

## RESEARCH COMMUNICATION

# MTHFR Polymorphisms and Pancreatic Cancer Risk: Lack of Evidence from a Meta-analysis

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### Abstract

**Objective:** Methylene tetrahydrofolate reductase (MTHFR) gene polymorphisms have been reported to be associated with pancreatic cancer, but the published studies had yielded inconsistent results. We therefore performed the present meta-analysis. **Methods:** A search of Google scholar, PubMed, Cochrane Library and CNKI databases before April 2012 was conducted to summarize associations of MTHFR polymorphisms with pancreatic cancer risk. Assessment was with odds ratios (ORs) and 95% confidence intervals (CIs). Publication bias were also calculated. **Results:** Four relative studies on MTHFR gene polymorphisms (C667T and A1298C) were involved 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. **Conclusion:** This meta-analysis indicated that MTHFR polymorphisms (C667T and A1298C) were not associated with pancreatic cancer risk.

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

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### 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 (<http://seer.cancer.gov/statfacts/html/pancreas.html>). 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 (Jang et al., 2012; Genkinger et al., 2012). In addition, up to 5% to 10% of pancreatic cancer cases are believed to be caused by genetic factors (Lam et al., 2012; Klein, 2012).

There is growing evidence that folate deficiency (a low normal level) is associated with risk of pancreatic cancer (Nikfam et al., 2012; Sanchez et al., 2012). The methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism that converts 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (Papandreou et al., 2012). 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, however the published results have been inconsistent (Frosst et al., 1995; Weisberg et al., 1998). 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 search

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: "methylene tetrahydrofolate 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 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.

#### Eligible Studies

Two investigators 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

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

| SNPs   | Author             | Year | Area     | cases | controls | Genotypes for cases |     |    | Genotypes for controls |     |     | P for HWE |
|--------|--------------------|------|----------|-------|----------|---------------------|-----|----|------------------------|-----|-----|-----------|
|        |                    |      |          |       |          | CC                  | CT  | TT | CC                     | CT  | TT  |           |
| C667T  | Li et al           | 2005 | American | 347   | 348      | 150                 | 117 | 36 | 149                    | 138 | 20  | 0.1       |
|        | 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 | 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   | 0.9       |
|        | Matsubayashi et al | 2005 | American | 303   | 305      | 133                 | 134 | 36 | 144                    | 140 | 21  | 0.09      |

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

| Genetic model   | Sample size |         | Type of model | Test of heterogeneity |      | Test of association |           | Begg's test |      |
|-----------------|-------------|---------|---------------|-----------------------|------|---------------------|-----------|-------------|------|
|                 | Case        | Control |               | I <sup>2</sup>        | P    | OR                  | 95% CI    | 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 |

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).

**Data Extraction**

Two investigators extracted the data independently, and the result was reviewed by a third investigator. 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**

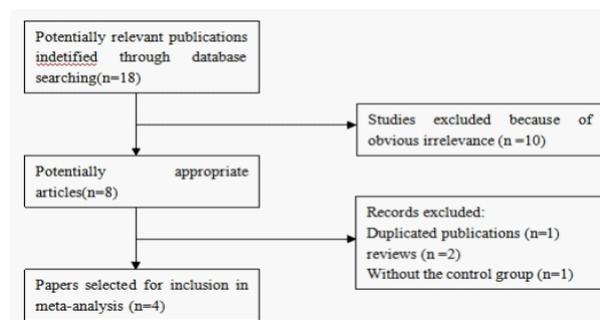
The strength of the association between MTHFR polymorphisms and pancreatic cancer risk was estimated by ORs with 95% CI under a homozygote comparison (AA vs aa), a heterozygote comparison (AA vs Aa), a dominant model and a recessive mode between groups. In this study, the dominant model was defined as 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  $\chi^2$  test. We also quantified the effect of heterogeneity by the Q-test and I<sup>2</sup> test. I<sup>2</sup> ranges between 0 and 100%. and I<sup>2</sup> 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<sup>2</sup> > 50% indicated heterogeneity across studies, the random 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.

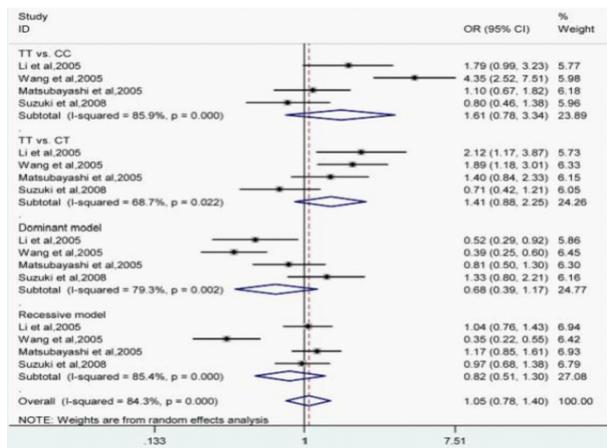
**Results**

*Characteristics of the 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 et al., 2005; Wang et al., 2005; Matsubayashi et al., 2005; Suzuki et al., 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 et al., 2005; Wang et al., 2005), 1 used real-time polymerase chain reaction method (Matsubayashi et



**Figure 1. Flow Diagram of Study Searching and Selection Process**



**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

al., 2005) and 1 used TaqMan Assays (Suzuki et al., 2008). All studies were consistent with HWE law in controls ( $P > 0.05$ ).

