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Reduced folate carrier gene G80A polymorphism is associated with an increased risk of gastroesophageal cancers in a chinese population

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ABSTRACT

Low folate intake has been associated with an increased risk of both oesophageal and gastric cancers. Reduced folate carrier (*RFC1*, also named *SLC19A1*) is an essential folate transporter and functions as a bidirectional anion exchanger, taking up folate cofactors and exporting various organic anions. A G80A polymorphism in *RFC1* gene has been shown to be associated with alterations in folate and homocysteine metabolism in healthy individuals. In this study, we hypothesised that genetic variants in *RFC1* may modulate risk of oesophageal cancer (EC) and gastric cancer (GC). To test this hypothesis, we evaluated the associations of the G80A polymorphism of *RFC1* with EC and GC risk in a case-control study of 216 EC and 633 GC cases and 673 cancer-free controls in a Chinese population. We found that compared with the 80GG/GA genotypes in the recessive model, the variant homozygote *RFC1* 80AA was associated with a significantly increased risk of EC (adjusted odds ratio (OR) = 1.80, 95% confidence interval (CI) = 1.29–2.51), GC (adjusted OR = 1.59, 95% CI = 1.25–2.02) and EC and GC combined (adjusted OR = 1.63, 95% CI = 1.30–2.04). In the dominant model, the risk associated with *RFC1* 80AA was also elevated in EC (OR = 1.35, 95% CI = 0.91–1.99), GC (OR = 1.43, 95% CI = 1.07–1.91) and EC and GC combined (adjusted OR = 1.40, 95% CI = 1.07–1.83), compared with the 80GG genotype. The stratification analyses showed that effects of the *RFC1* 80AA genotype were more evident in subgroups of relatively older (⩾60 years), female, non-smokers, and non-drinkers both in EC and GC. Although the exact biological mechanism of this association remains to be explored, our findings suggest possible involvement of *RFC1* variant in the susceptibility of EC and GC. Further large and functional studies are needed to confirm our findings.

1. Introduction

Gastric cancer (GC) and oesophageal cancer (EC) are the major causes of cancer-related deaths worldwide, ranking the second for GC and eighth for EC, although the incidence and mortality of both EC and GC have been declining in recent years.[1–3] In China, both GC and EC are the leading causes of cancer-related mortality among men and women,[4] and

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accounted for about 38% GC cases and 48% EC cases of the world in 1990.[5] In Yangzhong county, the high-incidence area of China, the incidence rates of GC (the world standardised rate) were 145.26 per 10^5 person-years in males and 74.59 per 10^5 person-years in females in 2003, and that of the EC were 93.84 per 10^5 person-years in males and 73.73 per 10^5 person-years in females.[6] Epidemiological studies have demonstrated that dietary factors and/or *helicobacter pylori* (HP) infection play an important role in the development of gastroesophageal cancers.[7] Studies have suggested that high consumption of vegetables and fruits is associated with a reduced risk of cancer, including EC and GC.[8,9]

Folate is one of the constituents in fruits and vegetables and may provide protection against gastroesophageal cancers.[10] Folate status is influenced by many exogenous and endogenous factors. The common exogenous factors include diet, smoking, alcohol consumption and some kind of drugs, while the endogenous factors include ethnicity, functional status of bowel and liver, and the presence of genetic variants.[11,12] Folic acid is essential for both the synthesis of nucleotide precursors of DNA and cellular methylation reactions. Low folate intake has been associated with an increased risk of both oesophageal[13] and gastric cancers.[14,15] Genes involved in the folate metabolism pathway are widely studied for their role in human diseases.[16–18] We previously reported that genetic variants of methylene-tetrahydrofolate reductase (*MTHFR*) and thymidylate synthase (*TS*) were associated with the increased risk of gastric cancer.[19,20] In addition to *MTHFR* and *TS* genes in the folate metabolism pathway, the reduced folate carrier gene (*RFC1*), also named solute carrier family 19 member 1 (*SLC19A1*), coding a typical facilitative transmembrane protein with 12 predicted transmembrane domains in the major route of folate delivery into cells,[21] has been shown to be associated with alterations in folate and homocysteine (Hcy) metabolism in healthy individuals.[22] Odin and colleagues reported that mean expression levels of *RFC1* gene were significantly higher in tumour tissues compared with mucosa of colorectal cancer,[23] indicating the importance of this gene in cancer development.

