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Polymorphisms of mismatch repair gene *hMLH1* and *hMSH2* and risk of gastric cancer in a Chinese population

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Abstract. The purpose of this study was to determine the genotype and allele frequencies of *hMLH1* (-93G>A and I219V) and *hMSH2* (-118T>C and IVS12-6T>C) polymorphisms in patients with gastric carcinoma and normal controls, and to evaluate the association between these polymorphisms and the risk of gastric cancer in a hospital-based Chinese population. Genomic DNA was extracted from peripheral blood lymphocytes. A TaqMan assay was used to determine the genotype and allele frequencies of *hMLH1* and *hMSH2* polymorphisms in data obtained from 554 gastric cancer cases and 592 controls. Unconditional logistic regression was used to assess the association between the four single nucleotide polymorphisms (SNPs) and gastric carcinoma risk. No evidence of an association among any of the four polymorphisms and the risk of gastric cancer was observed. However, when gastric cancer patients were further stratified by age, gender, smoking status, alcohol use and clinicopathological characteristics, and compared with the control populations, the combined variant genotype *hMSH2* -118T>C (TC+CC) was not only associated with an increased risk of gastric cancer in subgroups of younger subjects [ages ≤63years; adjusted odds ratio (OR)=1.51, 95% confidence interval (CI), 1.05-2.16], but also with diffuse tumors (adjusted OR=1.41, 95% CI, 1.01-1.96). These data indicate that the polymorphisms of -93G>A, I219V and IVS12-6T>C are not associated with the risk of gastric cancer. However, *hMSH2*-118T>C combined with variant genotypes (TC+CC) may confer a potential risk of gastric cancer in the Chinese population.

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Introduction

Although a decrease has been observed in the incidence and mortality of gastric cancer patients, it remains one of the major causes of cancer-related mortality worldwide (1-3). In China, gastric cancer is one of the most common malignant tumors, and the number of new cases per year is higher than that in other countries (4). Epidemiological and pathological studies have shown that gastric cancer is a complex, multistep and multifactorial process, involving numerous genetic and epigenetic alterations, such as abnormalities in growth factors/receptors, angiogenic factors, cell cycle regulators and DNA mismatch repair (MMR) genes. These abnormalities also define biological characteristics of gastric cancer cells, which are capable of serving as therapeutic targets for gastric cancer (5).

Gastric cancer is the second most common extracolonic malignancy in individuals with hereditary non-polyposis colorectal cancer (HNPCC) (6,7). HNPCC is among the most prevalent hereditary cancers in humans and is associated with germline mutations in DNA MMR genes, including human mutL homolog 1 (*hMLH1*) or human mutS homolog 2 (*hMSH2*), and microsatellite instability (MSI) in tumor tissue (8). The MMR genes *hMLH1* and *hMSH2* are integral components of the DNA MMR pathway, which recognizes and replaces mispaired nucleotides in DNA. The inactivation of these genes results in increased genetic instability, and in turn leads to an increased rate of mutation in 'gatekeeper' genes that regulate cell proliferation and death. Currently, over 200 allelic variants have been identified for each gene and most of these mutations result in inactivation of the MMR system (9).

The *hMLH1* and *hMSH2* genes encode DNA MMR enzymes, which identify and repair incompatible DNA base pairings that commonly occur during the replication of repetitive genomic tracts due to the slippage of DNA polymerase. Thus, *hMLH1* and *hMSH2* genes are thought to play significant roles in DNA MMR (10). However, to the best of our knowledge, the association of *hMLH1* or *hMSH2* polymorphisms with gastric cancer risk has not been investigated in the Chinese population. Therefore, a further assessment of the correlation between the risk of gastric cancer and the

polymorphisms of the MMR genes *hMLH1* and *hMSH2* is required. The aim of this study was to determine whether the polymorphisms of the *hMLH1* (-93G>A and I219V) and *hMSH2* (-118T>C and IVS12-6T>C) genes were associated with the risk of gastric cancer in the Chinese population.

Materials and methods

Single-nucleotide polymorphism (SNP) selection criteria. The four polymorphisms examined in this study were selected on the basis of the InSiGHT database (http://www.insight-group.org/mutations) that included exon, intron, and promoter, the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php), the Human Genome Variation Database (http://www.gwascentral.org/) and retrieved references that reported those mutations from PubMed (http://www.ncbi.nlm.nih.gov/pubmed). We selected validated SNPs with a minor allele frequency >1% that had multiple independent submissions to the SNP databases and/or multiple citations in the literature. The SNPs were confirmed by frequency assessment or genotype data in which all alleles had been observed in at least two chromosomes, and were located in putative functional domains and regulatory regions. We then extracted cancer information from the references for all the mutations and focused our attention on the association between mutations of *hMLH1* (-93G>A and I219V) and *hMSH2* (-118T>C and IVS12-6T>C) genes and gastric cancer.

