Biomarkers, November–December 2007; 12(6): 635–644

informa
healthcare

Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and susceptibility to gastric adenocarcinoma in an Italian population

STEFANIA BOCCIA[1], FRANCESCO GIANFAGNA[1], ROBERTO PERSIANI[2], ANTONIO LA GRECA[2], DARIO ARZANI[1], STEFANO RAUSEI[2], DOMENICO D'UGO[2], PAOLO MAGISTRELLI[2], PAOLO VILLARI[3], CORNELIA M. VAN DUIJN[4], & GUALTIERO RICCIARDI[1]

[1]*Institute of Hygiene and* [2]*Department of Surgery, Università Cattolica del Sacro Cuore, Rome, Italy,* [3]*Hygiene Section, Department of Experimental Medicine and Pathology, University "La Sapienza", Rome, Italy and* [4]*Department of Epidemiology & Biostatistics, Erasmus Medical Center, Rotterdam, the Netherlands*

Abstract

Methylenetetrahydrofolate reductase (MTHFR) plays a central role in the metabolism of folate, which provides a methyl donor for DNA methylation and deoxynucleoside synthesis. We performed a case–control study to explore the relationship between two common *MTHFR* polymorphisms (C677T and A1298C), their combination and interaction with environmental exposures, on gastric adenocarcinoma susceptibility and progression in an Italian population. One hundred and two cases and 254 hospital controls, matched by age and gender, were enrolled. Individuals carrying the *MTHFR* 677T allele showed an increased risk of gastric cancer (odds ratio (OR) 1.62, 95% confidence interval (CI) 0.98–2.67), particularly among ever smokers (OR 2.10, 95% CI 1.07–5.33) and, among 677 TT individuals, those with a low intake of fruit and vegetables (OR 2.18, 95% CI 1.05–4.54). The strongest effect, however, was noted for the *MTHFR* 677 TT genotype among the diffuse gastric cancer histotype (OR 2.92, 95% CI 1.12–7.60). No association was detected for the effect of *MTHFR* A1298C polymorphism. Survival analysis did not show any association between each polymorphism on the overall survival, although when the analysis was restricted to the first year of follow-up after the surgical intervention an improved survival was noted among *MTHFR* 677 CC subjects compared with the T allele carriers (p value for log-rank test 0.02). In conclusion, *MTHFR* 677 (any T genotype) appears to modulate an individual's susceptibility to gastric cancer, particularly when combined with cigarette smoking and among those with a low intake of fruit and vegetables. Our results also suggest that an aberrant DNA methylation pattern, through impaired folate metabolism, might play a key role in gastric carcinogenesis. A possible survival effect of the *MTHFR* C677T genotype in gastric cancer patients deserves further investigations with larger sample sizes.

Correspondence: Stefania Boccia, Assistant Professor of Hygiene and Public Health, Genetic Epidemiology and Molecular Biology Unit, Institute of Hygiene, Catholic University of Sacred Heart, L.go F.Vito 1, 00168 Rome, Italy. Tel: 0039-06-35001527. Fax: 0039-06-35001522. E-mail: sboccia@rm.unicatt.it

ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.1080/13547500701546766

Keywords: *Methylenetetrahydrofolate reductase (MTHFR), gastric cancer, genetic susceptibility, effect modification smoking, diffuse gastric cancer, survival*

(Received 17 March 2007; accepted 29 June 2007)

Introduction

Fruit and vegetable intake has been repeatedly reported as protective against cancer occurrence, including gastric cancer (Key et al. 2004). A recent meta-analysis of prospective studies showed an inverse association between fruit and vegetables intake and gastric cancer incidence, particularly for follow-up periods at least of 10 years (Lunet et al. 2005). The protective effect against cancer may be due to the combined action of a number of the antioxidant micronutrients, such as β-carotene, vitamins C and E, retinol and the folate content (Correa & Schneider 2005). Folate is a water-soluble B vitamin that plays the fundamental role of providing methyl groups for intracellular methylation reactions and *de novo* deoxynucleoside synthesis (Wagner 1995). Two prominent mechanisms whereby folate deficiency may influence cancer risk have been described (Larsson et al. 2006): low folate levels may induce misincorporation of uracil into DNA, which could lead to chromosomal breaks and mutations; and/or by causing DNA hypomethylation, resulting in altered gene expression (Duthie 1999).

