etiology. On the other hand, to avoid dietary interference in women who participate in such studies, it might be better to consider folate intake while designing the study. Additionally, some genes are known to have altered expression levels in PCOS (9). Since the MTHFR deficiency may cause impairment in the DNA methylation process, which is also one of the most important mechanisms in the regulation of gene expression (25), MTHFR genotypes and folate mechanisms may be considered to have an indirect effect on PCOS etiology. However, laboratory studies are needed to support this hypothesis.

Limitations

The study has several limitations; firstly TT genotypes were identified only in three volunteers. Secondly, there was a lack of information about Hcy and folic acid levels in the blood. Thirdly, the study group was formed with volunteers from the Central Anatolia region of Turkey.

Conclusion

This study demonstrated that neither T nor C allele of MTHFR C677T polymorphism contributed to PCOS risk in our study population. Further studies are required to conclude the impact of folate metabolism on PCOS etiology more accurately using a larger study group and detailed individual data.

Ethical Approval

The study was approved by the Ethics Committee of Medical School at Erzincan University. All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and/or family members of the scientific and medical committee members or members of the potential conflicts of interest, counsel-

ing, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Seher Polat; Design: Seher Polat; Control/Supervision: Seher Polat; Data Collection and/or Processing: Yasin Şimşek; Analysis and/or Interpretation: Seher Polat Yasin Şimşek; Literature Review: Yasin Şimşek, Seher Polat; Writing the Article: Seher Polat; Critical Review: Seher Polat Yasin Şimşek; Materials:Yasin Şimşek, Seher Polat.

