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CASE CONTROL STUDY

Polymorphisms of MTHFR and susceptibility to oesophageal adenocarcinoma in a Caucasian United Kingdom population

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Abstract

AIM: To identify if methylene tetra-hydrofolatereductase (*MTHFR*) *C677T* polymorphisms are associated with oesophageal adenocarcnomas in a Caucasian population and to test whether folic acid and homocysteine levels are linked with cancer risk.

METHODS: A case control study comprising of 58 non cancer and 48 cancer patients, *MTHFR C667T* genotyping was made and serum folate, homocysteine and vitamin B12 levels were made. Tumour stage, differentiation and survival was recorded. A *P* value of less than 0.05 was taken to be significant. The χ^2 used to compare discrete variables and the Mantel-Cox was used to compare survival. A *P* value less than 0.05 was deemed to be significant.

RESULTS: *MTHFR* polymorphisms is associated with an increased risk of several cancers. A link between *MTHFR C677T* polymorphisms and oesophageal squamous cell carcinoma and gastric cardia adenocarcinoma has been demonstrated in at risk Chinese populations. In a Western European population the role of the *MTHFR* gene has not previously been investigated in the setting of oesophageal adenocarcinoma. No association between folic acid levels and cancer patients was found. The unstable *MTHFR 667 TT* genotype occurred in 11% cancers and 7% controls, but statistical significance was not reached, homocysteine levels and folic acid levels were not affected, cancer patients with TT genotype displayed a trend for a shorter survival 7 mo *vs* 20 mo. Serum vitamin B12 levels were higher in the cancer group. The *MTHFR 667 TT* genotype is much lower than previous population studies.

CONCLUSION: We conclude that serum folic acid and MTHFR polymorphisms are not associated with an increased risk of oesophageal adenocarcinoma, although cancers with unstable TT genotype may indicate a more aggressive disease course.

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Key words: Polymorphisms of 5,10-methylenetetrahydrofolate reductase; Oesophageal adenocarcinoma; Caucasian population; *Helicobacter pylori*; Polymorphism

Core tip: Our paper is the first Western population study and shows that methylene tetra-hydrofolatereductase (*MTHFR*) *C677T* polymorphisms is not associated with risk of oesophageal adenocarcinoma in contrast to Chinese and perhaps Far East populations. This highlights the difference in terms of the biology, genetics and epigenetics between Western and Eastern cancer populations and adds to our understanding of the etiology of

oesophagogastric cancers.

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INTRODUCTION

The incidence of oesophageal adenocarcinoma has been reported to be increasing dramatically over the past 30 years[1,2]. This rise has largely been attributed to gastro-oesophageal reflux disease, an obesity epidemic and health care policies to reduce *Helicobacter pylori* infection[3-8]. The rising incidence is a great concern due to the high mortality rate, partly because the majority of patients present with an advanced disease stage. Barrett's oesophagus is an established risk factor for the development of oesophageal adenocarcinoma[9] but only a relatively small proportion are diagnosed and surveyed.

Folic acid is a major component of green leafy vegetables and citrus fruit, it's deficiency has been implicated in the development of a variety of gastro intestinal cancers. Folic acid is thought to have a central role in DNA synthesis and gene activation by promoting *de novo* deoxynucleoside synthesis and influencing DNA methylation[10]. In contrast, folic acid deficiency is thought to be carcinogenic, through the disruption of DNA repair and secondly, by altering DNA methylation, resulting in gene silencing. As folic acid deficiency is common, in the United Kingdom, 20% of the elderly population are thought to be deficient[11], it is plausible that additional factors are necessary in the development of carcinogenesis.

The enzyme methylene tetra-hydrofolatereductase (MTHFR) is central for the metabolism of folic acid by regulating the production of its circulating form: 5-methyl-tetrahydrofolate (5MTHF). The precursor of 5MTHF: 5,10 MTHF is important for DNA repair by providing a supply of the precursors for DNA synthesis. Two common functional polymorphisms of the *MTHFR* gene have been identified, C677T and A1298C[12]. These polymorphisms are associated with increased thermolability and significantly diminished specific activity of the MTHFR enzyme[13-15]. The *MTHFR 677 TT* genotype occur in approximately 10% of the Caucasian United Kingdom population, and lower in Indian Asians. Individuals with the *MTHFR 677 TT* have higher plasma homocysteine levels in comparison with the wild type *MTHFR 677* CC and thus associated with diminished genomic DNA methylation especially when folic acid intake is insufficient[14-20].