Study subjects. The study subjects consisted of 554 histopathologically confirmed gastric cancer cases and 592 cancer-free controls. Participants were from the cities of Yixing and Yangzhong, two regions with a high incidence and mortality rate of gastric cancer in the Jiangsu province, China. Eligibility criteria for those cases included diagnosis of primary incident gastric cancer between 1 October 2006 and 30 September 2009, and age 30-79 years at the time of diagnosis. Cases were excluded in the case that they had been diagnosed with previous cancer, recurrent cancer or metastasized cancer from another origin. Only Han Chinese patients were included in this study and 95% of the contacted cases agreed to participate in the study. Control subjects were recruited from individuals who were genetically unrelated to the cases, had no individual history of cancer, and were undergoing routine health examinations or had accompanied the patients, and were randomly selected in proportion to the geographic location of the cases. Controls were frequency-matched to cases by gender and 5-year age group, and excluded if they had significant mental impairment or blood transfusion in the previous few months. A total of 82% of the contacted controls participated in this study. Informed consent was obtained from each of the eligible subjects prior to recruitment.

In-person interviews were conducted by trained staff to collect diet and lifestyle data. Each eligible subject was interviewed in person to obtain demographic factors including age, gender, physical activity, height and weight during the referent period, regular use of aspirin and/or non-steroidal anti-inflammatory drugs (NSAIDs), alcohol use, cigarette smoking history, medical history and family history of cancer in first-degree relatives. In this study, the response rate of the eligible controls was approximately 88%. Following the interview, an approximately 5 ml venous blood sample was collected from each participant.

For gastric cancer patients, we collected data on tumor site, tumor histotype, invasion, lymph node status and distant metastasis status, when available, through review of the pathologic and surgical reports. None of the patients had undergone radiotherapy or chemotherapy prior to surgery. All types of gastric cancer were classified according to the tumor-node-metastasis (TNM) classification criteria of the International Union Against Cancer (UICC) 2002 (sixth edition). Lauren's criteria were used to classify the tumors into diffuse or intestinal types. This study was approved by the Ethics Committee of the Affiliated Yixing People's Hospital of Jiangsu University in accordance with the current Chinese rules, and informed consent was obtained from each participant.

SNP genotyping with the TaqMan assay. *hMLH1* (-93G>A, I219V) and *hMSH2* (-118T>C, IVS12-6T>C) genotyping was conducted at the Molecular Epidemiology Laboratory, Public Health Sciences Division, School of Public Health, Nanjing Medical University, China. Of the 1305 cases and controls with valid study data, 1146 (88% overall, 95% of cases and 82% of controls) provided additional blood samples during the in-person interview. Of the 1146 subjects who provided a blood sample, we were able to successfully genotype 1143 individuals for *hMLH1* -93G>A, 1146 individuals for *hMLH1* I219V, 1144 individuals for *hMSH2* IVS12-6C>T and 1136 individuals for *hMSH2* -118T>C. Genotypes were less than the total number (554 cases and 592 controls), since some genotypes were undetermined. Those cases that could not be genotyped had insufficient DNA or DNA of poor quality, or opted out of laboratory testing. Blood samples were collected in EDTA-containing tubes prior to surgery or therapy. Genomic DNA was extracted from peripheral blood lymphocytes using phenol-chloroform. DNA purity and concentrations were determined by measurement of absorbance at 260 and 280 nm by a UV spectrophotometer.

The TaqMan assay was used to genotype each of the four SNPs: *hMLH1* -93G>A, I219V and *hMSH2* -118T>C, IVS12-6T>C. Forward and reverse primers and FAM- and HEX-labeled probes were designed and synthesized by Gene Core Bio Technologies Company (Shanghai, China). Primer sequences and probes are shown in Table I. For the four SNPs, the master reaction mixture was made in a total volume of 10 μl that contained 2.8 μl ddH_2O, 5 μl probe qPCR mix (Toyobo, Japan), 0.3 μl forward primer, 0.3 μl reverse primer, 0.2 μl FAM-labeled probe, 0.2 μl HEX-labeled probe, 0.2 μl 50X Rox reference dye and 1 μl template DNA (10 pmol/μl of each prime and each probe). The PCR conditions were: 10 minutes at 95˚C (DNA pre-denaturation), 15 sec at 95˚C (DNA denaturation) and 1 min at 60˚C (primer-probe annealing and prime extension). The denaturation-annealing-extension sequence (15 sec at 95˚C and 1 min at 60˚C) was repeated for 45 cycles. The samples were run in 96-well polypropylene plates and analyzed by a 7300 detection system (Applied Biosystems, Foster City, CA, USA) with SDS software, version 1.3.2 (Applied Biosystems). Positive and negative controls were included on each plate. For additional quality control, genotyping of 85 randomly selected samples was repeated with no discrepancies between runs.