References

1. Azziz R. Introduction: determinants of polycystic ovary syndrome. Fertil Steril. 2016;106:4-5.[PubMed]
2. Pasquali R, Gambineri A. New perspectives on the definition and management of polycystic ovary syndrome. J Endocrinol Invest. 2018;41:1123-1135.[Crossref] [PubMed]
3. Fauser BC, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, Carmina E, Chang J, Yildiz BO, Laven JS, Boivin J, Petraglia F, Wijeyeratne CN, Norman RJ, Dunaif A, Franks S, Wild RA, Dumesic D, Barnhart K. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Fertil Steril. 2012;97:28-38.e25.[Crossref] [PubMed]
4. Murri M, Luque-Ramírez M, Insenser M, Ojeda-Ojeda M, Escobar-Morreale HF. Circulating markers of oxidative stress and polycystic ovary syndrome (PCOS): a systematic review and meta-analysis. Hum Reprod Update. 2013;19:268-288.[Crossref] [PubMed]
5. Rashad NM, El-Shal AS, Abdelaziz AM. Association between inflammatory biomarker serum procalcitonin and obesity in women with polycystic ovary syndrome. J Reprod Immunol. 2013;97:232-239.[Crossref] [PubMed]
6. Behboudi-Gandevani S, Ramezani Tehrani F, Bidhendi Yarandi R, Noroozzadeh M, Hedayati M, Azizi F. The association between polycystic ovary syndrome, obesity, and the serum concentration of adipokines. J Endocrinol Invest. 2017;40:859-866.[Crossref] [PubMed]
7. Filippou P, Homburg R. Is foetal hyperexposure to androgens a cause of PCOS? Hum Reprod Update. 2017;1;23:421-432.[Crossref] [PubMed]
8. Merkin SS, Phy JL, Sites CK, Yang D. Environmental determinants of polycystic ovary syndrome. Fertil Steril. 2016;106:16-24.[Crossref] [PubMed]
9. Mykhalchenko K, Lizneva D, Trofimova T, Walker W, Suturina L, Diamond MP, Azziz R. Genetics of polycystic ovary syndrome. Expert Rev Mol Diagn. 2017;17:723-733.[Crossref] [PubMed]
10. Sóter MO, Ferreira CN, Sales MF, Candido AL, Reis FM, Milagres KS, Ronda C, Silva IO, Sousa MO, Gomes KB. Peripheral blood-derived cytokine gene polymorphisms and metabolic profile in women with polycystic ovary syndrome. Cytokine. 2015;76:227-235.[Crossref] [PubMed]
11. Branavan U, Muneeswaran K, Wijesundera S, Jayakody S, Chandrasekharan V, Wijeyaratne C. Identification of selected genetic polymorphisms in polycystic ovary syndrome in Sri Lankan women using low cost genotyping techniques. PLoS One. 2018;31;13:e0209830.[Crossref] [PubMed] [PMC]
12. Tang W, Wang Y, Jiang H, Liu C, Dong C, Chen S, Kang M, Gu H. Insulin receptor substrate-1 (IRS-1) rs1801278G>a polymorphism is associated with polycystic ovary syndrome susceptibility: a meta-analysis. Int J Clin Exp Med. 2015;15;8:17451-17460.[PubMed] [PMC]
13. Kim JJ, Choi YM, Hong MA, Chae SJ, Hwang K, Yoon SH, Ku SY, Suh CS, Kim SH. FSH receptor gene p. Thr307Ala and p. Asn680Ser polymorphisms are associated with the risk of polycystic ovary syndrome. J Assist Reprod Genet. 2017;34:1087-1093.[Crossref] [PubMed] [PMC]
14. Song X, Sun X, Ma G, Sun Y, Shi Y, Du Y, Chen ZJ. Family association study between melatonin receptor gene polymorphisms and polycystic ovary syndrome in Han Chinese. Eur J Obstet Gynecol Reprod Biol. 2015;195:108-112.[Crossref] [PubMed]
15. Polat S, Şimşek Y. Five variants of the superoxide dismutase genes in Turkish women with polycystic ovary syndrome. Free Radic Res. 2020;54:467-476.[Crossref] [PubMed]
16. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet. 1995;10:111-113.[Crossref] [PubMed]
17. Huang L, Song XM, Zhu WL, Li Y. Plasma homocysteine and gene polymorphisms associated with the risk of hyperlipidemia in northern Chinese subjects. Biomed Environ Sci. 2008;21:514-520.[Crossref] [PubMed]
18. Refsum H, Ueland PM, Nygård O, Vollset SE. Homocysteine and cardiovascular disease. Annu Rev Med. 1998;49:31-62.[Crossref] [PubMed]
19. Hankey GJ, Eikelboom JW. Homocysteine and vascular disease. Indian Heart J. 2000;52:S18-26.[PubMed]
20. Giltay EJ, Hoogeveen EK, Elbers JM, Gooren LJ, Asscheman H, Stehouwer CD. Insulin resistance is associated with elevated plasma total homocysteine levels in healthy, non-obese subjects. Atherosclerosis. 1998;139:197-198.[PubMed]
21. Badawy A, State O, El Gawad SSA, El Aziz OA. Plasma homocysteine and polycystic ovary syndrome: the missed link. Eur J Obstet Gynecol Reprod Biol. 2007;131:68-72.[Crossref] [PubMed]
22. Ratnam S, Maclean KN, Jacobs RL, Brosnan ME, Kraus JP, Brosnan JT. Hormonal regulation of cystathionine beta-synthase expression in liver. J Biol Chem. 2002;8;277:42912-42918.[Crossref] [PubMed]