The role of *MTHFR* polymorphisms is associated with an increased risk of several cancers[11-13,15,18-20]. A link between *MTHFR C677T* polymorphisms and oesophageal squamous cell carcinoma and gastric cardia adenocarcinoma has been demonstrated in at risk Chinese populations[17,21]. In a Western European population the role of the *MTHFR* gene has not previously been investigated in the setting of oesophageal adenocarcinoma. Here we have tested the association of *MTHFR C677T* polymorphisms in a population with oesophageal adenocarcinoma. The intention of this study was to identify if *MTHFR C677T* polymorphisms are associated with oesophageal adenocarcnomas and to test whether folic acid and homocysteine levels are linked with cancer risk.

MATERIALS AND METHODS

Ethical approval for this study was granted from Wrightington, Wigan and Leigh ethics committee in 2003. A trial of 102 Caucasian patients were recruited from the Royal Albert Edward Infirmary, Wigan, Greater Manchester in North West England. There were 44 patients with oesophageal adenocarcinoma (recruited at the time of diagnosis) and 58 patients without cancer. Exclusion criteria included a previous diagnosis of other cancers and previous or current use of anti folate medication including anticonvulsants and antibiotics. Age, gender, smoking status, tumour differentiation and survival from diagnosis was recorded. Survival (mo) was determined from the time of cancer diagnosis to date of death or date of cessation for the study. Serum analysis of folic acid, red cell folate, and vitamin B12 were measured in selected cases at the time of recruitment. Folate levels were determined by measuring serum or red cell folate levels and according to laboratory references. Groups were divided into "normal", "low" or "high" levels if the determined level was within normal range, lower or higher respectively. Serum Vitamin B12 levels were determined and according to laboratory references. Groups were divided in to "normal", "low" or "high" levels if the recorded level was within range, lower or higher respectively. Homocysteine was analysed in 41 patients in total, 12 had oesophageal adenocarcinoma. Blood samples were stored at 4 ℃ until analysed for serum homocysteine and MTHFR C677T genotyping [KBioscience Ltd, competitive allele-specific PCR system (KASPar) for SNP analysis].

Statistical analysis

SPSS version 14 was used for statistical analysis and to calculate survival curves, the *t* test was used to compare continuous variables. The χ^2 used to compare discrete variables and the Mantel-Cox was used to compare survival. A *P* value less than 0.05 was deemed to be significant.

RESULTS

Results from 102 cases were analysed. Age and gender were similar in the cancer and non cancer groups. The results are summarised in Table 1. We did not identify any difference in the proportion of cases with low folic acid levels between the cancer and control groups. Specifi-

Table 1 Study group characteristics *n* (%)

	Cancer	Control	Significance
Male	36 (82)	38 (65)	
Female	8 (18)	20 (35)	
Age (yr)	69 ± 12	64 ± 12	$P = 0.230$
Folate level			$P = 0.735$
Normal	26 (84)	42 (82)	
Low	0	8 (16)	
High	5 (16)	1 (2)	
Vitamin B12 level			$P = 0.010$
Normal	23 (72)	48 (89)	High *vs* normal + low
Low	1 (3)	4 (7)	
High	8 (25)	2 (4)	
Homocysteine level μmol/L	10.3 ± 4.4	12.0 ± 6.0	$P = 0.959$
MTHFR C677T			Total TT *vs* CT and CC
TT	5 (11)	4 (7)	$P = 0.329$
CT	20 (46)	27 (47)	
CC	19 (43)	27 (47)	
MTHFR C677T	Normal folate	Low folate	High folate
TT	3 (10)	0	0
CT	11 (36)	0	1 (3)
CC	12 (38)	0	4 (13)
Survival (mo)			$P = 0.160$
TT	7 ± 1.4		TT *vs* CT + CC
CT	17 ± 4.4		
CC	21 ± 7.0		

The characteristics of the cancer and control groups are displayed, patient numbers are shown and percentages or SD are indicated in brackets. The gender and average age of the study population at the time of study are shown. Mean levels of homocysteine are presented with standard deviations in brackets. Methylene tetra-hydrofolatereductase (MTHFR) C667T polymorphisms, TT, CT and CT were determined, the percentage in the cancer and control groups are displayed. Mean survival times in months and standard errors are displayed.