Besides an inadequate folate intake, functional polymorphisms in key enzymes involved in folate metabolic pathway are supposed to modify the risk of cancer. Among them, the methylenetetrahydrofolate reductase (MTHFR) enzyme irreversibly converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulating form of folate. Two functional polymorphisms of *MTHFR*, C677T and A1298C, have been identified (Frosst et al. 1995), so that heterozygotes (CT) and homozygotes (TT) for the mutant allele of 677, respectively, have 65% and 30% of the enzyme activity of individuals with wild-type genotype, while CC homozygotes for the *MTHFR* 1298 polymorphism have an enzyme activity around 50–60% of those without the variant allele (Frosst et al. 1995, Weisberg et al. 1998). Individuals with the TT genotype for the *MTHFR* 677 have significantly lower plasma folate levels than those with the wild-type genotype, while for the 1298 variant the evidence is inconsistent (Friedman et al. 1999). Some nutrients (e.g. vitamins B_6 and B_{12}, and methionine) involved in the folate metabolic pathway, as well as alcohol (a folate antagonist) and smoking (which impairs folate status), may interact with folate and the *MTHFR* polymorphisms in relation to cancer risk (Bailey 1990, 2003).

A recently published meta-analysis shows no significant protective effect of dietary folate intake on gastric cancer, while an increased risk associated with the *MTHFR* 677 TT genotype has been detected (Larsson et al. 2006). Few studies, however, explored a possible effect modification of the *MTHFR* C677T polymorphism on gastric cancer risk by environmental exposures affecting folate status (Stolzenberg-Solomon et al. 2003, Gao et al. 2004, Graziano et al. 2006), and no one has ever explored this aspect in relation to *MTHFR* A1298C polymorphism. Furthermore, nothing is known about the influence of these genetic variants on the survival of gastric cancer patients. In the present study we aimed to investigate the effect of both *MTHFR* C677T and A1298C polymorphisms, their combination and the interaction with lifestyle exposure that might affect plasma folate levels, on gastric cancer development and progression in an Italian population.

Materials and methods

Study population and genotyping

The study subjects were selected according to a case–control study design as previously described (Boccia et al. 2005). Briefly, cases were consecutive primary gastric adenocarcinoma patients, with histological confirmation, who underwent a curative gastrectomy in the 'A. Gemelli' teaching hospital, located within the Università Cattolica del Sacro Cuore in Rome. Controls were selected from cancer-free patients, with a broad range of diagnoses, admitted to the same hospital during the identical time period and were frequency matched to cases for age ($\pm$5 years) and gender. All subjects were Caucasians born in Italy. According to the Lauren classification, the majority (57.8%) of the gastric cancer cases were intestinal (Lauren 1965). The tumours were located in the antrum (39.3%), the corpus (14.8%), the antrum/corpus (28.0%), in the cardia (10.3%), stumps (5.6%) and in the fundum (2.0%). Based on the cytological and architectural atypisms, as well as the histopathological reports (Sobin & Wittekind 2002), patients' tumours were classified accordingly: 68.3% scarcely differentiated, 29.2% moderately differentiated, 2.5% well differentiated, while 53.8% were staged I–II and 46.2% were staged III–IV. With a response rate of 95% and 90%, respectively, for cases and controls, 102 gastric cancer and 254 controls were recruited.

A venous blood sample was drawn from each participant, collected into EDTA-coated tubes from which DNA was isolated from peripheral blood lymphocytes. Genotyping for *MTHFR* C677T and A1298C polymorphisms was performed using a restriction-fragment length polymorphism-based method, as already described by Yi et al. (2002). Quality control for each genotyping was performed in each experiment, and 10% of the total samples were randomly selected and reanalyzed with 100% concordance. The analyst was blinded to the case or control status of the samples. The study was approved by the local review board and written informed consent was obtained from each subject. The procedures followed were in accordance with the Helsinki Declaration.

