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Riboflavin intake, *MTRR* genetic polymorphism (rs1532268) and gastric cancer risk in a Korean population: a case–control study

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Abstract

The vitamin B group, including riboflavin, plays paramount roles in one-carbon metabolism (OCM), and disorders related to this pathway have been linked to cancer development. The variants of genes encoding OCM enzymes and the insufficiency of B vitamins could contribute to carcinogenesis. Very few observational studies have revealed a relationship between riboflavin and gastric cancer (GC), especially under conditions of modified genetic factors. We carried out a study examining the association of riboflavin intake and its interaction with *MTRR* (rs1532268) genetic variants with GC risk among 756 controls and 377 cases. The OR and 95 % CI were evaluated using unconditional logistic regression models. We observed protective effects of riboflavin intake against GC, particularly in the female subgroup (OR = 0·52, 95 % CI 0·28, 0·97, P_{trend} = 0·031). In the *MTRR* (rs1532268) genotypes analysis, the dominant model showed that the effects of riboflavin differed between the CC and CT + TT genotypes. Compared with CC carriers, low riboflavin intake in T^+ carriers was significantly associated with a 93 % higher GC risk (OR = 1·93, 95 % CI 1·09, 3·42, $P_{interaction}$ = 0·037). In general, higher riboflavin intake might help reduce the risk of GC in both CC and TC + TT carriers, particularly the T^+ carriers, with marginal significance (OR = 0·54, 95 % CI 0·28, 1·02, $P_{interaction}$ = 0·037). Our study indicates a protective effect of riboflavin intake against GC. Those who carry at least one minor allele and have low riboflavin intake could modify this association to increase GC risk in the Korean population.

Key words: **Gastric cancer: Riboflavin: One-carbon metabolism: *MTRR* C524T: rs1532268**

In recent decades, the incidence of gastric cancer (GC) has tended to decrease, especially in developed countries. Nevertheless, it is still an important cause of the global cancer burden. According to GLOBOCAN 2018[1], GC ranked as the fifth most frequently diagnosed cancer and third leading cause of cancer death worldwide. In general, the incidence rate is higher in East Asia than in North America. In South Korea, newly updated data show that GC accounted for the highest cancer incidence and the fourth most frequent cause of cancer-related death in 2018[2].

The stomach plays a vital role in the intestinal system, and it helps digest and absorb nutrients from food to the bloodstream[3,4]. It also functions as a stable barrier to protect the body against harmful substances from the outside environment[5–7]. The risk of GC is greater among individuals in lower socio-economic classes, and environmental exposures appear to play an important part in its aetiology[8]. *Helicobacter pylori* infection is a potential risk factor for GC, especially in noncardia cancer; moreover, other contributors such as smoking, alcohol consumption, occupational exposure and diet have probable impacts on the incidence of GC[9]. According to the World Cancer Research Fund and the American Institute for Cancer Research, dietary factors such as preserved/high-salt foods, grilled/barbecued or processed meat and overweight/obesity status can increase the risk of GC, while high consumption of fruit/citrus fruit and vegetables is believed to have a protective effect for GC. Limited evidence has shown the effect of riboflavin on GC[9].

Riboflavin is an essential water-soluble vitamin that is absorbed from food and is needed for the normal functional processes of cells, including growth and development[10–12]. Furthermore, riboflavin, together with other B vitamins, serves as a cofactor for the enzyme methionine synthase reductase

Abbreviations: GC, gastric cancer; MTRR, methionine synthase reductase; OCM, one-carbon metabolism.

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(MTRR) in one-carbon metabolism (OCM). Riboflavin deficiency may be implicated in the aetiology of human cancers, but the exact underlying mechanism is not clearly understood. Some observational studies have shown that riboflavin has protective effects against GC. However, other conflicting studies did not recommend using B vitamins or riboflavin for cancer prevention[13–16]. In experimental studies, riboflavin-deficient rats showed increases in carcinogenesis, induction of DNA repair enzymes and carcinogen binding to DNA[17–19]. These studies have suggested that a higher intake of riboflavin especially in a deficient population would help reduce the risk of cancer.