Recently, a G80A polymorphism in exon 2 (rs1051266), which replaces Arg by His at position 27 of the *RFC1* protein, was identified,[22] and the G variant was correlated with lower plasma folate and higher homocysteine levels in healthy persons.[22] This G80A polymorphism in *RFC1* was extensively studied in birth defects (e.g. neural tube defects) but few in the association with risk of cancers. Some of the studies on birth defects reported that the allele G frequency was significantly higher in children with neural tube defects than in the controls,[24–26] while other studies on childhood acute lymphoblastic leukaemia reported that children with the 80 AA variant genotype had worse prognoses than patients with the GG genotype ($P = 0.04$).[18] However, there were no additional studies on *RFC1* polymorphism and risk of other cancers.

Based on the fact that low folate intake was associated with an increased risk of gastroesophageal cancers and that *RFC1* G80A variant was correlated with lower plasma folate, we hypothesised that genetic variants of *RFC1* (G80A, R27H) are associated with increased susceptibility for oesophageal and gastric cancers. To test this hypothesis, we performed genotyping analyses on this single nucleotide polymorphism (SNP) in a population-based case-control study of 633 GC, 216 EC patients and 673 frequency-matched cancer-free controls in a high-risk area of gastroesophageal cancers in Jiangsu Province, China.

2. Patients and methods

2.1. Study population

This population-based case-control study consisted of 633 patients with GC, 216 patients with EC and 673 cancer-free population controls. The GC and EC cases were consecutively recruited from Yang-Zhong and Yi-Xing counties, the areas of high gastroesophageal cancer mortality, in Jiangsu Province, People's Republic of China, between January 2003 and July 2005. All the cases were incident patients and were histopathologically diagnosed as gastric adenocarcinoma (GC) and oesophageal squamous cell carcinoma (EC). The population controls were selected from cancer-free individuals living in the same residential areas as the cases and were frequency-matched to the cases on age and sex. All the subjects consented to participate in the study and donated a blood sample. Trained interviewers used a pre-tested questionnaire to determine demographic and lifestyle characteristics such as sex, age and related factors including smoking tobacco and drinking alcohol. After interviews, approximately 5 ml venous blood sample was collected from each subject. Individuals that smoked once a day for over 1 year were defined as smokers, and those who consumed three or more alcoholic drinks a week for over 6 months were considered drinkers. The study was approved by the institutional review board of Nanjing Medical University.

2.2. Genotype analyses

Genomic DNA was isolated from leucocytes of venous blood by proteinase K digestion followed by phenol-chloroform extraction and ethanol precipitation. The PCR-restriction fragment length polymorphism (RFLP) assay was used to detect *RFC1* G80A polymorphism. The primers were 5′-GCACAGCGTCACCTTCGTC-3′ (forward) and 5′-CTCCCGCGTGAAGTTCTTGT-3′ (reverse), which generated a 235-bp fragment. The 20 μl PCR mixture contained approximately 50 ng of genomic DNA, 12.5 pmol of each primer, 0.1 mM each dNTP, 1× PCR buffer (50 mM KCl, 10 mM Tris HCl, and 0.1% Triton X-100), 1.5 mM $MgCl_2$, and 1.0 unit of *Taq* polymerase. The PCR profile consisted of an initial melting step of 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 60 °C for 40 s and 72 °C for 40 s; and a final extension step of 72 °C for 10 min. The fragment was then digested by *HhaI* (New England BioLabs, Beverly, MA). The wide-type (80G) allele produces three fragments of 129, 69 and 37-bp and the polymorphic (80A) allele produces two fragments of 166 and 69-bp. The digestion products were separated on a 3% agarose gel at 80 V for 40 min and stained with ethidium bromide (Fig. 1).