Data collection

Cases and controls were interviewed by trained medical doctors using a standard questionnaire to elicit information on demographic variables, cigarette smoking and drinking history, dietary habits and family history of cancer. Questions pertaining to lifestyle focused on the time period ending 1 year prior to diagnosis. Smoking status was categorized as never and ever smokers (including both current and former smokers). Pack-years were calculated as years smoked multiplied by the current number (or previous number, for those who had quit) of cigarettes smoked per day divided by 20. Fruit and vegetable intake was classified as high if the individual consumed at least two portions of fruit and vegetables per day. Family history of cancer referred to parents, siblings and offspring. Cases were actively followed up after the day of surgical intervention with a median follow-up time of 19.5 months, and information on all-cause mortality was collected. The proportion of lost to follow-up was 8.5% (12/102).

Statistical analysis

The relationship between gastric cancer and putative risk factors were measured using the adjusted odds ratios (ORs) and their 95% confidence interval (CI) derived from logistic regression analysis using STATA software (version 8.2). We considered possible risk factors for gastric cancer as potential confounders if the addition of that variable to the model changed the OR by 10% or greater. Confounding checks were performed in both of the univariate and final multivariate models. If a factor was identified as a confounder of any estimated main effect, it was kept in all models. Based on these criteria, we controlled for age, gender, alcohol consumption and family history of cancer, when appropriate. In the multivariable model, we adjusted for the continuous variables of age and alcohol. The Hardy–Weinberg equilibrium (HWE) was tested for separately in all of the case and control polymorphisms.

In order to assess if the effect of the studied polymorphisms is modified by lifestyle exposures that might affect folate metabolism, we performed a logistic regression analysis stratified for alcohol, smoking status, and fruit and vegetable intake. A homogeneity test was then used to test differences among the strata. In this analysis we used as a reference group those homozygous wild-type individuals who had not been exposed to environmental factors; smoking status was here considered as ever/never cigarette smokers and alcohol consumption as users/non-users (the latter including individuals whose alcohol intake was less than 7 g daily).

Finally, the log-rank test was used to evaluate the association between both *MTHFR* polymorphisms and the survival at 1 year, 3 years and overall survival after gastric surgery intervention. The risk of death was also estimated by Cox's proportional hazards model, when applicable. Hazard ratios (HR) were adjusted for age and gender, with the wild-type genotypes as the reference group.

Results

General characteristics of the study population are presented in Table I. Alcohol consumption and family history of cancer were associated with an increased risk of gastric cancer, with ORs of 2.10 (95% CI 1.21–3.67) and 3.74 (95% CI 1.13–12.45) for moderate and heavy drinkers, respectively, and an OR of 1.80 (95% CI 1.04–3.06) for individuals with a familial history of cancer (see Table I). The genotype frequencies of our control group were in line with those for Caucasians (Botto & Yang 2000) and were in HWE both for cases and controls ($p > 0.05$). As shown in Table II, we found a significant difference in the distribution of *MTHFR* 677T carriers among cases and controls: 71.6% vs 61.4%, respectively, with an OR of 1.62 (95% CI 0.98–2.67). When results were stratified according to tumour histology, the strongest effect was noted among the diffuse type, with an OR of 2.92 (95% CI 1.12–7.60) for *MTHFR* 677 TT (Table II).

From the analysis of the combined effect of the *MTHFR* C677T and A1298C, no one subject was homozygous for the mutant allele at both sites (data not shown). Among subjects with both *MTHFR* 677 TT and 1298 AA, the OR was 2.21 (95% CI 0.84–5.80), and similarly, individuals with both *MTHFR* 677 CT and 1298 AC genotypes had an OR for gastric cancer of 1.95 (95% CI 0.80–4.81) compared with those with combined 677 CC/1298 AA (data not shown). A homogeneity test, however, showed that none of these differences was statistically significant, probably due to the very small number of both variant alleles in the analysis.

Table I. Odds ratios (OR) (95% confidence interval (CI)) for gastric cancer according to selected variables and their frequency distribution among 102 gastric cancer cases and 254 controls.