As OCM is a key essential pathway for the processes of DNA synthesis, methylation and DNA repair, the impairment of OCM may lead to several disease outcomes and abnormal organ development[20]. The *MTRR* gene is positioned on the short arm of chromosome 5, region 5p15.2–15.3[21] and encodes for the MTRR enzyme, which is involved in the metabolic pathway of homocysteine[22]. At a located point, the *MTRR* gene can have its nucleotides replaced to make genetic polymorphisms, leading to varying genotypes. The polymorphism C524T is the substitution of a C allele with a T allele (C and T are defined as the wild-type and mutant/minor allele, respectively), which leads to a change in amino acids from serine to leucine. According to several literatures, *MTRR* C524T is one of the common SNP that can modify the level of the MTRR enzyme in the plasma and then interfere with its function. The results of studies investigating the effects of the *MTRR* C524T genetic polymorphism in the induction of cancers are also in disagreement[23–32].

The genetic variants of *MTRR* C524T in conjunction with riboflavin insufficiency can lead to the derangement of OCM and the induction of carcinogenesis. No previous study has measured the modification effects of *MTRR* C524T genetic variants and riboflavin intake in GC. The main purpose of our study was to reinvestigate the relationship among riboflavin intake, a SNP of *MTRR* C524T and GC risk. Simultaneously, we measured the interaction between riboflavin and genetic factors in the context of incident.

Materials and methods

Subject and data collection

A case–control study recruitment for GC research project was initiated in March 2011 and finished in December 2014 at the National Cancer Center Hospital in Korea. The Institutional Review Board of National Cancer Center (NCCNCS 11-438) has already approved the study protocol.

Individuals who had been diagnosed with early-stage GC approximately 3 months before the study and who did not change their dietary pattern for any reason were included in the case group. Patients who had developed other cancers within 5 years, had an advanced stage of GC, were pregnant or breast-feeding or suffered from chronic diseases (diabetes mellitus, systemic or mental disorders) were excluded. Simultaneously, the controls were recruited from the pool of Cancer Screenee Cohort Study subjects[33], who visited the Center for Cancer Prevention and Detection at the National Cancer Center for a health screening. They were confirmed as not having a medical history of cancer, other stomach damage, diabetes mellitus or treatment for *H. pylori*.

All participants were asked to complete the surveys by themselves; the survey included questions about demographics, lifestyle, dietary habits and medical history. We categorised BMI into three ranges according to the WHO classification of weight in adult Asians: normal range (18·5–22·9 kg/m^2), overweight at risk (23·0–24·9 kg/m^2) and obese I–II (25–29·9 kg/m^2 and ≥30 kg/m^2)[34]. We confirmed *H. pylori* infection mainly by using the rapid urease test (Pronto Dry, Medical Instruments Corp.) and assessing histology and serology. All participants were required to provide written informed consent.

Dietary intake assessment

Data on dietary food intake were collected by a semi-quantitative FFQ consisting of 106 food items[35]. Information regarding the portion size and average daily frequency of food intake was recalled from the past 12 months before the study was conducted. We used CAN-PRO 4.0 (Computer Aided Nutritional Analysis Program, Korea Nutrition Society) to extract the nutritional components from the daily food intake collected by the semi-quantitative FFQ. Then, the total riboflavin intake from daily food was summed and displayed for study.

Genotype measurement

Whole-blood samples were taken from participants and then isolated to obtain peripheral blood leukocytes. After that, the genomic DNA was extracted. The Affymetrix Axiom Exome 319 Array (Affymetrix Inc.) platform, which included 318 983 variants, was used for genotyping. Genetic markers that did not fulfil the quality control criteria of a minor allele frequency < 0·05, Hardy–Weinberg equilibrium's deviation *P*-value < 1×10^{-6} and low call rate (< 98 %) were rejected. The performance of the genotype imputation, an essential process for predicting unobserved genotypes in the SNP data, was assessed using the Asian population (n 504) in the 1000 Genomes Project phase III haplotypes panel from the integrated variant set release GRch37/hg19 (http://www.1000genomes.org/), which was used as a reference panel. SHAPIT (v2.r837) was used to perform phasing, and IMPUTE2 (2.3.2) was used to complete the imputation of SNP. The accuracy of those imputed genotypes was called the quality of imputation INFO score. We filtered the imputed genotypes with a stringent threshold INFO score of >0·6 to achieve the highest accuracy[36–39]. Finally, the genetic polymorphism of the *MTRR* gene (rs1532268) was selected.