Genotyping was performed without knowing the subjects' case and control status and an approximately equal number of the cases and the controls were assayed in each 96-well PCR plate with a positive control of a DNA sample with known heterozygous genotype. If a consensus on the

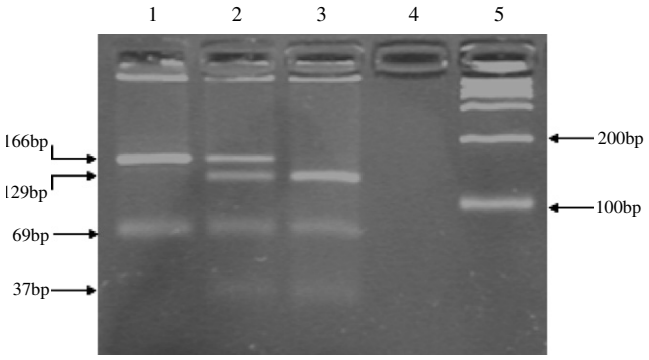

Fig. 1 – PCR-RFLP assay was performed to genotype the *RFC1* G80A. Electrophoresis in 3% agarose gel showed genotype patterns of 80AA (166-bp + 69-bp) (Lane1), 80AG (166-bp + 129-bp + 69-bp + 37-bp) (Lane2) and 80GG (129-bp + 69-bp + 37-bp) (Lane3). Lane 4: water control. Lane5: DNA marker.

tested genotype was not reached, two research assistants independently performed the repeated assays to achieve 100% concordance.

2.3. Statistical analyses

Differences in demographic characteristics, selected variables and frequencies of the genotypes of *RFC1* polymorphism between the cases and controls were evaluated using the χ^2 test. The associations between *RFC1* G80A genotypes and the risk of GC and EC were estimated by computing the odds ratios (ORs) and their 95% confidence intervals (CIs) from logistic regression analyses with adjustment for age, sex, tobacco smoking and alcohol drinking. Hardy–Weinberg equilibrium was tested by a goodness-of-fit χ^2 test to compare the observed genotype frequencies with the expected ones among the control subjects. All of the statistical analyses were performed with Statistical Analysis System software (v.9.1.3e; SAS Institute, Cary, NC).

3. Results

3.1. Characteristics of the study population

Selected characteristics of the 633 GC and 216 EC cases and 673 cancer-free controls included in the study are summarised in Table 1. The frequency-matching on age and sex between both GC and EC cases and controls were adequate as suggested by the χ^2 tests ($P = 0.243$ for GC *versus* controls and $P = 0.583$ for EC *versus* controls on age, and $P = 0.408$ for GC *versus* controls and $P = 0.869$ for EC *versus* controls on sex, respectively). The mean age was 60.5 years (±9.4 years) for the GC patients, 61.3 years (±8.1 years) for the EC cases, and 59.6 years (±10.3 years) for the controls. In addition, there were no significant differences between GC patients and controls in terms of tobacco smoking ($P = 0.734$) and alcohol drinking ($P = 0.067$). However, there were significantly more smokers and alcohol drinkers among EC patients than among controls ($P < 0.001$ for smoking and $P = 0.026$ for alcohol drinking) (Table 1).

3.2. Genotype distribution of *RFC1* polymorphism among the EC and GC cases and controls

The *RFC1* G80A genotype distribution in the cases and controls is shown in Table 2. The observed genotype frequencies for the polymorphism were in Hardy–Weinberg equilibrium in the controls ($\chi^2 = 3.153$, $P = 0.076$). The *RFC1* G80A genotype frequencies were 28.0% (GG), 38.2% (GA) and 33.8% (AA) in the GC cases, 30.6% (GG), 31.0% (AG) and 38.4% (AA) in the EC cases, and 28.7% (GG), 46.5% (GA) and 24.8%(AA) in the controls ($P = 0.001$ for GC *versus* controls and $P < 0.001$ for EC *versus* controls), suggesting that the AA genotype may be a risk genotype against both GC and EC. When the *RFC1* 80GG homozygote genotype was used as the reference group, the 80AA genotype was associated with an increased risk of both GC (adjusted OR = 1.43, 95% CI = 1.07–1.91) and EC (adjusted OR = 1.35, 95% CI = 0.91–1.99), although the association with EC was not

