

Red Meat, Family History, and Increased Risk of Gastric Cancer with Microsatellite Instability¹

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ABSTRACT

Microsatellite instability (MSI) occurs frequently in sporadic gastric cancer (GC) and may define a distinctive molecular pathway of carcinogenesis. We evaluated the role of dietary risk factors in GC according to MSI status. A large series of 382 GC cases and 561 controls were originally identified in a population-based case-control study carried out in the high-risk area around Florence, Italy; 126 GC patients were typed for MSI status. A MSI+ phenotype was detected in 43 of 126 cases (34.1%), whereas 83 cases were classified as MSI-. A multinomial logistic regression model was used to compare the two subgroups of GC classified according to MSI status in the same analysis, with all of the available population controls. A case-case approach was also used. The risk of MSI+ tumors was positively associated with high consumption of red meat and meat sauce and negatively associated with consumption of white meat. A positive association was also seen with total protein and nitrite intake, whereas no relation was found with micronutrient intake. Risk was especially high among subjects reporting both a positive GC family history and a high consumption of red meat (odds ratio, 25.7; 95% confidence interval, 6.4–102.8). For MSI- tumors, a significant protective effect was associated with frequent consumption of citrus and other fresh fruit, garlic, legumes, vegetables, and olive oil and with high intake of β -carotene and other antioxidants and sugar, whereas positive associations were seen with protein and sodium intake. In summary, a specific dietary pattern emerged for MSI+ gastric tumors, suggesting that factors related to red meat consumption are involved in this pathway, particularly among individuals with a positive family history. In contrast, the risk of MSI- tumors was strongly reduced by the frequent consumption of fresh fruit and vegetables.

INTRODUCTION

Genomic instability is thought to play a key role in the multistage process leading to cancer because it may generate the mutational variability that underlies tumor progression (1). Most tumors associated with the hereditary nonpolyposis colorectal cancer syndrome and a subset of sporadic gastrointestinal cancers exhibit a specific type of genomic instability, characterized by the accumulation of ubiquitous deletion/insertion mutations within repetitive microsatellite DNA (2–4). Widespread tumor-associated MSI³ is believed to be caused by altered repair of spontaneous DNA replication errors after mutational inactivation or epigenetic silencing of at least one of various MMR genes, including *hMLH1*, *hMSH2*, *hPMS1*, and *hPMS2* (2, 3, 5–10). Yeast and mammalian cell studies suggest that MMR acts not only on

base/base mismatches or small insertion/deletion loops that escape proofreading by the replicating DNA polymerase, but also on chemically altered bp (5). Studies of MMR genes in murine and human tumorigenesis suggest that MMR defects underlie the development of several types of cancer (5). Thus, MSI status represents a key molecular variable that can distinguish MMR-proficient from MMR-deficient cases. Tumors with a MSI+ phenotype have diploid DNA and follow a distinctive pathway of molecular progression, including frameshift mutations at mononucleotide runs within key cancer-related genes (2, 11–15). These cancers also differ from MSI- tumors in several clinicopathological features (12, 14, 16–18).

Although GC incidence and mortality rates have shown a consistent decline over several decades in most countries, GC still represents the fourth most common cancer in the world and is the second leading cause of cancer death (19). Because MSI occurs frequently in sporadic GC (in up to 33% of the cases), and appears to be associated with *hMLH1* inactivation by methylation rather than with MMR gene mutations (20–22), it would seem important to assess the epidemiological patterns of GC associated with MSI status. We have previously shown that MSI is significantly associated with distal (antral) tumors and with positive family history of GC (23). The present report evaluates the relation between dietary habits and MSI status using 126 GC cases and 561 population controls identified in a case-control study carried out in a high-incidence area around Florence, Italy (24, 25).

