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Original Article

Single nucleotide polymorphisms of DNA mismatch repair genes *MSH2* and *MLH1* confer susceptibility to esophageal cancer

Ming-Zhong Sun, Hui-Xiang Ju, Zhong-Wei Zhou, Hao Jin, Rong Zhu

Department of Clinical Laboratory, Affiliated Yancheng Hospital, School of Medicine, Southeast University, Yancheng 224001, Jiangsu, China

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Abstract: Defects in DNA mismatch repair genes like *MSH2* and *MLH1* confer increased risk of cancers. Here, single nucleotide polymorphisms (SNPs) in *MSH2* and *MLH1* were investigated for their potential contribution to the risk of esophageal cancer. This study recruited 614 participants from Affiliated Yancheng Hospital, School of Medicine, Southeast University, of which 289 were patients with esophageal cancer, and the remainder was healthy individuals who served as a control group. Two SNPs, *MSH2* c.2063T>G and *MLH1* IVS14-19A>G, were genotyped using PCR-RFLP. Statistical analysis was performed using chi-square test and logistic regression analysis. Carriers of the *MSH2* c.2063G allele were at significantly higher risk for esophageal cancer compared to individuals with the TT genotype [*OR* = 3.36, 95% confidence interval (CI): 1.18-11.03]. The *MLH1* IVS14-19A>G allele also conferred significantly increased (1.70-fold) for esophageal cancer compared to the AA genotype (*OR* = 1.70, 95% CI: 1.13-5.06). Further, the variant alleles interacted such that individuals with the susceptible genotypes at both *MSH2* and *MLH1* had a significantly exacerbated risk for esophageal cancer (*OR* = 12.38, 95% CI: 3.09-63.11). In brief, SNPs in the DNA mismatch repair genes *MSH2* and *MLH1* increase the risk of esophageal cancer. Molecular investigations are needed to uncover the mechanism behind their interaction effect.

Keywords: Esophageal cancer, DNA mismatch repair, MSH2, MLH1, single nucleotide polymorphism

Introduction

Esophageal cancer is a common digestive system malignancy [1]. Its occurrence has been associated with defects in many genes, including those involving ethanol metabolism, folate metabolism, cell cycle regulation, DNA repair, as well as known oncogenes [2]. Further, single nucleotide polymorphisms (SNPs) in DNA repair genes have been shown to cause genetic instability that promotes tumorigenesis [3]. Some work suggests that polymorphisms in specific repair genes are correlated with individual differences in the risk of cancer [2].

Particular genes of interest are those involved in DNA mismatch repair (MMR). The MMR system plays an important role in maintaining the integrity and stability of genetic information. Repair proteins involved in MMR include the MutS and MutL homologs MSH2 and MLH1 [4]. Mutations in *MSH2* cause hereditary non polyposis colorectal cancer, Muir-Torre syndrome, and rectal cancer. Moreover, *MSH2* variants have been identified in some patients with esophageal cancer, and under-expression of MSH2 correlates with the occurrence of esophageal cancer [5]. *MLH1* mutation produces DNA microsatellite instability, a common feature of sporadic tumors, and is common in stomach, rectal, and endometrial cancers [6]. In addition, both MSH2 and MLH1 are involved in mitotic recombination events, during which they repair the DNA strand breaks related to genetic exchange [7].

MSH2 and *MLH1* were previously reported to contribute to the occurrence of esophageal cancer [8]. To better understand the contribution of variants in MMR genes to esophageal cancer, we investigated the role of MMR-related genes, specifically *MSH2* and *MLH1,* in the

Table 1. The primer sequences, PCR reaction conditions and enzyme digestion products

SNP	Primers sequences	PCR products length (bp)	PCR reaction conditions	Restriction Enzyme	Genotype	Enzyme digested product length
MSH2	F: CGCGATTAATCATCAGTG	183	95°C 3 min	Hsp 92 II	TT	25, 158
c.2063T>G	R: ATGGCACAAAACACCCAA		30 cycles: 94°C 60 s, 55°C 60 s, 72°C 60 s		GG	183
			72°C 5 min		TG	25, 158, 183
MLH1	F: TCTTCTCATGCTGTCCCCT	181	94°C 5 min	Mae III	GG	181
IVS14-19A>G	R: ATAATAGAGAAGCTAAGTTAAAC		35 cycles: 94°C 50 s, 55°C 50s, 72°C 60 s		AG	53, 128, 181
			72°C 10 min		AA	53, 128

F: forward primer; R: reverse primer.

occurrence of esophageal cancer. The study genotyped samples from 289 patients with esophageal cancer and 325 healthy individuals for SNPs in *MSH2* (c.2063T>G) and *MLH1* (IVS14-19A>G) and determined the risk of esophageal cancer by genotype.

