RESEARCH ARTICLE

MTHFR C677T Polymorphism and Pancreatic Cancer Risk: a Meta-analysis

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Abstract

Background: Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in the metabolism of folate, and the role of the MTHFR C677T polymorphism in pancreatic carcinogenesis is still controversial. Method: A literature search was performed using Pubmed and CNKI databases for published studies through May 2012. We performed a meta-analysis of all relevant case-control studies that examined the association between MTHFR C677T polymorphism and pancreatic cancer risk. Results: Finally, 9 individual case-control studies with a total of 1,299 pancreatic cancer cases and 2,473 controls were included into this meta-analysis. Results: This meta-analysis showed there was an obvious association between MTHFR C677T polymorphism and pancreatic cancer risk in East Asians (for allele model, OR = 1.67, 95%CI 1.11-2.51; For homozygote model, OR = 2.77, 95%CI 1.40-5.48; for recessive model, OR = 1.96, 95%CI 1.54-2.50; for dominant model, OR = 2.11, 95%CI 1.01-4.41). However, no significant association was found in Caucasians. Conclusion: The MTHFR C677T polymorphism is associated with pancreatic cancer risk, and a race-specific effect may exist in this association. More studies with a larger sample size are needed to further clarify this association.

Keywords: Methylenetetrahydrofolate reductase - genetic polymorphism - pancreatic cancer - meta-analysis

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Introduction

Pancreatic cancer has an exceptionally high mortality rate, making it one of the four or five most common causes of cancer mortality in developed countries (Jemal et al., 2011). The incidence of pancreatic cancer varies greatly across regions, which suggests roles for lifestyle factors, such as smoking, diet and alcohol, or environmental factors (Raimondi et al., 2009; Hidalgo, 2010). Rising prevalence of smoking in developing countries, improved diagnosis and increasing population longevity are all likely to increase the global burden of pancreatic cancer in the coming decades (Taghavi et al., 2011). Besides, long-standing diabetes increases the risk of pancreatic cancer, but can also be an early manifestation of pancreatic tumors (Chiou et al., 2011). Therefore, understanding the etiology and identifying the risk factors are essential for the primary prevention of this deadly disease (Hidalgo, 2010). Some individual studies suggest that mutations in various polymorphic genes can lead to small increases in the risk of pancreatic cancer, but these findings need to be replicated (Dong et al., 2008; Milne et al., 2009; Lin et al., 2011).

Methylenetetrahydrofolate reductase (MTHFR) plays a central role in folate metabolism by irreversibly catalyzing the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (Goyette et al., 1998). Many rare mutations of the MTHFR gene have been described in individuals, resulting in very low enzymatic activity, whereas the most common polymorphism is a C to T mutation in exon 4 at nucleotide 677, leading to Ala222Val and lower enzyme activity (Goyette et al., 1998). Recent studies have shown that the MTHFR 677 TT genotype is associated with an increased risk of cancer risk, suggesting an important role of folate levels and subsequent impaired chromosomal DNA synthesis and aberrant DNA methylation in carcinogenesis (Dong et al., 2008). Besides, there were also many published studies investigating the association between MTHFR C677T polymorphism and pancreatic cancer risk, but this possible association remains controversial owing to the conflicting results from those studies (Li et al., 2005; Matsubayashi et al., 2005; Wang et al., 2005; Nisevic et al., 2008; Suzuki et al., 2008). In the present meta-analyses, we aimed to assess the effect of MTHFR C677T polymorphism on pancreatic cancer risk by performing a meta-analysis of all available published and unpublished studies.

Materials and Methods

Study identification and selection criteria

We searched PubMed and CNKI database using the following search strategy: ('pancreatic carcinoma' or 'pancreatic cancer' or 'pancreatic adenocarcinoma') and

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('Methylenetetrahydrofolate reductase' or 'MTHFR' or 'C677T') for published and unpublished studies (last search was done on May 20, 2012). The language of the papers was not restricted. All searched studies were retrieved, and their bibliographies were checked for other relevant publications. The following criteria were used to select the eligible studies: (1) case-control studies; (2) evaluation of MTHFR C677T polymorphism and pancreatic cancer risk; (3) identification of pancreatic cancer was confirmed histologically or pathologically; (4) sufficient reported genotypic frequencies in both case and control populations for estimating an odds ratio (OR) with a 95% confidence interval (95%CI); (5) the genotype distribution among the control population was consistent with Hardy-Weinberg Equilibrium (HWE). The major reasons for exclusion of studies were: (1) case only studies; (2) review papers; (3) containing overlapping data. When more than one of the same patient population was included in several publications, only the most recent or complete study was used in this meta-analysis.