MATERIALS AND METHODS

Study Population and Data Collection. The current series of GC cases was identified in 1985–1987 in Florence, Italy, where the coordinating center of a population-based multicenter case-control study was located (25–27). All GC cases were histologically confirmed and originally classified according to Lauren's classification by review of all available surgical pathology specimens (24). Computerized lists of residents were used to identify a random sample of eligible population controls; overall, 382 GC cases and 561 controls with complete data were available from the original study. In a previous investigation, a nonrandom sample of 108 of the original 382 GC cases was tested for MSI (23). To expand this sample, the histological specimens of an additional 18 GC cases were identified from the original population-based series, retrieved, and tested for MSI status in the same laboratory. Overall, MSI status was investigated in 126 of the original 382 GC cases.

Microsatellite Analysis. Formalin-fixed, paraffin-embedded blocks were retrieved from the archival files of the Pathology Department, University of Florence. Several 5- μ m-thick sections were cut for DNA extraction. For each case, matched DNAs from GC and normal tissue were extracted as reported previously (28). MSI was initially evaluated at six dinucleotide repeats (D1S104, D2S123, D3S1611, D5S107, D17S261, and D18S342) and then evaluated at seven mononucleotide repeats [BAT25, BAT26, TGF β RII poly(A)10, IGF1R poly(G)8, BAX poly(G)8, hMSH3 poly(A)8, and hMSH6 poly(C)8]. PCRs, electrophoretic separation, and autoradiography were as described previously (23, 28, 29). Paired genotypings of all cases positive for microsatellite alterations were confirmed in duplicate experiments, using independently extracted DNA samples.

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³ The abbreviations used are: MSI, microsatellite instability; MSI-H, high frequency MSI; MSI-L, low frequency MSI; MMR, mismatch repair; BMI, body mass index; OR, odds ratio; CI, confidence interval; GC, gastric cancer.

Overall, 43 GCs were classified as MSI+, and 83 were classified as MSI-. The 43 MSI+ GCs included 17 cases (17 of 126; 13.5%) that showed instability at both mono- and dinucleotide repeats (classified as MSI-H) and 26 cases (26 of 126; 20.6%) with instability at two or more of the six dinucleotide repeats and no instability at mononucleotide repeats (classified as MSI-L; Refs. 17 and 30). The MSI-H and MSI-L subsets showed similar characteristics with regard to the association with first-degree GC family history, which was reported by 8 of 17 MSI-H cases (47%) and 10 of 26 MSI-L cases (38.5%) versus 22 of 83 MSI- cases (26.5%).

Dietary and Other Interview Data. A detailed description of the questionnaire has been published elsewhere (26). Briefly, the questionnaire recorded demographic, anthropometric, socioeconomic, residential, occupational, smoking, medical, family, and dietary information. Diet was assessed by asking the usual frequency of consumption of 181 food items and beverages. With the aid of an instruction manual and an atlas depicting the most frequently consumed food items, the usual portion size (small, medium, and large) in a 12-month period before the interview was assessed for 146 food and beverage items. A standard portion size was assumed for the remaining items. Amounts of nutrients and energy provided by each food were estimated using newly updated Italian food composition tables (31) supplemented with other published data. When not available in tables, the amount of different food components in complex dishes was defined on the basis of traditional Italian recipes. Thermolability was taken into account by reducing the estimated contents of ascorbic acid by 50% and β -carotene by 15% in cooked foods. For all subjects, a cumulative daily average intake for each nutrient was computed by summing the values for each food. Use of vitamin supplements was shown to be uncommon during a pilot phase carried out in 1985 and was not considered in the original study questionnaire. BMI, calculated as weight in kilograms divided by height in square meters, was used as a measure of obesity. Average daily ethanol intake was computed by multiplying the amount of ethanol present in each alcoholic beverage reported at interview by the reported frequency of consumption; drinkers were grouped into four categories according to daily intake (<20, 20–40, 41–60, and 60+ g/day).