Table 1 – Distributions of select variables in oesophageal and gastric cancer cases and cancer-free controls

Variable	Control ($n = 673$)		Esophageal cancer ($n = 216$)			Gastric cancer ($n = 633$)		
	n	%	*n*	%	*P* value[a]	*n*	%	*P* value[a]
Age (years)					0.583			0.243
<60	315	46.8	96	44.4		275	43.4	
⩾60	358	53.2	120	55.6		358	56.6	
Sex					0.869			0.408
Male	448	66.6	142	65.7		435	68.7	
Female	225	33.4	74	34.3		198	31.3	
Smoking status					<0.001			0.734
Non-smokers	410	60.9	102	47.2		379	59.9	
Smokers	263	39.1	114	52.8		254	40.1	
Drink consumption					0.026			0.067
Never	408	60.6	112	51.9		415	65.6	
Ever	265	39.4	104	48.1		218	34.4	

a Two-sided χ^2 test.

Table 2 – Logistic regression analysis of associations between *RFC1* polymorphism and risk of GC, EC and gastroesophageal cancer

Genotypes	Controls (n = 673)		Oesophageal cancer cases (n = 216)			Gastric cancer cases (n = 633)			GC + EC cases (n = 849)		
	n	%[a]	n	%	OR[b] (95% CI)	n	%	OR[b] (95% CI)	n	%	OR[b] (95% CI)
RFC1 G80A											
GG	193	28.7	66	30.6	1.00	177	28.0	1.00	243	28.6	1.00
GA	313	46.5	67	31.0	0.60(0.41–0.88)	242	38.2	0.84(0.64–1.10)	309	36.4	0.78(0.61–0.99)
AA	167	24.8	83	38.4	1.35(0.91–1.99)	214	33.8	1.43(1.07–1.91)	297	35.0	1.40(1.07–1.83)
GG + GA	506	75.2	133	61.6	1.00	419	66.2	1.00	552	65.0	1.00
AA	167	24.8	83	38.4	1.80(1.29–2.51)	214	33.8	1.59(1.25–2.02)	297	35.0	1.63(1.30–2.04)
A allele		48.1		53.9			52.9			53.2	

a The observed genotype frequency among the control subjects was in agreement with the Hardy–Weinberg equilibrium (χ^2 = 3.153, P = 0.076).
b Adjusted for age, sex, smoking status, drink consumption.

Table 3 – Stratified analyses between *RFC1* polymorphism and oesophageal and gastric cancer risk by sex, age, smoking status, drink consumption

Variable	Control (n, %)		Esophageal cancer (n, %)		OR (95% CI)[a]		Gastric cancer (n, %)		OR (95% CI)[a]	
	GG + GA	AA	GG + GA	AA	GG + GA	AA	GG + GA	AA	GG + GA	AA
Sex										
Male	325(72.5)	123(27.5)	90(63.4)	52(36.6)	1.00	1.38(0.92–2.08)	294(67.6)	141(32.4)	1.00	1.30(0.97–1.74)
Female	181(80.4)	44(19.6)	43(58.1)	31(41.9)	1.00	2.87(1.61–5.12)	125(63.1)	73(36.9)	1.00	2.46(1.58–3.82)
Age (years)										
<60	232(73.7)	83(26.3)	65(67.7)	31(32.3)	1.00	1.26(0.76–2.11)	183(66.5)	92(33.5)	1.00	1.43(1.00–2.05)
⩾60	274(76.5)	84(23.5)	68(56.7)	52(43.3)	1.00	2.28(1.46–3.55)	236(65.9)	122(34.1)	1.00	1.73(1.24–2.40)
Smoking										
Never	322(78.5)	88(21.5)	62(60.8)	40(39.2)	1.00	2.44(1.52–3.90)	257(67.8)	122(32.2)	1.00	1.92(1.39–2.67)
Ever	184(70.0)	79(30.0)	71(62.3)	43(37.7)	1.00	1.35(0.85–2.16)	162(63.8)	92(36.2)	1.00	1.31(0.90–1.89)
Alcohol drinking										
Never	323(79.2)	85(20.8)	66(58.9)	46(41.1)	1.00	2.69(1.72–4.22)	282(68.0)	133(32.0)	1.00	1.87(1.36–2.57)
Ever	183(69.1)	82(30.9)	67(64.4)	37(35.6)	1.00	1.09(0.66–1.79)	137(62.8)	81(37.2)	1.00	1.30(0.89–1.92)

a Adjusted for age, sex, smoking and alcohol drinking.

