RESEARCH ARTICLE

MTHFR Gene Polymorphisms are Not Involved in Pancreatic Cancer Risk: A Meta-analysis

Yu-Liang Tu[1], Shi-Bin Wang[2]*, Xiang-Long Tan[1]

Abstract

Purpose: Methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms have been reported to be associated with pancreatic cancer, but the published studies have yielded inconsistent results. This study assessed the relationship between MTHFR gene polymorphisms and the risk for pancreatic cancer using a meta-analysis approach. Methods: A search of Google scholar, PubMed, Cochrane Library and CNKI databases before April 2012 was performed, and then associations of the MTHFR polymorphisms with pancreatic cancer risk were summarized. The association was assessed by odds ratios (ORs) with 95% confidence intervals (CIs). Publication bias was also calculated. Results: Four relative studies on MTHFR gene polymorphisms (C667T and A1298C) were included in this meta-analysis. Overall, C667T (TT vs. CC:OR=1.61,95%CI=0.78-3.34; TT vs. CT: OR=1.41,95%CI=0.88-2.25; Dominant model:OR=0.68,95%CI=0.40-1.17; Recessive model: OR=0.82,95%CI=0.52-1.30) and A1298C (CC vs. AA:OR=1.01,95%CI=0.47-2.17; CC vs. AC: OR=0.99,95%CI=0.46-2.14; Dominant model:OR=1.01, 95%CI=0.47-2.20; Recessive model: OR=1.01,95%CI=0.80-1.26) did not increase pancreatic cancer risk. Conclusions: This meta-analysis indicated that MTHFR polymorphisms (C667T and A1298C) are not associated with pancreatic cancer risk.

Keywords: Pancreatic cancer - MTHFR - gene polymorphism - meta-analysis

Asian Pacific J Cancer Prev, **13 (9)**, 4627-4630

Introduction

Pancreatic cancer is the tenth most common cause of cancer in the United States and is apparently the fourth leading cause of cancer mortality, it is estimated that 44030 new cases and 37660 associated deaths of the pancreatic cancer in 2011 (National Cancer Institute, 2012). Both genetic and environmental factors play a role in the in the development of the disorder. There are some factors may potentially result in pancreatic cancer: cigarette, smoking, age, race, gender, religious background, chronic pancreatitis, diabetes, peptic ulcer surgery, diet (Vimalachandran et al., 2004). In addition, up to 5% to 10% of pancreatic cancer cases are believed to be caused by genetic factors (Danes et al., 1982; Lynch et al., 1990; Ghadirian et al., 1991).

There is growing evidence that folate deficiency (a low normal level) is associated with risk of pancreatic cancer (Stolzenberg-Solomon et al., 2001; Stolzenberg-Solomon, 1999). MTHFR (5,10-methylenetetrahydrofolate reductase) plays a central role in folate metabolism. This enzyme catalyzes the irreversible reaction of 5,10-methylene-tetrahydrofolate to 5-methyl tetrahydrofolate, which serves as a substrate for the remethylation of homocysteine to methionine, with the subsequent synthesis of S-adenosylmethionine (Heijmans et al., 2003). Two significant functional polymorphisms of the MTHFR gene (C677T and A1298C) have been related to a reduced enzyme activity and increased the risk of pancreatic cancer, Heterozygotes (CT) and homozygotes (TT) for the C677T polymorphism have about 65% and 30%, respectively, of the MTHFR activity of individuals with the wild-type (CC) genotype (Frosst.,1995), For A1298C, homozygotes (CC) have about 60% of normal MTHFR activity (Weisberg, 1998). However the published results have been inconsistent (Li, 2005; Matsubayashi, 2005; Wang, 2005; Suzuki, 2008). In the present study, we investigated whether or not the MTHFR gene polymorphisms is associated with pancreatic cancer by performing a meta-analysis.

