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Role of methylenetetrahydrofolate reductase C677T and A1298C polymorphisms in polycystic ovary syndrome risk

J.B. Wu[1], J.F. Zhai[2] and J. Yang[3]

[1]Department of Perinatal Medical Center, Xuzhou Central Hospital, Xuzhou, Jiangsu, China
[2]Department of Obstetrics and Gynecology, Xuzhou Central Hospital, Xuzhou, Jiangsu, China
[3]People's Hospital of Wuhan University, Reproductive Medicine Center, Wuhan, Hubei, China

Corresponding author: J.F. Zhai
E-mail: zhangsw_zsw@126.com / jingyang559@126.com

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ABSTRACT. Polycystic ovary syndrome is one of the most frequently encountered endocrine malfunctions. Methylenetetrahydrofolate reductase (MTHFR) plays a vital role in folate metabolism, DNA methylation, and RNA synthesis. We carried out a study to investigate the association between *MTHFR* C677T and A1298C genetic variations and the risk of polycystic ovary syndrome in a Chinese population. We recruited 244 patients and 257 control subjects from an Inner Mongolian Medical University to this hospital-based, case-control study. The genotyping of the *MTHFR* C677T and A1298C polymorphisms was carried out using polymerase chain reaction coupled with restriction fragment length polymorphism. Using multiple logistic regression

analysis, we found that the TT genotype and the T allele of *MTHFR* C677T carriers showed increased risk of polycystic ovary syndrome compared with the wild-type genotype or allele carriers. The adjusted ORs for the TT genotype and the T allele of *MTHFR* C677T were 1.84 (1.05-3.26) and 1.38 (1.06-1.81), respectively. Subjects carrying the CC genotype (OR = 3.98, 95%CI = 1.60-11.23) and the C allele (OR = 1.46, 95%CI = 1.07-2.00) of *MTHFR* A1298C had an elevated risk of polycystic ovary syndrome compared with the AA genotype and A allele carriers. In conclusion, our study suggests that the *MTHFR* C677T and A1298C polymorphisms may have contributed to the risk of polycystic ovary syndrome in the Chinese women investigated. Further research involving a greater number of individuals is warranted to confirm our results.

Key words: *MTHFR*; C677T; A1298C; Polycystic ovary syndrome; Polymorphism

INTRODUCTION

Polycystic ovary syndrome is one of the most frequently encountered endocrine malfunctions, and is characterized by menstrual abnormalities, hair growth, obesity, high blood insulin, and insulin resistance. The main clinical manifestations of the disease are irregular menstruation (or even amenorrhea) and multicystic ovaries (Legro, 2003; Chang, 2004). It is estimated that 15% of women of reproductive age suffer from polycystic ovary syndrome. Long-term polycystic ovary syndrome is accompanied by complications such as type 2 diabetes and cardiovascular disease, and most patients eventually die from cardiovascular diseases (Wild, 1995; Holte et al., 1998; Chambers and Kooner, 2001). The etiology of polycystic ovary syndrome is not clearly understood, and it is caused by multiple environmental and lifestyle factors such as obesity, adrenal dysfunction, and hyperprolactinemia (Goodarzi et al., 2011; Zuo et al., 2016). However, hereditary factors such as insulin receptor substrate-1 (*IRS-1*), insulin-like factor 3, melatonin receptor gene, vitamin D receptor gene, peripheral blood-derived cytokine gene, and follicle-stimulating hormone receptor gene also contribute to the development of polycystic ovary syndrome (Dasgupta et al., 2015; Qiu et al., 2015; Song et al., 2015; Sóter et al., 2015; Tang et al., 2015; Shaikh et al., 2016).

Methylenetetrahydrofolate reductase (MTHFR) plays a vital role in folate metabolism, DNA methylation, and RNA synthesis (Bai et al., 2009; Muslumanoglu et al., 2009). MTHFR irreversibly catalyzes the conversion of 5, 10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, which is the main form of folic acid in plasma and tissues, and is involved in the conversion of homocysteine into S2 adenosine methionine. S2 adenosine methionine plays an important role in DNA methylation, nucleic acid synthesis, and metabolism. Genetic polymorphisms in the *MTHFR* gene can change the expression and activity of the protein it encodes (Jacques and Desreux, 1996; Bagley and Selhub, 1998), and therefore play an important role in the development of several kinds of diseases, such as breast cancer (Zhang et al., 2015). Two common genetic polymorphisms have been observed in *MTHFR*: C677T and A1298C. To date, several studies have investigated the correlation between *MTHFR* C677T and A1298C genetic polymorphisms and the development of

polycystic ovary syndrome, but the results are contradictory (Palep-Singh et al., 2007; Choi et al., 2009; Karadeniz et al., 2010; Jain et al., 2012; Qi et al., 2015). We carried out a study to investigate the association between *MTHFR* C677T and A1298C genetic variations and the risk of polycystic ovary syndrome in a Chinese population.