cally, none of the cancer cases displayed low folate levels, on the contrary, cancer patients tended to have higher serum folate levels compared to controls. Conversely, homocysteine levels are not elevated in the cancer group compared to controls. Vitamin B12 levels are higher in the cancer group compared to the non cancer group (25% *vs* 4%, $P = 0.010$). Analysis of the *MTHFR C667T* genotype identified that the cancer group have a slightly higher prevalence of the *TT* variant compared to the non cancer group (11% *vs* 7%) but this did not reach significance. Serum folic acid and vitamin B12 levels were not altered by the *MTHFR C677T* genotype, 31 cancer cases had both Folic acid level and MTHFR C677T measurements. The MTHFR genotype did not significantly influence survival, mean survival of the *TT, CT* and *CC* genotypes was 7, 17, and 21 mo respectively, a trend for a poor survival was observed in cancer patients with the *TT* genotype compared to *CT* and *CC* genotypes, 7 mo compared to 19 mo respectively ($P = 0.16$)

DISCUSSION

This study is limited by its small sample size in a case-control setting and hence the possibility of a type 2 statistical error. Our results are in keeping with an established male preponderance to oesophageal adenocarcinoma. Whilst there have been numerous studies investigating the association between folic acid consumption and risk of oesophageal adenocarcinoma[22-24], no previous study has investigated the role of *MTHFR C677T* polymorphisms and oesophageal adenocarcinoma in a western European population. Vitamin B12 is a co enzyme in the synthesis of DNA from folic acid, however, vitamin B12 levels are significantly higher in the oesophageal adenocarcinoma population. The cause of high vitamin B12 levels is not clear, it may be explained by the presence of liver metastases, vitamin B12 is normally stored in the liver. High vitamin B12 levels have previously been reported in oesophageal, gastric and prostate cancer[23,25], this has traditionally been thought to be a marker of a diet high in animal protein, an alternative theory is an aggressive cancer cell growth is stimulated by high levels of vitamin B12. Conversely, vitamin B12 deficiency is associated with reduced proliferation of neuroblastoma cells[26]. Never the less our study is in keeping with previous studies which indicate that high vitamin B12 levels display a more frequent occurrence in cancer, however as this occurs in only 25% of cancer cases, it can not be advocated as a predictive test in a clinical setting.

The role of folic acid and the pathogenesis of gastrointestinal cancers has been extensively studied[27]. It appears that a diet low in folic acid has the potential to increase the risk of carcinogensis[22-24]. Assessment of folic acid consumption by measurement of serum folic acid levels has been investigated in pancreatic adenocarcinoma[28] and squamous cell carcinoma of the oesophagus, a low serum folic acid level is associated with a higher risk of cancer[29]. Recently a study in a Turkish population has identified low serum folic acid levels in oesophageal adenocarcinoma patients compared to a normal control population, low serum folic acid levels were also seen in patients with reflux oesophagitis and Barrett's oesophagus[30]. In gastric and oesophageal squamous cell carcinoma, the risk of carcinogenesis is also associated with *MTHFR 677 TT* polymorphisms, however this association is more apparent in Chinese populations that display a higher prevalence of the dysfunctional TT phenotype in the general population. The increased risk of oesophageal squamous cell carcinoma also seems to be associated in combination with low folic acid consumption/levels[31]. A combination of a diet low in folic acid and an impaired folic acid metabolism may reduce DNA methylation and DNA repair and subsequently increase cancer risk. In oesophageal adenocarcinomas in North West England, we identified that the unstable *TT* polymorphism is more frequently seen compared to non cancer population, however this only accounts for 10% of cancer cases and the frequency of the *TT* polymorphism did not reach statistical significance when compared between cancer and control populations. The frequency of the *TT* polymorphism occurrence in the oesophageal adenocarcinoma cancer population in North West England is similar to previous studies in non cancer populations, this is in contrast to the higher prevalence in China, Japan and Germa-

