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Methylenetetrahydrofolate reductase 677C>T polymorphism and gastric cancer susceptibility in Mexico

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ABSTRACT

This study investigated whether methylenetetrahydrofolate reductase *MTHFR 677C>T* polymorphism modified gastric cancer (GC) risk independently as well as in combination with folate intake and alcohol consumption. A hospital-based case-control study of 201 cases and 427 controls was conducted in three geographical areas of Mexico, between 1994 and 1996. The *MTHFR 677T* allele frequency was 51.0% in cases compared with 45.3% in controls. After controlling for dietary sources of folate, alcohol intake and other selected variables, a significant increase in GC risk was found among carriers of the *677TT* genotype compared with those with the *677CC* genotype (odds ratio (OR) 1.62, 95% confidence interval (CI) 1.00–2.59), with a significant trend ($P = 0.048$). There were no significant interactions between the *MTHFR* polymorphism and consumption of folate and alcohol. Our results suggest that the high prevalence of *MTHFR 677T* allele may be a contributor to the high rate of morbidity and mortality in GC in Mexico.

1. Introduction

Low dietary intake of folate has been associated with increased risk of gastric cancer (GC).[1] Methylenetetrahydrofolate reductase (MTHFR) is a key folate-metabolising enzyme involved in the generation of the universal methyl donor S-adenosylmethionine (SAM) and in DNA synthesis.[2] A common polymorphism, *MTHFR 677C>T* (alanine to valine) results a thermolabile variant of the enzyme, with a reduced activity. The MTHFR activities of the *677CT* and *677TT* genotypes were only 70% and 30% of activities observed in the wildtype *677CC* genotype.[3] Low folate intake and high level of alcohol consumption was capable of reducing the concentrations of SAM which was required for DNA methylation,[4,5] the variant *MTHFR* genotype has also been shown to result in DNA hypomethylation.[6] Hypomethylation of DNA occurs early in the carcinogenesis of gastric cancer.[6,7]

In contrast to the downward trend of GC incidence worldwide, GC remains steady as the second leading cause of cancer mortality in Mexico.[8,9] The Mexican population had the highest frequency (>50%) of the *MTHFR 677T* allele compared with other world populations[10]: they may have enhanced susceptibility to GC due to this genetic makeup. The purpose of this study was to examine the relationship between *MTHFR* and GC in a Mexican population. We also examined whether the *MTHFR 677C>T* polymorphism modified relationships between folate-containing diet, alcohol consumption and risk of GC.

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2. Materials and methods

Between 1994 and 1996 a hospital-based case-control study on the aetiology of GC was undertaken in three geographical areas of Mexico, with low (Mexico City), medium (Puebla) and high mortality (Yucatán) rates due to GC.[11]

2.1. Cases

All cases were patients histologically confirmed with adenocarcinoma of the stomach with no other personal antecedents of cancer: with a minimum age of 20 years and who had resided for the previous 6 months at least in the study areas. Thirteen hospitals participated in the study (7 in Mexico City, 3 in the city of Puebla and 3 in the city of Mérida). Histological types for the tumours (intestinal or diffuse) were assigned by a single pathologist specialising in GC, according to Lauren Criteria.[12] A total of 281 eligible patients were identified: 261 agreed to participate in the study, giving a participation rate of 92.9%. Information on *MTHFR* genotype was available for 201 cases (68 intestinal, 111 diffuse and 22 atypical). Information regarding anatomical site of the tumour origin was not available.

2.2. Controls

For each case, 2 hospital controls were selected, who were matched by age (±5 years) and gender of the index case. The exclusion were: personal antecedents of cancer, illnesses related to diet (principally gastritis, peptic ulcer, hepatic cirrhosis and diabetes mellitus), or from immunosuppressive complaints, and having resided in the same area for less than 6 months prior to recruitment. The most frequent diagnoses in the control group were: failings of the circulatory system for reasons other than hypertension (18.5%), illnesses of the nervous system and the sensory organs, other than psychiatric syndromes (15.7%): osteo-muscular and related tissue failings (14.5%): wounds and poisonings (10.8%): diseases of the respiratory tract (9.6%): diseases of the genital-urinary tract (8.4%): diseases of the skin (5.9%): individuals referring to the hospital for preventative services (9.8%): and a lesser percentage (6.8%) of patients with a variety of illnesses which included infectious illnesses or parasites, endocrine and metabolic disorders, complications from giving birth and related to post-partum, as well as congenital disorders. The response rate for the control group was 94.6%.

