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Association between a single nucleotide polymorphism in *MTHFR* gene and polycystic ovary syndrome

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ABSTRACT

Objective: The aim of the present study was to investigate whether there is an association between the C677T polymorphism in *MTHFR* and PCOS in a Korean population.
Study design: The prevalence of *MTHFR* gene was compared between women with PCOS ($n = 227$) and normal patients ($n = 115$) using restriction fragment length polymorphism (RFLP) analysis. The HapAnalyzer was used to analyze the genotype of *MTHFR* polymorphism in PCOS and control subjects. We considered a p-value less than 0.05 as statistically significant.
Results: The frequency of *C*/*C*, *C*/*T*, and *T*/*T* genotype showed similar proportion between PCOS and control subjects. In addition, the frequencies of co-dominant (p-value = 0.8334, odds ratio (OR) = 1.04), dominant (p-value = 0.8749, OR = 0.96) and recessive alleles (p-value = 0.5574, OR = 1.22) did not show any association between PCOS and control subjects.
Conclusion: Our data demonstrate that the C677T polymorphism of *MTHFR* gene is not associated with PCOS in a Korean population, suggesting that the C677T polymorphism in *MTHFR* may have different influences in various ethnic groups and diseases.

1. Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine–metabolic disorder, affecting 5–10% of reproductive age women. According to the American Society for Reproductive Medicine/European Society for Human Reproduction and Embryology (ASRM/ESHRE) Rotterdam consensus in 2003, polycystic ovaries, oligo/anovulation or hyperandrogenism are often features of PCOS [1–4]. Like other endocrine–metabolic disorders, PCOS is also affected by environmental and genetic factors. Therefore, it is difficult to construe the pathological milieu of PCOS. It is well known that obesity and insulin resistance are reported as major symptoms in patients with PCOS [5]. Several studies were focused on the association between PCOS and genetic factors related to insulin resistance and type 2 diabetes mellitus such as *INSR* [1]. In addition, a high risk of premature coronary artery disease (CAD) has been reported in patients with PCOS [6]. However, the exact etiology has not yet been fully identified. To identify the pathogenesis for CAD in PCOS patients, several genetic studies have been performed relating to risk factors of CAD [7,8]. The risk factors associated with CAD are smoking, diabetes, family history, and increased homocysteine levels in PCOS [9]. Among the risk factors, it was reported that the homocysteine level is highly related with insulin resistance in PCOS patients [10].

Homocysteine (Hcy) is produced in the conversion of methionine, and it is maintained at low levels (5–15 μmol/l) in the plasma. Many genetic factors including vitamin B_{12} and folate are involved in the regulation of Hcy metabolism pathway [11]. In elevated plasma homocysteine concentrations, the transmethylation of homocysteine to methionine is regulated by *methylenetetrahydrofolate reductase* (*MTHFR*), and its deficiency causes hyperhomocysteinemia [12].

MTHFR is an important regulatory enzyme in folate and homocysteine metabolism, which is necessary for a number of biological cellular mechanisms [12,14]. The methionine supplies the methyl groups for the formation of DNA and protein methylation [13]. Furthermore, the C677T missense variant in the *MTHFR*, changing from alanine to valine residue, results in perturbation in normal enzymatic activities and leads to high homocysteine and low folate levels in the plasma [14–18].

Although the mechanism is unknown at present, strong association has been found between Hcy levels and CAD, which

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is found often in PCOS women. The correlation provokes possible association between the C677T mutation in *MTHFR* and PCOS [19,20]. Also, it has been reported that the C677T variant of *MTHFR* gene decreased the stimulation of ovary and contributes to early menopause [17]. Recently, Palep-Singh and colleagues performed a study to identify the association between the prevalence of the C677T and A1298C single nucleotide polymorphisms (SNPs) in the *MTHFR* gene and folate and Hcy metabolism in Caucasian and South Asian women. The result suggests that the mechanisms may behave independently in the two different ethnic groups [21]. However, the association has not been investigated in any other ethnic groups. The goal of this study was to investigate whether there is an association between the C677T polymorphism in *MTHFR* and PCOS in a Korean population.

