RESEARCH COMMUNICATION

Methylenetetrahydrofolate Reductase Gene Polymorphisms as Predictive and Prognostic Biomarkers in Ovarian Cancer Risk

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Abstract

Early diagnosis and better prognosis of ovarian cancer is still a challenge. Besides environmental risk factors, genetic factors have established a role in pathogenesis of ovarian cancer. Methods: A case-control and a prospective study design conducted in 224 ovarian cancer patients and 432 controls in Chinese population. MTHFR C677T genotyping was done by PCR-RFLP. Results: Patients with ovarian cancer is associated with a higher less number of delivery and less frequent oral contraceptive use. When potential confounding factors adjusted logistic regression analysis between cases and controls were performed, significant association was obtained for 677T/T genotype and ovarian cancer (OR=3.13, 95% CI=1.59-5.72). Cox regression survival analysis showed individuals carrying T/T genotype had significantly increased HR for death in ovarian cancer patients (HR=2.86, 95% CI=1.27-7.93). In conclusion, we observed that the MTHFR C677T polymorphism is associated with the susceptibility and survival of ovarian cancer in Chinese population.

Keywords: Methylenetetrahydrofolate reductase gene - polymorphism - ovarian cancer risk

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Introduction

Ovarian cancer is the seventh most common cancer in women and leading the cause of death among gynecological cancers (Kristensen and Trope, 1997). The incidence rate vary in both sexes and geographic worldwide. China has a relatively low incidence of 3-5/105 females, which is about one-fourth of the incidence in northern European countries (Jin et al., 1993). The wide geographic variation at an international levels of ovarian cancer in terms of incidence and mortality suggested the role of genetic and environmental factors in the pathogenesis of this cancer.

Little is known concerning the etiological factors of the malignancy. Most of the known risk factors are related to parity and family history of ovarian cancer. Reduced ovarian cancer risks are associated with oral contraceptive use and fewer menstrual cycles, while increased risks are associated with infertility, low parity and hormone replacement therapy (Kristensen and Trope, 1997; La, 2001). To our knowledge, folate deficiency resulting from low consumption of vegetables and fruits is related to increased risk of several cancers, including ovarian cancer (Zhang et al., 2004). The carcinogenesis of ovarian cancer by folate deficiency is through two mechanisms ways: one is inducing misincorporation of uracil into DNA to lead to disruption of DNA integrity and DNA repair. Another is causing the alteration in DNA methylation, which could induce the altering expression of critical tumor suppressor genes and proto-oncogenes (Choi and Mason, 2000; Blount et al., 2007; Gallus and Vecchia, 2007;).

MTHFR is a central enzyme in folate metabolism which catalyzes the reduction of 5, 10-methylene-tetrahydrofolate to 5-methyltetrahydrofolate, and then methionine synthase catalyzed the reaction of 5-methyltetrahydrofolate and homocysteine to generate methionine and tetrahydrofolate. Under the condition of folate deficiency, MTHFR may result in point mutations and/or chromosomal breaks, facilitate the conversion of 5,10-methylene THF to 5-methyl THF, and cause decline of 5-methyl THF to decrease the conversion of homocysteine to methionine. Ultimately, MTHFR plays a role in the carcinogenesis process of DNA hypomethylation (Bailey and Gregory, 1999).

There were about 20 kinds of genetic polymorphisms of MTHFR, and non-synonymous C677T is the most studies genetic polymorphisms. The C677T variant (Ala 222 Val, rs 1801133) has been associated with a decreased activity of MTHFR, an increased level of homcysteine and an altered distribution of folate (Frosst et al., 2008). Therefore, the inactive MTHFR C677T may play a role in the carcinogenesis process of DNA hypomethylation. Several studies have explore the impact of MTHFR C677T polymorphism in various cancer including lung, gastric, breast, colorectal and esophageal cancer. However, association studies of MTHFR C677T polymorphism in ovarian cancer have been conflicting (Zhang et al., 2004; Terry et al., 2010).

