Scholars LITERATURE

La Prensa Medica Argentina

Research Article

DOI: https://doi.org/10.47275/0032-745X-213
Volume 106 Issue 3

Methylenetetrahydrofolate Reductase C677T Polymorphisms as a Risk Factor for Polycystic Ovarian Syndrome in a Sample of Jordanian Women

Azzam OA[1]*, Mahgoub SS[2,3], Alrawashdeh HM[4], Farhan SS[5], Al-Kharabsheh AM[6], Ramadan BK[7], Ghoul I[8] and Abd El-Kareem HM[9,10]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Mutah University, Jordan
[2]Department of Biochemistry, Molecular Biology, Faculty of Medicine, Mutah University, Jordan
[3]Department of Biochemistry & Molecular Biology, Faculty of Medicine, Al-Minia University, Egypt
[4]Department of Ophthalmology, Ibn-Alhaytham Hospital, Jordan
[5]Department of Basic Sciences, Faculty of Pharmacy, Al-Rafidain University College, Iraq.
[6]Department of Obstetrics and Gynecology, College of Medicine, Mutah University, Jordan.
[7]Faculty of Medicine, Mutah University, Jordan.
[8]Department of Pediatric, Ibn-Alhaytham Hospital, Jordan.
[9]Department of Biochemistry and Molecular Biology, Faculty of Medicine, Mutah University, Jordan. 10Department of Biochemistry and Molecular Biology, Benha Faculty of Medicine, Egypt

Abstract

Background: Polycystic ovary syndrome (PCOS) is a common endocrine reproductive disorder, it can be identified by hyperandrogenism, oligomenorrhea or anovulation and polycystic ovaries on ultrasound. Methylenetetrahydrofolate Reductase (MTHFR) C677T polymorphisms associated with hyperhomocysteinemia are among the risk factors for PCOS.

Objective: The present case control study aims to explore the relationship between Methylenetetrahydrofolate Reductase (MTHFR) C677T polymorphisms as a risk factor and PCOS among Jordanian patients suffering from this disease.

Methods: 306 subjects (146 PCOS patients and 160 healthy subjects as a control group) were enrolled in the study. DNA was extracted from venous blood sample withdrawn from each participant for analyzing MTHFR C677T polymorphisms using Polymerase Chain Reaction (PCR) in combination with restriction enzyme fragment length polymorphism (PCR-RFLP). Later, PCR-RFLP products were digested with hinfI enzyme, then, electrophoresed on a 2% agarose gel, stained and examined under UV light. Plasma homocysteine levels were assayed using ELISA method.

Results: A significant difference was observed in plasma homocysteine levels among PCOS patients versus the control subjects and in between the different polymorphisms of PCOS patients. No significant difference was detected in the distribution and allelic frequency of MTHFR C677T polymorphisms in PCOS patients compared to the controls. 677/TT genotype and T allele were associated with 1.54 and 1.46 folds increase in the susceptibility for PCOS.

Conclusion: The study has shown that MTHFR T677T polymorphism and T allele are possible risk factors for PCOS among Jordanian women and may play a role in the pathogenesis of the disease.

Keywords: Homocysteine; Polycystic Ovarian Syndrome; Hyperandrogenism; MTHFR C677T Polymorphisms; Risk factor; PCR-RFLP

Correspondence to: Omar A Azzam, Department of Obstetrics and Gynecology, Faculty of Medicine, Mutah University, Jordan, E-mail: oabuazzam@yahoo.com

Citation: Azzam OA, Mahgoub SS, Alrawashdeh HM, et al. (2020) Methylenetetrahydrofolate Reductase C677T Polymorphisms as a Risk Factor for Polycystic Ovarian Syndrome in a Sample of Jordanian Women. Prensa Med Argent (Genetics), Volume 106:3. 213. DOI: https://doi.org/10.47275/0032-745X-213

Received: February 27, 2020; **Accepted:** March 03, 2020; **Published:** April 02, 2020

Introduction

According to Polycystic Ovary Syndrome (PCOS) Consensus Workshop Group criteria, PCOS is the most common heterogeneous endocrine disorder among women throughout the reproductive life in agreement with those of the National Institute of Health for diagnosis of PCOS, it is characterized by a confirmed hyperandrogenism by clinical, laboratory investigations and an ultrasound image revealing polycystic ovaries with the exclusion of the other pathologies having similar characters, not having excess androgen secretion or those who are not showing typical ultrasonographic evidence of polycystic ovaries [1]. It is defined as a multifactorial disorder with various metabolic, endocrine, environmental and partly genetic factors. However, more than a hundred candidate genes have been investigated to play significant roles in the pathogenesis of PCOS on one hand and in improving the diagnosis and treatment of the disease on the other hand [2].