Table I. Primer and probe sequences for the four SNPs.

SNP		Primer sequence	Probe sequence
hMLH1			
-93G>A	Forward	5'-GCCAATCACCTCAGTGCCTC-3'	A 5'F-TCTTCCTTTAGCTGTAGCT-P3'
(rs1800734)	Reverse	5'-TGAGAAATTTGACTGGCATTCAAG-3'	G 5'H-TCTTCCTTCAGCTGTAG-P3'
I219V	Forward	5'-CACTACCCAATGCCTCAACC-3'	A 5'F-TCGCTCCTTCTTT-P3'
(rs1799977)	Reverse	5'-TGAAAGGTTCCAAAATAATGTGA-3'	G 5'H-TCGCTCCCTCTTT-P3'
hMSH2			
-118T>C	Forward	5'-TGCTTATGATTGGTTGCCG-3'	T 5'F-ACACCCAATCAGCTT-P3'
(rs2303425)	Reverse	5'-CCTGGTTGAAGAAAATGCG-3'	C 5'H-ACACCCAGTCAGCTT-P3'
IVS12-6T>C	Forward	5'-GAATATATGTTGATTTACCTCCCATATTG-3'	T 5'F-CCTACAAAACAAATTA-P3'
(rs2303428)	Reverse	5'-CAATCCATTTATTAGTAGCAGAAAGAAGTT-3'	C 5'H-CCTACAGAACAAATTA-P3'

SNP, single nucleotide polymorphisms; F, FAM; H, HEX; P, TaqMan-MGB.

Statistical analysis. Pearson's χ^2-test was used to evaluate the difference in the frequency distributions of demographic variables and the selected risk factors between cases and controls. The Hardy-Weinberg equilibrium of the genotype frequencies of the controls was tested by a goodness-of-fit χ^2 test. The odds ratio (OR) and 95% confidence interval (CI) were calculated from unconditional univariate and multivariate logistic regression models to estimate the risk of gastric cancer associated with genotype, while adjusting for confounding variables including age at diagnosis or selection, gender, alcohol use and smoking status on a regular basis. Age was dichotomized according to the median age (63 years) of the controls. In addition to the overall association analysis, stratified analyses were performed according to age, gender, smoking status, alcohol use and tumor clinicopathological characteristics to further examine the association between the four SNPs and the risk of gastric cancer in each stratum. Analyses were performed using SAS software (version 9.1.3; SAS Institute, Cary, NC, USA) and statistical tests were two-sided. P<0.05 was considered to be statistically significant.

Results

Gastric cancer patients and control subjects. We genotyped a total of 554 gastric cancer patients and 592 control subjects for all four SNPs in a Chinese population. The age at diagnosis for gastric cancer patients and control subjects was 59.8±9 years. Selected variables of the study subjects are shown in Table II. No significant differences in age (P=0.219) and gender (P=0.397) distributions were observed, but there were more regular smokers and drinkers in the gastric cancer patients (45.2 and 33.4%, respectively) than in the controls (29.1 and 25.0%, respectively), and the differences were significant (P<0.001 and P=0.002, respectively). These differences were later controlled by the multivariate analyses. Furthermore, the number of patients with cancer of the gastric cardia and non-cardia were 212 (41.9%) and 294 (58.1%), respectively. As for the histological types, >50% of the gastric cancer cases were of the intestinal type and approximately 46.3% (240) were of the diffuse type. Positive lymph nodes and distant metastasis (M1) were identified in 291 (57.1%) and 42 (8.3%) cases. For depth of tumor infiltration, 105 (20.5%), 95 (18.5%), 197 (38.5%) and 115 (22.5%) were T1, T2, T3 and T4, respectively.