2. Materials and methods

2.1. Subjects

All study subjects were recruited from Fertility Center of CHA General Hospital in Seoul, Korea. The study included 227 PCOS patients and 115 healthy Korean women as case and control groups based on the revised diagnostic criteria according to the 2003 ASRM/ESHRE Rotterdam consensus [3,4]. Table 1 shows the clinical and biochemical characteristics of women with PCOS and control groups. The control group was healthy on the basis of medical history, complete blood chemistry, pelvic and physical examination, but they had faced problems in pregnancy due to infertility of their partners. The diagnosis of the disease was determined by medical history, physical and pelvic examination, and complete blood chemistry. For the diagnostic criteria of PCOS, oligomenorrhea was defined as a reduction in the frequency of menses with intervals between 40 days and 6 months and hyperandrogenism was defined as serum testosterone (T) >0.6 ng/ml and/or serum DHEA-S ≥300 μg/dl [22,23].

Blood samples for molecular genetic study were collected in tubes containing EDTA as an anticoagulant and were stored at 4 °C. Genomic DNA was then extracted from the blood of women with PCOS and normal control groups in Korean women. This SNP study with human blood samples were approved by the Institutional Review Board (IRB).

2.2. Biochemical determinations

Blood samples from PCOS patient and control groups were analyzed for plasma FSH, LH, E_2, prolactin, TSH, DHEA-S, and T [3,4].

2.3. Genetic analysis

The C677T variant of *MTHFR* was amplified using a forward primer 5′-TGAAGGAGAAGGTGTCTGCGGGA-3′ and a reverse primer 5′-AGGACGGTGCGGTGAGAGTG-3′ by polymerase chain reaction (PCR). Cycling parameters were as follows: denaturation at 94 °C for 5 min, 30 cycles at 94 °C for 30 s, 65.5 °C for 30 s, 72 °C for 30 s, and finally at 72 °C for 5 min.

The PCR products were digested with HinfI (New England Biolabs, Beverly, MA, USA) for 3 h at 37 °C. The single 198-bp band from the digested product indicates homozygosity for the *C* allele. The two fragments, 175- and 23-bp bands indicate homozygosity for the *T* allele, and the presence of three fragments, 198-, 175- and 23-bp bands indicates heterozygosity for the *C* allele and the *T* allele.

2.4. Statistical analysis

Statistical analysis was performed using HapAnalysis [1,2]. Logistic regression and test were used to analyze the association between two groups. A $p < 0.05$ value was considered statistically significant.

3. Results

For the diagnostic criteria of PCOS, we followed the instructions of the ASRM/ESHRE Rotterdam consensus in 2003 [3,4]. Accordingly, PCOS is diagnosed in patients who showed phenotypes of any two of the three criteria including oligo- or amenorrhea, clinical or biochemical hyperandrogenism and ultrasonographic ovarian morphology (Fig. 1). The clinical and biochemical features of patients showed significant difference between PCOS and control groups in the level of LH, TSH, DHEA-S and testosterone (Table 1). Among PCOS patients, 31 (13.65%) patients had hyperandrogenism and oligo- or amenorrhea, 22 (9.69%) patients had hyperandrogenism and polycystic ovary, 152 (66.97%) patients had oligo- or amenorrhea and polycystic ovaries, and 22 (9.69%) showed hyperandrogenism, oligo- or amenorrhea and polycystic ovaries (Fig. 1). A SNP in *MTHFR* gene at a nucleotide 677 leads to change in amino acid from alanine to valine. This variation was identified by a restriction digestion method using HinfI enzyme (Fig. 2A). Therefore, we investigated the frequency of three bands: 198-, 175-, and 23-bp obtained in PCOS samples using restriction fragment length polymorphism (RFLP) analysis (Fig. 2B).

For the genotypic analysis of the SNP in C677T *MTHFR* gene, we recruited 227 PCOS and 115 control samples. In this study, the frequency of *C*/*C*, *C*/*T*, and *T*/*T* genotype showed similar proportion

Table 1
Clinical and biochemical characteristics of PCOS patients (n = 227) and normal controls (n = 115).