Statistical Analysis. A multinomial logistic regression model was used to permit the simultaneous analysis of two subgroups of GC cases classified according to MSI status (MSI+ or MSI-) and the series of population controls. All of the regression equations included terms for nondietary variables (age, sex, social class, family history of GC, area of residence, and BMI tertiles) and total energy intake. The frequencies of consumption of food items or food groups were introduced in the models as tertiles. Nutrient intake tertiles were calculated on the residual of the regression of the nutrient on energy, according to Willett (32). Maximum likelihood estimates of OR and 95% CI were calculated. Tests for trend for variables on more than two levels were based on the likelihood ratio test between the models with and without a linear term for each variable. Tests of homogeneity of the OR for the two subgroups of GC cases were made. Interaction terms according to MSI status were calculated by means of the Wald χ^2 test with one degree of freedom. As a supplementary analysis, a case-case approach used the unconditional logistic regression analysis including the same covariate pattern used in the multinomial approach, directly comparing the two subgroups of GC cases according to MSI status.

According to the original study design, all of the analyses were carried out comparing the two subgroups of GCs classified as MSI+ ($n = 43$) and MSI- ($n = 83$) with the large series of population controls and with each other. In addition, we also performed a few separate analyses for the MSI-H subset ($n = 17$). These analyses showed no material differences when compared with the MSI+ subgroup, although the smaller sample size precluded meaningful comparisons based on the use of complex models. Only the results for the two larger categories of MSI+/MSI- gastric tumors and the series of population controls are presented here.

RESULTS

Among 126 GC cases tested for MSI, 43 were classified as MSI+ (34.1%), and 83 were classified as MSI- (65.9%). Table 1 shows the distribution of study subjects according to sociodemographic variables, family history, BMI, smoking, and alcohol drinking compared with controls. Both subgroups of GC cases showed a higher frequency

Table 1 Distribution of 561 population controls and 126 GC cases classified by MSI phenotype and epidemiologic variables

	Controls (561)		GC cases (126)		Exact P
	N (%)	MSI+ N (%)	MSI- N (%)		
Gender					
Male	328 (58.5)	26 (60.5)	56 (67.5)		
Female	233 (41.5)	17 (39.5)	27 (32.5)		0.3
Age group (yrs)					
<50	122 (21.8)	3 (7.0)	11 (13.3)		
50–64	188 (33.5)	16 (37.2)	26 (31.3)		
>64	251 (44.7)	24 (55.8)	46 (55.4)		0.04
Residence					
Urban	478 (85.2)	29 (67.4)	58 (69.9)		
Rural	83 (14.8)	14 (32.6)	25 (30.1)		<0.001
Migration from southern Italy					
No	501 (89.3)	40 (93.0)	80 (96.4)		
Yes	60 (10.7)	3 (7.0)	3 (3.6)		<0.001
Social class					
Low	323 (57.6)	34 (79.1)	60 (72.3)		
Medium	154 (27.5)	7 (16.3)	18 (21.7)		
High	84 (15.0)	2 (4.7)	5 (6.0)		0.08
No. of 1st-degree relatives with GC					
0	487 (86.8)	25 (58.1)	61 (73.5)		
1	66 (11.8)	15 (34.9)	17 (20.5)		
2+	8 (1.4)	3 (7.0)	5 (6.0)		0.006
BMI					
Low	203 (36.2)	12 (27.9)	36 (43.4)		
Medium	190 (33.9)	18 (41.9)	30 (36.1)		
High	168 (30.0)	13 (30.2)	17 (20.5)		0.3
Smoking history					
Never smoker	210 (37.4)	18 (41.9)	27 (32.5)		
Former	171 (30.5)	15 (34.9)	23 (27.7)		
Current	180 (32.1)	10 (23.2)	33 (39.8)		0.4
Alcohol (gs/day)					
Never	76 (13.6)	7 (16.3)	7 (8.4)		
1–20	76 (13.6)	8 (18.6)	5 (6.0)		
21–40	128 (22.8)	8 (18.6)	26 (31.3)		
41–60	167 (29.8)	7 (16.3)	22 (26.5)		
>60	114 (20.3)	13 (30.2)	23 (27.7)		0.04
Total	561	43	83		

of subjects with positive family history for GC among parents or siblings, indices of lower social class, and rural residence. Neither subgroup of GC was significantly associated with BMI, alcohol drinking, or cigarette smoking.