statistically significant. Interestingly, the 80GA heterozygote genotype was shown to have a protective effect on EC (adjusted OR = 0.60, 95% CI = 0.41–0.88) and GC (adjusted OR = 0.84, 95% CI = 0.64–1.10) with adjustment for age, sex, smoking and alcohol drinking in the logistic regression analyses. In the recessive model, when the 80GG/GA genotypes were used as the reference group, the 80AA homozygote genotype was associated with a significantly 80% increased risk of EC and 59% elevated risk of GC, respectively (adjusted OR = 1.80, 95% CI = 1.29–2.51 for EC, adjusted OR = 1.59, 95% CI = 1.25–2.02 for GC). Because the GC and EC may share the similar exposures and genetic factors, we combined the GC and EC cases as one case group. When the 80GG genotype was used as the reference group, the 80AA was associated with a 40% increased risk of gastroesophageal cancers (adjusted OR = 1.40, 95% CI = 1.07–1.83), and in the recession model, the 80AA was associated with a significantly 63% increased risk of gastroesophageal cancers compared with the 80GG/GA genotypes (adjusted OR = 1.63, 95% CI = 1.30–2.04).

3.3. *Stratification analyses of RFC1 polymorphism*

To evaluate the effects of *RFC1* G80A polymorphism on EC and GC risk according to sex, age, smoking, and alcohol drinking, we performed stratification analyses. As shown in Table 3, the risk of both EC and GC associated with the *RFC1* 80AA genotype were more evident among women (adjust OR = 2.87, 95% CI = 1.61–5.12 for EC, adjust OR = 2.46, 95% CI = 1.58–3.82 for GC), older subjects (⩾60 years) (adjusted OR = 2.28, 95% CI = 1.46–3.55 for EC, adjusted OR = 1.73, 95% CI = 1.24–2.40 for GC), non-smokers (adjusted OR = 2.44, 95% CI = 1.52–3.90 for EC, adjusted OR = 1.92, 95% CI = 1.39–2.67 for GC) and non-drinkers (adjusted OR = 2.69, 95% CI = 1.72–4.22 for EC, adjusted OR = 1.87, 95% CI = 1.36–2.57 for GC, respectively) compared with the 80GG/GA genotypes.

4. Discussion

In this population-based case-control study in a high-risk Chinese population, we investigated the association between

RFC1 G80A polymorphism and risk of EC and GC. We found a significantly increased risk of both EC and GC associated with the *RFC1* 80AA homozygote, and these significant associations were more evident among women, older subjects, non-smokers and non-drinkers. These consistent results both from EC and GC support our hypothesis that the *RFC1* G80A variant may play a role in the folate metabolism and therefore might be involved in the gastroesophageal carcinogenesis. To the best of our knowledge, this is the first molecular epidemiological study of this *RFC1* polymorphism in gastric and oesophageal cancers.

Folate intake has been associated with a reduced risk for a number of cancers,[27–29] including gastroesophageal cancers,[13,14] particularly in China.[30,31] Mayne and colleagues reported that dietary intake of folate was inversely associated with the risk of both oesophageal and gastric cancers (OR = 0.58, 95% CI = 0.39–0.85 for oesophageal squamous cell carcinoma and OR = 0.73, 95% CI = 0.55–0.97 for gastric cardia adenocarcinoma) (the 75th percentile *versus* the 25th percentile of folate intake),[14] indicating the similar mechanisms of GC and EC. There are two principal mechanisms through which low folate status may increase the risk of cancers.[32] Firstly, folate deficiency, by reducing intracellular S-adenosylmethionine, can alter cytosine methylation in DNA, leading to inappropriate activation of proto-oncogenes and induction of malignant transformation. Secondly, folate is essential for normal DNA synthesis and repair. Chronic folate deficiency *in vivo* and *in vitro* has been associated with DNA strand breaks and chromosomal damage.[32,33] It is likely that not only folate deficiency but also functional polymorphisms in genes associated with impaired folate metabolism may contribute to the risk of gastroesophageal cancers.