	Cases (%)	Controls (%)	OR (95% CI)[a]
Age (years ± SD)	66.3 ± 12.1	64.0 ± 13.0	–
Male gender	54 (53.0)	141 (55.5)	–
Alcohol drinkers			
0–6 g daily	40 (39.6)	150 (59.3)	1[b]
7–29 g daily	53 (52.5)	96 (37.9)	2.10 (1.21–3.67)
>30 g daily	8 (7.9)	7 (2.8)	3.74 (1.13–12.45)
Smoking status			
Never	54 (53.0)	146 (57.5)	1[b]
Ever	48 (47.1)	108 (42.5)	1.09 (0.63–1.90)
Pack-years of smoking			
0	55 (55.0)	146 (57.7)	1[b]
1–25	21 (21.0)	62 (24.5)	0.97 (0.50–1.92)
>25	24 (24.0)	45 (17.8)	1.05 (0.53–2.12)
Fruit and vegetable intake			
High[c]	19 (18.8)	40 (15.9)	1[b]
Low	82 (81.2)	212 (84.1)	0.96 (0.50–1.85)
Meal salt addition			
No	88 (86.3)	235 (92.9)	1[b]
Yes	14 (13.7)	18 (7.1)	1.52 (0.68–3.41)
Family history of cancer			
No	59 (62.8)	192 (78.4)	1[b]
Yes	35 (37.2)	53 (21.6)	1.80 (1.04–3.06)

[a]OR adjusted by age, gender, alcohol consumption (as continuous variable) and family history of cancer; [b]Reference category; [c]at least two portions of fruit and vegetables per day (see methods).

From our analysis there was no evidence of effect modification of *MTHFR* 677 and 1298 polymorphisms by the lifestyle exposures (Table III); however, ever smokers carrying the *MTHFR* 677 T allele showed a significant increased risk (OR 2.40, 95% CI 1.07–5.33) of gastric cancer, while among never smokers that risk appeared not

Table II. Distribution of the studied polymorphisms in 102 gastric cancer cases and 254 controls.

	Cases (%)	Controls (%)	All cases OR (95% CI)[a]	Intestinal OR (95% CI)[a]	Diffuse OR (95% CI)[a]
MTHFR 677					
CC	29 (28.4)	98 (38.6)	1[b]	1[b]	1[b]
CT	51 (50.0)	115 (45.3)	1.53 (0.90–2.62)	1.27 (0.67–2.41)	2.45 (1.09–5.50)
TT	22 (21.6)	41 (16.1)	1.84 (0.95–3.59)	1.27 (0.55–2.92)	2.92 (1.12–7.60)
T carriers	73 (71.6)	156 (61.4)	1.62 (0.98–2.67)	1.27 (0.70–2.32)	2.58 (1.19–5.58)
MTHFR 1298					
AA	50 (49.0)	125 (49.2)	1[b]	1[b]	1[b]
AC	43 (42.2)	107 (42.1)	0.98 (0.60–1.59)	0.47 (0.33–1.63)	0.72 (0.36–1.45)
CC	9 (8.8)	22 (8.7)	0.97 (0.42–2.27)	–	1.50 (0.52–3.91)
C carriers	52 (51.0)	129 (50.8)	0.98 (0.62–1.55)	1.06 (0.60–1.88)	0.84 (0.44–1.60)

[a]Odds ratio (OR) adjusted by age and gender; [b]reference category. CI, confidence interval; MTHFR, methylenetetrahydrofolate reductase.

Table III. Risk of gastric cancer associated with the methylenetetrahydrofolate reductase *(MTHFR)* C677T and A1298C genotypes according to selected lifestyle exposures.

	MTHFR 677 T carriers[a]		*p* Value	*MTHFR 1298* C carriers[a]		*p* Value
	No. cases/ no. controls	OR (95% CI)[b]	for for homogeneity	No. cases/ no. controls	OR (95% CI)[b]	for for homogeneity
Smoking status						
Never	35/90	1.16 (0.60–2.52)		27/81	0.78 (0.41–1.48)	
Ever	38/66	2.40 (1.07–5.33)	0.17	25/48	1.36 (0.68–2.70)	0.22
Alcohol drinkers						
No	21/71	1.76 (0.71–4.34)		21/75	1.16 (0.51–2.66)	
Yes	52/85	1.46 (0.78–2.73)	0.67	31/54	0.87 (0.49–1.54)	0.61
Fruit and vegetable intake						
High[c]	12/21	1.48 (0.48–4.60)		9/22	0.68 (0.22–2.11)	
Low	61/135	1.68 (0.95–3.00)	0.94	43/106	1.06 (0.63–1.77)	0.51

[a]Reference category is the homozygous wild-type genotype; [b]Odds ratio (OR) adjusted by age and gender; [c]At least two portions of fruit and vegetables per day; CI, confidence interval.

significant (OR 1.16, 95% CI 0.60–2.52). Additionally, when the *MTHFR* 677 TT genotype was stratified according to fruit and vegetable intake (data not shown), ORs of 0.49 (95% CI 0.04–5.9) and 2.18 (95% CI 1.05–4.54) resulted among high and low consumers, respectively (data not shown; *p* value of homogeneity among the estimates = 0.09).