Materials and Methods

Literature review

We searched the Google scholar, PubMed, Cochrane Library and CNKI (China National Knowledge Infrastructure) databases for all studies on the association between MTHFR polymorphisms and pancreatic cancer risk before April 2012. The following key words were used: "methylenetetrahydrofolate reductase", "MTHFR", "pancreatic adenocarcinoma", "pancreatic cancer", "polymorphism", "mutation" and "variant". We recruited data from published papers and abstracts without restriction of language.The reference lists of reviews and

*[1]Department of Hepatobiliary Surgery, [2]Department of General Surgery, the First Affiliated Hostpial of PLA General Hospital, Beijing, China *For correspondence: wang.shibin@yahoo.cn*

Table 1. Characteristics of the Included Studies for Meta-analysis

Author	Year	Area	cases	controls	Genotypes for cases	Genotypes for controls	P for HWE
C667T					CC CT TT	CC CT TT	
Li et al	2005	American	347	348	150 117 36	149 138 20	0.10
Wang et al	2005	China	163	337	31 79 53	135 149 53	0.27
Matsubayashi et al	2005	American	303	305	145 115 43	134 135 36	0.82
Suzuki et al	2008	Japan	157	785	57 80 20	291 366 128	0.47
A1298C					AA AC CC	AA AC CC	
Li et al	2005	American	347	348	129 145 29	133 137 40	0.61
Wang et al	2005	China	163	337	124 37 2	243 86 8	0.90
Matsubayashi et al	2005	American	303	305	133 134 36	144 140 21	0.09

retrieved articles were hand searched at the same time. In the case of more than one article was published by the same author using the same case series, the latest published results were used.

Inclusion and exclusion criteria

Two investigators (Yuliang Tu and Shibin Wang) reviewed all identified studies independently to determine whether an individual study was eligible for inclusion. The following criteria were used to include published studies: (1) case-control studies were included to evaluate the association between MTHFR polymorphism and pancreatic cancer risk; (2) sufficient genotype data were presented to calculate the odds ratios (ORs) and 95% confidence intervals (CIs); (3) Genotype distribution of the pancreatic cancer patients and the controls must be in Hardy-Weinberg equilibrium (HWE). The exclusion criteria were as follows: (1) not case-control stud¬ies that evaluated the association between MTHFR polymorphism and pancreatic cancer risk; (2) case reports, letters, reviews, meta-analysis and editorial articles; (3) studies that were based on incomplete raw data and those with no usable data reported; (4) duplicate data were included in the studies; (5) healthy controls were not in HWE.

Data extraction

Two investigators (Yuliang Tu and Shibin Wang) extracted the data independently, and the result was reviewed by a third investigator (Xianglong Tan). The following characteristics were collected from each study:first author, years of publication, ethnicity (country) of study population, the number of patients and controls for a study, and evidence of HWE.

Statistical analysis

The strength of the association between MTHFR polymorphisms and pancreatic cancer risk was estimated by ORs with 95% CI under a homozygote comparison (AA vs aa), a heterozygote comparison (AA vs Aa), a dominant model and a recessive mode between groups. In this study, the dominant model was defined as Aa+aa vs AA, where "A" and "a" are major and minor alleles, respectively, and the recessive model as aa vs AA+Aa. The distribution of genotypes in the included studies was tested for HWE using the χ^2 test. We also quantified the effect of heterogeneity by the Q-test and I^2 test. I^2 ranges between 0 and 100%, and I^2 values of 25, 50 and 75% were defined as low, moderate and high estimates, respectively. When a significant Q-test ($P<0.10$) or $I^2 >$ 50% indicated heterogeneity across studies, the random

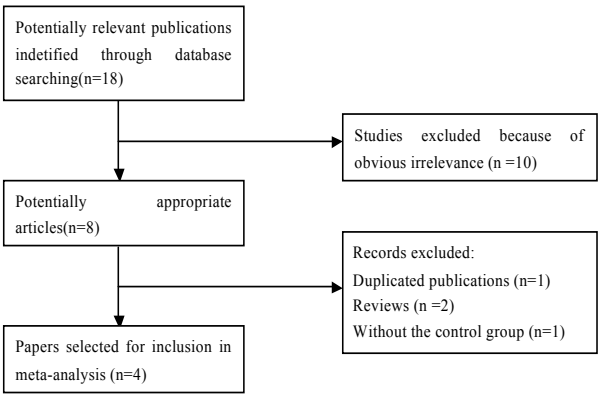

Figure 1. Flow Diagram of Study Searching and Selection Process

effects model was used for meta-analysis, or else the fixed effects model was calculated. Begg's test was used to provide evidence of publication bias,which was shown as a funnel plot ($P<0.05$ was considered a significant publication bias). Analyses were conducted using Stata 12.0 (Stata Corporation, College Station, TX, USA). All P values are two-tailed.