MATERIAL AND METHODS

Subjects

We recruited 244 patients and 257 control subjects from Xuzhou Central Hospital and Inner Mongolia Medical University between January 1, 2014 and May 30, 2015 to this hospital-based, case-control study. Polycystic ovary syndrome was newly diagnosed based on the criteria proposed by the Rotterdam PCOS consensus in 2004 (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004). Patients who had high blood prolactin, late-onset adrenal cortex hyperplasia, primary decreased ovarian reserve, premature ovary failure, malignant tumor, or who used exogenous androgen were excluded from this study.

Between January 2014 and May 2015, the control subjects were women of reproductive age who received regular gynecological health examinations in outpatient clinics. Women with any history of polycystic ovary syndrome, malignant tumors, or gynecological or endocrine diseases were excluded as controls. The clinical characteristics of all patients and controls were selected from medical records, including clinical stage, age, age of menarche, age of menopause, tobacco smoking, alcohol consumption, body mass index, and family history of polycystic ovary syndrome. All the polycystic ovary syndrome patients and control subjects signed an informed consent before enrollment, and the performance of our study was approved by the Ethics Committee of Xuzhou Central Hospital and the Affiliated Hospital of Inner Mongolia Medical University.

DNA extraction and genotyping

Each study subject was asked to provide a 5-mL peripheral blood sample for DNA extraction. The samples were stored in vacuum tubes coated with 5% and kept at -20°C until required. The genomic DNA was extracted using a DNA Blood Mini Kit (Tiangen Biotech, Beijing, China). The genotyping of the *MTHFR* C677T and A1298C polymorphisms was carried out using polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism. The primer sequences, restriction enzymes, and digestive fragments of *MTHFR* C677T and A1298C are provided in Table 1. The PCR cycles for *MTHFR* C677T were as follows: an initial denaturation at 95°C for 3 min; followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 45 s, and extension at 72°C for 60 s; and a final extension at 72°C for 10 min. The PCR cycles for *MTHFR* A1298C were as follows: an initial denaturation at 92°C for 2 min; followed by 35 cycles of denaturation at 92°C for 60 s, annealing at 60°C for 60 s, and extension at 72°C for 60 s; and a final extension at 72°C for 10 min. A 4% agarose gel glue product was used to examine the PCR products and analyze the enzyme digestion.

Statistical analysis

The Student *t*-test was used to compare the differences between baseline and clinical information, as well as the genotype frequencies of *MTHFR* between polycystic ovary

syndrome patients and controls. Deviation from the Hardy-Weinberg equilibrium of *MTHFR* C677T and A1298C in the controls was analyzed using the chi-square test (χ^2). Multiple logistic regression analyses were used to estimate the relationship between *MTHFR* C677T and A1298C polymorphisms and polycystic ovary syndrome risk. The adjusted odds ratio (ORs) and 95% confidence intervals (95%CIs) were used to describe the results. The statistical analyses were conducted using the SPSS software version 16.0 (SPSS, Inc., Chicago, IL, USA). $P < 0.05$ was considered a statistically significant difference.

Table 1. Primers sequences, restriction enzymes and digestive fragments of *MTHFR* C677T and A1298C.

MTHFR	Primer sequences (5'-3')	PCR products	Restriction enzymes	Digestive fragments
C677T	Forward: TGAAGGAGAAGGTGTCTGCGGA Reverse: AGGACGGTGGGTGAGAGTG	198 bp	*Hin*fI	TT: 175 and 23 bp CT: 198, 175, and 23 bp CC: 198 bp
A1298C	Forward: CTTTGGGGAGCTGAAGGACTACTAC Reverse: CACTTTGACCATTCCGGTTTG	163 bp	*Mbo*II	CC: 84, 31 and 30 bp AC: 84, 56, 30, and 28 bp AA: 56, 31, 30, and 28 bp

RESULTS

Compared with those of the control subjects, patients with polycystic ovary syndrome had higher values of body mass index ($t = 8.96$, $P < 0.001$), HOMA-IR ($t = 13.30$, $P < 0.001$), luteinizing hormone ($t = 14.80$, $P < 0.001$), T-testosterone ($t = 16.73$, $P < 0.001$), fasting plasma glucose ($t = 34.94$, $P < 0.001$), fasting insulin ($t = 18.20$, $P < 0.001$), total cholesterol ($t = 11.20$, $P < 0.001$), and low-density lipoprotein (LDL)-cholesterol ($t = 11.20$, $P < 0.001$); and lower levels of progesterone ($t = 6.07$, $P < 0.001$) and high-density lipoprotein (HDL)-cholesterol ($t = 3.18$, $P = 0.001$) (Table 2). The polycystic ovary syndrome patients were comparable with the control subjects in terms of age ($t = 0.79$, $P = 0.22$), follicle-stimulating hormone ($t = 0.48$, $P = 0.31$), prolactin ($t = 0.96$, $P = 0.17$), estradiol ($t = 1.02$, $P = 0.15$), and triglyceride ($t = 0.35$, $P = 0.36$).