Overall, only participants who completed the self-administered surveys and the semi-quantitative FFQ and had available data on genetic characteristics were eligible for inclusion in the final analyses. The case and control groups were then frequency-matched for sex and 5-year age distributions (2 controls per case). Finally, 756 healthy controls and 377 GC cases were selected for the analyses of the study.

Statistical analyses

We used *t* test for continuous variables and χ^2 test for categorical variables to compare the general characteristics of the case and control groups.

The riboflavin intake was initially adjusted for total daily energy intake using the residual method[40], and then we categorised riboflavin intake into tertile groups based on the distribution in the control group. To explore the association of riboflavin intake with GC, we computed OR with 95 % CI for each group through multiple logistic regression using the lowest group as a reference. The multivariable models were the models controlled for age, sex and energy intake (model 1) and further adjusted for first-degree family history of GC, level of education, occupation, income, smoking status and physical activity (model 2). Moreover, *H. pylori* was added to the fully adjusted model (model 3). A stratified analysis by *H. pylori* status was performed; furthermore, the median value of riboflavin intake in each tertile group was used as a continuous variable to test for trends.

The association between *MTRR* (rs1532268) genetic variants and GC risk was determined in the dominant model with CC as the reference group. The interaction between riboflavin intake and SNP was measured by a likelihood ratio test applying the multiplicative interaction term (SNP × riboflavin) to the multivariate logistic regression model. All analyses were performed using SAS software (version 9.4, SAS Institute).

Results

General characteristics of study participants

The general characteristics of the study participants showed some notable distinctions between the cancer and non-cancer patients (Table 1). In the cancer group, the prevalence of *H. pylori*-positive infection, smoking, low level of education and poor income status was higher than that in the control group. Cancer patients were more likely to have a family history of GC (20·5 %) than non-cancer patients (12·6 %). Moreover, the amount of daily riboflavin intake in mg was greater in the controls ($P_{trend} < 0·001$). In the subgroups of men and women, the differences were in the same direction as in the total population analysis.

Association between riboflavin intake and gastric cancer risk

The tertile ranges of riboflavin intake were significantly associated with GC risk in the total population of 1133 individuals and in the subgroup of 390 women (Table 2). Compared with the lowest tertile, the highest tertile exhibited a remarkable reduction in the risk of GC by approximately 54 % in the general population (T1 as reference) (OR = 0·46, 95 % CI = 0·33, 0·63, $P_{trend} < 0·001$), even after adjusting for age, sex, total energy intake, first-degree family history of GC, occupation, level of education, smoking status, income and physical activity (OR = 0·57, 95 % CI = 0·40, 0·82, $P_{trend} = 0·002$) as well as *H. pylori* infection status (OR = 0·56, 95 % CI = 0·39, 0·81, $P_{trend} = 0·002$). Further analyses in the sex subgroups showed significant results only in females in the three aforementioned adjusted models (OR = 0·39, 95 % CI = 0·23, 0·67; OR = 0·54, 95 % CI = 0·30, 0·98; OR = 0·52, 95 % CI = 0·28, 0·97, respectively, all $P_{trend} < 0·05$). As shown in Table 3, the results were in the same direction in the *H. pylori* infection subgroups after controlling for potential confounding factors. A higher intake of riboflavin was related to a reduction in GC risk in both the positive and negative infection groups (OR = 0·60, 95 % CI = 0·40, 0·89, $P_{trend} = 0·009$; OR = 0·20, 95 % CI = 0·05, 0·78, $P_{trend} = 0·019$, respectively).

