Int J Clin Exp Pathol 2016;9(8):8532-8537
www.ijcep.com /ISSN:1936-2625/IJCEP0026962

Original Article

Role of methylenetetrahydrofolate reductase genetic polymorphisms in polycystic ovary syndrome risk

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Received February 18, 2016; Accepted August 1, 2016; Epub August 1, 2016; Published August 15, 2016

Abstract: We carried out a study to investigate the association between *MTHFR* C677T and A1298C genetic variations and risk of polycystic ovary syndrome. With a hospital-based case-control design, 175 patients and 236 control subjects were recruited in Reproductive Medical Center of Henan provincial People's Hospital between January 2014 and March 2015. The genotyping of the *MTHFR* C677T and A1298C was carried out using polymerase chain reaction (PCR)-coupled with restriction fragment length polymorphism (RFLP). A significant difference was observed between the two study groups in terms of the genotype frequencies of *MTHFR* C677T (χ^2=15.23, P<0.001). Using multiple logistic regression analysis, we observed that the CT (OR=1.65, 95% CI=1.03-2.65) and TT (OR=2.84, 95% CI=1.61-5.02) genotypes of *MTHFR* C677T and A1298C carriers showed increased risk to polycystic ovary syndrome when compared to the CC genotype carriers. Subjects with T allele of *MTHFR* C677T had an elevated risk of polycystic ovary syndrome in comparison to the C allele (OR=1.80, 95% CI=1.35-2.40). However, no significant was observed between *MTHFR* A1298C genotype and allele polymorphisms and risk of polycystic ovary syndrome. In summary, our study suggests that the *THFR* C677T may contribute to polycystic ovary syndrome risk in the Chinese women under investigation. Further research employing a greater number of study subjects is greatly required to corroborate our results.

Keywords: *MTHFR*, C677T, A1298C, polymorphism, polycystic ovary syndrome

Introduction

Polycystic ovary syndrome is one of the most encountered endocrine malfunctions. The type 2 diabetes and cardiovascular diseases complications would occur due to long term suffering from polycystic ovary syndrome, and most patients eventually die of cardiovascular diseases [1-4]. 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 [5, 6]. However, hereditary factors contribute to the development of polycystic ovary syndrome, 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 [7-12].

Methylene tetrahydrofolate reductase (MTHFR) plays a vital role in folate metabolism, and MTHFR is an important part for the DNA methylation and RNA synthesis [13, 14]. The *MTHFR* catalyzes an irreversible change of -5,10-methlenetetrahydrofolate (THF) into 5-methylenetetrahydrofolate. The 5-methylenetetrahydrofolate is the main form of folic acid in plasma and tissues, and is involved in the process of homocysteine into S2 adenosine methionine. S2 adenosine methionine plays an important role in the process of DNA methylation and nucleic acid synthesis as well as metabolism processes. The genetic polymorphisms in *MTHFR* could change the enzyme expression and activity of this protein [15]. Two common genetic polymorphisms were observed in *MTHFR*, including C677T and A1298C. Currently, several studies have been investigated the correlation between *MTHFR* C677T and A1298C genetic polymor-

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	*Hinf*I	TT: 175 bp and 23 bp CT: 198 bp, 175 bp and 23 bp CC: 198 bp
A1298C	Forward: CTTTGGGGAGCTGAAGGACTACTAC Reverse: CACTTTGACCATTCCGGTTTG	163 bp	*Mbo*II	CC: 84 bp, 31 bp, 30 bp AC: 84 bp, 56 bp, 30 bp and 28 bp AA: 56 bp, 31 bp, 30 bp and 28 bp

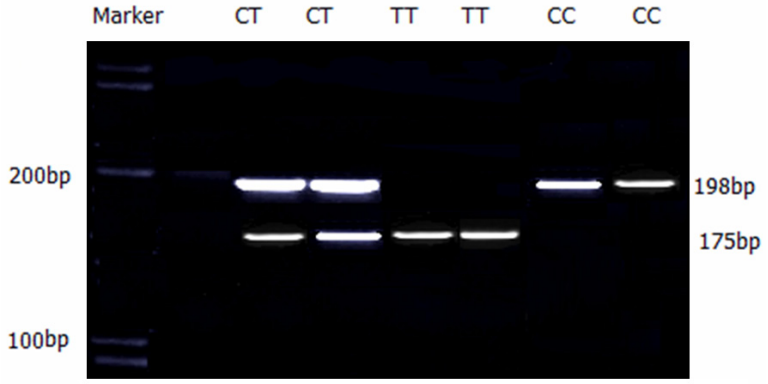

Figure 1. Agarose gel electrophoresis images for *MTHFR* C677T. TT: 175 bp and 23 bp; CT: 198 bp, 175 bp and 23 bp; CC: 198 bp.