Methods

Subjects

The subjects included a case group and control group. The case group included 289 cases with esophageal cancer histopathologically confirmed in Affiliated Yancheng Hospital, School of Medicine, Southeast University (Yancheng City, Jiangsu Province, P. R. China) between January and December 2013; selection was not limited by the sex, age, and histological type, and participants did not undergo preoperative treatment involving anticancer drugs or radiotherapy. The group comprised 224 males and 65 females (mean age 59.78 ± 8.10 years); there were 89 cases of grade I, 103 cases of grade II, and 97 cases of grade III according to the histological grading. The control group comprised 325 healthy individuals who underwent physical examinations in our hospital in the same period, who were not blood relatives and who were paired with the case group according to age and sex. The group comprised 238 males and 87 females (mean age 60.17 ± 9.04 years); there were 102 cases of grade I, 115 cases of grade II, and 108 cases of grade III according to the histological grading. The patients' records included age, sex, history of smoking and alcohol consumption, and family history. All participants provided informed consent. This study was approved by the Ethics Committee of Affiliated Yancheng Hospital, School of Medicine, Southeast University (Yancheng City, Jiangsu Province, P. R. China).

Genotyping

Genomic DNA was extracted from whole blood using "a kit (solution type) for rapid genomic DNA extraction from whole blood" (BioTeke Corporation, Beijing, China). Genotypes of SNP sites in the target genes were detected by PCR and restriction fragment length polymorphism (RFLP). The amplification reaction was performed in the PCR Thermal Cycler Dice (TaKaRa Biotech Co. Led, Code TP600, Dalian, China). The primer sequences, reaction conditions and enzyme digestion results were listed in **Table 1**. The PCR products were separated and detected using 8.0% agarose gel electrophoresis, ethidium bromide staining, and ultraviolet radiation.

Statistical methods

Epidata 3.1 version (Odense, Denmark) software was used to create a data bank through double data entry and logic checks. SAS v9.2 (SAS Institute, Cary, NC, USA) was used to analyze the data using X^2 and unconditional logistic regression; the former was used to assess the difference between the demographic characteristics in the case group and control groups, and the latter was used to analyze the correlation between genotype and the risk of occurrence of esophageal cancer. Odds ratios (OR) with 95% confidence intervals (CI) are presented. When $P < 0.05$, the difference was considered to be statistically significant.

Results

Demographic characteristics

Demographic characteristics are presented in **Table 2**. The differences in age and sex between the case group and control group were not statistically significant ($P > 0.05$). In contrast, there

Table 2. Basic demographic characteristics

Characteristic		Case group (n = 289) (n, %)	Control group (n = 325) (n, %)	X^2	*P* value
Sex	Male	224 (77.51)	238 (73.23)	1.50	0.220
	Female	65 (22.49)	87 (26.77)		
Age (years)	<60	158 (54.67)	188 (57.85)	0.63	0.429
	≥60	131 (45.33)	137 (42.15)		
Smoking	No	96 (33.22)	201 (61.85)	50.20	< 0.001
	Yes	193 (66.78)	124 (38.15)		
Drinking	No	108 (37.37)	209 (64.31)	44.45	< 0.001
	Yes	181 (62.63)	116 (35.69)		
Family history	Esophageal cancer	21 (7.27)	16 (4.92)	4.72	0.094
	Other tumors	23 (7.96)	15 (4.62)		
	No	245 (84.78)	294 (90.46)		

Table 3. Correlation between genotypes at *MSH2* and *MLH1* and the risk of esophageal cancer

Genotype	Case group, n (%)	Control group, n (%)	*OR* (95% *CI*)
MSH2c.2063T>G			
TT	198 (68.51)	299 (92.00)	1.00
TG	59 (20.42)	26 (8.00)	3.36 (1.18-11.03)*
GG	32 (11.07)	0 (0.00)	
TG+GG	91 (31.49)	26 (8.00)	4.36 (1.27-9.50)*
T	453 (78.37)	621 (95.54)	
G	125 (21.63)	29 (4.46)	
MLH1 IVS14-19A>G			
AA	201 (69.55)	264 (81.23)	1.00
AG	83 (28.72)	58 (17.85)	1.68 (1.12-4.93)*
GG	5 (1.73)	3 (0.92)	1.91 (1.08-37.91)*
AG+GG	88 (30.45)	61 (18.77)	1.70 (1.13-5.06)*
A	481 (83.22)	584 (89.85)	
G	97 (16.78)	66 (10.15)	

Note: *denotes the comparison with the control group, *P* < 0.05.