The mortality rate in our gastric cancer cases was 1.01/100 person-months (95% CI 0.70–1.45). Patients carrying at least one *MTHFR* 677 T allele did not show a different median survival time (*p* value for log-rank test = 0.49), with an HR of 1.19 (95% CI 0.49–2.92) and 1.79 (95% CI 0.67–4.78) for *MTHFR* CT and TT genotypes, respectively. Similar results were obtained for *MTHFR* 1298T carriers, with an HR of 1.05 (95% CI 0.50–2.22, *p* value for log-rank test = 0.82) compared with the homozygous wild-type. When the analysis was restricted to 1-year survival, *MTHFR* 677 CC subjects during that period all resulted alive with respect to those carrying at least one 677 T allele (*p* value for log-rank test = 0.02) (Figure 1). However, when the time period was extended to 3-years the effect was no longer detected (*p* value for log-rank test = 0.60). Absence of a survival affect was noted for *MTHFR* 1298C carriers when restricting the analysis to the first year and three years after the surgical intervention (data not shown).

Discussion

This case–control study of 102 surgical cases of gastric adenocarcinoma and 254 controls evaluated the effect on gastric cancer risk of two common *MTHFR* polymorphisms. Results showed an increased risk for *MTHFR* 677T carriers, with a growing trend from individuals carrying only one variant allele to those carrying two (*p* value for trend = 0.05). That risk became higher in patients with the diffuse gastric cancer histological type. No association was detected for the effect of *MTHFR* A1298C polymorphism on gastric cancer risk; also, there was no evidence of effect modification by the lifestyle exposures. An increased risk was detected among ever

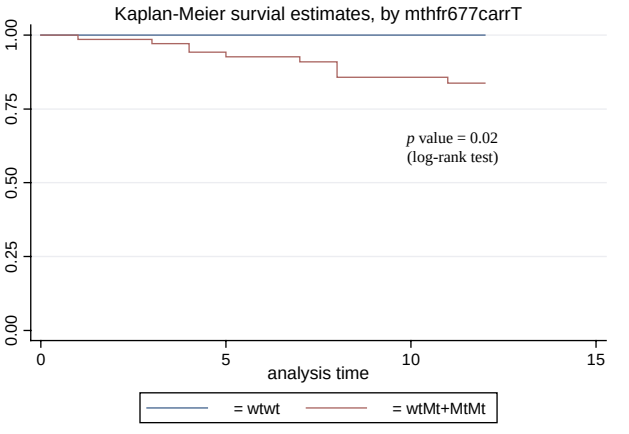

Figure 1. Association between methylenetetrahydrofolate reductase (*MTHFR*) C677T genotypes and 1-year survival after gastric surgery intervention.

smokers carrying at least one *MTHFR* 677 variant allele and among those with *MTHFR* 677 TT and a low intake of fruit and vegetables, despite the results of the homogeneity test this did not support a true effect modification in both instances. Our results also confirm previous findings of gastric cancer risk to be increased by alcohol intake and familial clustering (Bagnardi et al. 2001, Aoki et al. 2005). In addition, from the survival analysis a possible effect was noted when considering only the first year of follow-up, with all subjects who died among those carrying at least one 677 T allele.

Some limitations of the study need to be considered before interpreting the results. Firstly, based on the prevalence of the analyzed genotypic variants in our control population (see Table II), our study is powered to detect a minimum OR of 2.0 for the effect of *MTHFR* 677T carriers and *MTHFR* 1298C carriers (with a significance level of 0.05); however, the power is lower for the homozygote variants of both genotypes. The study sample size also limits the ability to explore the effect modification of the lifestyle exposures, or the combined effects of both genotypes, which highlights the need to increase the sample size in order to confirm our results. However, when appropriately conducted, large and small studies should, theoretically, give the same results, with just a more precise effect measure estimate from the larger ones (Ioannidis 2006). Secondly, as in all case–control studies information bias may exist, leading to biased ORs related to the lifestyle exposures.