Distribution of genotypes and risk of gastric cancer. Four SNPs in *hMLH1* (-93G>A and I219V) and *hMSH2* (-118T>C and IVS12-6T>C) were included in the analysis. The allele distributions in cases and controls were consistent with the Hardy-Weinberg equilibrium (all P>0.05). The genotype and allele distributions of the four SNPs between the cases and controls are shown in Table III. No statistically significant differences were observed in genotype frequency distribution between the case patients and control subjects. However, when the case patients were stratified by age, gender, smoking status and alcohol use and compared with the control subjects (Table IV), we observed a statistically significant association between the combined -118TC+CC variant genotypes and the risk of gastric cancer in subgroups of younger subjects (ages ≤63 years; adjusted OR=1.51, 95% CI, 1.05-2.16), but not in older subjects (ages >63 years, Table IV). This type of significant association was not found for the two *hMLH1* SNPs and the *hMSH2* SNPs (data not shown).

Genotypes and clinicopathological characteristics of gastric cancer. Since low-penetrance alleles may, not only be associated with cancer incidence, but also affect cancer phenotype and prognosis, we examined the associations among each of the four variant SNPs and the available clinicopathological characteristics of gastric cancer, which included the tumor position (cardia and non-cardia), histological types (diffuse and intestinal), tumor infiltration (T1, T2, T3 and T4), lymph node metastasis (negative and positive) and distant metastasis (M0 and M1). We found that when case patients were stratified by clinicopathological parameters of gastric cancer, only the *hMSH2* combined -118TC+CC variants genotypes were associated with diffuse tumors (adjusted OR=1.41, 95% CI, 1.01-1.96). For the remaining three SNPs, we found no association between any clinicopathological characteristics and the variant alleles of SNPs (Table V).

Table II. Distribution of selected variables between gastric cancer cases and control subjects.

Variables	Cases (n=554) n (%)	Controls (n=592) n (%)	P[b]
Age (years)			0.219
≤63	299 (54.0)	298 (50.3)	
>63	255 (46.0)	294 (49.7)	
Gender			0.397
Male	378 (68.2)	390 (65.9)	
Female	176 (31.8)	202 (34.1)	
Smoking status[a]			<0.001
Never	281 (54.8)	415 (70.9)	
Regular	232 (45.2)	170 (29.1)	
Alcohol use[a]			0.002
Never	339 (66.6)	438 (75.0)	
Regular	170 (33.4)	146 (25.0)	
Tumor site[a]			
Cardia	212 (41.9)		
Non-cardia	294 (58.1)		
Histological type[a]			
Diffuse	240 (46.3)		
Intestinal	278 (53.7)		
Depth of tumor infiltration[a]			
T1	105 (20.5)		
T2	95 (18.5)		
T3	197 (38.5)		
T4	115 (22.5)		
Lymph node metastasis[a]			
Negative (N0)	219 (42.9)		
Positive (N1-3)	291 (57.1)		
Distant metastasis[a]			
M0	467 (91.7)		
M1	42 (8.3)		

[a]The number of subjects in cases or controls was less than the total number (554 cases, 592 controls) as certain information could not be obtained. [b]Two-sided χ^2-test for the distribution of selected variables between gastric cancer cases and controls.

Discussion

HNPCC is an autosomal dominant inheritance syndrome that confers an elevated risk of early-onset colorectal cancer (CRC) and an increased lifetime risk for other types of cancer of the endometrium, small intestine, kidney and ureter (11-14). There is also a high incidence of gastric cancer in HPNCC patients, suggesting a correlation between HPNCC and the development of gastric cancer (6,15). HNPCC is associated with heritable defects in DNA MMR. The mutations of *hMLH1* and *hMSH2* underlie 90% of the germline HNPCC mutations (16,17). HNPCC mutation carriers have a substantial risk for gastric cancer, particularly in patients with a *hMLH1* or *hMSH2* mutation (7). However, whether or not a *hMLH1* or *hMSH2* mutation was associated with stomach cancer in the Chinese population is unknown. Thus, in this study, the allele frequencies of four *hMLH1*/*hMSH2* (-93G>A; I219V; -118T>C; and IVS12-6T>C) polymorphisms in MMR genes and their association with the incidence of gastric cancer in a Chinese population were examined. The allele and genotype frequencies of the four SNPs exhibited no statistically significant differences between the case patients and control subjects. However, when the case patients were stratified by age, gender, smoking status and alcohol use, or clinicopathological parameters of gastric cancer, we observed that the *hMSH2* combined -118TC+CC variant genotype was not only associated with subgroups of younger subjects (ages ≤63 years; adjusted OR=1.51, 95% CI, 1.05-2.16) but also with diffuse tumors (adjusted OR=1.41, 95% CI, 1.01-1.96).