Many important genes are involved in folate metabolism, including *MTHFR*, *TS* and *RFC1* etc.[19,20] The *RFC1* gene is located at the end of the long arm of chromosome 21q22.3,[34] and functions as a bidirectional anion exchanger, taking up folate cofactors and exporting various organic anions, including thiamine pyrophosphate.[22,35] Because *RFC1* is one of the key enzymes in folate metabolism pathway, it is biologically plausible that the increased cancer risk associated *RFC1* 80AA be explained by the genetic polymorphism modulating folate deficiency. A non-synonymous polymorphism, G80A, in the *RFC1* gene has been successively identified at codon 27 (Arg27His), causing an Arg to His change.[22] Although the exact functional relevance of this variant is unknown, it has been demonstrated that individuals carrying two copies of the rare *RFC1* 80A alleles had significantly lower plasma folate levels and homocysteinemia than individuals with wild-type alleles 80G.[22] Therefore, it is likely that this variant genotype may be involved in gastroesophageal carcinogenesis by affecting plasma folate and Hcy levels, which is associated with suboptimal DNA methylation, DNA repair capacity and cancer susceptibility.

In our present association study, we confirmed that the *RFC1* 80AA genotype was associated with a 43% increased risk of GC and a 35% elevated risk of EC, compared with 80GG, and the risk was more pronounced in the recessive model by using the 80GA/GG as the reference group. In addition, the *RFC1* G80A polymorphism associated with an increased risk of both EC and GC was consistently more evident among women, relatively older subjects, non-smokers and non-alcoholic drinkers, indicating that it might play an important role in carcinogenesis of both EC and GC. Although smoking and alcohol drinking are established risk factors for gastroesophageal cancers, particularly EC, we did not find any evidence for interactions between *RFC1* polymorphism and these risk factors. This observed association in subgroups might suggest that the cancer patients without the established risk factors may have other unknown exposures. Although this *RFC1* variant did not adequately explain the risk for those non-smokers and non-drinkers, the observed effect may be attributed to other folate metabolism enzymes encoded by genes in this same pathway such as *MTHFR* and *TS*, as a result of cosegregation with a polymorphism in one of these genes. This hypothesis needs to be tested in future studies. Another possibility is that the results are due to chance because the sample size in the subgroups was relatively small.

Although this is a population-based case-control study which has the advantage compared with hospital-based ones in terms of selection bias in estimating exposure effects, there may still be information bias, as seen in other case-control studies. The major shortcoming of this study is the lack of data on detailed dietary intake of folate, plasma folate or homocysteine levels, because the effect of genetic variants in folate metabolic genes on cancer risk will depend on folate intake status.[36,37] Therefore, our study could underestimate the risk in the presence of low dietary folate intake and could not evaluate possible gene-nutrient interaction. Another limitation of the study is that there may be other polymorphisms in the *RFC1* gene that are in linkage disequilibrium with *RFC1* G80A, because the function of this polymorphism has not been known. Larger studies with multiple polymorphisms are needed to verify these findings, in which the potential gene-gene and gene-environmental interactions on gastroesophageal carcinogenesis could be further examined.

In conclusion, our results suggest that the *RFC1* G80A polymorphism may be functionally relevant as evidenced by their associations with risk of developing gastroesophageal cancers in the Chinese population. This finding is an important addition to previously published works on the folate metabolism pathway and needs to be further validated in larger studies in different populations and other types of tumours. Therefore, future studies incorporating information of dietary folate intake and other potential exposure variables are needed to evaluate gene-environment or gene-nutrient interactions.

Conflict of interest statement

None declared.