Moreover, the lack of screening tests for early detection

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and limited treatment options for the ovarian cancer may induce the poor prognosis of this cancer. The role of MTHFR C677T polymorphism on the survival of patients has also been reported in different types of malignancies with inconsistent results (Jain et al., 2008; Upadhyay et al., 2008; Upadhyay et al., 2009). Also, population specific differences was found in cancer susceptibility as well as allele frequency of gene polymorphism by several studies (Miller et al., 1988; Shen et al., 2001; Wilcken et al., 2003; Reddy and Jamil., 2006; Sarbia et al., 2006; Jain et al., 2008; Upadhyay et al., 2008; Joshi et al., 2009; Upadhyay et al., 2009). However, there is no study explore the association between MTHFR C677T polymorphism and survival of ovarian cancer in Chinese population.

Therefore, the current study is aimed to investigate the association between MTHFR C677T polymorphism and ovarian cancer risk, as well as explore the role of this genotype on the survival of this cancer.

Materials and Methods

Study population

This study is a case-control and a prospective study design conducted in the Affiliated Hospital of Sichuan University, China. All Chinese female cases with newly diagnosed primary ovarian cancer between May. 2006 and May. 2011 in the hospital were invited for face-to-face interviews within two months after diagnosis. All cases recruited in this study were examined histologically confirmed. Among a total of 224 eligible cases, 206 were interviewed with a participation rate of 91.9%. Controls were randomly selected from hospital visitors and outpatients from the same hospital. Controls were required to be without any history of any type of cancer and frequency matched by five-year age groups, with a control to case ratio of two, whenever possible. Among a total of 459 eligible controls, 432 were successfully interviewed with a participation rate of 94.1%. All the patients were followed up since the endpoint of study, and we recorded the deaths of all patients.

Data collection

A face to face interview was conducted using a structured questionnaire to collect information on clinic characteristics and potential confounding factors of ovarian cancer by four trained interviewers. The collected potential confounders mainly included body mass index, tobacco smoking, alcohol consumption, number of delivery, menopausal status, oral contraceptive use, tumor history and tumor type. In order to avoid reverse causality, subjects were asked to report their anthropometric measures five years before the diagnosis (for cases) or the interview (for controls). Approval to conduct this study was granted by the Ethics Committee of the Affiliated Hospital of Sichuan University. Informed consent was obtained before each interview.

Genotyping

A total of 5 ml venous blood was collected from each study and kept frozen at -20°C until DNA extraction. Genomic DNA was extracted, using standard methods, from peripheral blood of the controls or patients with esophageal cancer. The MTHFR genotypes at the C677T site were analyzed by PCR-based RFLP methods. The primers sequences were: forward 5'-TGAAGGAGAAGGTGTCTGCGGGA-3' and 5'-AGGACGGTGCGGTGAGAGTG-3' (Skibola et al., 1999). Each 25.0 μL reaction mixture contained 2.5 μL 10 × PCR buffer, 1.5 μL MgCl2 (25 mmol/L), 0.5 μL dNTP (10 mmol/L), 0.5 μL forprimer (20 μmol/L), 0.5 μL revprimer (20 μmol/L), 0.2 μL Taq DNA polymerase (5 U/μL), 1.5 μL template DNA, and 17.8 μL nuclease free water. The PCR conditions were as follows: initial denaturation at 95°C for 5 min, followed by 30 cycles at 95°C for 30 s, at 60°C for 30 s, at 72°C for 60 s, and a final extension at 72°C for 10 min. After transient centrifugation, agarose electrophoresis was conducted. The PCR products included 173-bp fragments of 677C/C wild-type homozygotes; 175-bp and 23-bp fragments of polymorphic T, and 198-bp for wide type C allele.

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software 13.0 for windows. Differences in demographic and clinic characteristics as well as potential confounding factors were tested using Chi-squared test. Unconditional logistic regression was used to estimate the odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for each exposure variables. The associations between exposure variables and risk of ovarian cancer were further examined after adjusting for potential confounders using multivariate logistic regression models. The outcome for the study was overall survival, which was estimated using the Kaplan-Meir method. A log rank test was used to assess the association between the factors and overall survival. A univariate Cox's regression analysis was used to assess association between each potential prognostic factor and calculated from the Cox regression model for all significant predictors from cancer diagnosis to the endpoint of this study.