The full manifestation of PCOS can be detected at adolescence when the hypothalamic-pituitary-gonadal axis becomes functioning, which is associated with some metabolic changes. The metabolic changes are associated with fat distribution within the body that increases insulin levels as a result of the increased impact of circulating androgens and stimulation of steroidogenesis in ovaries [3]. Such a condition of increased insulin in women with PCOS resulting in hyperandrogenism and anovulation. Overweight girls with insulin-resistance are subjected to early adrenal overactivity and PCOS at adulthood [3].

PCOS is also known as a familial disorder to be inherited from one generation to the next one. The genetic effects on PCOS pathogenesis include those revealing high familial heritability of the disease [4]. Recently, various loci associated with PCOS risk had been identified by genome-wide association studies (GWASs) [5] that included; acute regulatory gene of steroidogenesis polymorphisms, gonadotropin-releasing hormone receptor gene polymorphisms, follicle-stimulating hormone receptor gene polymorphisms, insulin receptor polymorphisms, vitamin D3 receptor polymorphisms, methylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms [6].

MTHFR is the enzyme that catalyzes the conversion of 5, 10-methylenetetrahydrofolate into 5-methyltetrahydrofolate for the remethylation of homocysteine into methionine [7]. Few studies have investigated the effect of low folates levels in inhibiting the ovulation in immature super-ovulated rats and the degradation of Griffin follicles with an increased number of cystic follicles in female rhesus monkeys [8]. Among humans, PCOS and the high plasma homocysteine level are correlated, which can be corrected by supplementing the patients with folic acid [9].

MTHFR C677T polymorphism (cytosine to thymine transition at nucleotide 677 resulting in an alanine to valine substitution at codon 222) are involved in regulating folate metabolism and affecting homocysteine pathway, furthermore was associated with 50% decrease in the activity of the enzyme (thermolabile MTHFR) and elevated levels of plasma homocysteine [10].

This is suggesting MTHFR as a candidate gene for increasing the risk for PCOS [11]. Homocysteine is a cysteine homolog, which can be converted into methionine or cysteine for the reactions that require B-complex vitamins especially B6. There is an inverse relationship between high plasma and follicular fluid levels of homocysteine, oocyte, and embryo quality, suppressing the expression of the gene encoding for elongation factor 2 (E2) which participates in the elongation stage of protein synthesis resulting in impaired follicle growth and oocyte maturation among the PCOS patients [12].

More recently, a meta-analysis was performed to evaluate the effect of MTHFR C677T polymorphisms as risk factors for PCOS, the results obtained from the analysis of 1478 PCOS cases from 16 different ethnicities showed that the MTHFR C667T genotypes had variable effects that may lead to increase the risk of PCOS [13].

The present study aims to explore the possible correlation between MTHFR C677T polymorphisms as a risk factor and PCOS among the Jordanian women having the disease. To the best of our knowledge, this is the first time for such a study to be conducted in Jordan.

Methodology

Subject

A case-control study was conducted on 146 Jordanian patients with PCOS, who attended the Gynecology and Obstetrics outpatient clinics of the Health center, Mutah University, and Al-Karak Governmental Hospital, Jordan. Rotterdam consensus criteria [14] was applied to confirm the diagnosis of PCOS. 160 normal healthy women, age-matched, free of menstrual cycle disturbances with no PCOS symptoms, no history of autoimmune or endocrine disorders and no surgery in the pelvis were enrolled in the study as a control group.

Exclusion criteria

All patients with diabetes mellitus, hypertension, thyroid diseases, Cushing,s syndrome, ovarian tumors, acromegaly, and oral contraceptive pills use during the last 6 months prior to the study were excluded.

Blood sampling

A 5 ml venous blood sample was withdrawn from each participant in the study in EDTA treated tubes after overnight fasting. Plasma was separated immediately by centrifugation at 1800 xg for 15 minutes, then stored at -20°C for homocysteine assay. The whole EDTA blood was used for DNA extraction.

Assay of plasma homocysteine concentration

It was done by commercially Enzyme-Linked Immunosorbent Assay (ELISA) supplied by Diagnostic Automation/USA according to the method of Engvall E, et al. (1977) [15].