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REFERENCES

1. Devesa SS, Blot WJ, Fraumeni Jr JF. Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. *Cancer* 1998;**83**:2049–53.
2. Plummer M, Franceschi S, Munoz N. Epidemiology of gastric cancer. *IARC Sci Publ* 2004;(157):311-26.
3. Jemal A, Tiwari RC, Murray T, et al. Cancer statistics, 2004. *CA Cancer J Clin* 2004;**54**:8–29.
4. Sun X, Mu R, Zhou Y, et al. 1990–1992 mortality of stomach cancer in China. *Zhonghua Zhong Liu Za Zhi* 2002;**24**:4–8.
5. Pisani P, Parkin DM, Bray F, Ferlay J. Estimates of the worldwide mortality from 25 cancers in 1990. *Int J Cancer* 1999;**83**:18–29;
   Pisani P, Parkin DM, Bray F, Ferlay J. Estimates of the worldwide mortality from 25 cancers in 1990. *Erratum in: Int J Cancer* 1999;**83**:870–3.
6. Wang JM, Xu B, Hsieh CC, Jiang QW. Longitudinal trends of stomach cancer and esophageal cancer in Yangzhong County: a high-incidence rural area of China. *Eur J Gastroenterol Hepato* 2005;**17**:1339–44.
7. Crew KD, Neugut AI. Epidemiology of upper gastrointestinal malignancies. *Semin Oncol* 2004;**31**:450–64.
8. Kobayashi M, Tsubono Y, Sasazuki S, Sasaki S, Tsugane S. JPHC Study Group. Vegetables, fruit and risk of gastric cancer in Japan: a 10-year follow-up of the JPHC study cohort I. *Int J cancer* 2002;**102**:39–44.
9. Kelley JR, Duggan JM. Gastric cancer epidemiology and risk factors. *Journal of Clinical Epidemiology* 2003;**56**:1–9.
10. Mayne ST, Navarro SA. Diet, obesity and reflux in the etiology of adenocarcinomas of the esophagus and gastric cardia in humans. *J Nutr* 2002;**132**:3467S–70S.
11. Ganji V, Kafai MR. Demographic, health, lifestyle, and blood vitamin determinants of serum total homocysteine concentrations in the third National Health and Nutrition Examination Survey, 1988-1994. *Am J Clin Nutr* 2003;**77**:826–33.
12. Nakano E, Taylor CJ, Chada L, Mcgaw J, Powers HJ. Hyperhomocystinemia in children with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 2003;**37**:586–90.
13. Zhang ZF, Kurtz RC, Yu GP, et al. Adenocarcinomas of the esophagus and gastric cardia: the role of diet. *Nutr Cancer* 1997;**27**:298–309.
14. Mayne ST, Risch HA, Dubrow R, et al. Nutrient intake and risk of subtypes of esophageal and gastric cancer. *Cancer Epidemiol Biomark Prev* 2001;**10**:1055–62.
15. Bailey LB, Gregory JF, Caudill M, Cruz A. The relationship between increased folate stetabolism and the increased requirement for folate in pregnancy. *BJOG* 2001;**108**:772–3.
16. James SJ, Pogribna M, Pogribny IP, et al. Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down syndrome. *Am J Clin Nutr* 1999;**70**:495–501.
17. Lin J, Spitz MR, Wang Y, et al. Polymorphisms of folate metabolic genes and susceptibility to bladder cancer: a case-control study. *Carcinogenesis* 2004;**25**:1639–47.
18. Laverdiere C, Chiasson S, Costea I, Moghrabi A, Krajinovic M. Polymorphism G80A in the reduced folate carrier gene and its relationship to methotrexate plasma levels and outcome of childhood acute lymphoblastic leukemia. *Blood* 2002;**100**:3832–4.
19. Shen H, Newmann AS, Hu Z, et al. Methylenetetrahydrofolate reductase polymorphisms/haplotypes and risk of gastric cancer: a case-control analysis in China. *Oncol Rep* 2005;**13**:355–60.
20. Zhang Z, Xu Y, Zhou J, et al. Polymorphisms of thymidylate synthase in the 5′- and 3′-untranslated regions associated with risk of gastric cancer in South China: a case-control analysis. *Carcinogenesis* 2005;**26**:1764–9.