Association between the MTRR (rs1532268) C524T genetic polymorphism and gastric cancer risk

As shown in Table 4, *MTRR* C524T was individually analysed in the dominant model (CC *v.* TC + TT genotypes). A significant association was not observed between the *MTRR* C524T genetic polymorphism and the risk of GC. However, when we investigated the interaction of the *MTRR* gene at the C524T SNP and riboflavin intake in GC risk, *MTRR* C524T and riboflavin intake was found to have synergistic carcinogenic effects (Table 5). Compared with carriers of the CC genotype, low riboflavin intake in T^+ carriers was significantly associated with a 93 % higher risk of GC (OR = 1·93, 95 % CI 1·09, 3·42, $P_{for\ interaction} = 0·037$). In general, higher riboflavin intake may help reduce the risk of GC in both CC and TC + TT carriers, particularly in T^+ carriers with borderline significance (OR = 0·54, 95 % CI 0·28, 1·02, $P_{for\ interaction} = 0·037$).

Discussion

In our study, which comprised 377 cases and 756 controls, we observed a protective impact of daily riboflavin intake against GC and the genetic variants of *MTRR* C524T also played a role in this association. At *MTRR* C524T, the T allele was considered to be a risk allele because, among individuals with the same low level of riboflavin intake, T^+ carriers tended to have a higher risk of GC than CC carriers. In other words, high riboflavin consumption significantly decreased the risk of GC in the TC and TT carriers and this decrease in risk was more apparent than in the CC carriers.

A cohort study including 323 GC patients (*n* 73 009) in 2014 explored the significant protective association of riboflavin against GC in premenopausal women[15]. In 1993, a randomised controlled trial with 29 584 participants conducted over 5 years of follow-up identified the anticancer effect of some nutritional supplements, including combined riboflavin–niacin tablets[41]. Moreover, two case–control studies displayed the same correlation direction as our studies, with sample sizes of 272 and 2575[42,43]. However, contrary results were revealed in other studies with limited study populations ranging from 318 to 777, in which riboflavin intake had no association with the risk of GC[13,44,45], or it could somehow increase GC risk in varied study sample sizes ranging from 492 to 3152 subjects[46,47]. The inconsistency could be explained by the limited sample sizes in almost all previous case–control studies and the differences in dietary pattern collection and measurement methods. Importantly, the differences could also be due to the difference in regional distribution. All of the results that were in

Table 1. General characteristics of the study subjects
(Mean values and standard deviations; numbers and percentages)