#### Meta-analysis results

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 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).

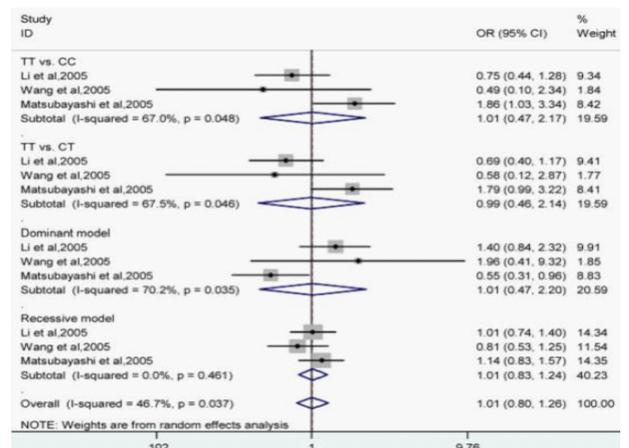
#### Publication Bias

Begg's test showed no evidence of publication bias in the present meta-analysis of the MTHFR polymorphisms (Table 2).

#### 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.

Previous meta-analyses have shown the MTHFR 677TT genotype increase gastric cancer risk (Boccia et al., 2008), the possible mechanism is 677T allele



**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

contributes to DNA hypomethylation, which in turn may lead to altered gene expression. And the polymorphism decrease the risk of colorectal cancer (Botto et al., 2000; Taioli et al., 2009; Lee et al., 2012; Ramsey et al., 2012). 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 in different study populations because of gene-folate interaction as discussed above. 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 et al., 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 provided no evidence that MTHFR polymorphisms (C677T, and A1298C) are associated with pancreatic cancer risk.

#### 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.
- 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.
- Frost 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.
- Genkinger JM, Li R, Spiegelman D et al (2012). Coffee, tea, and sugar-sweetened carbonated soft drink intake and pancreatic cancer risk: a pooled analysis of 14 cohort studies. *Cancer Epidemiol Biomarkers Prev*, **21**, 305-18.
- Jang JH, Cotterchio M, Borgida A et al (2012). Genetic variants in carcinogen-metabolizing enzymes, cigarette smoking and pancreatic cancer risk. *Carcinogenesis*, **33**, 818-27.
- Klein AP (2012). Genetic susceptibility to pancreatic cancer. *Mol Carcinog*, **51**, 14-24.
- Lam ET, Bracci PM, Holly EA, et al (2012). Mitochondrial DNA sequence variation and risk of pancreatic cancer. *Cancer Res*, **72**, 686-95.
- Lee JE, Wei EK, Fuchs CS, et al (2012). Plasma folate, methylenetetrahydrofolate reductase (MTHFR), and colorectal cancer risk in three large nested case-control studies. *Cancer Causes Control*, **23**, 537-45.
- Li D, Ahmed M, Li Y 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. Available from: URL: <http://seer.cancer.gov/statfacts/html/pancreas.html>.
- Nikfam S, Pourshams A (2012). A systematic review of environmental risk factors for pancreatic cancer. *Govaresh*, **16**, 258-65.
- Papandreou CN, Doxani C, Zdoukopoulos N, et al (2012). Evidence of association between methylenetetrahydrofolate reductase gene and susceptibility to breast cancer: a candidate-gene association study in a South-eastern European population. *DNA Cell Biol*, **31**, 193-8.
- Ramsey SD, Holmes RS, McDermott CL (2012). A comparison of approaches for association studies of polymorphisms and colorectal cancer risk. *Colorectal Dis*. doi: 10.1111/j.1463-318.
- Sanchez GV, Weinstein SJ, Stolzenberg-Solomon RZ (2012). Folate associated with pancreatic cancer? *Mol Carcinog*, **51**, 119-27.
- Suzuki T, Matsuo K, Sawaki A et al (2008). Alcohol drinking and one-carbon metabolism-related gene polymorphisms 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.
- Wang L, Miao X, Tan W et al (2005). Genetic polymorphisms in methylenetetrahydrofolate reductase and thymidylate synthase and risk of pancreatic cancer. *Clin Gastroenterol Hepatol*, **3**, 743-51.
- 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.