MMR plays a crucial role in maintaining genome stability. Defects in MMR genes have been involved in a number of types of sporadic and hereditary cancer (18). The *hMLH1* gene is considered one of the key members of the MMR pathway. The *hMLH1* -93G>A polymorphism (rs1800734) variant may

Table III. Genotype and allele frequencies of the *hMLH1* and *hMSH2* polymorphisms among cases and controls and the association with risk of gastric cancer.

Genotype	Cases (n=554) n (%)	Controls (n=592) n (%)	P[b]	Adjusted OR (95% CI)[b]
hMLH1				
-93G>A[a]				
GG	104 (18.8)	124 (21.1)		1.00 (reference)
GA	262 (47.3)	271 (46.1)	0.598	1.09 (0.79-1.51)
AA	188 (33.9)	193 (32.8)	0.368	1.17 (0.83-1.65)
GA+AA	450 (81.2)	464 (78.9)	0.450	1.12 (0.83-1.52)
A allele	0.576	0.559	0.313[c]	
I219V				
AA	522 (94.2)	568 (95.9)		1.00 (reference)
AG+GG[d]	32 (5.8)	24 (4.1)	0.129	1.54 (0.88-2.67)
G allele	0.030	0.021	0.187[c]	
hMSH2				
IVS12-6C>T[a]				
CC	64 (11.6)	73 (12.3)		1.00 (reference)
CT	258 (46.7)	279 (47.1)	0.631	1.10 (0.74-1.64)
TT	230 (41.7)	240 (40.6)	0.569	1.12 (0.75-1.68)
CT+TT	488 (88.4)	519 (87.7)	0.581	1.11 (0.76-1.63)
C allele	0.650	0.641	0.728[c]	
-118T>C[a]				
TT	367 (66.9)	422 (71.4)		1.00 (reference)
TC	163 (29.7)	154 (26.1)	0.169	1.21 (0.92-1.59)
CC	19 (3.4)	15 (2.5)	0.383	1.39 (0.67-2.89)
TC+CC	182 (33.1)	169 (28.6)	0.128	1.23 (0.94-1.60)
C allele	0.183	0.156	0.096[c]	

[a]Genotypes were less than the total number (554 cases, 592 controls) since some genotypes were undetermined. [b]Adjusted for age, gender, smoking status and alcohol use in the logistic regression model. [c]Two-sided χ^2-test for the differences in the minor allele frequencies between cases and controls. [d]Heterozygous (AG) and homozygous (GG) variants were collapsed due to the low variant G allele frequency.

reduce *hMLH1* transcription and expression, thereby reducing the overall DNA repair capability. The *hMLH1* -93G>A variant allele, in the homozygous or heterozygous state, was associated with a higher risk of developing MSI-H tumors than the wild-type allele (19). Other studies, however, have shown that the *hMLH1* -93G>A variant allele has a positive association with risks of colorectal cancer in a large-scale genome-wide study, and with an increased risk of a number of types of cancer, including endometrial and breast cancer (20). In addition, no significant association was found between the *hMLH1* -93G>A genotype and the risk for adenocarcinoma or small cell carcinoma in a Korean population. However, the AA genotype was associated with a significantly increased risk for squamous cell carcinoma compared with the GG genotype and the combined GG and GA genotype (21). Homozygosity for the *hMLH1* I219V polymorphism (rs1799977) variant was statistically significantly correlated with a reduced *hMLH1* protein expression in sporadic colorectal cancer cases. Furthermore, the *hMLH1* I219V variant has been associated with the risk of lung cancer and an almost 5-fold increased risk of ulcerative colitis, a major risk factor for colon cancer (22,23). The *hMLH1* I219V polymorphism was capable of affecting the clinical course of the disease and leading to resistance to therapy (24). However, in our study, the allele and genotype frequencies of the *hMLH1* -93G>A and the *hMLH1* I219V SNPs were similar between gastric cancer patients and healthy controls; thus, the *hMLH1* -93G>A or the *hMLH1* I219V gene polymorphism is not significantly associated with gastric cancer risk in the Chinese population.

The *hMSH2* gene is one of the MMR genes that encodes the human homolog of the bacterial MutS protein, which is responsible for recognizing DNA mismatches (25). A number of polymorphisms in the *hMSH2* gene have been reported thus far (26). Whereas the functional effects of these polymorphisms are less known, the *hMSH2* IVS12-6T>C polymorphism (rs2303428) was shown to be correlated to non-Hodgkin's lymphoma, sporadic and familial colorectal cancer, ulcerative colitis cancer and acute myeloid leukemia (27-32). It is noteworthy that the presence of at least one *hMSH2* IVS12-6C allele was associated with a significantly increased risk of lung adenocarcinoma, as compared with the homozygous IVS12-6T wild-type (26). The *hMSH2* -118T>C polymorphism

Table IV. Stratification analyses between *hMSH2* -118T>C genotypes and risk of gastric cancer in cases and controls.