Our study supports the evidence of an increased risk for gastric cancer among individuals carrying the unfavourable variant of *MTHFR* 677, thus confirming the results of the two recently published meta-analyses (Larsson et al. 2006, Zintzaras 2006). Individuals who are *MTHFR* 677T carriers have reduced enzyme activity and, particularly among those with inadequate folate intake (Graziano et al. 2006), subsequent aberrant genomic DNA methylation. We also observed that the risk for *MTHFR* 677T carriers is higher among those with a diffuse gastric cancer histotype, which is the most deadly form of gastric cancer (Wu et al. 1997), as already noted by Lacasana-Navarro et al. (2006). Recently, aberrant methylation of proto-oncogenes has been explored as both a mechanism and marker of carcinoma progression (Dunn 2003), with some papers reporting a different methylation pattern

between the intestinal and diffuse gastric cancer histotype (Tahara 2004, Yamashita et al. 2006). Taken together, these results suggest that a global aberrant DNA methylation pattern may play a key role in gastric cancer susceptibility and progression, with the MTHFR enzyme playing a central part.

In the present study, we observed that the effect of the *MTHFR* 677 variant genotype is particularly strong among ever smokers, which is in keeping with the results from Gao et al. (2004). As for the negative effect of smoking on folate status, some authors reported that elevated folate turnover in response to rapid tissue proliferation in aerodigestive tissues among people exposed to tobacco smoke might partially explain this phenomenon, which might even be worsened among individuals carrying the unfavourable *MTHFR* genotype variant (Heimburger 1992). We cannot ignore, however, that this effect might be confused by alcohol intake or dietary habits (Tungtrongchitr et al. 2003).

To our knowledge, no study has ever explored whether or not the effect of the *MTHFR* 677 TT genotype on gastric cancer is modified by folate levels; however, two studies reported a strong association between *MTHFR* 677 TT and gastric cancer in populations with folate deficiency (Miao et al. 2002, Graziano et al. 2006). Also, a prospective study on colorectal cancer risk reported that the protective effect of the *MTHFR* 677 TT genotype disappears among those with folate deficiency (Ma et al. 1997), so we would expect a partial reduction of the negative effect on gastric cancer risk by the *MTHFR* 677 variant among those with adequate folate intake. From our study it appears that the effect of the *MTHFR* 677 variant genotypes on gastric cancer might be modified by fruit and vegetable intake, the main source of dietary folate, particularly among TT individuals. The lack of statistical power, however, limits our results which need to be confirmed by increasing the sample size.

As for the combined inheritance of the two *MTHFR* variants, although all the reported results are not statistically significant for an $\alpha = 0.05$, there is slight evidence of an increased risk in individuals carrying at least one T allele of *MTHFR* C677T, which is in keeping with the results of Miao (2002).

Finally, we were not able to detect any effect of the studied polymorphisms on the overall survival after surgical gastrectomy intervention; however, when considering the first year of follow-up an increased mortality rate was experienced from *MTHFR* 677T carriers when compared with the CC individuals. Despite the result being based on a very small number of subjects, it suggests that a different pathway of tumour progression might be experienced from gastric cancer patients based on their *MTHFR* 677 polymorphism, which eventually affects folate blood levels and DNA methylation status. A recent study on the assessment of folic acid supplementation on colorectal adenomas failed to detect a preventive effect, while an increased risk of colorectal neoplasia was revealed among treated individuals (Cole et al. 2007). To our knowledge, similar trials have never been conducted on individuals with gastritis or gastric cancer; therefore, the exact effect on gastric carcinogenesis of folic acid levels, affected by *MTHFR* status, deserves additional investigations in order to integrate all the results into one coherent picture.