Results

Eligible studies

Based on the search criteria, 18 articles were found. Of these, 10 papers were excluded after reading the title or abstract because of obvious irrelevance to our study aim. In addition,1 duplicated publications and 2 reviews were excluded. And 1 paper did not have the control group were further excluded. Therefore, Only 4 studies for the association between MTHFR polymorphisms and pancreatic cancer were included in the final meta-analysis (Li, 2005; Wang, 2005; Matsubayashi, 2005; Suzuki, 2008). A flow chart summarizing the process of study inclusion/exclusion is depicted (Figure 1). The characteristics of the included studies are listed in Table 1. All the 4 eligible studies were hospital-based case-control studies. Of the 4 included studies, 2 used restriction fragment length polymorphism (PCR-RFLP) method (Li, 2005; Wang, 2005), 1 used real-time polymerase chain reaction method (Matsubayashi, 2005) and 1 used TaqMan Assays (Suzuki, 2008). All studies were consistent with HWE law in controls ($P>0.05$).

Meta-analysis

For MTHFR C677T polymorphism, a total of 970 cases and 1,775 controls were identified. The C677T polymorphism were not associated with the risk of

Table 2. Summary ORs and 95%CI of the Included Studies for Meta-analysis

Genetic model	Sample size Case	Control	Type of model	Test of heterogeneity I^2	P	Test of association OR	95% CI	Begg's test z	P
C667T									
TT vs. CC	970	1775	Random	85.9%	0.00	1.61	0.78-3.34	0.00	0.91
TT vs. CT			Random	68.7%	0.02	1.41	0.88-2.25	0.00	1.00
Dominant model			Random	79.3%	0.00	0.68	0.40-1.17	0.00	1.00
Recessive model			Random	85.4%	0.00	0.82	0.52-1.30	0.00	1.00
A1298C									
CC vs. AA	813	990	Random	67.0%	0.05	1.01	0.47-2.17	0.00	1.00
CC vs. AC			Random	67.5%	0.05	0.99	0.46-2.14	0.00	1.00
Dominant model			Random	70.2%	0.04	1.01	0.47-2.20	0.00	1.00
Recessive model			Fixed	0..0%	0.46	1.01	0.80-1.26	1.04	0.30

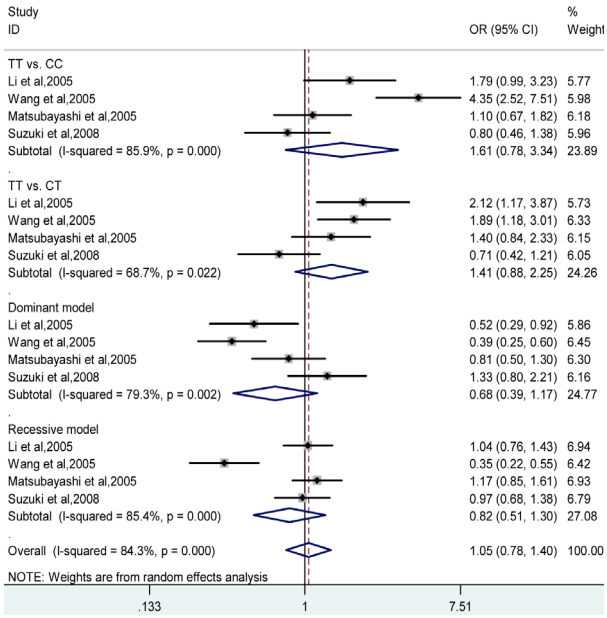

Figure 2. Forest Plots of ORs with 95% CI for MTHFR C677T Polymorphism and Risk for Pancreatic Cancer. Studies were ordered by year of publication. Square sizes are proportional to the weight of each study in the meta-analysis