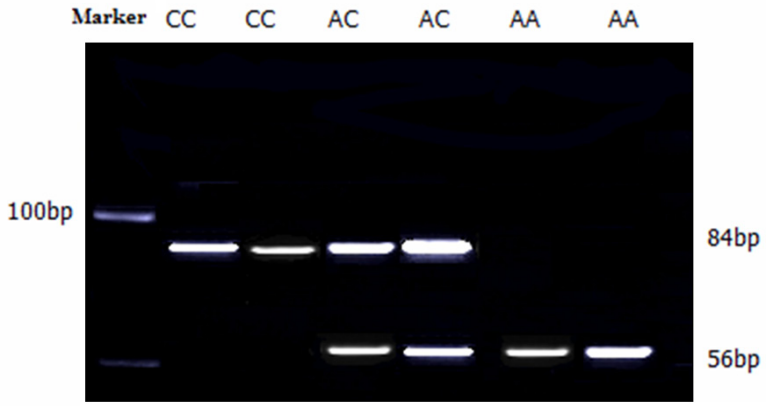

Figure 2. Agarose gel electrophoresis images for MTHFR C677T. CC: 84 bp, 31 bp, 30 bp; AC: 84 bp, 56 bp, 30 bp and 28 bp; AA: 56 bp, 31 bp, 30 bp and 28 bp.

phisms and development of polycystic ovary syndrome, but the results are conflicting [16-19]. In our study, we carried out a study to investigate the association between *MTHFR* C677T and A1298C genetic variations and risk of polycystic ovary syndrome in a Chinese population.

Patients and methods

Study subjects

With a hospital-based case-control design, 175 patients and 236 control subjects were recruited in Reproductive Medical Center of Henan provincial People's Hospital between January 2014 and March 2015. Polycystic ovary syndrome was newly diagnosed based on the criteria of Rotterdam PCOS consensus in 2004 [20]. Patients who had primary decreased ovarian reserve, premature ovary failure, and malignant tumor were excluded from this study.

Between January 2014 and March 2015, the control subjects were women of reproductive age who received regular health gynecologic examination in outpatient clinics. Women with any history of polycystic ovary syndrome, malignant tumors, gynecological diseases and 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. Our study was approved by the ethics committee of Reproductive Medical Center of Henan provincial People's Hospital.

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

Variables	Patients N=175	Controls N=236	t test	*P* value
Age, years	26.50±2.43	26.84±2.31	1.44	0.07
Body mass index, kg/m^2	24.35±3.64	20.65±3.75	10.01	<0.001
HOMA-IR	2.54±1.70	1.23±0.54	11.11	<0.001
Luteinizing hormone, mIU/L	10.77±7.58	4.52±1.83	12.20	<0.001
Follicle stimulating hormone, mIU/L	7.56±2.05	7.42±1.83	0.73	0.23
Prolactin, mIU/L	14.25±6.45	14.65±5.70	0.66	0.25
Progestone, ng/ml	0.51±0.36	1.38±2.32	4.92	<0.001
Estradiol, ng/ml	84.64±50.41	80.53±45.53	0.85	0.20
T-testosterone, ng/ml	0.66±0.36	0.22±0.15	16.91	<0.001
Fasting plasma glucose, mmol/L	5.64±0.31	4.13±0.56	32.19	<0.001
Fasting insulin, mmol/L	11.23±6.35	5.24±2.53	13.16	<0.001