	Total (*n* 1133)					Men (*n* 743)					Women (*n* 390)				
	Control (%) (*n* 756)		Case (%) (*n* 377)			Control (%) (*n* 497)		Case (%) (*n* 246)			Control (%) (*n* 259)		Case (%) (*n* 131)		
Category	*n*	%	*n*	%	*P**	*n*	%	*n*	%	*P**	*n*	%	*n*	%	*P**
Age (years)															
Mean[a]	53·8		53·9		0·663	54·8		55·0		0·758	51·9		51·6		0·826
SD	9·0		9·3			8·4		8·6			9·7		10·1		
Sex															
Men	497	65·7	246	65·3	0·920										
Women	259	34·3	131	34·8											
H. pylori infection															
Negative	292	38·6	28	7·4	< 0·001	175	35·2	16	6·5	< 0·001	117	45·2	12	9·2	< 0·001
Positive	464	61·4	349	92·6		322	64·8	230	93·5		142	54·8	119	90·8	
Smoking status															
Current smoker	154	20·4	116	30·8	< 0·001	150	30·2	109	44·3	< 0·001	4	1·5	7	5·3	0·038
Former smoker	258	34·1	110	29·2		251	50·5	103	41·9		7	2·7	7	5·3	
Non-smoker	344	45·5	151	40·1		96	19·3	34	13·8		248	95·8	117	89·3	
Alcohol consumption															
Current drinker	486	64·3	228	60·5	0·333	370	74·5	173	70·3	0·330	116	44·8	55	42·0	0·863
Former drinker	58	7·7	37	9·8		46	9·3	31	12·6		12	4·6	6	4·6	
Non-drinker	212	28·0	112	29·7		81	16·3	42	17·1		131	50·6	70	53·4	
First-degree family history of GC															
Yes	95	12·6	77	20·5	< 0·001	71	14·3	55	22·5	0·008	24	9·3	22	16·8	0·044
No	659	87·4	299	79·5		424	85·7	190	77·6		235	90·7	109	83·2	
Total energy intake (kcal/d)															
Mean[a]	1717		1925		< 0·001	1766		2034		< 0·001	1625		1721		0·093
SD	547		612			542		636			545		506		
Riboflavin intake (mg/d)															
Mean[a]	0·98		0·9		< 0·001	0·9		0·9		0·017	1·1		0·94		< 0·001
SD	0·3		0·3			0·3		0·2			0·3		0·3		
BMI (kg/m^2)															
Mean[a]	24·0		23·9		0·389	24·5		24·3		0·288	23·1		23·1		0·984
SD	2·9		3·0			2·7		3·0			3·1		3·0		
<23	276	36·6	147	39·1	0·673	140	28·2	84	34·2	0·207	136	52·7	63	48·5	0·727
23–25	230	30·5	107	28·5		160	32·2	68	27·6		70	27·1	39	30·0	
≥25	249	33·0	122	32·5		197	39·6	94	38·2		52	20·2	28	21·5	
Physical exercise															
Yes	424	56·3	136	36·1	< 0·001	279	56·5	100	40·7	< 0·001	145	56·0	36	27·5	< 0·001
No	329	43·7	241	63·9		215	43·5	146	59·4		114	44·0	95	72·5	
Marital status															
Married	652	86·4	327	87·0	0·849	441	88·9	221	90·2	0·682	211	81·5	106	80·9	1·000
Others	103	13·6	49	13·0		55	11·1	24	9·8		48	18·5	25	19·1	
Education															
Under middle school	109	15·0	126	33·5	< 0·001	64	13·7	81	33·1	< 0·001	45	17·5	45	34·4	< 0·001
High school	225	31·0	163	43·4		124	26·4	106	43·3		101	39·3	57	43·5	
Above college	392	54·0	87	23·1		281	60·0	58	23·7		111	43·2	29	22·1	
Occupation															
Professional, administrative	144	19·1	65	17·3	< 0·001	108	21·9	54	22·0	0·004	36	13·9	11	8·4	0·006
Office, service and sales	240	31·9	108	28·7		186	37·7	72	29·4		54	20·9	36	27·5	
Labourer, agricultural	117	15·5	98	26·1		100	20·2	78	31·8		17	6·6	20	15·3	
Unemployed and others	252	33·5	105	27·9		100	20·2	41	16·7		152	58·7	64	48·9	
Monthly income (million won)															
<2	132	19·1	120	35·3	< 0·001	74	16·7	78	35·1	< 0·001	58	23·4	42	35·6	0·049
2-4	313	45·2	132	38·8		217	48·9	94	42·3		96	38·7	38	32·2	
>4	247	35·7	88	25·9		153	34·5	50	22·5		94	37·9	38	32·2	
MTRR (rs1532268)															
CC	585	77·4	288	76·4	0·766	386	77·7	186	75·6	0·593	199	76·8	102	77·9	0·920
CT + TT	171	22·6	89	23·6		111	22·3	60	24·4		60	23·2	29	22·1	
The classification subtype of GC															
Cardia classification															
Non-cardia	–		361	97·0		–		233	95·9		–		128	99·2	
Cardia	–		11	3·0		–		10	4·1		–		1	0·8	
Lauren's classification															
Intestinal	–		145	41·6		–		119	52·0		–		26	21·7	
Diffuse	–		149	42·7		–		70	30·6		–		79	65·8	
Mixed	–		51	14·6		–		37	16·2		–		14	11·7	
Indeterminate	–		4	1·2		–		3	1·3		–		1	0·8	

Categorical variables are summarised as the number (%) and were compared using χ^2 test.
[a]Continuous variables are summarised as the mean and standard deviation (SD) and were compared using *t* test.
**P* values denote the difference between the cases and controls at the 95 % confidence level. Significant *P* value < 0.05.