Data extraction

Two reviewers independently evaluated the final articles included into this meta-analysis, and disagreements were resolved by reaching a consensus among all authors. Data retrieved from the articles included the following: first author's name, publication year, country of origin, racial decent of the study population (categorized as Caucasians, East Asians and others), the frequency for MTHFR C677T genotypes and the allele frequency of MTHFR C677T polymorphism.

Statistical analysis

The association between MTHFR C677T polymorphism and pancreatic cancer risk was estimated by OR with its 95%CI. Five different comparison models of ORs were calculated: the allele model (T vs. C), the homozygote model (TT versus CC), the heterozygote model (CT versus CC), the recessive genetic model (TT versus T/C+CC), and the dominant genetic model (TT+T/C versus CC). For the control group of each study, the distribution of genotypes was tested for HWE using the Chi-square test. If controls of studies were found not to be in HWE, sensitivity analyses were performed with and without these studies to test the robustness of the pooled ORs. The χ^2-based Q statistic was used to investigate the heterogeneity between the studies, and a P value < 0.05 was interpreted as significant heterogeneity among the studies (Cochran, 1954). If heterogeneity existed, the random effects model (the DerSimonian and Laird method) was adopted to calculate the overall OR (DerSimonian and Laird, 1986). Otherwise, the fixed effects model (the Mantel-Haenszel method) was used (Mantel and Haenszel, 1959). We also calculated separate pooled estimates for different ethnic groups (Caucasians, East Asians and the others). In order to assess the stability of the results, sensitivity analyses were performed by reanalyzing the significance of ORs after omitting each study in turn. An estimate of potential publication bias was carried out by the Begg's funnel plot, and an asymmetric plot suggested a possible publication bias. Besides, the funnel plot asymmetry was further assessed by the method of Egger's linear regression test, a linear regression approach to measure funnel plot asymmetry on the natural logarithm scale of the OR (Egger et al., 1997). The analysis was conducted using Stata (Version 12.0; College Station, TX). All P values were two-sided and a P value of less than 0.05 was deemed statistically significant.

Results

Studies Characteristics

17 unique references were initially identified by the search. After excluding those which did not meet the selection criteria, 7 publications were finally included into this meta-analysis (Li et al., 2005; Matsubayashi et al., 2005; Wang et al., 2005; Wang et al., 2006; Nisevic et al., 2008; Suzuki et al., 2008; Yang and Qian, 2009). The study by Li D et al reported three individual case-control studies from different ethnicity (Li et al., 2005). Thus, 9 individual case-control studies with a total of 1299 pancreatic cancer cases and 2473 controls were included into this meta-analysis. There were four studies from East Asians (Matsubayashi et al., 2005; Wang et al., 2005; Wang et al., 2006; Yang and Qian, 2009) and three studies from Caucasians (Li et al., 2005; Nisevic et al., 2008; Suzuki et al., 2008). The distribution of MTHFR C677T genotypes was all consistent with HWE in the controls of those 9 studies (All P values for HWE were more than 0.05). The sample size varied from 33 (Li et al., 2005) to 942 (Suzuki et al., 2008), with a mean of 419.

Meta-analysis

Table 1 showed the pooled results of this meta-analysis.