2.3. Interviews

Socio-demographic, clinical and dietary information was obtained by means of personal structured interviews, which were carried out in the various hospitals, after the participants had signed their consent forms. Trained interviewers as well as study participants were blinded to the study hypothesis. The interviews were undertaken over a short period between histological confirmation of the diagnosis and eventual hospitalisation. The participants were questioned about their dietary habits 3 years prior to the diagnosis (cases) and their respective illnesses (controls).

2.4. Folate and alcohol intake

The intake of foods containing folate was used as a proxy estimation of folate intake. Dietary folate sources included selected vegetables, fruits and meat. The frequency of consumption of individual foods as well as alcoholic beverages was determined with a previously validated questionnaire.[13,14] The questionnaire included 133 foods and drinks in Mexico City, 134 foods and drinks in Puebla and 147 foods and drinks in Yucatán. This instrument contains predetermined portions of each food and drink and 10 options for the frequency of consumption, ranging from none to 6 times a d. In the case of consumption of fruit and vegetables, the month and the seasonable availability of foods were taken into account for the calculation.

The vegetables that were considered as source of folate were: tomatoes, potatoes, carrots, lettuce, spinach, onion, avocado, garlic, cabbage, white corn, string beans and squash. Fruit sources of folate included: banana, orange, melon,

Table 1 – Characteristics of the study population according to the genotype methylenetetrahydrofolate reductase MTHFR 677C>T

Characteristic	*677CC* (200)	*677CT* (264)	*677TT* (164)
Age (years)			
X	57.1	58.7	58.3
(Min–max)	(28–85)	(28–79)	(28–80)
Sex (%)			
Male	55.5	57.6	58.5
Female	44.5	42.4	41.5
Residence (%)			
DF	22.5	28.4	35.4
Puebla	32.5	27.6	28.7
Yucatán	45.0	43.9	35.9
H. pylori (CagA+) (%)			
Positive	68.2	71.1	69.0
Negative	31.8	28.9	30.9
Vegetable intake[a] (portions/d)			
X	3.9	3.9	3.6
(10–90‰)	2.3–5.8	2.3–5.7	2.3–5.2
Fruit intake[b] (portions/d)			
X	2.25	1.90	1.81[d]
(10–90‰)	0.6–4.5	0.5–3.9	0.4–3.7
Animal sources intake[c] (portions/d)			
X	1.19	1.29	1.24
(10–90‰)	0.5–1.9	0.7–1.9	0.6–2.0
Alcohol consumption (%)			
Ever	54.0	52.6	51.2
Never	46.0	47.3	48.8

X, Mean; DF, Mexico City (Federal district).
a Banana, orange, melon, apple, watermelon, pineapple, pear, tangerine, strawberries, peach, grapes and lime.
b Tomatoes, potatoes, carrots, lettuce, spinach, onion, avocado, garlic, cauliflower, white corn, cabbage, string beans and squash.
c Eggs, chicken, beef, bacon, beef and pork liver, pork breakfast sausage, tuna and sardines.
d ANOVA test $P < 0.05$.

apple, watermelon, pineapple, pear, tangerine, strawberries, peach, grapes and lime. The following animal sources of folate were considered: eggs, chicken, beef, bacon, beef and pork liver, pork sausage, tuna and sardines.

Those individuals who reported a daily calorie intake of less than 700 kcal ($n = 9$) or above 4500 kcal ($n = 73$) were not included in the analysis, because these values indicate a doubtful quality of reporting of food consumption.

2.5. Blood samples

For each individual a 10 ml blood sample was taken, from which genomic DNA was isolated and kept until further analyses.

2.6. Genotyping

The genotyping of the *MTHFR 677C>T* polymorphism was successfully ascertained for 427 controls and 201 cases (final sample size for this report) using a method previously described.[15]

2.7. Seropositive to Helicobater pylori (CagA+)

To control for the presence of IgG antibodies against CagA+ protein those were determined by the enzyme-linked immunosorbent assay (ELISA) method according to the methodology described by Torres and colleagues.[16]

2.8. Statistical analysis

General characteristics of the study population were compared with respect to *MTHFR* genotypes, using χ^2 test and analysis of variance (ANOVA). The main effect of dietary sources of folate (i.e., vegetables, fruits and meat), alcohol consumption, and the *MTHFR* polymorphism on GC risk was estimated by unconditional logistical regression in terms of odds ratio (OR) and 95% confidence interval (95% CI). Multivariate analyses were performed by including the following covariants in the model: age, sex, total caloric and capsaicin intake, years of education, and *H. pylori* CagA+ status. To assess a potential interaction between the *MTHFR* polymorphism and dietary folate and alcohol intake, respectively,