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Table 2. Association between riboflavin intake stratified by tertiles and gastric cancer risk (Numbers and percentages; odds ratio and 95 % confidence intervals)

	Control		Case		Model 1		Model 2		Model 3	
Riboflavin (mg/d)	*n*	%	*n*	%	OR	95 % CI	OR	95 % CI	OR	95 % CI
Total (*n* 1133)										
T1 (<0·84)	251	33·2	175	46·4	1·00		1·00		1·00	
T2 (0·84–1·06)	253	33·5	113	30·0	0·56	0·42, 0·76	0·63	0·46, 0·88	0·67	0·47, 0·95
T3 (>1·06)	252	33·3	89	23·6	0·46	0·33, 0·63	0·57	0·40, 0·82	0·56	0·39, 0·81
P_{trend}*						< 0·001		0·002		0·002
Male (*n* 743)										
T1 (<0·82)	165	33·2	108	43·9	1·00		1·00		1·00	
T2 (0·82–1·01)	166	33·4	72	29·3	0·60	0·41, 0·88	0·71	0·47, 1·09	0·74	0·47, 1·16
T3 (>1·01)	166	33·4	66	26·8	0·56	0·38, 0·82	0·73	0·47, 1·13	0·75	0·48, 1·18
P_{trend}*						0·002		0·135		0·194
Female (*n* 390)										
T1 (<0·91)	86	33·2	70	53·4	1·00		1·00		1·00	
T2 (0·91–1·18)	86	33·2	33	25·2	0·43	0·25, 0·72	0·49	0·27, 0·87	0·50	0·27, 0·93
T3 (>1·18)	87	33·6	28	21·4	0·39	0·23, 0·67	0·54	0·30, 0·98	0·52	0·28, 0·97
P_{trend}*						< 0·001		0·031		0·031

Riboflavin was categorised into tertiles (T1, T2, T3) based on the distribution of the control group. Multiple logistic regression for the association, using the lowest group as a reference. Model 1: adjusted by age, sex, energy intake. Model 2: adjusted by age, sex, energy intake, first-degree family history of gastric cancer, education level, job, household income, smoking status, regular exercise. Model 3: additional adjustment for *H. pylori* infection status. **P*-trends < 0·05 denote significant level.

Table 3. Association between riboflavin intake by tertiles and gastric cancer risk, stratified by *H. pylori* infection status (Odds ratio and 95 % confidence intervals)

		H. pylori - Positive (*n* 813)					*H. pylori* - Negative (*n* 320)				
			Model 1		Model 2			Model 1		Model 2	
Riboflavin (mg/d)	Median intake (mg/d)	Control/Case	OR	95 % CI	OR	95 % CI	Control/Case	OR	95 % CI	OR	95 % CI
T1 (<0·84)	0·70	165/161	1·00		1·00		86/14	1·00		1·00	
T2 (0·84–1·06)	0·94	148/103	0·62	0·44, 0·88	0·64	0·44, 0·94	105/10	0·55	0·23, 1·33	0·65	0·23, 1·80
T3 (>1·06)	1·23	151/85	0·52	0·36, 0·74	0·60	0·40, 0·89	101/4	0·20	0·06, 0·65	0·20	0·05, 0·78
P_{trend}*				< 0·001		0·009			0·006		0·019

Riboflavin was categorised into tertiles (T1, T2, T3) based on the distribution of control group. Multiple logistic regression for the association, using the lowest group as a reference. Model 1: adjusted by age, sex, energy intake. Model 2: adjusted by age, sex, energy intake, first-degree family history of gastric cancer, education level, job, household-income, smoking status, regular exercise. **P*-trends < 0·05 denote significant level.

Table 4. Associations of *MTRR* (rs1532268) genetic polymorphisms and gastric cancer risk in the dominant model (Numbers and percentages)

		Control		Case		Model 1		Model 2		Model 3	
MTRR (rs1532268)	Allele	*n*	%	*n*	%	OR	95 % CIs	OR	95 % CIs	OR	95 % CIs
Total (*n* 1133)	CC	585	77·4	288	76·4	1·00		1·00		1·00	
	TC + TT	171	22·6	89	23·6	1·06	0·79, 1·43	1·02	0·73, 1·41	1·1	0·78, 1·56
Male (*n* 743)	CC	386	77·7	186	75·6	1·00		1·00		1·00	
	TC + TT	111	22·3	60	24·4	1·14	0·79, 1·64	1·11	0·73, 1·68	1·21	0·78, 1·89
Female (*n* 390)	CC	199	76·8	102	77·9	1·00		1·00		1·00	
	TC + TT	60	23·2	29	22·1	0·95	0·57, 1·58	0·87	0·49, 1·54	0·95	0·51, 1·75