Analysis of MTHFR C677T polymorphisms

DNA was extracted from all samples using the method of Sambrook J, et al. (2006) [16]. They were analyzed by the PCR method coupled with the restriction enzyme fragment length polymorphism (PCR-RFLP) as described by Jacques PF, et al. (1996) (17). The sequences of primers for MTHFR C677T polymorphisms were as follows: sense-5'-TGA AGG AGA AGG TGT CTG CGG GA -3' and antisense-5'- AGG ACG GTG CGG TGA GAG TG -3'. These primers amplified 198 bp fragment of DNA. The PCR began with an initial denaturation at 94°C for two minutes, then, 35 cycles of denaturation at 94°C for one minute, annealing at 62°C for one minute, and extension at 72°C for one minute. The final extension was at 72°C for 10 minutes. The amplicons were digested with 5 units of hinfI restriction enzyme (Promega, Madison, WI, USA) at 37°C for two hours. The digested amplicons were separated by electrophoresis on 2% agarose gel, stained with ethidium bromide. Later, the amplicons were visualized using a UV transilluminator, which resulted in a 198 bp band for the homozygous wild type (C677C), 175 bp and 23 bp bands for the heterozygous mutants (C677T), and 198, 175 and 23 bp bands for the homozygous mutants (T677T) of MTHFR C677T polymorphisms. The 23 bp band was not seen because of its small size.

Statistical analysis

Data were analyzed using SPSS statistics version 21 software (IBM Corp. Armonk, NY). The numerical data were expressed as mean ± SD and the difference between groups was assessed using ANOVA. The qualitative data were expressed as frequency, percentage, and odds ratio. The possible connection between the two variables was assessed using Pearson's χ^2. Allele and genotype frequencies were determined by allele counting. The concordance of the polymorphisms frequencies with the Hardy-Weinberg equilibrium was evaluated using Pearson's χ^2.

Results

The results have shown significant difference between the mean

values of plasma homocysteine level in the group of PCOS patients when compared to the control subjects (Table 1). The distribution of MTHFR C677T polymorphisms and the frequency of C and T alleles have been shown in (Table 2).

Regarding the prevalence of MTHFR C677T polymorphisms, there was no significant difference between PCOS patients compared to the control subjects, there was an increased prevalence of the heterozygous polymorphism (677/CT) in the PCOS group. No statistically significant association between the different polymorphisms was detected in PCOS patients; however, the homozygous genotype (677/TT) and T allele were associated with increased risk for PCOS (OR=1.54 and 1.46, respectively) (Table 3). A statistically significant association was found between the mean homocysteine level of PCOS patients with the different MTHFR C677T polymorphisms (p=0.039).

Discussion

PCOS is the most prevalent multifactorial endocrine pathology, associated with infertility, obesity, and insulin resistance caused by complex interactions between environmental and predisposing polygenic background [18]. However, the heterogeneity and the inability to realize the exact etiology and pathophysiology of PCOS have made it difficult to identify the candidate genes with understandable clinical significance involved in causing PCOS [19]. HapMap project of the human genome was used to describe the pattern of human genetic variation, especially those affecting health. Several candidate genes play a role in PCOS development, including genes involved in homocysteine-methionine metabolism particularly, the MTHFR gene [20].

Table 1: Mean value ± SD of the age and the plasma homocysteine level among PCOS patients and the controls.

	PCOS patients (no. 146)	Control group (no. 160)	P value
Age	26.1 ± 2.34	26.9 ± 3.01	0.655
Homocysteine (µmol/l)	23.2 ± 0.877	6.42 ± 0.532	<0.001*

*P<0.001 is significant versus the control subjects.

Table 2: MTHFR C677T polymorphisms distribution and allele frequency between PCOS patients and the controls.

Polymorphism/ allele	PCOS patients (no. 146)	Control (no. 160) OR (95% CI)		P vaule
T677T	49 (33.56)	78 (48.75)	1.54 (1.11-2.56)	0.43
C677T	67 (45.89)	52 (32.50)	0.78 (0.89-1.78)	0.66
C677C	30 (20.55)	30 (18.75)	0.72 (0.91-1.74)	0.71
C	127 (43.49)	124 (38.75)	0.89 (0.87-1.94)	0.86
T	165 (56.51)	196 (61.25)	1.46 (1.13-1.91)	0.39

Table 3: Association between the mean values of plasma homocysteine levels among PCOS patients with the different types of MTHFR C677T polymorphisms.