Dietary analyses for food groups are shown in Table 2. The risk of MSI+ tumors increased 4-fold with increasing consumption of red meat and meat sauce, whereas an inverse relation was evident with consumption of white meat (OR, 0.3; 95% CI, 0.1–0.8). Other foods (including fresh fruit, raw and cooked vegetables, and olive oil) tended to be negatively associated with risk but failed to reach statistical significance. Citrus fruit consumption was unrelated to the risk of MSI+ tumors.

On the other hand, the risk of MSI- tumors was positively associated with frequent consumption of soups (OR, 3.0; 95% CI, 1.5–5.9), red and cured/canned meats, and cheese (P for trend <0.01, 0.05, and 0.04, respectively). Risk was significantly reduced by high consumption of legumes, cooked vegetables, garlic and onion, olive oil, citrus, and other types of fresh fruit (except apples and pears).

When consumption tertiles of both meat types were introduced simultaneously in a multinomial model, the risk of MSI+ tumors was positively associated with red meat consumption (OR, 3.9; 95% CI, 1.6–9.9) and negatively associated with white meat consumption (OR, 0.4; 95% CI, 0.2–1.0), whereas no significant associations were found with MSI- tumors.

Table 3 presents the ORs and 95% CIs associated with intake of specific nutrients. High intake of total protein (OR, 3.3; 95% CI, 1.1–10.1) and nitrites (OR, 2.9; 95% CI, 1.1–8.0) was positively

Table 2 ORs^a and 95% CIs for MSI+ and MSI- GC according to tertiles of consumption of selected foods, based on comparison with 561 population controls