21. Gorlick R, Goker E, Trippett T, et al. Defective transport is a common mechanism of acquired methotrexate resistance in acute lymphocytic leukemia and is associated with decreased reduced folate carrier expression. *Blood* 1997;**89**:1013–8.
22. Chango A, Emery-fillon N, DE Courcy GP, et al. A polymorphism (80G->A) in the reduced folate carrier gene and its associations with folate status and homocysteinemia. *Mol Genet Metab* 2000;**70**:310–5.
23. Odin E, Wettergren Y, Nilsson S, et al. Altered gene expression of folate enzymes in adjacent mucosa is associated with outcome of colorectal cancer patients. *Clin Cancer Res* 2003;**9**:6012–9.
24. De Marco P, Calevo MG, Moroni A, et al. Polymorphisms in genes involved in folate metabolism as risk factors for NTDs. *Eur J Pediatr Surg* 2001;**11**:S7–S14.
25. Shaw GM, Lammer EJ, Zhu H, Baker MW, Neri E, Finnell RH. Maternal periconceptional vitamin use, genetic variation of infant reduced folate carrier (A80G) and risk of spina bifida. *Am J Med Genet*. 2002;**108**:1–6;
   Shaw GM, Lammer EJ, Zhu H, Baker MW, Neri E, Finnell RH. Maternal periconceptional vitamin use, genetic variation of infant reduced folate carrier (A80G) and risk of spina bifida. *Erratum in: Am J Med Genet* 2002;**113**:392.
26. Pei L, Zhu H, Ren A, et al. Reduced folate carrier gene is a risk factor for neural tube defects in a Chinese population. *Birth Defects Res A Clin Mol Teratol* 2005;**73**:430–3.
27. Rampersaud GC, Kauwell GP, Bailey LB. Folate: a key to optimizing health and reducing disease risk in the elderly. *J Am Coll Nutr* 2003;**22**:1–8.
28. Bailey LB. Folate, methyl-related nutrients, alcohol, and the MTHFR 677C–>T polymorphism affect cancer risk: intake recommendations. *J Nutr* 2003;**133**:3748S–53S.
29. Engel LS, Chow WH, Vaughan TL, et al. Population attributable risks of esophageal and gastric cancers. *J Natl Cancer Inst* 2003;**95**:1403–4.
30. Stolzenberg-Solomon RZ, Qiao YL, Abnet CC, et al. Esophageal and gastric cardia cancer risk and folate- and vitamin B (12)-related polymorphisms in Linxian, China. *Cancer Epidemiol Biomarkers Prev* 2003;**12**:1222–6.
31. Takezaki T, Gao CM, Wu JZ, et al. Dietary protective and risk factors for esophageal and stomach cancers in a low-epidemic area for stomach cancer in Jiangsu Province, China: comparison with those in a high-epidemic area. *Jpn J Cancer Res* 2001;**92**:1157–65.
32. Duthie SJ. Folic acid deficiency and cancer: mechanisms of DNA instability. *Br Med Bull* 1999;**55**:578–92.
33. Blount BC, Mack MM, Wehr CM, et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. *Proc Natl Acad Sci USA* 1997;**94**:3290–5.
34. Moscow JA, Gong M, He R, et al. Isolation of a gene encoding a human reduced folate carrier (RFC1) and analysis of its expression in transport-deficient, methotrexate-resistant human breast cancer cells. *Cancer Res* 1995;**55**:3790–4.
35. Zhao R, Gao F, Wang Y, Diaz GA, Gelb BD, Goldman ID. Impact of the reduced folate carrier on the accumulation of active thiamin metabolites in murine leukemia cells. *J Biol Chem* 2001;**276**:1114–8.
36. Giovannucci E. Alcohol, one-carbon metabolism, and colorectal cancer: recent insights from molecular studies. *J Nutr* 2004;**134**:2475S–81S.
37. Ma J, Stampfer MJ, Christensen B, et al. A polymorphism of the methionine synthase gene: association with plasma folate, vitamin B12, homocyst(e)ine, and colorectal cancer risk. *Cancer Epidemiol. Biomarkers Prev* 1999;**8**:825–9.