Variables	Cases/controls	Genotype (cases/controls)				P[c]	Adjusted OR (95% CI)[c] TC+CC vs TT
		TT		TC+CC			
		n	%	n	%		
Total[a]	549/591	367/422	66.8/71.4	182/169	33.2/28.6	0.128	1.23 (0.94-1.60)
Age (years)							
≤63	297/298	185/208	62.3/69.8	112/90	37.7/30.2	0.026	1.51 (1.05-2.16)
>63	252/293	182/214	72.2/73.0	70/79	27.8/27.0	0.961	0.99 (0.66-1.48)
Gender							
Male	374/390	258/281	69.0/72.0	116/109	31.0/28.0	0.355	1.17 (0.84-1.63)
Female	175/201	109/141	62.3/70.1	66/60	37.7/29.9	0.120	1.44 (0.91-2.28)
Smoking status[b]							
Never	280/412	190/292	67.9/70.9	90/120	32.1/29.1	0.441	1.14 (0.82-1.60)
Regular	228/172	151/126	66.2/73.3	77/46	33.8/26.7	0.142	1.39 (0.90-2.16)
Alcohol use[b]							
Never	338/437	227/316	67.2/72.3	111/121	32.8/27.7	0.154	1.26 (0.92-1.72)
Regular	166/146	110/101	66.3/69.2	56/45	33.7/30.8	0.689	1.11 (0.67-1.84)

[a]Genotypes were less than the total number (554 cases, 592 controls) since some genotypes were undetermined. [b]The numbers of subjects in cases or controls were less than the total number (554 cases, 592 controls) as certain information could not be obtained. [c]Adjusted for age, gender, smoking status and alcohol use in the logistic regression model.

Table V. Association between *hMSH2* -118T>C genotypes and clinicopathological characteristics of gastric cancer.

Variables	Genotype, n (%)		P[c]	Adjusted OR (95% CI)[c] TC+CC vs TT
	TT	TC+CC		
Controls (n=591)[a]	422 (71.4)	169 (28.6)		1.00 (reference)
Cases (n=549)[a]	367 (66.9)	182 (33.1)	0.128	1.23 (0.94-1.60)
Tumor site[b]				
Cardia	144 (68.3)	67 (31.7)	0.593	1.10 (0.78-1.55)
Non-cardia	190 (65.4)	100 (34.6)	0.085	1.31 (0.96-1.78)
Histological[b]				
Diffuse	148 (62.4)	89 (37.6)	0.043	1.41 (1.01-1.96)
Intestinal	195 (70.6)	81 (29.4)	0.565	1.10 (0.80-1.51)
Depth of tumor infiltration[b]				
T1	71 (67.6)	34 (32.4)	0.430	1.19 (0.77-1.84)
T2	58 (62.4)	35 (37.6)	0.165	1.38 (0.88-2.16)
T3	131 (66.8)	65 (33.2)	0.227	1.24 (0.87-1.76)
T4	79 (69.3)	35 (30.7)	0.786	1.06 (0.69-1.64)
Lymph node metastasis[b]				
Negative (N0)	150 (69.1)	67 (30.9)	0.371	1.17 (0.83-1.64)
Positive (N1-3)	190 (65.7)	99 (34.3)	0.080	1.32 (0.97-1.79)
Distant metastasis[b]				
M0	308 (66.5)	155 (33.5)	0.090	1.26 (0.96-1.65)
M1	31 (73.8)	11 (26.2)	0.743	1.10 (0.61-1.99)

[a]Genotypes were less than the total number (554 cases, 592 controls) since some genotypes were undetermined. [b]The numbers of subjects in cases or controls was less than the total number (554 cases, 592 controls) since certain. [c]Adjusted for age, gender, smoking status and alcohol use in the logistic regression model.

(rs2303425) was associated with a strong family history of colorectal cancer in patients from Ontario, Canada (33). The contribution of the *hMSH2* -118T>C SNP to lung cancer was also examined in another Korean case-control population, with no significant results (26). Similarly, in our study, the clinicopathological correlation analysis of our data showed that the four SNP genotypes were not associated with disease characteristics, including tumor site, histological type, depth of tumor infiltration, lymph node metastasis and distant metastasis. However, when case patients were stratified in analyses according to age, gender, smoking status, alcohol use and clinicopathological characteristics of gastric cancer, the *hMSH2* combined -118TC+CC variant genotypes were associated with a significantly increased risk for gastric cancer in younger subjects (ages ≤63 years; adjusted odds ratio OR=1.51, 95% CI, 1.05-2.16) compared with the older subjects, and with the diffuse tumors (adjusted OR=1.41, 95% CI, 1.01-1.96) compared with the intestinal tumors.