Polymorphisms	Plasma homocysteine level (µmol/l) (Mean ± SD)	P value
677/CC	18.32 ± 2.01	-
677/CT	24.31 ± 1.93	0.039*
677/TT	18.73 ± 1.66	-

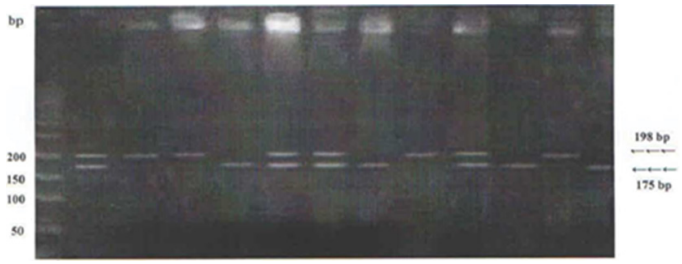

Figure 1: PCOS patients with the different MTHFR C677T polymorphisms.

The present study has investigated the level of plasma homocysteine as a consequence for MTHFR C677T polymorphisms resulting in a reduction of enzyme activity among the patients with PCOS. The results have revealed a statistically significant difference in plasma homocysteine levels among PCOS patients as compared to the control subjects. Hyperhomocysteinemia is associated with atherogenesis and chronic vascular damage leading to arterial stiffness [21], which could be a risk factor for PCOS. Li D, et al. (2018) showed that serum homocysteine levels were greater among PCOS patients as compared to control subjects, which provides evidence for playing the role of impaired liver function in PCOS [22-24] have shown that the mean homocysteine levels were significantly higher among PCOS patients as compared to the healthy control subjects. The treatment of hyperhomocysteinemia in those patients is essential to improve reproductive functions. Gallardo T, et al. (2007) reported an increase in the levels of homocysteine in PCOS patients, which is negatively associated with the levels of folic acid [25].

In the present study, the comparison of homocysteine levels of PCOS patients within different MTHFR C677T polymorphisms revealed a statistically significant difference (p=0.039), which is confirmed by the study of [26]. The genetic variants of MTHFR could affect the metabolism of folate and homocysteine resulting in elevated plasma levels of homocysteine, and playing an important role in PCOS pathogenesis [27]. Reported in a study including women with PCOS and healthy controls that the polymorphisms of MTHFR C677T do not influence the serum levels of homocysteine and PCOS development [28].

The evaluation of MTHFR C677T polymorphisms frequency between the two study groups in our study revealed no statistically significant difference. In the PCOS group, there was an increased frequency of the heterozygous polymorphism (677C/T), but no statistically significant association between C677T genotype and the increase in the susceptibility for PCOS (OR=0.78). The homozygous genotype (T677T) and T allele had elevated risk of PCOS when compared to the other genotypes (C677T and C677C) and C allele (OR=1.54 and 1.46, respectively). Those findings are consistent with the results of [10], who reported statistically insignificant difference among the different MTHFR C677T polymorphisms in PCOS patients with a slightly higher prevalence of heterozygous (C677T) polymorphism among the women with PCOS.

The genetic association studies are essential as they provide a clear idea about the role MTHFR C677T polymorphism in the pathogenesis of PCOS [29]. An Indian study shows a relative risk of PCOS (OR=1.32) for the heterozygous (C677T) genotype. Another study found a relatively high risk for PCOS [30]. The majority of the studies did not find any significant association between PCOS and MTHFR C677T polymorphisms [28]. It was noticed that high plasma levels of homocysteine were observed among PCOS patients; however, the presence of MTHFR C677T polymorphisms was not found to affect the high levels of homocysteine [30]. Palep-Singh M, et al. (2001) found that Caucasian women with PCOS had higher plasma levels of homocysteine and a 1.9 times higher frequency of the T allele as compared to the South Asian PCOS group [31].

The obtained results in the present study revealed that the frequency was higher for the heterozygous model C677T than the other two models

C677C and T677T which is inconsistent with the results obtained by Naghavi A, et al. (2015) who reported that the homozygous model C677C was higher in prevalence among Iranian patients with PCOS when compared to the other two models [18]. Wang L, et al. (2017) studied the association between MTHFR C677T polymorphisms and the risk of PCOS, the study results revealed that the T allele was not significantly associated with the risk of PCOS [13]. The analysis of the association by ethnicity showed that the T allele significantly increases the risk of PCOS among the Asian population. There was no association for the Middle Eastern population and interestingly the T allele was found to be protective against PCOS among the Caucasian population. Moreover, the combined genotypes form (CT+TT) was significantly associated with increased risk of PCOS among the Asian and Middle Eastern populations.