Results

Demographical and clinical characteristics of patients and controls are showed in Table 1. There were no difference in mean age, BMI (5 years ago), smoking, alcohol consumption and menopausal status between cases and controls. Compared with controls, patients with ovarian cancer tended to have a higher less number of delivery and less frequent oral contraceptive use. Also, most of the ovarian cancer had apparent family susceptibility.

Genotype frequencies of ovarian cancer patients and controls are shown in Table 2. Frequency of the 677T/T genotype was higher in controls than patients (12.2% vs. 5.2%), while almost similar for the 677C/T genotype (44.7 vs. 41.2). When potential confounding factors adjusted logistic regression analysis between cases and controls were performed, significant association was obtained for 677T/T genotype and ovarian cancer (OR=3.13, 95% CI=1.59-5.72).

Table 1. Demographical and Clinical Characteristics of Ovarian Cancer Patients

Variable	Cases, N(%)	Controls, N(%)	P value
Age, years [mean, (sd)]	48.4, 9.6	48.7, 10.1	0.36
Body mass index(kg/m2) (5 years ago)			
<25	165(73.6)	334(77.4)	0.29
≥25	59(26.4)	98(22.6)	
Tobacco smoking			
Never	215(95.9)	422(97.8)	0.22
Ever	9(4.1)	10(2.2)	
Alcohol consumption			
Never	176(78.5)	334(77.3)	0.71
Ever	48(21.5)	98(22.7)	
Number of deliveries			
0	28(12.6)	20(4.7)	<0.001
1	93(41.7)	157(36.4)	
2	83(37.2)	183(42.3)	
≥3	19(8.5)	72(16.6)	
Menopausal status			
No	122(54.6)	246(56.9)	0.54
Yes	102(45.4)	186(43.1)	
Oral contraceptive use			
Never	177(79.2)	267(61.8)	<0.001
Ever	47(20.8)	165(38.2)	
Ovarian cancer in first-degree relatives			
No	210(93.7)	430(99.6)	<0.001
Yes	14(6.3)	2(0.4)	
Tumor type			
Invasive	142(63.5)		
Borderline	81(36.2)		
Missing	1(0.3)		

Table 2. Frequency Distribution and Association of MTHFR C677T Genotypes with Ovarian Cancer

Genotype/Allele	Cases, N(%)	Controls, N(%)	OR[1](95% CI), P value
MTHFR C677T			
C/C	97(43.1)	232(53.6)	1.0 (Reference)
C/T	100(44.7)	178(41.2)	1.44(0.97-2.21)
T/T	27(12.2)	22(5.2)	3.13(1.59-5.72)
C	294(65.6)	642(74.3)	1.0 (Reference)
T	154(34.4)	222(25.7)	1.68(1.27-2.05)

[1]Adjusted for age, tobacco smoking, alcohol consumption, number of delivery, menopausal status, oral contraceptive and ovarian cancer history

Table 3. Kaplan-Meier Survival Estimation of Median Survival and HRs with MTHFR C677T Gene Polymorphism

	n (%)	Mean Survival, 95% CI (month)	HR(95% CI), P value
MTHFR C677T			
C/C	97(43.1)	34.7 (31.9-37.4)	1.0 (reference)
C/T	100(44.7)	32.5 (29.3-35.7)	1.25(0.86-2.85), 0.28
T/T	27(12.2)	23.7 (17.3-30.1)	2.86(1.27-7.93), <0.05
C/T+T/T	127(56.9)	27.1(21.4-35.9)	1.57(0.96-3.68), 0.07

The media survival rate of patients was 32.61 month. A five year survival rate was found only in 19% cases with ovarian cancer. When the survival time of the patients was compared among MTHFR C677T genotypes. Individuals carrying the 677T/T genotype had significantly less mean survival than 677C/C genotype. Furthermore, individuals carrying T/T genotype had significantly increased HR for death in ovarian cancer patients (HR=2.86, 95% CI=1.27-7.93) (Table 3 and Figure 1).