Table 2 – Adjusted odds ratios (OR) for dietary sources of folate (vegetable, fruit and animal sources) and alcohol consumption in relation to gastric cancer risk

Food/alcohol	Cases	Controls	OR	OR
Vegetable intake (portions/d)				
0.00/3.05	67	140	1.0[a]	1.0[e]
3.06/3.97	59	142	0.76 (0.48–1.20)	0.78 (0.49–1.23)
3.98/29.14	75	145	0.92 (0.55–1.54)	0.97 (0.58–1.62)
P for trend			0.724	0.864
Fruit intake (portions/d)				
0.02/1.13	66	139	1.0[b]	1.0[f]
1.14/2.14	46	143	0.77 (0.48–1.22)	0.78 (0.49–1.24)
2.15/14.79	79	145	0.87 (0.52–1.43)	0.90 (0.55–1.50)
P for trend			0.581	0.705
Animal sources intake				
0.00/0.95	68	146	1.0[c]	1.0[g]
0.96/1.39	62	138	0.90 (0.58–1.40)	0.90 (0.58–1.41)
1.40/6.66	71	143	0.94 (0.60–1.46)	0.90 (0.58–1.42)
P for trend			0.779	0.659
Alcohol intake				
No	94	203	1.0[d]	1.0[h]
Yes	107	224	0.98 (0.67–1.42)	0.98 (0.67–1.44)

a Adjusted by age, sex, energy intake, level of education, *H. pylori* CagA + status, capsaicin intake, alcohol intake and dietary intake of animal and fruit sources of folate.
b Adjusted by age, sex, energy intake, level of education, *H. pylori* CagA + status, capsaicin intake, alcohol intake and dietary intake of vegetable and animal sources of folate.
c Adjusted by age, sex, energy intake, level of education, *H. pylori* CagA + status, capsaicin intake, alcohol intake and dietary intake of fruit and vegetable sources of folate.
d Adjusted by age, sex, energy intake, level of education, *H. pylori* CagA + status, capsaicin intake and dietary intake of fruit, vegetable and animal sources of folate.
e Adjusted by age, sex, energy intake, level of education, *H. pylori CagA* + status, capsaicin intake, alcohol intake, methylenetetrahydrofolate reductase (MTHFR) and dietary intake of animal and fruit sources of folate.
f Adjusted by age, sex, energy intake, level of education, *H. pylori* CagA + status, capsaicin intake, alcohol intake, MTHFR and dietary intake of vegetable and animal sources of folate.
g Adjusted by age, sex, energy intake, level of education, *H. pylori* CagA + status, capsaicin intake, alcohol intake, MTHFR and dietary intake of fruit and vegetable sources of folate.
h Adjusted by age, sex, energy intake, scholarship, *H. pylori* CagA + status, capsaicin intake, MTHFR and dietary intake of fruit, vegetable and animal sources of folate.

we used the likelihood ratio test (LRT) incorporating the product of the three categories of the *MTHFR* genotype and the tertile distribution of dietary folate and the dichotomous values for alcohol intake (yes/no). All the analyses were performed using the statistical software Stata 7.0 (Stata, College Station, TX, United States of America (USA)).

3. Results

Table 1 represents the general characteristics of the study population according to the *MTHFR* polymorphism. No significant differences were observed with relation to age, gender, residence, seropositivity to *H. pylori* CagA+, consumption of folate-containing vegetables and animal foods, and alcohol intake. A significantly lower consumption of folate-containing fruits was observed among *677TT* individuals compared with other *MTHFR* genotypes. The *MTHFR 677C>T* genotype distribution in the study population followed the Hardy–Weinberg equilibrium ($P = 0.17$).

No significant protective effects on GC were observed with respect to dietary consumption of folate-containing vegetables, fruits and animal foods, nor was there any association between GC risk and alcohol consumption before and after adjusting by *MTHFR* genotypes (Table 2).