Table 2. Association between baseline characteristics of polycystic ovary syndrome patients and controls.

Variables	Patients (N = 244)	Controls (N = 257)	*t*-test	P value
Age, years	26.45 ± 2.43	26.62 ± 2.40	0.79	0.22
Body mass index, kg/m^2	24.14 ± 3.71	21.20 ± 3.63	8.96	<0.001
HOMA-IR	2.61 ± 1.55	1.17 ± 0.76	13.30	<0.001
Luteinizing hormone, mIU/L	11.55 ± 5.86	5.63 ± 2.54	14.80	<0.001
Follicle stimulating hormone, mIU/L	7.61 ± 2.03	7.52 ± 2.10	0.48	0.31
Prolactin, mIU/L	14.31 ± 6.15	14.83 ± 5.96	0.96	0.17
Progestone, ng/mL	0.58 ± 0.24	1.42 ± 2.15	6.07	<0.001
Estradiol, ng/mL	83.77 ± 46.72	79.64 ± 43.56	1.02	0.15
T-testosterone, ng/mL	0.71 ± 0.33	0.31 ± 0.19	16.73	<0.001
Fasting plasma glucose, mM	5.75 ± 0.42	4.27 ± 0.52	34.94	<0.001
Fasting insulin, mM	12.64 ± 5.74	5.26 ± 2.97	18.20	<0.001
Triglyceride, mM	1.34 ± 0.66	1.32 ± 0.63	0.35	0.36
Total cholesterol, mM	5.14 ± 1.03	4.25 ± 0.73	11.20	<0.001
LDL-cholesterol, mM	3.06 ± 0.75	2.89 ± 0.67	2.68	0.004
HCL-cholesterol, mM	1.45 ± 0.36	1.54 ± 0.27	3.18	0.001

The chi-square test revealed a significant difference between the two study groups in terms of the genotype frequencies of *MTHFR* A1298C ($\chi^2 = 11.01$, $P = 0.004$), whereas no significant difference was observed in relation to *MTHFR* C677T ($\chi^2 = 5.57$, $P = 0.07$) (Table 3). The genotype distributions of *MTHFR* C677T ($\chi^2 = 1.43$, $P = 0.23$) and A1298C ($\chi^2 = 0.90$, $P = 0.34$) did not deviate from the Hardy-Weinberg equilibrium in the controls.

Table 3. Genotype distributions of *MTHFR* C677T and A1298C of the two study groups.

MTHFR	Patients (N = 244)	%	Controls (N = 257)	%	χ^2 test	P value	χ^2 for HWE	P value
C677T								
CC	94	38.52	122	47.08				
CT	106	43.44	104	40.86				
TT	44	18.03	31	12.06	5.57	0.07	1.43	0.23
A1298C								
AA	143	58.61	166	64.59				
AC	77	31.56	84	32.68				
CC	24	9.84	7	2.72	11.01	0.004	0.90	0.34

HWE: Hardy-Weinberg equilibrium.

Using multiple logistic regression analysis, we observed that the TT genotype and the T allele of *MTHFR* C677T carriers showed increased risk of polycystic ovary syndrome compared with the wild-type genotype or allele carriers (Table 4). The adjusted ORs for the TT genotype and the T allele of *MTHFR* C677T were 1.84 (1.05-3.26) and 1.38 (1.06-1.81), respectively. Subjects with the CC genotype (OR = 3.98, 95%CI = 1.60-11.23) and the C allele (OR = 1.46, 95%CI = 1.07-2.00) of *MTHFR* A1298C had an elevated risk of polycystic ovary syndrome in comparison with the AA genotype and the A allele carriers.

Table 4. Relationship between *MTHFR* C677T and A1298C polymorphisms and polycystic ovary syndrome risk.