The frequencies of the polymorphic alleles are known to vary by ethnic background, due to the gene-gene interactions and gene-environment interactions. The frequency of the *hMSH2* -118T>C polymorphism variant C allele in the general populations of Ontario and Newfoundland were 13.8 and 12.5%, respectively. These values are lower than those found in Asian populations, i.e., 20% in Japanese and Korean populations, respectively (34). Among patients in Ontario, a statistically significant association was found between the MLH1-93G>A promoter variant allele and a strong family history of colorectal cancer, as defined by the Amsterdam criteria (35). This association was not observed in the Newfoundland case patients. Consistent with our findings, no differences in variant allele frequencies between gastric cancer cases and controls were observed, perhaps due to the small number of patients. However, other factors that may play a role have yet to be determined.

In conclusion, our data show that the polymorphic variants of four SNPs are not significant markers for determining patient risk of developing gastric cancer or for prognosis, at least in a hospital-based Chinese population. It is possible that these findings, particularly those findings from the stratified analyses, may be to the relatively small number of cases in the subgroups. Therefore, additional studies with larger sample sizes are required to confirm our findings.

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References

1. Jemal A, Siegel R, Xu J and Ward E: Cancer statistics. CA Cancer J Clin 60: 277-300, 2010.
2. Afuwape OO, Irabor DO, Ladipo JK and Ayandipo B: A Review of the Current Profile of Gastric Cancer Presentation in the University College Hospital Ibadan, a Tertiary Health Care Institution in the Tropics. J Gastrointest Cancer Feb, 2011 (Epub ahead of print).
3. Herszényi L and Tulassay Z: Epidemiology of gastrointestinal and liver tumors. Eur Rev Med Pharmacol Sci 14: 249-258, 2010.
4. Wang W, Li YF, Sun XW, *et al*: Prognosis of 980 patients with gastric cancer after surgical resection. Chin J Cancer 29: 923-930, 2010.
5. Arkenau HT: Gastric cancer in the era of molecularly targeted agents: current drug development strategies. J Cancer Res Clin Oncol 135: 855-866, 2009.
6. Aarnio M, Sankila R, Pukkala E, *et al*: Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer 81: 214-218, 1999.
7. Goecke T, Schulmann K, Engel C, *et al*: Genotype-phenotype comparison of German MLH1 and MSH2 mutation carriers clinically affected with Lynch syndrome: a report by the German HNPCC consortium. J Clin Oncol 24: 4285-4292, 2006.
8. Abdel-Rahman WM, Mecklin JP and Peltomaki P: The genetics of HNPCC: application to diagnosis and screening. Crit Rev Oncol Hematol 58: 208-220, 2006.
9. Liu T: Mutational screening of hMLH1 and hMSH2 that confer inherited colorectal cancer susceptibility using denature gradient gel electrophoresis (DGGE). Methods Mol Biol 653: 193-205, 2010.
10. Vasen HF, Stormorken A, Menko FH, *et al*: MSH2 mutation carriers are at higher risk of cancer than MLH1 mutation carriers: a study of hereditary nonpolyposis colorectal cancer families. J Clin Oncol 19: 4074-4080, 2001.
11. Haghighi MM, Javadi GR, Parivar K, Milanizadeh S, Zali N, Fatemi SR and Zali MR: Frequent MSI mononucleotide markers for diagnosis of hereditary nonpolyposis colorectal cancer. Asian Pac J Cancer Prev 11: 1033-1035, 2010.
12. Scheuner MT, McNeel TS and Freedman AN: Population prevalence of familial cancer and common hereditary cancer syndromes. The 2005 California Health Interview Survey. Genet Med 12: 726-735, 2010.
13. Power DG, Gloglowski E and Lipkin SM: Clinical genetics of hereditary colorectal cancer. Hematol Oncol Clin North Am 24: 837-859, 2010.
14. Sheng X, Zhou HH, Zhou XY, *et al*: Germline mutation analysis of hPMS2 gene in Chinese families with hereditary nonpolyposis colorectal cancer. World J Gastroenterol 16: 3847-3852, 2010.
15. Corso G, Marrelli D and Roviello F: Familial gastric cancer: update for practice management. Fam Cancer 10: 391-396, 2011.
16. Yamasaki Y, Matsushima M, Tanaka H, *et al*: Patient with eight metachronous gastrointestinal cancers thought to be hereditary nonpolyposis colorectal cancer (HNPCC). Intern Med 49: 209-213, 2010.
17. Capelle LG, Van Grieken NC, Lingsma HF, *et al*: Risk and epidemiological time trends of gastric cancer in Lynch syndrome carriers in the Netherlands. Gastroenterology 138: 487-492, 2010.
18. Xie J, Guillemette S, Peng M, Gilbert C, Buermeyer A and Cantor SB. An MLH1 mutation links BACH1/FANCJ to colon cancer, signaling, and insight toward directed therapy. Cancer Prev Res (Phila) 3: 1409-1416, 2010.
19. van Roon EH, van Puijenbroek M, Middeldorp A, *et al*: Early onset MSI-H colon cancer with MLH1 promoter methylation, is there a genetic predisposition? BMC Cancer 10: 180, 2010.
20. Allan JM, Shorto J, Adlard J, *et al*: MLH1 -93G>A promoter polymorphism and risk of mismatch repair deficient colorectal cancer. Int J Cancer 123: 2456-2459, 2008.
21. Park SH, Lee GY, Jeon HS, *et al*: -93G-->A polymorphism of hMLH1 and risk of primary lung cancer. Int J Cancer 112: 678-682, 2004.
22. Wang H, Wang Y, Liu H, *et al*: Polymorphisms in hMLH1 and risk of early-onset lung cancer in a southeast Chinese population. Lung Cancer 59: 164-170, 2008.
23. Vietri MT, Riegler G, De Paola M, *et al*: I219V polymorphism in hMLH1 gene in patients affected with ulcerative colitis. Genet Test Mol Biomarkers 13: 193-197, 2009.
24. Trojan J, Zeuzem S, Randolph A, *et al*: Functional analysis of hMLH1 variants and HNPCC-related mutations using a human expression system. Gastroenterology 122: 211-219, 2002.
25. Bellacosa A: Functional interactions and signaling properties of mammalian DNA mismatch repair proteins. Cell Death Differ 8: 1076-1092, 2001.
26. Jung CY, Choi JE, Park JM, *et al*: Polymorphisms in the hMSH2 gene and the risk of primary lung cancer. Cancer Epidemiol Biomarkers Prev 15: 762-768, 2006.
27. Worrillow LJ, Travis LB, Smith AG, Sara R, Andrew JS and Christopher PW: An intron splice acceptor polymorphism in hMSH2 and risk of leukemia after treatment with chemotherapeutic alkylating agents. Clin Cancer Res 9: 3012-3020, 2003.