Table 4. Interaction effect of *MSH2* and *MLH1* variants on the risk of esophageal cancer

MSH2 c.2063T>G Genotype	*MLH1* IVS14-19A>G Genotype	Case group, n (%)	Control group, n (%)	*OR* (95% CI)
TT	AA	155 (53.64)	252 (77.54)	1.00
TT	AG+GG	43 (14.88)	38 (11.69)	1.21 (1.03-6.41)*
TG+GG	AA	47 (16.26)	31 (9.54)	1.96 (1.12-13.05)*
TG+GG	AG+GG	44 (15.22)	4 (1.23)	12.38 (3.09-63.11)*

Note: *denotes the comparison with the control group, *P* < 0.05.

were significant differences in smoking history and alcohol consumption (*P* < 0.05). Family history was not statistically different between groups (*P* > 0.05).

Risk of esophageal cancer by genotype

Hardy-Weinberg equilibrium analysis indicated that the genotypes for both *MSH2* and *MLH1* in the control groups met genetic equilibrium (*P* > 0.05) and were representative of the population (**Table 3**). In the case group, however, there were differences in allelic and genotypic frequencies; for both *MSH2* and *MLH1*, the G alleles were more frequently represented (*P* < 0.001). For *MSH2*, compared with the common TT genotype, the TG genotype conferred a significantly higher risk of esophageal cancer (*OR* = 3.36, 95% CI: 1.18-11.03, *P* < 0.001). For *MLH1*, the risk of esophageal cancer in those with the GG genotype was significantly higher than that of those with the common AA genotype (*OR* = 1.91, 95% CI: 1.08-37.91, P = 0.001).

Interaction between SNPs and the risk of esophageal cancer

To determine whether the combination of variants of both *MSH2* and *MLH1* interact to confer risk of esophageal cancer, the susceptible genotypes were analyzed together (**Table 4**). Individuals with a "susceptible" genotype (those conferring higher risk in the analysis above)

at just one of the two genes had a risk of esophageal cancer 1.21-1.96 times that of individuals with the common genotypes at both genes. Individuals with susceptible genotypes at both genes had a 12.38 times higher risk of esophageal cancer that individuals with the common genotypes at both genes (*OR* = 12.38, 95% *CI*: 3.09-63.11, $P < 0.001$).

Discussion

The expression and function of DNA repair genes can be altered by the presence of SNPs, and reduced DNA repair capacity is associated with an increased risk of cancer [3, 9-13]. Esophageal cancer can arise from various gene mutations that involve dysregulation of the cell cycle and DNA repair [14]. In this study, we analyzed the correlation between variants in two genes involved in DNA repair, *MSH2* c.2063T>G and *MLH1* IVS14-19A>G, and the risk of occurrence of esophageal cancer. The results indicate that both SNPs confer increased susceptibility to esophageal cancer, a finding consistent a previous study [8]. Indeed, these SNPs interact to further increase the risk of esophageal cancer.

Defects in mismatch repair genes lead to microsatellite instability. More seriously, the functional defects of MMR can lead to rapid accumulation of mutations in proto-oncogenes and anti-oncogenes, eventually affecting the proliferation and regulation of normal cells and thereby promoting oncogenesis [15]. MSH2 is an important component of the mismatch repair pathway, repairing specific types of errors. The MSH2c.2063T>G SNP site is located in the Walker motif A, a region that interacts with the α- and β-phosphate groups in the V-shaped domain of ATPases, which are necessary for the repair activity of MSH2 [16]. Mutations in this motif have been shown experimentally to reduce hydrolysis and binding capacity of the enzyme. A non-conservative amino acid substitution causes reduced MMR capacity [17]. Thus, the MSH2c.2063T>G variant may code for a structurally-different enzyme due to the introduction of positively-charged residues with the substitution of positively-charged arginine for the nonpolar hydrophobic methionine. In an analysis of host cell reactivation, the Arg at position 688 could be related to the reduced damage repair capacity caused by endogenous and exogenous carcinogens [18].

The apparent interaction effect of *MSH2* and *MLH1* variants could result from functional interaction of the enzymes to maintain stability of MMR [19], and defects may promote cancer. In short, this study shows that MSH2c.2063G and MLH1IVS14-19G alleles confer susceptibility to esophageal cancer; the risk is exacerbated in the presence of susceptible alleles at both genes. Further research is needed to understand the molecular mechanism responsible for this result, and to enable clinical application of this information to the detection and prevention of esophageal cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Ming-Zhong Sun, Department of Clinical Laboratory, Affiliated Yancheng Hospital, School of Medicine, Southeast University, Yancheng 224001, Jiangsu, China. E-mail: sunmzhyan@126.com

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