Model 1: adjusted by age, sex, energy intake. Model 2: adjusted by age, sex, energy intake, first-degree family history of gastric cancer, education level, job, household-income, smoking status, regular exercise. Model 3: additional adjustment for *H. pylori* infection status.

agreement with our studies were conducted on Asian populations (China, Korea)[15,41–43], while the others were in Belgium, Australia, northern Italy, Canada and the USA[13,44–47], where distinct gaps with the Asian countries in terms of dietary patterns/habits, culture, genes and other potential characteristics exist. In addition to riboflavin consumed in food, riboflavin supplements might contribute to the overall protective effect of riboflavin. Hence, the inconsistent results in the above studies could be driven by the difference in vitamin supplement habits between individuals or populations, which was not assessed in this study. One possible hypothesis regarding the increase in GC risk is that milk is rich in riboflavin, and people who have preceding cancer symptoms tend to drink more milk to relieve discomfort. While milk/dairy product consumption is positively associated with GC in some studies[48], this hypothesis indicates the inability to eliminate the possibility of a spurious association (harmful effect of

Table 5. Interaction between *MTRR* (rs1532268) genetic polymorphisms and riboflavin intake in gastric cancer risk (Numbers and percentages)

		Control		Case		Model 1		Model 2		Model 3	
MTRR (rs1532268) Allele	Riboflavin intake (mg/d)	*n*	%	*n*	%	OR	95 % CI	OR	95 % CI	OR	95 % CI
CC	T1 (< 0·84)	202	26·7	132	35·0	1·00		1·00		1·00	
	T2 (0·84–1·06)	190	25·1	87	23·1	0·63	0·45, 0·89	0·74	0·51, 1·08	0·80	0·53, 1·19
	T3 (> 1·06)	193	25·5	69	18·3	0·50	0·35, 0·73	0·68	0·45, 1·01	0·67	0·44, 1·03
CT + TT	T1 (< 0·84)	49	6·5	43	11·4	1·45	0·90, 2·32	1·62	0·95, 2·76	1·93	1·09, 3·42
	T2 (0·84–1·06)	63	8·3	26	6·9	0·55	0·33, 0·92	0·59	0·34, 1·05	0·67	0·37, 1·22
	T3 (> 1·06)	59	7·8	20	5·3	0·48	0·27, 0·84	0·53	0·28, 0·97	0·54	0·28, 1·02
$P_{interaction}$*							0·226		0·066		0·037

Riboflavin was categorised into tertiles (T1, T2, T3) based on the distribution of control group. Multiple logistic regression for the association, using the lowest riboflavin level in CC group as a reference. Model 1: adjusted by age, sex, energy intake. Model 2: adjusted by age, sex, energy intake, first-degree family history of gastric cancer, education level, job, household income, smoking status, regular exercise. Model 3: additional adjustment for *H. pylori* infection status. *$P_{interaction}$ is the P_{trend} of the interaction between riboflavin intake and *MTRR* genotype.

riboflavin) based on a case–control study design. The results of this study showed that riboflavin intake was only negatively associated with GC risk in the female subgroup, whereas no significant association was found in the male subgroup. A reasonable explanation for this difference is that oestrogen exposure in females is protectively associated with GC risk as found in previous studies[49,50]. Moreover, the oestrogen hormone and its receptors, which are present in gastric tissue, were shown to interfere with GC development and prognosis in some studies[51,52]. However, the difference in sample size, average energy intake and mean riboflavin consumption between the two subgroups, which could not be eliminated in our study, could have led the existence of a spurious association.