MTHFR	Patients (N = 244)	%	Controls (N = 257)	%	Adjusted OR (95%CI)[1]	P value
C677T						
CC	94	38.52	122	47.08	Reference	
CT	106	43.44	104	40.86	1.32 (0.89-1.97)	0.15
TT	44	18.03	31	12.06	1.84 (1.05-3.26)	0.02
Allele						
C	294	60.25	348	67.51	Reference	
T	194	39.75	166	32.49	1.38 (1.06-1.81)	0.01
A1298C						
AA	143	58.61	166	64.59	Reference	
AC	77	31.56	84	32.68	1.06 (0.71-1.59)	0.75
CC	24	9.84	7	2.72	3.98 (1.60-11.23)	0.001
Allele						
A	363	74.39	416	80.93	Reference	
C	125	25.61	98	19.07	1.46 (1.07-2.00)	0.01

[1]Adjusted for body mass index, HOMA-IR, luteinizing hormone, T-testosterone, fasting plasma glucose, fasting insulin, total cholesterol, LDL-cholesterol, and lower levels of progestone and HCL-cholesterol.

DISCUSSION

We carried out a clinical study to investigate the relationship between *MTHFR* C677T and A1298C polymorphisms and susceptibility to polycystic ovary syndrome risk in a female Chinese population. We found that individuals harboring the TT genotype and the T allele of *MTHFR* C677T and the CC genotype and the C allele of *MTHFR* A1298C had an increased risk of polycystic ovary syndrome compared with individuals harboring the wild-type genotype or allele.

Single nucleotide polymorphisms (SNPs) are DNA sequence polymorphisms caused by a single nucleotide variation, and the frequency of genetic polymorphisms is at least 1% in a population. The mutations include the transformation of a single base by transversion, insertion, or deletion, and SNPs are thought to affect susceptibility to human diseases (De

Gobbi et al., 2006; Keeling, 2008). Genetic variations could influence the function of the MTHFR protein, thereby affecting susceptibility to diseases.

A high level of plasma homocysteine is associated with the risk of polycystic ovary syndrome (Loverro et al., 2002; Yilmaz et al., 2008; Salehpour et al., 2011). Loverro et al. (2002) carried out a study on 53 women with polycystic ovary syndrome and 20 healthy subjects, and found higher mean plasma homocysteine concentrations in the polycystic ovary syndrome patients (10.4 ± 4.4 ng/dL) compared with the healthy women (7.2 ± 1.5 ng/dL). Salehpour et al. (2011) carried out a study on 85 polycystic ovarian syndrome patients and 83 healthy controls, and discovered an increased level of plasma homocysteine in patients with polycystic ovary syndrome. Yilmaz et al. (2008) reported that the level of serum homocysteine is associated with increased risk of polycystic ovary syndrome in Turkish women. Moreover, the authors of previous experimental studies have reported that genetic variations in *MTHFR* may influence the metabolism of folate and homocysteine in humans, and may be associated with high plasma homocysteine levels (Park and Chang, 2014; Santilli et al., 2016). Therefore, the genetic variations in *MTHFR* could play an important role in the pathogenesis of polycystic ovary syndrome.

The authors of previous studies have reported an association between *MTHFR* genetic polymorphisms and the development of polycystic ovary syndrome, but the results are contradictory (Orio et al., 2003; Palep-Singh et al., 2007; Choi et al., 2009; Karadeniz et al., 2010; Jain et al., 2012; Qi et al., 2015). Qi et al. (2015) carried out a study on 115 polycystic ovary syndrome patients and 58 fertile women in China, and reported that the *MTHFR* C677T mutation can influence the occurrence of polycystic ovary syndrome risk, whereas the *MTHFR* A1298C mutation is not associated with the onset of the disease. Jain et al. (2012) carried out a study in an Indian population, and reported that the CT genotype of *MTHFR* C677T confers a 1.32-fold risk of developing polycystic ovary syndrome. However, the authors of other studies have reported contradictory results. Orio et al. (2003) carried out a study on 70 young women with polycystic ovary syndrome and 70 healthy women, and reported that the *MTHFR* C677T polymorphism did not influence serum homocysteine levels and the development of polycystic ovary syndrome. Choi et al. (2009) reported that the *MTHFR* C677T polymorphism is not correlated with polycystic ovary syndrome in the Korean population. Karadeniz et al. (2010) suggested that *MTHFR* C677T gene variations do not affect homocysteine levels in patients with polycystic ovary syndrome in Turkish women. These inconsistent results may be caused by differences in ethnicity, selection of study subjects, and sample size.

In summary, our study suggests that the *MTHFR* C677T and A1298C polymorphisms may have contributed to the risk of polycystic ovary syndrome in the Chinese women investigated. Further research involving a greater number of individuals is warranted to confirm our results.

Conflicts of interest

The authors declare no conflict of interest.

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