	PCOS patient group		Control group
No.	227		115
Body mass index (kg/m^2)	22.96 ± 3.86 (16.36–37.32)		20.95 ± 2.49 (16.40–28.65)
Waist/hip ratio (WHR)	0.82 ± 0.06 (0.69–0.95)		0.80 ± 0.05 (0.70–0.91)
FSH levels (mIU/ml)	5.40 ± 1.10 (3.20–8.49)		6.42 ± 1.82 (3.00–11.50)
LH levels (mIU/ml)	6.05 ± 3.46 (1.00–17.02)		3.30 ± 1.62 (1.00–7.10)
		p-Value < 0.001	
E_2 levels (pg/ml)	33.17 ± 15.19 (9.10–81.40)		36.93 ± 34.58 (5.00–219.90)
Prolactin levels (pg/ml)	10.63 ± 3.80 (2.30–20.90)		13.24 ± 7.48 (4.10–46.40)
TSH levels (μIU/ml)	2.10 ± 1.08 (0.46–5.47)		1.88 ± 0.94 (0.03–4.06)
		p-Value = 0.629	
DHEA-S levels (μg/dl)	194.07 ± 75.54 (45.30–377.20)		153.03 ± 56.46 (67.20–257.40)
		p-Value = 0.011	
Testosterone (mg/ml)	0.38 ± 0.21 (0.06–0.86)		0.21 ± 0.14 (0.01–0.54)
		p-Value < 0.001	

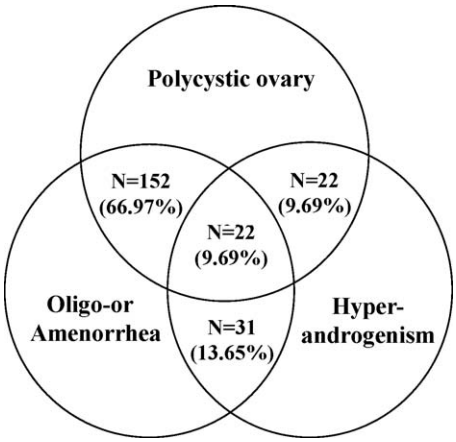

Fig. 1. Diagnostic criteria for PCOS patients consist of three symptoms: oligo- or amenorrhea, hyperandrogenism, and polycystic ovary (PCO). According to the criteria of the 2003 ESHRE/ASRM Rotterdam consensus, PCOS patients were enrolled when subjects were diagnosed with two out of three symptoms.

between PCOS and control subjects (Table 1). In addition, the frequencies of co-dominant (p-value = 0.8334, odds ratio (OR) = 1.04), dominant (p-value = 0.8749, OR = 0.96) and recessive alleles (p-value = 0.5574, OR = 1.22) did not show any association between PCOS and control subjects (Table 2). Thus, our present study indicates that a C677T variant of *MTHFR* is not associated with PCOS patients in a Korean population.

4. Comment

A number of SNPs in the *MTHFR* gene are known to be associated with various multifactorial disorders [19]. Aberration in *MTHFR* gene, substituting alanine to valine at nucleotide 677 causes change in enzymatic activity [19]. Moreover, homozygote state of *MTHFR* mutation leads to an elevated level of circulating Hcy [24]. A recent study has shown that there was a high association between Hcy and CVD in women with PCOS and a high Hcy level is related to the prevalence of CVD in PCOS women [25]. According to earlier studies, Koreans, along with Indian, Western European, and German populations, have shown association between the C677T in the *MTHFR* gene and male fertility [24,26,27]. Previously, the prevalence of the C677T and A1298C SNPs in the *MTHFR* gene was compared in Caucasian and South Asian women [21]. The study showed that PCOS recruits with the variant *T* allele had higher Hcy concentrations but among PCOS women there was no significant difference in C677T SNPs and Hcy levels between the Caucasian and South Asian groups. Interestingly, the study also revealed that *C*/*T* heterozygote at base position 677 and *A*/*A* homozygote at position 1298 had significantly higher values of Hcy levels in Caucasian women. In South Asian women, a significant observation was that the *T* allele is confined only in PCOS group with *C*/*T* heterozygote but not in *T*/*T* homozygote at base position 677 [21].

Table 2
Allele frequencies of C677T *MTHFR* gene in PCOS group (n = 227) and control group (n = 115).