Overall, there was obvious heterogeneity in all the genetic models (All P values for Q statistic were less than 0.05), and the random effect model was used to pool those data. The pooled outcomes showed there was no association between MTHFR C677T polymorphism and

Table 1. Association Between MTHFR C677T Polymorphism and Pancreatic Cancer Risk

Genetic models	OR(95%CI)	P_{OR}	Pooled model	$P_{Q\ statistic}$
All studies				
Allele model	1.28(0.97-1.70)	0.077	Random	<0.001
Homozygote model	1.68(0.95-2.96)	0.074	Random	<0.001
Heterozygote model	1.33(0.95-1.86)	0.1	Random	<0.001
Recessive model	1.47(0.98-2.19)	0.061	Random	0.001
Dominant model	1.45(0.97-2.19)	0.072	Random	<0.001
East Asians				
Allele model	1.67(1.11-2.51)	0.014	Random	<0.001
Homozygote model	2.77(1.40-5.48)	0.004	Random	0.001
Heterozygote model	1.72(0.92-3.22)	0.09	Random	<0.001
Recessive model	1.96(1.54-2.50)	<0.001	Fixed	0.136
Dominant model	2.11(1.01-4.41)	0.047	Random	<0.001
Caucasians				
Allele model	1.01(0.86-1.18)	0.932	Fixed	0.579
Homozygote model	1.09(0.75-1.57)	0.66	Fixed	0.089
Heterozygote model	0.96(0.77-1.21)	0.756	Fixed	0.536
Recessive model	1.09(0.49-2.14)	0.951	Random	0.031
Dominant model	0.99(0.79-1.23)	0.913	Fixed	0.952

POR was the P value of pooled OR; PQ statistic was for the P value of Q statistic

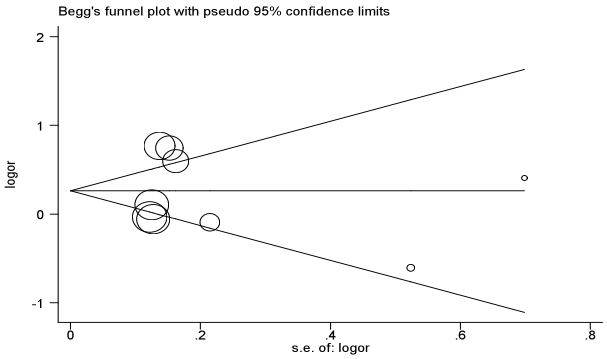

Figure 1. Begg's Funnel Plot Showed Low Risk of Publication Bias in this Meta-analysis

pancreatic cancer risk (All P values for pooled ORs were more than 0.05) (Table 1). However, sensitivity analyses by reanalyzing the significance of ORs after omitting each study in turn showed the significance of pooled ORs changed frequently, suggesting the lack of stability in the pooled results from this meta-analysis.

In the subgroup analyses of East Asians, the pooled outcomes showed there was an obvious association between MTHFR C677T polymorphism and pancreatic cancer risk in East Asians (For allele model, OR = 1.67, 95%CI 1.11-2.51; For homozygote model, OR = 2.77, 95%CI 1.40-5.48; For recessive model, OR = 1.96, 95%CI 1.54-2.50; For dominant model, OR = 2.11, 95%CI 1.01-4.41) (Table 1). Besides, sensitivity analyses by omitting each study in turn showed the pooled ORs were statistically robust, suggesting the stability in the pooled results from this subgroup analysis.

In the subgroup analyses of Caucasians, the pooled outcomes showed there was no association between MTHFR C677T polymorphism and pancreatic cancer risk in Caucasians (All P values for the pooled ORs were more than 0.05) (Table 1). Besides, sensitivity analyses by omitting each study in turn showed the pooled ORs were not statistically robust, suggesting the lack of stability in the pooled results from this subgroup analysis.

Publication bias

Begg's funnel plot and Egger's test were performed to assess the possible publication bias in this meta-analysis. The shape of the funnel plot did not reveal any evidence of obvious asymmetry, suggesting low risk of publication bias in this meta-analysis (Figure 1). Besides, the outcome of Egger's test provided statistical evidence for the symmetry of funnel plot (P = 0.901). Thus, there was no obvious risk of publication bias in this meta-analysis.