The results of the study of Carlus SJ, et al. (2016) had shown no association between MTHFR C667T polymorphisms and the susceptibility of PCOS [32]. which was inconsistent with the obtained results of Fu L, et al. (2014) who reported C667T polymorphism as a risk factor for PCOS [33]. Qi Q, et al. (2015) found that MTHFR C677T genetic mutation could influence the occurrence of PCOS risk among the Chinese population [34]. Jain M, et al. (2012) stated that the CT genotype of MTHFR C677T was associated with a 1.32-fold increase in the risk of developing PCOS [10]. Choi SW, et al. (2009) indicated that there was no correlation between the MTHFR C677T polymorphism and PCOS among the Korean population [29]. Among the Turkish population, Karadeniz M, et al. (2010) reported that MTHFR C677T gene variants have no effect on plasma homocysteine levels in PCOS patients [30]. The inconsistency of these results may be due to differences in ethnicities, selection of study subjects, and sample size.

Limitations of the study

The current findings mare limited by the relatively small study subjects, other factors that may play various roles in developing the disease including the environment, behavior, and diet were not evaluated, also, PCOS patients and the controls were selected from a health center and a hospital. Therefore multicenter studies should be included with recruiting more study subjects for verifying the obtained results in the current study and environmental-genetic interactions should be considered.

Conclusion

The study has investigated the correlation between MTHFR C677T polymorphisms as risk factors and PCOS among Jordanian patients suffering from this disease. There was a statistically significant difference (p=0.039) in homocysteine levels of PCOS patients with different genotypes. The results showed the association between homozygote mutant (TT) and T allele of MTHFR C677T polymorphism and increasing the susceptibility for PCOS among Jordanian women.