ny (13%-44%)[27]. This suggests that *TT* polymorphisms are unhelpful to identify patients at risk of oesophageal adenocarcinoma, at least in this particular population and this is in keeping with the population in Turkey. Adenocarcinoma patients with *TT* polymorphisms may indicate a poorer outcome in terms of survival but larger studies, that can adjust for tumour staging and treatment, are needed to confirm this. In contrast to the Turkish population, we did not find a link between low serum folic acid levels and oesophageal adenocarcinoma however this may be due to study design, due to less stringent exclusion criteria for the normal control population. This finding also differs with previous studies in oesophageal squamous cell carcinoma and proximal gastric adenocarcinoma in China[28] and pancreatic adenocarcinoma in Finland[29]. The majority of studies investigating the role of folic acid in oesophageal adenocarcinoma have suggested that low folate consumption (measured by dietary questionnaires rather than serum folic acid levels) are a significant risk factor for carcinogenesis.

Homocysteine is closely related to folic acid metabolism it is important for methlylation by supplying methionine. An inverse relationship with homocysteine and folic acid exists, with high homocysteine levels occurring when folic acid levels are low. Overall homocysteine levels did not differ between cancer and non cancer groups, this is consistent with the findings of similar serum folic acid levels in cancer and non cancer groups. In this particular study, the lack of correlation with oesophageal adenocarcinoma patients and serum low folic acid levels, together with a lack of a relationship with homocysteine and *MTHFR C677T* polymorphism, indicates that folic acid levels are unlikely to be an important factor in the pathogenesis or predictor in the of the mainstay of oesophageal adenocarcinoma cases in North West England. The lack of relationship may be accounted by the small sample size or the absence of knowledge of the dietary consumption of folic acid. Folic acid levels were only analysed once the diagnosis of cancer had been made therefore we have no information of the levels in the years leading up to the diagnosis. However by the nature of the disease, oesophageal adenocarcinoma is often associated with malnutrition and vitamin deficiency. Because folic acid levels were similar in the cancer and non cancer groups, this suggests that folic acid levels are unlikely to be lower in the cancer group in the mo prior to the diagnosis. Taken together with the low proportion of *MTHFR 677 TT* genotypes in the cancer group, this indicates that analysis of *MTHFR C677T* genotypes, folic acid and vitamin B12 levels is unlikely to predict at risk individuals that may develop oesophageal adenocarcinoma. Future studies should be conducted with similar designs but with much larger sample size and in a prospective manner. This will reduce the possibility of type 2 statistical bias and will allow a a significant conclusion in a gene polymorphism study. In addition, other MTHFR polymorphisms like A1298C should also be studied to see if there is any association with oesophageal adenocarcinoma in a Caucasian United Kingdom population.

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COMMENTS

Background

Methylene tetra-hydrofolatereductase (*MTHFR*) polymorphisms is associated with an increased risk of several cancers. A link between *MTHFR C677T* polymorphisms and oesophageal squamous cell carcinoma and gastric cardia adenocarcinoma has been demonstrated in at risk Chinese populations. In a Western European population the role of the *MTHFR* gene has not previously been investigated in the setting of oesophageal adenocarcinoma.

Research frontiers

Authors have tested the association of *MTHFR C677T* polymorphisms in a United Kingdom Caucasian population with oesophageal adenocarcinoma. The intention of this study was to identify if *MTHFR C677T* polymorphisms are associated with oesophageal adenocarcnomas and to test whether folic acid, B12 and homocysteine levels are linked with cancer risk.

Innovations and breakthroughs

No association between folic acid levels and cancer patients was found. The unstable MTHFR 667 TT genotype occurred in 11% cancers and 7% controls, but statistical significance was not reached. Homocysteine and folic acid levels were not affected, cancer patients with TT genotype displayed a trend for a shorter survival 7 mo *vs* 20 mo. Serum vitamin B12 levels were higher in the cancer group.

Applications

This indicates that analysis of *MTHFR C677T* genotypes, folic acid and vitamin B12 levels is unlikely to predict at risk individuals that may develop oesophageal adenocarcinoma. Future studies should be conducted with similar designs but with much larger sample size and in a prospective manner. Other *MTHFR* polymorphisms like A1298C should also be studied to see if there is any association with oesophageal adenocarcinoma in a Caucasian United Kingdom population.

Peer review

In this case-control study the authors report their interesting results of the risk of oesophageal adenocarcinoma in a United Kingdom Caucasian population with MTHFR genetic polymorphisms.

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