A significant increase in GC risk was found among the *MTHFR 677T* carriers: the *677T* allele frequency was 51.0% in cases compared with that of 45.3% in controls. Compared with the *677CC* genotype, the *677TT* genotype had a significant increase in GC risk (OR 1.62, 95% CI 1.00–2.59). A significant dose-dependent relationship was observed across the *677CC*, *677CT* and *677TT* genotypes (P trend = 0.048). These associations remained significant among those with diffuse type of GC (OR 1.81, 95% CI 1.00–3.26, P trend = 0.048). Similar point estimates were observed in intestinal cases, but results did not reach statistical significance (Table 3). No *MTHFR*–dietary folate and *MTHFR*–alcohol interactions were found.

4. Discussion

Our results showed that the *MTHFR 677T* allele increased susceptibility to GC, predisposing a large portion of the Mexican population to a higher risk of developing GC. This susceptibility effect is similar in both intestinal and diffuse types of the disease, although the relationship was statistically significant in the latter, possibly due to the small number in intestinal cases. Our findings are in line with other existing studies, all from Chinese populations, in which increased risks for GC,[17–22] cardias GC[23,24] and oesophageal cancer[24–26] were observed among *677TT* individuals. However, none of these studies have dietary folate information to examine gene–environment interactions: one of them reported a significant *MTHFR*–alcohol interaction.[24]

The elevated risk associated with the *677T* allele, which resulted in DNA hypomethylation in previous studies, supports the notion that aberrant DNA methylation is important in GC aetiology.[6,7] The lack of significant interactions between folate and alcohol intake and *MTHFR* genotypes on GC risk, respectively, in this population, needs further replication in larger studies.

In spite of the lack of information at a national level regarding the anatomical site of the GC tumours, results from a recent study in Mexico City showed that approximately 10%

Table 3 – Adjusted odds ratios (OR) for methylenetetrahydrofolate reductase MTHFR 677C>T polymorphisms and gastric cancer risk

Genotype	Cases	Controls	OR[a]	OR[b]
All				
677CC	56	144	1.0	1.0
677CT	85	179	1.28 (0.84–1.96)	1.24 (0.81–1.90)
677TT	60	104	1.67 (1.04–2.67)	1.62 (1.00–2.59)
P for trend			0.033	0.048
P for interaction with folate				0.160
P for interaction with alcohol				0.356
Intestinal				
677CC	17	144	1.0	1.0
677CT	32	179	1.53 (0.80–2.94)	1.42 (0.73–2.75)
677TT	19	104	1.61 (0.77–3.37)	1.53 (0.73–3.23)
P for trend			0.194	0.253
P for interaction with folate				0.511
P for interaction with alcohol				0.998
Diffuse				
677CC	32	144	1.0	1.0
677CT	44	179	1.20 (0.70–2.06)	1.21 (0.70–2.09)
677TT	35	104	1.81 (1.01– 3.23)	1.81 (1.00–3.26)
P for trend			0.047	0.048
P for interaction with folate				0.100
P for interaction with alcohol				0.361

a Adjusted by age, sex, energy intake, level of education, *H. pylori* CagA+ status and capsaicin intake.
b Adjusted by age, sex, energy intake, level of education, *H. pylori* CagA+ status, capsaicin intake, alcohol intake and dietary intake of folate (animal, vegetable and fruit sources).

of GC cases arose in the cardia.[26] If we had been able to stratify by the anatomic site of the GC tumour, the associations might have been found to be stronger, since previous studies showed stronger associations with cardias GC versus all types of GC.[17,18]

The limitation of the study is its case-control design, which is prone to 'recall bias'. However, such limitation is minimal in our study for various reasons: (i) both study population and interviewers were blinded to the study hypothesis; (ii) people in general are not aware of which foods contain folate, and much less of those associated with GC.

The selection of controls is a critical element in epidemiological studies. In this respect it is important to emphasise that despite the fact that the controls in this study may have suffered from other illnesses apart from GC, none of these illnesses were associated with the *MTHFR* genotype or with the consumption of folate and alcohol (data not shown). In accordance with a national survey of seroepidemiology in Mexico[16] the frequency of seropositive individuals *H. pylori* CagA+ was approximately 70% of controls in this study: thus our control group was not substantially different from the general population in Mexico.

Unlike most parts of the world, GC incidence and mortality remain high in Mexico. Our study showed that 1 in every 2 Mexicans carries the risk allele for GC, *MTHFR 677T*. Besides prevalent infection of *H. pylori*, unfavourable dietary habits and delayed diagnosis, the high prevalence of the *MTHFR 677C>T* polymorphism may be an additional contributor to the high rate of morbidity and mortality due to GC in Mexico.

Conflict of interest statement

None declared.

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