Discussion

Pancreatic cancer is considered to be one of the most lethal human cancers worldwide, and epidemiological studies have confirmed that some factors are etiological factors of pancreatic carcinogenesis. However, those factors only stay in the few individuals, which suggest the host's genetic factors may increase the host's susceptibility to pancreatic cancer (Dong et al., 2008; Lin et al., 2011). To test whether the MTHFR C667T polymorphism could modify the risk of pancreatic cancer, we conducted this meta-analysis. Finally, 9 individual case-control studies with a total of 1299 pancreatic cancer cases and 2473 controls were included into this meta-analysis. Results from this meta-analysis showed there was an obvious association between MTHFR C677T polymorphism and pancreatic cancer risk in East Asians (For allele model, OR = 1.67, 95%CI 1.11-2.51; For homozygote model, OR = 2.77, 95%CI 1.40-5.48; For recessive model, OR = 1.96, 95%CI 1.54-2.50; For dominant model, OR = 2.11, 95%CI 1.01-4.41). However, no significant association was found in Caucasians. Thus, MTHFR C677T polymorphism is associated with pancreatic cancer risk, and a race-specific effect may exist in this association.

Evidence from both experimental and epidemiologic studies support the hypothesis that folate maintains DNA stability and prevents cancer. Folate status is determined by both dietary folate intake and folate metabolism, and defects in folate metabolism have been linked to risk of a wide range of adverse health conditions. There is growing evidence that mild folate deficiency (a low normal level) is associated with an increased risk of developing certain cancers. Folate deficiency has been associated with certain types of human cancer (Crider et al., 2012). Numerous genes involved in the folate metabolism pathway and MTHFR is the most extensively studied gene among all. MTHFR is an important functional enzyme involved in folate and methyl group metabolism, and the polymorphisms in MTHFR gene were significantly associated with increased risk of several common cancers, such as esophageal carcinoma, and bladder cancer (Lin et al., 2007; Kouidhi et al., 2011; Liu et al., 2011). MTHFR C667T polymorphism is a 677C-to-T transition that causes an alanine-to-valine substitution in the MTHFR protein, with subsequent reduction in the enzyme activity and increase in its thermolability. Relative to the specific activity of MTHFR in the normal CC genotype, that of the homozygous TT genotype is reduced by 70%, and that of the heterozygous CT genotype is reduced by 35% (Goyette et al., 1994; Frosst et al., 1995). The TT genotype has been observed to increase plasma homocysteine levels in association with low folate intake and low level of serum folate (Goyette et al., 1994; Frosst et al., 1995). Thus, there is experimental evidence for the possible association between MTHFR C677T polymorphism and pancreatic cancer risk.

DNA methylation is an important epigenetic determinant in gene expression, maintenance of DNA integrity and stability, chromosomal modifications, and development of mutations. Aberrant DNA methylation is observed in a nonrandom, tumor type-specific manner. Furthermore, the regional hypermethylation is often associated with the inactivation of the tumor suppressor genes and hypomethylation is associated with activation of the oncogenes. The potential influence of MTHFR activity on the metabolism of methyl groups, DNA methylation, and the availability of uridylates and thymidylates for DNA synthesis and repair makes MTHFR attractive as a candidate cancer-modifying gene. Molecular epidemiologic studies have revealed that MTHFR polymorphisms are associated with an increased risk of various cancers (Lin et al., 2007; Dong et al.,

2008; Kouidhi et al., 2011). Besides, our meta-analysis adds a new evidence for the association between MTHFR 677C>T polymorphism and cancer.

Our analysis had several limitations to be considered when interpreting the findings. Firstly, the inclusion of few studies with relatively small sample size and poor validation was the main limitation of the meta-analysis. Thus, more studies with large sample size and careful design are needed to further identify this association more comprehensively. Secondly, the association between MTHFR 677C>T polymorphism and pancreatic cancer may be affected by the different histological types of pancreatic cancer. However, little data on this aspect were reported in those studies, and we were unable to make subgroup analyses by the different histological types of pancreatic cancer. Further studies are needed to identify this association in different histological types of pancreatic cancer. Finally, gene-gene and gene-environmental factors interactions were not fully addressed in this meta-analysis for the lack of sufficient data. Future studies may further assess the interactions among folate intake, folate status, smoking, alcohol, and MTHFR genotype in the association between MTHFR 677C>T polymorphism and pancreatic cancer.

In conclusion, our meta-analysis supports an association between MTHFR 677C>T polymorphism and pancreatic cancer, and there might be a race-specific effect in this association. Bedsides, further studies with large sample size and careful design are needed to identify this association more comprehensively.

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

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