Food items	MSI+ gastric tumors (N = 43)			MSI- gastric tumors (N = 83)		
	Tertiles		P for linear trend	Tertiles		P for linear trend
	2	3 (high)		2	3 (high)	
Pasta	0.8 (0.3-1.7)	0.9 (0.4-2.1)	0.9	0.9 (0.5-1.7)	1.0 (0.5-1.8)	0.8
Rice and polenta	0.9 (0.4-2.2)	1.6 (0.7-3.6)	0.2	1.0 (0.5-1.8)	1.4 (0.8-2.6)	0.2
Soups	1.0 (0.4-2.5)	1.5 (0.6-3.5)	0.2	2.0 (1.0-4.0)	3.0 (1.5-5.9)	0.002^b
Bread	0.9 (0.3-2.5)	1.2 (0.5-2.7)	0.5	2.5 (1.2-5.0)	1.6 (0.9-3.1)	0.2
Pizza/toast/sandwich	1.0 (0.4-2.2)	1.4 (0.6-3.4)	0.3	1.7 (0.9-3.1)	1.3 (0.7-2.6)	0.4
Red meat (beef/pork/lamb/game)	1.7 (0.6-4.6)	4.3 (1.8-10.8)	0.001	0.9 (0.4-1.7)	2.1 (1.2-3.7)	0.008
White meat (poultry/rabbit)	0.6 (0.3-1.3)	0.3 (0.1-0.8)	0.01	1.1 (0.6-1.9)	0.9 (0.5-1.6)	0.7
Other meats (offal/giblets/liver)	1.1 (0.5-2.6)	1.0 (0.4-2.2)	0.8	0.8 (0.4-1.5)	1.4 (0.8-2.5)	0.2
Cured & canned meats	1.0 (0.5-2.4)	1.0 (0.4-2.6)	0.1	1.2 (0.6-2.3)	1.9 (1.0-3.7)	0.05
Salted & dried fish	1.6 (0.7-3.8)	1.5 (0.6-3.7)	0.4	1.6 (0.8-3.0)	1.7 (0.9-3.3)	0.1
Fresh fish & seafood	0.5 (0.2-1.2)	0.9 (0.4-2.0)	0.8	0.9 (0.5-1.7)	0.8 (0.4-1.4)	0.4
Eggs	1.2 (0.6-2.7)	0.7 (0.3-1.6)	0.3	1.2 (0.7-2.2)	0.7 (0.4-1.3)	0.2
Cheese	0.8 (0.4-1.8)	1.0 (0.4-2.2)	0.9	1.0 (0.5-1.9)	1.8 (1.0-3.3)	0.04
Milk/yogurt/fresh cheese	1.4 (0.6-3.2)	1.4 (0.6-3.3)	0.4	1.3 (0.7-2.2)	1.0 (0.5-1.9)	0.9
Legumes (beans/lentils/peas)	0.7 (0.3-1.6)	0.6 (0.2-1.4)	0.2	0.9 (0.5-1.7)	0.5 (0.3-1.0)	0.04
Raw vegetables	0.5 (0.2-1.1)	0.6 (0.3-1.3)	0.1	0.7 (0.4-1.2)	0.8 (0.4-1.4)	0.3
Cooked vegetables	0.9 (0.4-1.9)	0.5 (0.2-1.1)	0.09	0.5 (0.3-0.9)	0.4 (0.2-0.8)	0.005
Potatoes	0.6 (0.2-1.7)	1.9 (0.8-4.2)	0.07	1.0 (0.5-1.9)	1.1 (0.6-2.1)	0.4
Pickled & canned vegetables, olives	1.1 (0.5-2.4)	0.7 (0.3-1.7)	0.4	1.0 (0.6-1.8)	0.9 (0.5-1.7)	0.8
Garlic & onion	0.6 (0.3-1.4)	0.6 (0.3-1.3)	0.1	0.8 (0.5-1.4)	0.5 (0.2-0.9)	0.02
Citrus fruit & fruit juices	1.3 (0.6-2.8)	1.0 (0.4-2.3)	0.9	0.6 (0.3-1.0)	0.5 (0.2-0.9)	0.01
Apples & pears	1.6 (0.7-3.9)	1.6 (0.7-3.8)	0.3	0.5 (0.3-1.0)	1.0 (0.6-1.7)	0.9
Fresh fruit (all other types)	0.6 (0.3-1.4)	0.5 (0.2-1.2)	0.1	0.9 (0.5-1.5)	0.4 (0.2-0.7)	0.006
Dessert and pastry (all types)	0.7 (0.3-1.5)	1.1 (0.5-2.4)	0.8	0.8 (0.5-1.5)	0.6 (0.3-1.2)	0.1
Coffee and tea	0.5 (0.2-1.1)	0.8 (0.3-1.9)	0.5	0.8 (0.4-1.4)	0.6 (0.3-1.2)	0.1
Sugar/honey/jam	0.8 (0.4-1.8)	0.9 (0.4-2.2)	0.8	1.1 (0.6-2.0)	0.5 (0.3-1.1)	0.1
Olive oil	0.4 (0.2-1.0)	0.5 (0.2-1.1)	0.07	0.7 (0.4-1.2)	0.6 (0.3-1.0)	0.05
Tomato sauce	1.3 (0.5-3.5)	1.2 (0.4-3.0)	0.8	1.6 (0.8-3.4)	1.2 (0.6-2.4)	0.9
Meat sauce	2.0 (0.5-7.4)	4.2 (1.2-14.9)	0.01	0.5 (0.3-1.1)	1.4 (0.7-2.8)	0.07

^a Estimates from separate multinomial logistic regression models including terms for nondietary variables (age, sex, social class, family history of GC, area of residence, and BMI tertiles), total energy, and consumption tertiles of each food of interest (reference, lowest tertile).