References

1. Franks S (2006) Diagnosis of polycystic ovarian syndrome: in defense of the Rotterdam criteria. J Clin Endocrinol Metab 91: 786-789.
2. Jones MR, Goodarzi MO (2016) Genetic determinants of polycystic ovary syndrome: progress and future directions. Fertil Steril 106: 25-32.
3. Lewy VD, Danadian K, Witchel SF, Arslanian S (2001) Early metabolic abnormalities in adolescent girls with polycystic ovarian syndrome. J Pediatr 138: 38-44.
4. Vink JM, Sadrzadeh S, Lambalk CB, Boomsma DI (2006) Heritability of polycystic ovary syndrome in a Dutch twin-family study. J Clin Endocrinol Metab 91: 2100-2104.
5. Lee H, Oh JY, Sung YA, Chung H, Kim HL, et al. (2015) Genome-wide association study identified new susceptibility loci for polycystic ovary syndrome. Hum Reprod 30: 723-731.
6. Chen Y, Fang S (2018) Potential genetic polymorphisms predicting polycystic ovary syndrome. Endocr Connect 7: R187-R195.
7. Reilly R, McNulty H, Pentieva K, Strain JJ, Ward M (2014) MTHFR 677TT genotype and disease risk: is there a modulating role for B-vitamins? Proc Nutr Soc 73: 47-56.
8. Mohanty D, Das KC (1982) Effect of folate deficiency on the reproductive organs of female rhesus monkeys: a cytomorphological and cytokinetic study. J Nutr 112: 1565-1576.
9. Kazerooni T, Asadi N, Dehbashi S, Zolghadri J (2008) Effect of folic acid in women with and without insulin resistance who have hyperhomocysteinemic polycystic ovary syndrome. Int J Gynecol Obstet 101: 156-160.
10. Jain M, Pandey P, Tiwary NK, Jain S (2012) MTHFR C677T polymorphism is associated with hyperlipidemia in women with polycystic ovary syndrome. J Hum Reprod Sci 5: 52-56.
11. Szafarowska M, Segiet A, Jerzak MM (2016) Methylenotetrahydrololate reductase A1298C and C677T polymorphisms and adverse pregnancy outcome in women with PCOS. Neuroendocrinol Lett 37: 141-146.
12. Qiao J, Feng HL (2011) Extra-and intra-ovarian factors in polycystic ovary syndrome: impact on oocyte maturation and embryo developmental competence. Hum Reprod Update 17: 17-33.
13. Wang L, Xu W, Wang C, Tang M, Zhou Y (2017) Methylenetetrahydrofolate reductase C677T polymorphism and the risks of polycystic ovary syndrome: an updated meta-analysis of 14 studies. Oncotarget 8: 59509-59517.
14. Fauser BCJM, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, et al. (2012) Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Fertil Steril 97: 28-38.
15. Engvall E, Jonsson K, Perlmann P (1971) Enzyme-linked immunosorbent assay. II. Quantitative assay of protein antigen, immunoglobulin G, by means of enzyme-labelled antigen and antibody-coated tubes. Biochim Biophys Acta (BBA)-Protein Struct 251: 427-434.
16. Sambrook J, Russell DW, Sambrook J (2006) The condensed protocols: from molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press Cold Spring Harbor, USA.
17. Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, et al. (1996) Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. Circulation 93: 7-9.
18. Naghavi A, Mozdarani H, Garshasbi M, Yaghmaei M (2015) Prevalence of methylenetetrahydrofolate reductase C677T polymorphism in women with polycystic ovary syndrome in southeast of Iran. J Med Life 8: 229-232.
19. Franks S, McCarthy M (2004) Genetics of ovarian disorders: polycystic ovary syndrome. Rev Endocr Metab Disord 5: 69-76.
20. Dumesic DA, Abbott DH (2008) Implications of polycystic ovary syndrome on oocyte development. Semin Reprod Med 26:53-61.
21. Stracquadanio M, Ciotta L, Palumbo MA (2018) Effects of myo-inositol, gymnemic acid, and L-methylfolate in polycystic ovary syndrome patients. Gynecol Endocrinol 34: 495-501.
22. Li D, Liu HX, Fang YY, Huo JN, Wu QJ, et al. (2018) Hyperhomocysteinemia in polycystic ovary syndrome: decreased betaine-homocysteine methyltransferase and cystathionine β-synthase-mediated homocysteine metabolism. Reprod Biomed Online 37: 234-241.
23. Agilli M, Aydin FN, Cayci T, Kurt YG (2014) Homocysteine levels in Indian women with Polycystic Ovary Syndrome. J Clin diagnostic Res JCDR 8: CL01.
24. Salehpour S (2011) Evaluation of homocysteine levels in patients with polycystic ovarian syndrome. Int J Fertil Steril 4: 168-171.
25. Gallardo T, Diestro MD, Hernanz A, Pérez E, Fernández-Miranda C (2007) Increased homocysteine levels in polycystic ovary syndrome. Med Clin (Barc) 129: 292-294.
26. Grodnitskaya EE, Kurtser MA (2012) Homocysteine metabolism in polycystic ovary syndrome. Gynecol Endocrinol 28: 186-189.
27. Santilli F, Davi G, Patrono C (2016) Homocysteine, methylenetetrahydrofolate reductase, folate status and atherothrombosis: A mechanistic and clinical perspective. Vascul Pharmacol 78: 1-9.

28. Orio Jr F, Palomba S, Di Biase S, Colao A, Tauchmanova L, et al. (2003) Homocysteine levels and C677T polymorphism of methylenetetrahydrofolate reductase in women with polycystic ovary syndrome. J Clin Endocrinol Metab 88: 673-679.

29. Choi SW, Gu BH, Ramakrishna S, Park JM, Baek KH (2009) Association between a single nucleotide polymorphism in MTHFR gene and polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol 145: 85-88.

30. Karadeniz M, Erdogan M, Zengi A, Eroglu Z, Tamsel S, et al. (2010) Methylenetetrahydrofolate reductase C677T gene polymorphism in Turkish patients with polycystic ovary syndrome. Endocrine 38: 127-133.

31. Palep-Singh M, Picton HM, Yates ZR, Barth J, Balen AH (2007) Polycystic ovary syndrome and the single nucleotide polymorphisms of methylenetetrahydrofolate reductase: a pilot observational study. Hum Fertil 10: 33-41.

32. Carlus SJ, Sarkar S, Bansal SK, Singh V, Singh K, et al. (2016) 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 11: e0151510.

33. Fu L, Dai L, Li X, Zhang K, Bai Y (2014) Association of methylenetetrahydrofolate reductase gene C677T polymorphism with polycystic ovary syndrome risk: a systematic review and meta-analysis update. Eur J Obstet Gynecol Reprod Biol 172: 56-61.

34. Qi Q, Zhang H, Yu M, Wang X, Wang Z, et al. (2015) Association of methylenetetrahydrofolate reductase gene polymorphisms with polycystic ovary syndrome. Chinese J Med Genet 32: 400-404.