23. Harmon DL, Woodside JV, Yarnell JW, McMaster D, Young IS, McCrum EE, Gey KF, Whitehead AS, Evans AE. The common 'thermolabile' variant of methylene tetrahydrofolate reductase is a major determinant of mild hyperhomocysteinaemia. QJM. 1996;89:571-577.[Crossref] [PubMed]
24. Willmott M, Bartosik DB, Romanoff EB. The effect of folic acid on superovulation in the immature rat. J Endocrinol. 1968;41:439-445.[Crossref] [PubMed]
25. Stern LL, Mason JB, Selhub J, Choi SW. Genomic DNA hypomethylation, a characteristic of most cancers, is present in peripheral leukocytes of individuals who are homozygous for the C677T polymorphism in the methylenetetrahydrofolate reductase gene. Cancer Epidemiol Biomarkers Prev. 2000;9:849-853.[PubMed]
26. Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, Wickramasinghe SN, Everson RB, Ames BN. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci U S A. 1997;1;94:3290-3295.[Crossref] [PubMed] [PMC]
27. Zhang X, Li H, Jin H, Ebin Z, Brodsky S, Goligorsky MS. Effects of homocysteine on endothelial nitric oxide production. Am J Physiol Renal Physiol. 2000;279:F671-678.[Crossref] [PubMed]
28. Starkebaum G, Harlan JM. Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. J Clin Invest. 1986;77:1370-1376.[Crossref] [PubMed] [PMC]
29. Poddar R, Sivasubramanian N, DiBello PM, Robinson K, Jacobsen DW. Homocysteine induces expression and secretion of monocyte chemoattractant protein-1 and interleukin-8 in human aortic endothelial cells: implications for vascular disease. Circulation. 2001;5;103:2717-2723.[Crossref] [PubMed]
30. Mohanty D, Das KC. Effect of folate deficiency on the reproductive organs of female rhesus monkeys: a cytomorphological and cytokinetic study. J Nutr. 1982;112:1565-1576.[Crossref] [PubMed]
31. Szymański W, Kazdepka-Ziemińska A. [Effect of homocysteine concentration in follicular fluid on a degree of oocyte maturity]. Ginekol Pol. 2003;74:1392-1396.[PubMed]
32. Loscalzo J. The oxidant stress of hyperhomocyst(e)inemia. J Clin Invest. 1996;1;98:5-7.[Crossref] [PubMed] [PMC]
33. van der Put NM, Gabreëls F, Stevens EM, Smeitink JA, Trijbels FJ, Eskes TK, van den Heuvel LP, Blom HJ. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am J Hum Genet. 1998;62:1044-1051.[Crossref] [PubMed] [PMC]
34. Xiong Y, Bian C, Lin X, Wang X, Xu K, Zhao X. Methylenetetrahydrofolate reductase gene polymorphisms in the risk of polycystic ovary syndrome and ovarian cancer. Biosci Rep. 2020;31;40: BSR20200995. [Crossref] [PubMed] [PMC]
35. Gong JM, Shen Y, Shan WW, He YX. The association between MTHFR polymorphism and cervical cancer. Sci Rep. 2018;8;8:7244.[Crossref] [PubMed] [PMC]
36. Carlus SJ, Sarkar S, Bansal SK, Singh V, Singh K, Jha RK, Sadasivam N, Sadasivam SR, Gireesha PS, Thangaraj K, Rajender S. Is MTHFR 677 C>T polymorphism clinically important in polycystic ovarian syndrome (pcos)? a case-control study, meta-analysis and trial sequential analysis. PLoS One. 2016;16;11:e0151510.[Crossref] [PubMed] [PMC]
37. Zhu X, Hong X, Chen L, Xuan Y, Huang K, Wang B. Association of methylenetetrahydrofolate reductase C677T and A1298C polymorphisms with genetic susceptibility to polycystic ovary syndrome: A PRISMA-compliant meta-analysis. Gene. 2019;30;719:144079.[Crossref] [PubMed]
38. Xu L, Qin Z, Wang F, Si S, Li L, Lin P, Han X, Cai X, Yang H, Gu Y. Methylenetetrahydrofolate reductase C677T polymorphism and colorectal cancer susceptibility: a meta-analysis. Biosci Rep. 2017;7;37: BSR20170917. [Crossref] [PubMed] [PMC]
39. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, Janssen OE, Legro RS, Norman RJ, Taylor AE, Witchel SF; Task Force on the Phenotype of the Polycystic Ovary Syndrome of The Androgen Excess and PCOS Society. The androgen excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. Fertil Steril. 2009;91:456-488.[Crossref] [PubMed]
40. Hatch R, Rosenfield RL, Kim MH, Tredway D. Hirsutism: implications, etiology, and management. Am J Obstet Gynecol. 1981;1;140:815-830.[Crossref] [PubMed]
41. Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, Monauni T, Muggeo M. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care. 2000;23:57-63.[Crossref] [PubMed]
42. Lin S, Huiya Z, Bo L, Wei W, Yongmei G. The plasminogen activator inhibitor-1 (PAI-1) gene -844 A/G and -675 4G/5G promoter polymorphism significantly influences plasma PAI-1 levels in women with polycystic ovary syndrome. Endocrine. 2009;36:503-9.[Crossref] [PubMed]
43. Unsal T, Konac E, Yesilkaya E, Yilmaz A, Bideci A, Ilke Onen H, Cinaz P, Menevse A. Genetic polymorphisms of FSHR, CYP17, CYP1A1, CAPN10, INSR, SERPINE1 genes in adolescent girls with polycystic ovary syndrome. J Assist Reprod Genet. 2009;26:205-216.[Crossref] [PubMed] [PMC]
44. Prapas N, Karkanaki A, Prapas I, Kalogiannidis I, Katsikis I, Panidis D. Genetics of polycystic ovary syndrome. Hippokratia. 2009;13:216-223.[PubMed] [PMC]
45. Polat S, Karaburgu S, Unluhizarci K, Dündar M, Özkul Y, Arslan YK, Karaca Z, Kelestimur F. The role of androgen receptor CAG repeat polymorphism in androgen excess disorder and idiopathic hirsutism. J Endocrinol Invest. 2020;43:1271-1281.[Crossref] [PubMed]