DNA extraction and genotyping

Each study subject was asked to provide five ml peripheral blood sample for DNA extraction, and the blood samples were stored in vacuum tubes with 5% EDTA and kept at -20°C until using. The genomic DNA was extracted using DNA Blood Min Kit (Tiangen Biotech, Beijing, China). The genotyping of the *MTHFR* C677T and A1298C was carried out using polymerase chain reaction (PCR)-coupled with restriction fragment length polymorphism (RFLP). The primers sequences, restriction enzymes and digestive fragments of *MTHFR* C677T and A1298C were described in **Table 1** and **Figures 1**, **2**. The PCR cycles for *MTHFR* C677T were conducted as follows: an initial denaturation at 95°C for 3 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 45 seconds, extension at 72°C for 60 seconds, and a final extension at 72°C for 10 minutes; for the *MTHFR* A1298C, the reaction began at 92°C for 2 minutes, then 35 cycles of denaturation at 92°C for 60 seconds, annealing at 60°C for 60 seconds, extension at 72°C for 60 seconds, and a final extension at 72°C for 10 minutes. 3% agarose gel glue product was used for observing PCR products and analyzing enzyme digestion.

Statistical analysis

Student's t test was taken to compare the differences between demographic and lifestyle information as well as genotype frequencies of *MTHFR* between polycystic ovary syndrome patients and controls. Deviation from Hardy-Weinberg equilibrium of *MTHFR* C677T and A1298C in controls was analyzed by Chi-square test (χ^2). Multiple logistic regression analyses were taken to estimate the relationship between *MTHFR* C677T and A1298C polymorphisms and polycystic ovary syndrome risk. The adjusted odds ratio (AORs) along with 95% confidence Intervals (CI) was taken to describe the results. Statistic analyses were conducted using the software SPASS version 16.0 (SPSS, Inc., Chicago, IL, USA).

Results

The polycystic ovary syndrome patients and controls were comparable in respect to age (t=1.44, P=0.07), follicle stimulating hormone (t=0.73, P=0.23), prolactin (t=0.66, P=0.25) and estradiol (t=0.85, P=0.20) (**Table 2**). However, there was significant differences between polycystic ovary syndrome patients and controls in terms of body mass index (t=10.01, P<0.001), HOMA-IR (t=11.11, P<0.001), luteinizing hormone (t=12.20, P<0.001), progestone (t=4.92, P<0.001), T-testosterone (t=16.91, P<0.001), fasting plasma glucose (t=32.19, P<0.001) and fasting insulin (t=13.16, P< 0.001).

The *MTHFR* C677T and A1298C genotype distributions of polycystic ovary syndrome patients and healthy controls were compared using Chi-square test (**Table 3**). The results revealed a significant difference between the two study groups in terms of the genotype frequencies of *MTHFR* C677T (χ^2=15.23, P<0.001), while no significant association was seem in the genotype frequencies of *MTHFR* A1298C (χ^2=4.22, P=0.12). Genotype frequencies of *MTHFR*

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

MTHFR	Patients N=175	%	Controls N=236	%	χ^2 test	*P* value	χ^2 for HWE	*P* value
C677T								
CC	51	29.14	102	43.22				
CT	79	45.14	96	40.68				
TT	54	30.86	38	16.10	15.23	<0.001	3.51	0.06
A1298C								
AA	104	59.43	157	66.53				
AC	57	32.57	70	29.66				
CC	14	8.00	9	3.81	4.22	0.12	0.12	0.73

HWE: Hardy-Weinberg equilibrium.

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

MTHFR	Patients	%	Controls	%	Adjusted OR (95% CI)[1]	*P* value
C677T						
CC	51	29.14	102	43.22	Reference	
CT	79	45.14	96	40.68	1.65 (1.03-2.65)	0.03
TT	54	30.86	38	16.10	2.84 (1.61-5.02)	<0.001
Allele						
C	181	51.71	300	63.56	Reference	
T	187	53.43	172	36.44	1.80 (1.35-2.40)	<0.001
A1298C						
AA	104	59.43	157	66.53	Reference	
AC	57	32.57	70	29.66	1.23 (0.78-1.93)	0.34
CC	14	8.00	9	3.81	2.35 (0.91-6.37)	0.05
Allele						
A	265	75.71	384	81.36	Reference	
C	85	24.29	88	18.64	1.40 (0.98-1.99)	0.05

[1]Adjusted for body mass index, HOMA-IR, progestone, T-testosterone, fasting plasma glucose and fasting insulin.