Some experimental studies strongly supported the carcinogenic effect of insufficient riboflavin due to an increase in protein and DNA oxidative damage[17,19], an increase in carcinogen binding to DNA[18] and the over-induction of DNA repair enzymes[17]. The anticancer mechanism of riboflavin is unclear, but the effect may be related to its antioxidant effects and strong involvement in OCM. The antioxidant properties of riboflavin are mainly demonstrated by its role in the glutathione redox cycle, which therefore can lead to an increase in the dysfunction of the antioxidant-glutathione in some circumstances. Moreover, riboflavin also affected the activity of catalase, superoxide dismutase enzymes and some other antioxidant enzymes. Hence, insufficient consumption of riboflavin and the resulting reduction in enzyme activities would impair the antioxidant defence system of the body and lead to an imbalance in the oxidant–antioxidant system[53–55]. As an important cofactor in OCM, which is a critical network of several biological processes, such as DNA synthesis, methylation and DNA repair, riboflavin deficiency in humans may have consequences for DNA single-strand breaks and anomalous DNA repair[17,56], which contribute to the induction of carcinogenesis. Another mechanism identified in experimental studies is that riboflavin helps to maintain epithelial integrity. Hence, the lack of riboflavin could lead to a higher risk of epithelial dysplasia in the upper gastrointestinal tract[57,58].

In our study, *MTRR* C524T showed no significant association with GC, which is in agreement with two other studies conducted in 2012 and 2014[26,27]. In 2007, a study in Poland showed that the T allele in Ex5 + 123C > T (another name for C524T) marginally increased the risk of GC (OR = 1·30; 95 % CI 0·93, 1·82)[25]. Regarding other cancer types, the T allele in *MTRR* C524T was a harmful factor for prostate and breast cancer[23,24], while no association was found for oesophageal, colorectal, liver, lung or bladder cancer[26,28–32]. As no other study exists on *MTRR* C524T and GC, hypothesising about the reason for this discrepancy is challenging, with the exception of noting the regional characteristics, study designs and small sample sizes. Although an association between the rs1532268 SNP and GC was not observed in our study, a significant interaction was found between the candidate SNP and riboflavin intake that modifies the association between riboflavin and GC risk. Because the MTRR enzyme along with riboflavin is the crucial component in the OCM pathway, the impact of *MTRR* gene variants on the anticancer capacity of riboflavin is reasonable and explainable.

Because our research used a case–control design, several limitations still exist. We could not eliminate the possibility of selection bias and recall bias. Because this study had a hospital-based design, the controls were healthy people participating in the health screening who tended to have a higher awareness of illnesses and may have healthier lifestyles/habits; thus, they might not be representative of the general population. Additionally, the duration of riboflavin intake is very important: the long-term use of combined riboflavin tablet was found to result in a more substantial reduction in the cancer risk in a randomised controlled trial[41]; however, we could not clearly define the exposure time of our factor of interest. Additionally, as mentioned above, riboflavin supplements might also have contributed to the favourable effect of overall riboflavin, but this study did not assess this potential variable. However, the current study proposed to assess dietary riboflavin intake, not supplemental intake, and the evidence regarding the association of riboflavin supplements and GC is quite limited. Moreover, recall bias could have occurred due to the long period of dietary intake reported by participants (12 months). Temporal bias is also acknowledged because the dietary food assessment was performed after the disease diagnosis was confirmed. Certainly, even with our best recruitment effort, the sample size was relatively small to allow for sufficient statistical power.

Although there are unavoidable limitations, our study has some strengths that can inform future projects. (1) The current study is the first to address the interaction of *MTRR* rs1532268

variants with riboflavin intake in the context of GC risk. (2) We used a comprehensive and validated semi-quantitative FFQ with 106 items for dietary data collection and riboflavin intake calculation. (3) Additionally, the information of the case group was collected by well-trained interviewers using a self-reported method with strict rechecking of missing data in the control group. (4) Finally, almost all information about potential risk factors for GC, such as *H. pylori* infection, smoking and alcohol consumption, was completely available. Hence, the study had more advantages with regard to controlling for all possible confounders.

Conclusion

According to our research, riboflavin is a protective factor for GC. At *MTRR* C524T, the T allele was considered to be a risk allele for GC in the Korean population. Our study offers a hopeful direction for clinical practice in the future by demonstrating that T allele carriers should be encouraged to consume more riboflavin for better GC prevention. However, a larger sample size and better validity design are needed for the upcoming studies to reconfirm this interaction.

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