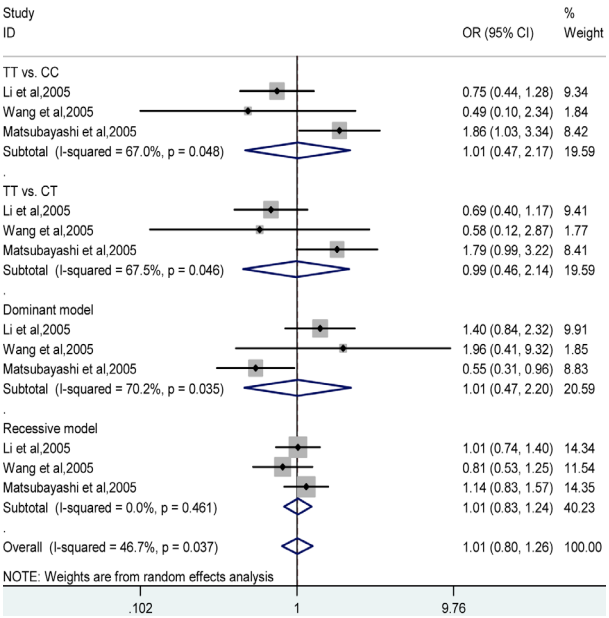

Figure 3. Forest Plots of ORs with 95% CI for MTHFR A1298C Polymorphism and Risk for Pancreatic Cancer. Studies were ordered by year of publication. Square sizes are proportional to the weight of each study in the meta-analysis

pancreatic cancer (TT vs. CC:OR=1.61,95%CI=0.78-3.34; TT vs. CT: OR=1.41,95%CI=0.88-2.25 Dominant model:OR=0.68,95%CI=0.40-1.17; Recessive model: OR=0.82,95%CI=0.52-1.30) (Figure 2, Table 2). A total of 813 cases and 990 controls were identified for MTHFR A1298C polymorphism. The A1298C polymorphism did not have an increased risk of pancreatic cancer (CC vs. AA:OR=1.01, 95%CI=0.47-2.17; CC vs. AC: OR=0.99, 95%CI=0.46-2.14; Dominant model: OR=1.01,95%CI=0.47-2.20; Recessive model: OR=1.01, 95%CI=0.80-1.26) (Figure 3,Table 2).

Publication bias

Begg's test showed no evidence of publication bias in the present meta-analysis of the MTHFR polymorphisms. (Table 2), which implied that the publication bias was low in the present meta-analysis.

Discussion

Although several research studies have evaluated the association between MTHFR polymorphisms and pancreatic cancer, the specific association is still controversial.Our meta-analysis quantitatively assessed the association between MTHFR polymorphisms and pancreatic cancer risk. In the current meta-analysis, we examined the association between MTHFR polymorphisms and the risk of pancreatic cancer by critically including all published studies. Finally,4 case-control studies were included and assessed,from which we selected 4 studies on MTHFR C677T polymorphism and 3 studies on MTHFR A1298C polymorphism.

To our knowledge, this is the first meta-analysis considering MTHFR polymorphisms and the risk of pancreatic cancer.Previous meta-analyses have shown the MTHFR 677TT genotype increase gastric cancer risk (Boccia, 2008),the possible mechanism is 677T allele contributes to DNA hypomethylation,which in turn may lead to altered gene expression and the polymorphism decrease the risk of colorectal cancer (Botto, 2000; Kono, 2005; Sanjoaquin, 2005; Taioli, 2009). It may be due to C677T polymorphism exert a protective effect by increasing the levels of the MTHFR substrate (essential for DNA synthesis). So it is not straightforward to interpret the MTHFR-cancer association.And our current pooled data suggested no evidence for a major role of MTHFR C677T polymorphism in the risk of pancreatic cancer.

These conflicting findings might reflect different folate status of ethnic differences in genetic backgrounds and the environment in which they lived in.In addition, genomic DNA hypomethylation does not always facilitate cancer development because in Apc min mice DNMT1 hypomorphs have a reduced risk of gastrointestinal neoplasia (Eads, 2002). As for the A1298C genotype, studies showed no important effects on pancreatic cancer. The number of studies in the literature on the association of MTHFR gene polymorphisms and pancreatic cancer was comparatively few, so we can not use subgroup analysis to investigate the confounding factors, pending further research.

There were still some limitations in our meta-analysis. First, the random effect model was partly used to calculate ORs, it may affect the precision of the result. Secondly, although all cases and controls of each study were well defined with similar inclusion criteria, there may be potential factors that were not taken into account that may have influenced our results. Finally, the genotype information stratified for the main confounding variables was not available in the original papers,such as age, sex, ethnicity and exposures.and the confounding factors might cause serious confounding bias.