C677T (χ^2=3.51, P=0.06) and A1298C (χ^2= 0.12, P=0.73) were not departure from Hardy-Weinberg equilibrium in controls.

Using multiple logistic regression analysis, we observed that the CT and TT of *MTHFR* C677T carriers showed increased risk to polycystic ovary syndrome when compared to the CC genotype carriers. The adjusted ORs for the CT and TT genotypes of *MTHFR* C677T were 1.65 (1.03-2.65) and 2.84 (1.61-5.02), respectively (**Table 4**). Subjects with T allele of *MTHFR* C677T had an elevated risk of polycystic ovary syndrome in comparison to the C allele (OR=1.80, 95% CI=1.35-2.40). However, no significant was observed between *MTHFR* A1298C genotype and allele polymorphisms and risk of polycystic ovary syndrome.

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 Chinese female population, and we observed that individuals harboring the CT and TT genotypes of *MTHFR* C677T had an elevated risk of polycystic ovary syndrome when compared to individuals harboring the CC genotype. However, we observed that the *MTHFR* A1298C could not influence the susceptibility to polycystic ovary syndrome development.

High plasma homocysteine level is associated with the risk of polycystic ovary syndrome [21-23]. Loverro et al. carried out a study with 53 women with polycystic ovary syndrome and 20 healthy subjects, and observed a higher mean plasma homocysteine concentrations in patients (10.4±4.4 ng/dl) with polycystic ovary syndrome as compared with healthy women (7.2±1.5 ng/dl) [22]. Salehpour et al. carried out a study with 85 polycystic ovarian syndrome and 83 healthy controls, and discovered that increased level of plasma homocysteine was seem in patients with polycystic ovary syndrome [23]. Yilmaz et al. reported that serum homocysteine level is associated increased risk of polycystic ovary syndrome in Turkish women [21]. However, the genetic variations in *MTHFR* could influence the metabolism of folate and homocysteine in human, and is associated with the high plasma homocysteine level [24, 25]. Therefore, the genetic variations in *MTHFR* could play an important role in pathogenesis of polycystic ovary syndrome.

Previous studies have reported the association between *MTHFR* genetic polymorphisms and development of polycystic ovary syndrome, but the results are conflicting [16-19, 26, 27]. Qi et al. carried out a study with 115 polycystic ovary syndrome patients and 58 fertile women in China, and they reported that *MTHFR* C677T genetic mutation could influence the occurrence of polycystic ovary syndrome risk, while *MTHFR* A1298C genetic variation is not associated with the onset of this disease [16]. Jain et al. carried out a study in an Indian population, and reported that the CT genotype of *MTHFR* C677T confer 1.32 fold risk of developing polycystic ovary syndrome [17]. However, some studies reported inconsistent results. Orio et al. carried out a study with 70 young women with polycystic ovary syndrome and 70 healthy women, and reported that *MTHFR* C677T genetic polymorphism do not influence the serum Hcy levels and development of polycystic ovary syndrome [26]. Choi et al. indicated that the *MTHFR* C677T polymorphism is not correlated with polycystic ovary syndrome in Korean population [27]. Karadeniz et al. suggested that *MTHFR* C677T gene variations do not affect homocysteine levels of patients with polycystic ovary syndrome in Turkish women [18]. The inconsistent of these results may be caused by differences in ethnicities, selection of study subjects and sample size.

One limitation to this study should be considered. First, the patients and controls were collected from a hospital, which would result in selection bias. However, the genotype frequencies of *MTHFR* C677T and A1298C polymorphisms tested conformed to HWE, suggesting that our investigated subjects are representative of the general population. Further multicenter studies are greatly needed to confirm our findings.

In summary, our study suggests that the *MTHFR* C677T may contribute to polycystic ovary syndrome risk in the Chinese women under investigation, while no such association was found in relation to *MTHFR* A1298C polymorphism. Further research employing a greater number of study subjects is greatly required to corroborate our results.

Acknowledgements

We thank for the funding from Medical science and technology research projects of Henan Province (201303138).

Disclosure of conflict of interest

None.

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