^b Significant values are shown in bold.

associated with MSI+ tumors, whereas no significant inverse association was evident.

On the other hand, the risk of MSI- tumors was positively associated with intake of total protein (OR, 2.4; 95% CI, 1.3-4.4), animal protein (OR, 2.2; 95% CI, 1.2-3.8), and sodium (OR, 2.1; 95% CI, 1.2-3.6), whereas protective effects were related to intake of sugar (OR, 0.5; 95% CI, 0.2-0.9), ascorbic acid (OR, 0.4; 95% CI, 0.2-0.8), β -carotene (OR, 0.2; 95% CI, 0.1-0.5), α -tocopherol (OR, 0.4; 95% CI, 0.2-0.7), and nitrates (OR, 0.4; 95% CI, 0.2-0.7).

In both subgroups of GC, a high consumption of grilled meat was associated with a 2-fold increased risk (data not shown). Subjects reporting both high consumption of red meat and high frequency of grilling showed a 5-fold increased risk of MSI+ tumors, with risk increasing steadily across tertiles for red meat consumption. Whereas the risk of MSI- tumors was elevated at high intakes of red meat, there was no clear evidence of a dose-response effect.

We also examined the combined effects of red meat consumption and first-degree family history of GC (Table 4). Subjects reporting both high consumption of red meat and a positive family history of GC showed a 25-fold increased risk of MSI+ tumors. Among subjects with a negative family history, risk increased steadily to reach 5-fold in the highest frequency tertile. In contrast, the risk of MSI- tumors was only moderately increased in the highest tertile of red meat consumption, with no clear evidence of a dose-response effect. No significant interaction emerged between red meat consumption (or frequency of grilling) and family history when cases were stratified by MSI status.

The case-case approach showed that the risk of MSI+ tumors was reduced with high consumption of white meat (OR, 0.3; 95% CI, 0.1-0.9) and elevated with high intake of carbohydrates (OR, 3.3; 95% CI, 1.2-9.2), whereas the risk of MSI- tumors was reduced with high β -carotene intake (OR, 0.3; 95% CI, 0.1-0.9).

DISCUSSION

In this case-control study, we compared a series of 126 GC cases classified by MSI status with a large group of population controls to evaluate the effects of dietary factors on GC risk according to molecular phenotypes, reflecting the MMR activity of tumors (2, 33, 34). The dietary profile associated with MSI+ tumors featured a high consumption of red meat and meat sauce as well as nitrites, whereas white meat consumption showed an inverse association. The risk associated with red meat consumption was especially pronounced in subjects with a family history of GC. The dietary patterns associated with MSI- tumors resembled those previously reported for GC, including protective effects of fresh fruit and vegetables as well as various micronutrients and increased risks from sodium.

A diet rich in meat-derived foods has been suggested to play a role in gastric carcinogenesis by several studies in Western populations (25, 35, 36). Consistent with studies in laboratory animals (37), an increased risk of GC has been linked to intake of highly grilled or well-done red meat (38). Although we had no information on the degree of meat doneness, our results suggest that high consumption of red meat, particularly when grilled, elevates the risk of MSI+ tumors. The underlying mechanism for this association is unclear but may involve increased tolerance to DNA damage associated with reduced MMR activity (5). Murine cell lines lacking normal *Msh2* alleles have been reported to escape apoptosis after chronic oxidative stress (39); this mechanism has been invoked to explain the increased cancer risk characteristic of hereditary nonpolyposis colorectal cancer and appears to be modulated by dietary factors such as red meat. It seems plausible that dietary mutagens or carcinogens in red meat increase GC risk in a subset of susceptible individuals with a low efficiency of MMR functions. When we compared MSI+ tumors with population controls, a greater than multiplicative effect was suggested when a