In conclusion, this study supports the role of *MTHFR* C677T polymorphism, but not A1298C, in gastric carcinogenesis, particularly for the diffuse histotype which is usually associated with a poorer prognosis. In order to gain a clearer picture of the events influencing gastric cancer susceptibility and progression through the folate metabolic pathway, it is critical that larger prospective studies with appropriate

collection of data on lifestyle habits and folate intake are implemented. This would lay the foundation for evaluating possible benefits from preventive nutritional intervention in at-risk individuals for gastric cancer.

Acknowledgements

We are grateful to Rosarita Amore for the processing of blood samples, and to Emma De Feo and Paola Gallì for the database management. This study was supported by a grant from Università Cattolica del Sacro Cuore (D1 projects 2006).

References

Aoki M, Yamamoto K, Noshiro H, Sakai K, Yokota J, Kohno T, Tokino T, Ishida S, Ohyama S, Ninomiya I, Uesaka K, Kitajima M, Shimada S, Matsuno S, Yano M, Hiratsuka M, Sugimura H, Itoh F, Minamoto T, Maehara Y, Takenoshita S, Aikou T, Katai H, Yoshimura K, Takahashi T, Akagi K, Sairenji M, Yamamura Y, Sasazuki T. 2005. A full genome scan for gastric cancer. Journal of Medical Genetics 42:83–87.

Bagnardi V, Blangiardo M, La Vecchia C, Corrao G. 2001. A meta-analysis of alcohol drinking and cancer risk. British Journal of Cancer 85:1700–1705.

Bailey LB. 1990. Folate status assessment. The Journal of Nutrition 120(Suppl. 11):1508–1511.

Bailey LB. 2003. Folate, methyl-related nutrients, alcohol, and the MTHFR 677C-->T polymorphism affect cancer risk: intake recommendations. The Journal of Nutrition 133:3748S–3753S.

Boccia S, Persiani R, La Torre G, Rausei S, Arzani D, Gianfagna F, Romano-Spica V, D'Ugo D, Ricciardi G. 2005. Sulfotransferase 1A1 polymorphism and gastric cancer risk: a pilot case–control study. Cancer Letters 229:235–243.

Botto LD, Yang Q. 2000. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. American Journal of Epidemiology 151:862–877.

Cole BF, Baron JA, Sandler RS, Haile RW, Ahnen DJ, Bresalier RS, McKeown-Eyssen G, Summers RW, Rothstein RI, Burke CA, Snover DC, Church TR, Allen JI, Robertson DJ, Beck GJ, Bond JH, Byers T, Mandel JS, Mott LA, Pearson LH, Barry EL, Rees JR, Marcon N, Saibil F, Ueland PM, Greenberg ER; Polyp Prevention Study Group. 2007. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. JAMA 297:2351–2359.

Correa P, Schneider BG. 2005. Etiology of gastric cancer: what is new? Cancer Epidemiology. Biomarkers and Prevention 14:1865–1868.

Dunn BK. 2003. Hypomethylation: one side of a larger picture. Annals of the New York Academy of Sciences 983:28–42.

Duthie SJ. 1999. Folic acid deficiency and cancer: mechanisms of DNA instability. British Medical Bulletin 55:578–592.

Friedman G, Goldschmidt N, Friedlander Y, Ben-Yehuda A, Selhub J, Babaey S, Mendel M, Kidron M, Bar-On H. 1999. A common mutation A1298C in human methylenetetrahydrofolate reductase gene: association with plasma total homocysteine and folate concentrations. The Journal of Nutrition 129:1656–1661.

Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP, Rozen R. 1995. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nature Genetics 10:111–113.

Gao CM, Takezaki T, Wu JZ, Liu YT, Ding JH, Li SP, Su P, Hu X, Kai HT, Li ZY, Matsuo K, Hamajima N, Sugimura H, Tajima K. 2004. Polymorphisms in thymidylate synthase and methylenetetrahydrofolate reductase genes and the susceptibility to esophageal and stomach cancer with smoking. Asian Pacific Journal of Cancer Prevention 5:133–138.

Graziano F, Kawakami K, Ruzzo A, Watanabe G, Santini D, Pizzagalli F, Bisonni R, Mari D, Floriani I, Catalano V, Silva R, Tonini G, Torri V, Giustini L, Magnani M. 2006. Methylenetetrahydrofolate reductase 677C/T gene polymorphism, gastric cancer susceptibility and genomic DNA hypomethylation in an at-risk Italian population. International Journal of Cancer 118:628–632.