In summary, this meta-analysis evaluated the effect of MTHFR polymorphisms on the risk of pancreatic cancer. And there is no evidence that MTHFR polymorphisms (C677T, and A1298C) are associated with pancreatic cancer risk.

Acknowledgements

The author(s) declare that they have no competing interests.

References

Boccia S, Hung R, Ricciardi G, et al (2008). Meta- and Pooled Analyses of the Methylenetetrahydrofolate Reductase C677T and A1298C Polymorphisms and Gastric Cancer Risk: A Huge-GSEC Review. *Am J Epidemiol*, **167**, 505-16.

Botto LD, Yang Q (2000). 5, 10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. *Am J Epidemiol*, **151**, 862-77.

Danes BS, Lynch HT (1982). A familial aggregation of pancreatic cancer: an in vitro study. *JAMA*, **247**, 2798-802.

Lynch HT, Fitzsimmons ML, Smyrk TC, et al (1990). Familial pancreatic cancer: clinicopathologic study of 18 nuclear families. *Am J Gastroenetrol*, **85**, 54-60.

Eads CA, Nickel AE, Laird PW (2002). Complete genetic suppression of polyp formation and reduction of CpG-island hypermethylation in Apc(Min/+) Dnmt1-hypomorphic Mice. *Cancer Res*, **62**, 1296-9.

Frosst P, Blom HJ, Milos R, et al (1995). A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet*, **10**, 111-3.

Ghadirian P, Boyle P, Simard, et al (1991). Reported family aggregation of pancreatic cancer within a population based case-control study in the Francophone community in Montreal, Canada. *Int J Pancreatol*, **10**, 183-96.

Heijmans BT, Boer JM, Suchiman HE, et al (2003). A common variant of the methylenetetrahydrofolate reductase gene (1p36) is associated with an increased risk of cancer. *Cancer Res*, **63**, 1249-53.

Kono S, Chen K (2005). Genetic polymorphisms of methylenetetrahydrofolate reductase and colorectal cancer and adenoma. *Cancer Sci*, **96**, 535-42.

LiDH,AhmedM,LiY, et al (2005). 5,10-methylenetetrahydrofolate reductase polymorphisms and the risk of pancreatic cancer. *Cancer Epidemiol Biomarkers Prev*, **14**, 1470-6.

Matsubayashi H, Skinner HG, Iacobuzio-Donahue C, et al (2005). Pancreaticobiliary cancers with deficient methylenetetrahydrofolate reductase genotypes. *Clin Gastroenterol Hepatol*, **3**, 752-60.

National Cancer Institute, Surveillance Epidemiology and End Results. Pancreas. SEER Stat Fact Sheets. Availble from: URL: http://seer.cancer.gov/statfacts/html/pancreas.html.

Sanjoaquin MA, Allen N, Couto E, et al (2005). Folate intake and colorectal cancer risk: a meta-analytical approach. *Int J Cancer*, **113**, 825-8.

Stolzenberg-Solomon RZ, Albanes D, Nieto FJ, et al (1999). Pancreatic cancer risk and nutrition-related methyl-group availability indicators in male smokers. *J Natl Cancer Inst*, **91**, 535-41.

Stolzenberg-Solomon RZ, Pietinen P, Barrett MJ, et al (2001). Dietary and other methyl-group availability factors and pancreatic cancer risk in a cohort of male smokers. *Am J Epidemiol*, **153**, 680-7.

Suzuki T, Matsuo K, Sawaki A, et al (2008). Alcohol drinking and one-carbon metabolism-related gene polymporphisms on pancreatic cancer risk. *Cancer Epidemiol Biomarkers Prev*, **17**, 2742-7.

Taioli E, Garza MA, Ahn YO, et al (2009). Meta- and pooled analyses of the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and colorectal cancer: a HuGE-GSEC review. *Am J Epidemiol*, **170**, 1207-21.

Vimalachandran D, Ghaneh P, Costello E, et al (2004). Genetics and prevention of pancreatic cancer. *Cancer Control*, **11**, 6-14.

Weisberg I, Tran P, Christensen B, et al (1998). A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. *Mol Genet Metab*, 64, 169-72.