Table 3 ORs^a and 95% CIs for MSI+ and MSI- GC according to tertiles of estimated intake of selected nutrients, based on comparison with 561 population controls

Nutrients	MSI+ gastric tumors (N = 43)			MSI- gastric tumors (N = 83)		
	Tertiles		P for linear trend	Tertiles		P for linear trend
	2	3 (high)		2	3 (high)	
Total protein	4.3 (1.5–12.1)	3.3 (1.1–10.1)	0.04^b	1.8 (1.0–3.3)	2.4 (1.3–4.4)	0.006
Animal protein	1.9 (0.8–4.5)	1.9 (0.8–4.8)	0.1	0.9 (0.5–1.3)	2.2 (1.2–3.8)	0.005
Vegetable protein	2.2 (0.9–5.4)	1.4 (0.5–3.6)	0.5	1.1 (0.6–1.9)	0.9 (0.5–1.7)	0.8
Total fat	0.8 (0.3–2.0)	1.0 (0.4–2.3)	0.9	1.1 (0.6–1.9)	1.1 (0.6–1.9)	0.8
Animal fat	0.5 (0.2–1.3)	1.0 (0.4–2.3)	0.8	1.3 (0.7–2.3)	1.5 (0.8–2.7)	0.1
Vegetable fat	0.7 (0.3–1.6)	0.6 (0.3–1.4)	0.2	0.8 (0.4–1.4)	0.7 (0.4–1.2)	0.2
FA ^c						
Total saturated FA	0.9 (0.4–2.2)	1.1 (0.5–2.7)	0.7	1.3 (0.7–2.3)	1.4 (0.7–2.5)	0.3
Total monounsaturated FA	0.6 (0.2–1.5)	0.9 (0.4–2.0)	0.7	1.0 (0.6–1.8)	0.9 (0.5–1.6)	0.7
Total polyunsaturated FA	0.9 (0.4–2.1)	0.7 (0.3–1.8)	0.5	0.7 (0.4–1.3)	0.9 (0.5–1.5)	0.5
Cholesterol	1.0 (0.5–2.4)	1.2 (0.5–2.8)	0.7	1.3 (0.7–2.4)	1.5 (0.8–2.8)	0.1
Carbohydrates	1.6 (0.6–4.1)	1.9 (0.8–4.8)	0.1	0.8 (0.4–1.4)	0.8 (0.5–1.4)	0.4
Starch	1.8 (0.7–4.8)	1.8 (0.7–4.6)	0.2	0.9 (0.5–1.7)	1.1 (0.6–1.9)	0.7
Sugar	0.9 (0.4–2.2)	1.0 (0.4–2.5)	0.9	0.9 (0.5–1.5)	0.5 (0.2–0.9)	0.03
Fiber	1.1 (0.4–2.6)	1.4 (0.6–3.4)	0.4	1.1 (0.6–1.9)	0.6 (0.3–1.2)	0.1
Alcohol	0.5 (0.2–1.1)	0.6 (0.3–1.6)	0.3	1.0 (0.6–1.8)	0.9 (0.5–1.7)	0.7
Total calories	1.1 (0.5–2.4)	0.6 (0.3–1.6)	0.3	0.5 (0.3–0.9)	0.7 (0.4–1.2)	0.1
Minerals						
Sodium	0.8 (0.3–2.1)	1.6 (0.7–3.6)	0.2	0.9 (0.5–1.7)	2.1 (1.2–3.6)	0.007
Potassium	1.2 (0.5–2.9)	1.7 (0.7–4.0)	0.2	0.7 (0.4–1.2)	0.7 (0.4–1.2)	0.1
Vitamins						
Vitamin C	1.9 (0.8–4.3)	1.3 (0.5–3.3)	0.5	0.6 (0.3–0.9)	0.4 (0.2–0.8)	0.003
Alpha-tocopherol	1.3 (0.6–2.9)	0.8 (0.3–2.1)	0.7	0.6 (0.3–1.0)	0.4 (0.2–0.7)	0.002
Beta carotene	1.0 (0.4–2.2)	1.1 (0.4–2.6)	0.8	0.4 (0.2–0.6)	0.2 (0.1–0.5)	0.0001
Retinol	0.9 (0.4–2.1)	0.8 (0.3–1.9)	0.6	1.3 (0.7–2.3)	1.6 (0.9–2.9)	0.08
Nitrates & nitrites						
Nitrates	0.5 (0.2–1.1)	0.6 (0.3–1.5)	0.2	0.6 (0.3–1.0)	0.4 (0.2–0.7)	0.002
Nitrites	2.6 (1.0–7.3)	2.9 (1.1–8.0)	0.04	1.6 (0.9–3.0)	1.7 (0.9–3.1)	0.09