	PCOS patient group	Control group
Genotype		
CC	67 (29.5%)	33 (28.7%)
CT	125 (55.1%)	67 (58.26%)
TT	35(15.4%)	15 (13.04%)
Co-dominant	OR (95% CI) = 1.04 (0.75–1.42), p-value = 0.8334	
Dominant	OR (95% CI) = 0.96 (0.59–1.58), p-value = 0.8749	
Recessive	OR (95% CI) = 1.22 (0.63–2.33), p-value = 0.5574	
Total	OR (95% CI) = 1.03 (0.75–1.42), p-value = 0.9105	

Furthermore, *MTHFR* polymorphism has been analyzed for Korean patients with various diseases. In Korean patients with ischemic stroke, it was reported that the plasma Hcy level was elevated by influence of MTR 2756A > G (methionine synthase) and MTHFR 677C > T [28]. In cases of osteonecrosis of the femoral head (ONFH), an ischemic injury, Korean patients were influenced by MTHFR C667T polymorphism [29]. On the other hand, Korean patients with adult acute leukemia were not associated with SNPs in *MTHFR* gene [30].

Therefore, this study aimed at investigating the association between the C677T of *MTHFR* gene and women with PCOS in a Korean population. However, our data showed no significant association between the C677T polymorphism in *MTHFR* and PCOS in this population. We did not study homocysteine levels in order to identify the relation between *MTHFR* polymorphism and homocysteine levels due to the difficulty in sample processing. Although the C677T variant of MTHFR is not associated with PCOS in a Korean population, development of therapeutic approaches is very necessary for PCOS patients. This study may be underpowered, but these data with genetic association studies will provide insights into the roles of *MTHFR* gene in the pathological milieu of PCOS in different ethnic groups and

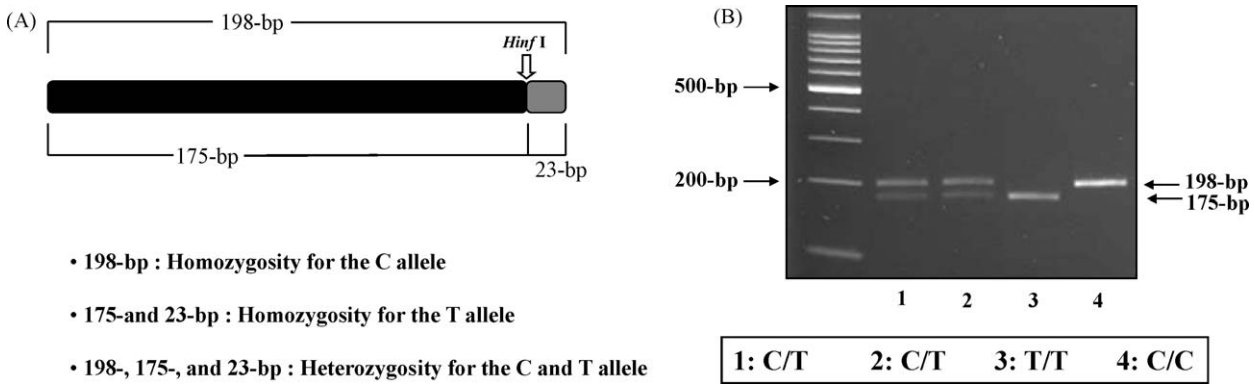

Fig. 2. (A) Structure of the *MTHFR* gene. Arrow indicates the restriction of the *MTHFR* gene. When the sequence has the *T* allele, Hinfl restricts the site. The products are three fragments, 198-, 175-, and 23-bp. The *C* allele makes only one fragment, 198-bp. (B) The *C*/*T* polymorphism in the *MTHFR* gene. The *T* allele has been restricted by Hinfl. Shown is 2% agarose gel electrophoresis with ethidium bromide staining following Hinfl digestion of the PCR product. The single band with 198-bp indicates a *C*/*C* genotype. Homozygosity of *T* allele has two bands with 175- and 23-bp. However, 23-bp band is not detected on the 2% agarose gel. The *C*/*T* type is indicated by three bands with 198-, 175-, and 23-bp.

diseases. Consequently, our association study may provide significant insight for investigating larger populations of PCOS patients in various ethnic groups.

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