28. Paz-y-Miño C, Pérez JC, Fiallo BF and Leone PE: A polymorphism in the hMSH2 gene (gIVS12-6T>C) associated with non-Hodgkin lymphomas. Cancer Genet Cytogenet 133: 29-33, 2002.
29. Palicio M, Blanco I, Tórtola S, *et al*: Intron splice acceptor site polymorphism in the hMSH2 gene in sporadic and familial colorectal cancer. Br J Cancer 82: 535-537, 2000.
30. Goessl C, Plaschke J, Pistorius S, Hahn M, Frank S and Hampl M: An intronic germline transition in the HNPCC gene hMSH2 is associated with sporadic colorectal cancer. Eur J Cancer 33: 1869-1874, 1997.
31. Brentnall TA, Rubin CE, Crispin DA, *et al*: A germline substitution in the human MSH2 gene is associated with high-grade dysplasia and cancer in ulcerative colitis. Gastroenterology 109: 151-155, 1995.
32. Cheng H, Sun N, Sun X, *et al*: Polymorphisms in hMSH2 and hMLH1 and response to platinum-based chemotherapy in advanced non-small-cell lung cancer patients. Acta Biochim Biophys Sin (Shanghai) 42: 311-317, 2010.
33. Mrkonjic M, Raptis S, Green RC, *et al*: MSH2 118T>C and MSH6 159C>T promoter polymorphisms and the risk of colorectal cancer. Carcinogenesis 28: 2575-2580, 2007.
34. Shin KH, Shin JH, Kim JH and Park JG: Mutational analysis of promoters of mismatch repair genes hMSH2 and hMLH1 in hereditary nonpolyposis colorectal cancer and early onset colorectal cancer patients: identification of three novel germ-line mutations in promoter of the hMSH2 gene. Cancer Res 62: 38-42, 2002.
35. Vasen HF, Watson P, Mecklin JP and Lynch HT: New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. Gastroenterology 116: 1453-1456, 1999.