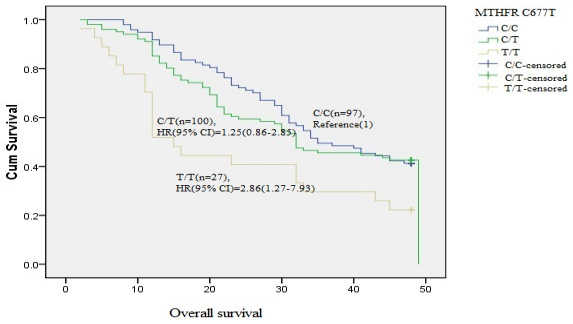

Figure 1. Overall Survival of MTHFR Gene Polymorphism of Ovarian Cancer

Discussion

In this study in Chinese population, we observed that MTHFR C677T polymorphism is associated with susceptibility to ovarian cancer. MTHFR 677T/T genotypes showed significant poor prognostic associations.

The prevalence of variant genotypes of the MTHFR C677T polymorphism varies to a great extent among different human populations. In Africans, the frequency of MTHFR677T/T is below 1%, whereas in Mexicans, it is above 30%, and frequency of MTHFR677T/T genotype in controls of our study was 12.2%, which is comparable to previous reports from Chinese populations (Yang et al., 2005; Wang et al., 2007).

We found a significant associations with any of MTHFR C677T polymorphism for developing ovarian cancer. In published literature, the role of MTHFR C677T polymorphism in relation to ovarian cancer susceptibility and survival and been inconsistent (Gershoni et al., 2000; Terry et al., 2010). A recent large sample multi-center studies in New England and United states showed a significant associations between MTHFR C677T polymorphism and serous ovarian cancer risk, while no association was observed in Americans (Terry et al., 2010). Another study conducted in Israel showed 677T allele is significantly more common in ovarian cancer (Gershoni et al., 2000). The MTHFR C677T polymorphism showed diversity in different populations, this may be due to genuine population-specific differences in the risk of ovarian cancer due to MTHFR C677T polymorphism. Moreover, our study showed the 677T/T genotype frequency in most Chinese population is higher than other populations, and this may be one of the probable reasons for the associations of this gene polymorphism with ovarian cancer.

Our study showed a significant association between MTHFR C677T polymorphism and ovarian cancer prognosis. However, previous studies showed inconsistent results between this gene polymorphism and ovarian cancer. Goode et al. reported an association between these MTHFR SNPs and ovarian cancer prognosis (Goode et al., 2010). While another study did not find a association maybe due to limited sample size (Terry et al., 2010). Some biologic data support a potential for survival bais for the MTHFR C677T polymorphism. The balance of

the 5,10-MTHF and 5-MTHF controlled by MTHFR may influence the efficacy of chemotherapy with 5-fluorouracil (5-FU). The inactivity MTHFR 677T/T could increase the amounts of 5,10-MTHF. Cell line experiments suggest that fluoropyrimidines like 5-FU exert their effect by inhibiting thymidylate synthetase through the formation of a ternary complex, involving 5-FU, thymidylate synthetase, and 5, 10-MTHF (Zhang et al., 1992). Therefore, these MTHFR polymorphisms may enhance the effect of 5-FU by increasing the amount of 5, 10-MTHF. In previous animal models study, the administration of 5, 10-MTHF could enhance the efficacy of 5-FU (Carlsson et al., 1997). Previously, Cohen and colleagues showed colon cancer cases with inactive MTHFR 677T/T need three times more likely to respond to 5-FU chemotherapy than the MTHFR 677C/C genotype (Cohen et al., 2003). In our study, we found individual with 677T/T had a poor survival compared with cases with 677C/C, and most cases in our study received chemotherapy, which indicated the MTHFR C677T polymorphism may have influence on the chemotherapy.

Our study has several major strengths. First, an extensive effort was made to collect information on major risk for ovarian cancer, which was further considered and adjusted throughout the analysis. Second, all cases in this study were histologically confirmed, which minimized misclassification. In addition, the controls in our study were selected from hospital visitors and outpatients in our hospital, which had a better representative than controls selected from hospitalized individuals, and could represent the general population.

In conclusion, we observed that the MTHFR C677T polymorphism is associated with the susceptibility and survival of ovarian cancer. The limitation of our study was the lower number of patients with survival data. In future, studies with a higher sample size are warranted.

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