^a Estimates from separate multinomial logistic regression models including terms for nondietary variables (age, sex, social class, family history of GC, area of residence, and BMI tertiles), total energy, and tertiles of the residuals of each nutrient of interest (reference, lowest tertile).

^b Significant values are shown in bold.

^c FA, fatty acids.

high frequency of red meat consumption was reported by subjects with a positive family history of GC. We have reported previously that the MSI+ phenotype was significantly associated with familial clustering of GC, which probably reflects increased genetic susceptibility (23). Furthermore, a 10-year follow-up study of our original series (24) revealed that GC prognosis was adversely affected by high intake of animal protein among cases with a positive family history. In susceptible individuals, it seems plausible that both tumor induction and tumor progression reflect MMR deficiency, with cell clones being more tolerant of genotoxic damage and likely to escape apoptosis. On the other hand, dietary haem, the iron carrier found in red meat, has

recently been suggested as a link between red meat consumption and colon cancer, consistent with its cytotoxic and proliferative effects on the colonic mucosa (40). The increased mitotic activity may then increase the frequency of spontaneous mutations in target genes, which are repaired less efficiently in susceptible individuals.

In summary, the usual risk factors reported for GC, particularly diets low in fresh fruit and vegetables and high in sodium, were related mainly to MSI- tumors in our study. The association of MSI+ tumors with red meat consumption, particularly among individuals with familial susceptibility, calls for further investigation into the nutritional and genetic determinants of molecular subtypes of GC.

Table 4 ORs^a and 95% CIs for MSI+ (n = 43) and MSI- (n = 83) gastric tumors according to consumption of red meat and a first-degree family history for gastric cancer, based on comparison with 561 population controls

Family history	Red meat consumption tertiles			Overall OR (95% CI)
	1 (low)	2	3 (high)	
MSI+ tumors				
Negative	167/3 ^b	160/8	160/14	1 ^c
Positive	24/4	2.8 (0.7–10.9)	5.1 (1.4–18.4)	4.1 (2.1–7.9)^d
	7.4 (1.5–36.4)	30/3	20/11	
		5.4 (1.0–28.8)	25.7 (6.4–102.8)	
Total OR (95% CI)	1 ^c	1.6 (0.6–4.4)	4.0 (1.6–9.7)	
MSI- tumors				
Negative	167/15	160/13	160/33	1 ^c
Positive	24/7	0.9 (0.4–2.0)	2.4 (1.2–4.7)	2.2 (1.2–3.8)
	3.1 (1.1–8.6)	30/6	20/9	
		2.2 (0.8–6.4)	4.3 (1.6–11.4)	
Total OR (95% CI)	1 ^c	0.9 (0.5–1.7)	2.1 (1.2–3.7)	

^a Estimates from separate unconditional logistic models. Differences from estimates and CIs reported in Table 2 are due to the different models used (i.e., multinomial versus binomial).

^b Study subjects (population controls/GC cases) in each category.

^c Reference category.

^d Bold values represent marginal ORs.

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