Heimburger DC. 1992. Localized deficiencies of folic acid in aerodigestive tissues. Annals of the New York Academy of Sciences 669:87–96.

Ioannidis JPA. 2006. Meta-analysis in public health: potential and problems. Italian Journal of Public Health 3:9–14.

Key TJ, Schatzkin A, Willett WC, Allen NE, Spencer EA, Travis RC. 2004. Diet, nutrition and the prevention of cancer. Public Health Nutrition 7:187–200.

Lacasana-Navarro M, Galvan-Portillo M, Chen J, Lopez-Cervantes M, Lopez-Carrillo L. 2006. Methylenetetrahydrofolate reductase 677C>T polymorphism and gastric cancer susceptibility in Mexico. European Journal of Cancer 42:528–533.

Larsson SC, Giovannucci E, Wolk A. 2006. Folate intake, MTHFR polymorphisms, and risk of esophageal, gastric, and pancreatic cancer: a meta-analysis. Gastroenterology 131:1271–1283.

Lauren P. 1965. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathologica et Microbiologica Scandinavica 64:31–49.

Lunet N, Lacerda-Vieira A, Barros H. 2005. Fruit and vegetables consumption and gastric cancer: a systematic review and meta-analysis of cohort studies. Nutrition and Cancer 53:1–10.

Ma J, Stampfer MJ, Giovannucci E, Artigas C, Hunter DJ, Fuchs C, Willett WC, Selhub J, Hennekens CH, Rozen R. 1997. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. Cancer Research 57:1098–1102.

Miao X, Xing D, Tan W, Qi J, Lu W, Lin D. 2002. Susceptibility to gastric cardia adenocarcinoma and genetic polymorphisms in methylenetetrahydrofolate reductase in an at-risk Chinese population. Cancer Epidemiology. Biomarkers and Prevention 11:1454–1458.

Sobin LH, Wittekind C. 2002. International Union Against Cancer (UICC). TNM classification of malignant tumors. 6th edn. New York: Wiley-Liss.

Stolzenberg-Solomon RZ, Qiao YL, Abnet CC, Ratnasinghe DL, Dawsey SM, Dong ZW, Taylor PR, Mark SD. 2003. Esophageal and gastric cardia cancer risk and folate- and vitamin B12-related polymorphisms in Linxian, China. Cancer Epidemiology. Biomarkers and Prevention 12:1222–1226.

Tahara E. 2004. Genetic pathways of two types of gastric cancer. IARC Scientific Publications 157: 327–349.

Tungtrongchitr R, Pongpaew P, Soonthornruengyot M, Viroonudomphol D, Vudhivai N, Tungtrongchitr A, Phonrat B, Pooudong S, Schelp FP. 2003. Relationship of tobacco smoking with serum vitamin B12, folic acid and haematological indices in healthy adults. Public Health Nutrition 6:675–681.

Wagner C. 1995. Biochemical role of folate in cellular metabolism. In: Bailey LB, editor. Folate in health and disease. New York: Marcel Dekker. p. 23–42.

Weisberg I, Tran P, Christensen B, Sibani S, Rozen R. 1998. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Molecular Genetics and Metabolism 64:169–172.

Wu MS, Yang KC, Shun CT, Hsiao TJ, Lin CC, Wang HP, Chuang SM, Lee WJ, Lin JT. 1997. Distinct clinicopathologic characteristics of diffuse- and intestinal-type gastric cancer in Taiwan. Journal of Clinical Gastroenterology 25:646–649.

Yamashita K, Park HL, Kim MS, Osada M, Tokumaru Y, Inoue H, Mori M, Sidransky D. 2006. PGP9.5 methylation in diffuse-type gastric cancer. Cancer Research 66:3921–3927.

Yi P, Pogribny I, Jill James S. 2002. Multiplex PCR for simultaneous detection of 677 C-->T and 1298 A-->C polymorphisms in methylenetetrahydrofolate reductase gene for population studies of cancer risk. Cancer Letters 181:209.

Zintzaras E. 2006. Association of methylenetetrahydrofolate reductase (MTHFR) polymorphisms with genetic susceptibility to gastric cancer: a meta-analysis. Journal of Human Genetics 51:618–624.