46. Polat S, Karaburgu S, Ünlühizarcı K, Dündar M, Özkul Y, Arslan YK, Karaca Z, Kelestimur F. Comprehensive genotyping of Turkish women with hirsutism. J Endocrinol Invest. 2019;42:1077-1087. [Crossref] [PubMed]
47. Tran P, Leclerc D, Chan M, Pai A, Hiou-Tim F, Wu Q, Goyette P, Artigas C, Milos R, Rozen R. Multiple transcription start sites and alternative splicing in the methylenetetrahydrofolate reductase gene result in two enzyme isoforms. Mamm Genome. 2002;13:483-492.[Crossref] [PubMed]
48. Liew SC, Gupta ED. Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism: epidemiology, metabolism and the associated diseases. Eur J Med Genet. 2015;58:1-10.[Crossref] [PubMed]
49. Gudnason V, Stansbie D, Scott J, Bowron A, Nicaud V, Humphries S. C677T (thermolabile alanine/valine) polymorphism in methylenetetrahydrofolate reductase (MTHFR): its frequency and impact on plasma homocysteine concentration in different European populations. EARS group. Atherosclerosis. 1998;136:347-354.[Crossref] [PubMed]
50. Kang SS, Zhou J, Wong PW, Kowalisyn J, Strokosch G. Intermediate homocysteinemia: a thermolabile variant of methylenetetrahydrofolate reductase. Am J Hum Genet. 1988;43:414-421.[PubMed] [PMC]
51. Motulsky AG. Nutritional ecogenetics: homocysteine-related arteriosclerotic vascular disease, neural tube defects, and folic acid. Am J Hum Genet. 1996;58:17-20. Erratum in: Am J Hum Genet 1996;58:648.[PubMed] [PMC]
52. Kang SS, Wong PW, Susmano A, Sora J, Norusis M, Ruggie N. Thermolabile methylenetetrahydrofolate reductase: an inherited risk factor for coronary artery disease. Am J Hum Genet. 1991;48:536-545.[PubMed] [PMC]
53. Engbersen AM, Franken DG, Boers GH, Stevens EM, Trijbels FJ, Blom HJ. Thermolabile 5,10-methylenetetrahydrofolate reductase as a cause of mild hyperhomocysteinemia. Am J Hum Genet. 1995;56:142-150.[PubMed] [PMC]
54. Karadeniz M, Erdogan M, Zengi A, Eroglu Z, Tamsel S, Olukman M, Saygili F, Yilmaz C. Methylenetetrahydrofolate reductase C677T gene polymorphism in Turkish patients with polycystic ovary syndrome. Endocrine. 2010;38:127-133.[Crossref] [PubMed]
55. Kim JJ, Choi YM. Phenotype and genotype of polycystic ovary syndrome in Asia: ethnic differences. J Obstet Gynaecol Res. 2019;45:2330-2337.[Crossref] [PubMed]
56. Williamson K, Gunn AJ, Johnson N, Milsom SR. The impact of ethnicity on the presentation of polycystic ovarian syndrome. Aust N Z J Obstet Gynaecol. 2001;41:202-206.[Crossref] [PubMed]
57. Bayraktar F, Dereli D, Ozgen AG, Yilmaz C. Plasma homocysteine levels in polycystic ovary syndrome and congenital adrenal hyperplasia. Endocr J. 2004;51:601-608.[Crossref] [PubMed]