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Research paper

Polymorphisms in mismatch repair genes are associated with risk and microsatellite instability of gastric cancer, and interact with life exposures

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ABSTRACT

Background: Epigenetic alterations of DNA mismatch repair (MMR) genes are associated with risk of gastric cancer (GC) by causing microsatellite instability (MSI). Less understood is the association of common polymorphisms in MMR genes with the risk and MSI phenotype of GC.
Methods: A hospital-based study was conducted in China with 423 cases and 454 matched controls. Four potentially functional polymorphisms were selected and analyzed: rs1800734 in *MLH1*, rs2303428 in *MSH2*, rs735943 in *EXO1*, and rs11797 in *TREX1*.
Results: The rs1800734 *G*-allele was associated with decreased risk of GC (*GA* or *GG* vs *AA*, OR = 0.72; 95% CI: 0.50–1.05; $P_{trend} = 0.029$). For combined effects, a dose–response manner was observed in which GC risk was increased with increasing number of at-risk genotypes ($P_{trend} = 0.039$); this manner mainly existed in MSI GC ($P_{trend} = 0.047$) rather than in microsatellite stability GC, though neither single polymorphism was linked with MSI. For exposures, modified effects were observed from green tea drinking and soy foods intake on rs11797 (*P* for interaction = 0.007 and 0.016, respectively).
Conclusions: The *MLH1* rs1800734 polymorphism is associated with GC risk. Those at-risk genotypes have a joint effect on GC risk, which contributes to the MSI phenotype of GC. Life exposures modify GC risk, stratified by MMR genotypes.

1. Introduction

Gastric cancer (GC) is a major public health problem worldwide. Malfunction of DNA mismatch repair (MMR) is known to play important roles in the onset of GC (Velho et al., 2014). The core MMR genes include *MSH2*, *MLH1*, *MSH3*, *MSH6*, *PMS1* and *PMS2*, other genes such as *EXO1*, *TREX1*, *TP73* and *MGMT* also take part in MMR. The main function of the MMR system is to identify and repair incompatible DNA base pairings that are generated during DNA replication and recombination (Li, 2008). Defects in the MMR system can cause microsatellite instability (MSI), which presents as a somatic gain or loss in simple repeat (microsatellite) sequences of DNA. The accumulation of such errors increases the spontaneous mutation rate secondary to genome-wide instability and inactivates tumor suppressor genes, facilitating carcinogenesis. The incidence of MSI in GC has been reported from 15 to 30%, and MSI is associated with gastric carcinogenesis (Velho et al., 2014).

There are large interindividual differences in MMR capacity, owing largely to genetic and epigenetic alterations in the MMR genes among individual. Previously, epigenetic silencing of *MLH1* by promoter hypermethylation was reported to be the main mechanism leading to MMR deficiency in GC, while which only partly explained the MSI phenotype of GC (Velho et al., 2014). In terms of somatic mutations in MMR genes, it was found to be rare in GC and did not explain its onset (Pinto et al., 2008). Less understood is the contribution of common polymorphisms in MMR genes to GC risk, though they have been associated with susceptibility and MSI phenotype to many cancers (Campbell et al., 2009; Nogueira et al., 2015; Lancaster et al., 2015; McCullough et al., 2014; Jung et al., 2006).

Recently, several studies have investigated the role of particular polymorphisms in MMR genes in gastric carcinogenesis; significant

Abbreviations: MMR, mismatch repair; GC, gastric cancer; Hp, *Helicobacter pylori*; MSI, microsatellite instability; MSS, microsatellite stability; LD, linkage disequilibrium; OR, odds ratios; CI, confidence intervals.

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disease associations were identified with rs1047840 in *EXO1* (Bau et al., 2009) and rs2303425, rs2303428 and rs3732183 in *MSH2* (Xiao et al., 2012; Wang et al., 2012), indicating the necessity to further explore potential additional effects of other polymorphisms in MMR genes. However, no study has analyzed the relationship between relevant polymorphisms and MSI phenotype of GC, and therefore these associations remain inconclusive. Besides, gene–gene and gene–exposure interactions in MMR system are still unknown in GC. We sought to explore these issues with a case–control study.

Four polymorphisms in four MMR genes were selected in this study: rs1800734 in *MLH1*, rs2303428 in *MSH2*, rs735943 in *EXO1*, and rs11797 in *TREX1*. They were nonsynonymous polymorphisms or polymorphisms that were located at the 3′UTR or the splicing site, which were found to have a high genotype frequency among Chinese patients with GC in our preliminary study (unpublished results). Both rs1800734 and rs2303428 have been found to be associated with either cancer risk or clinical outcomes in many cancers, while their roles in gastric carcinogenesis are still controversial (Xu et al., 2012; He et al., 2013). The association between rs735943 or rs11797 and GC risk has never been studied, though a potential biologic function of these two polymorphisms had been suggested previously (Dong et al., 2009).

2. Materials and methods

2.1. Subjects

The cases studied at the Affiliated Hospital of Jiangsu University were unrelated Han Chinese newly diagnosed with GC in the period from September 2008 to March 2012. A board-certified gastropathologist reviewed each GC diagnosis. The selection of controls and interview of subjects have been previously described in detail (Wang et al., 2012). For each case, up to 2 healthy controls were selected.

Initially, 446 GC patients and 895 controls agreed to participate in the study. Among the GC patients, 23 tumors other than adenocarcinoma led to exclusion. Among the controls, 141 were excluded because of a disease history that met our exclusion criteria (Wang et al., 2012). The final groupings for analysis consisted of 423 GC cases and 454 matched controls. Informed consent was obtained from all the subjects, and the research protocol was approved by the Clinical Research Ethics Committee of the Affiliated Hospital of Jiangsu University.

2.2. Genotyping

Genomic DNA was extracted using previously described methods (Wang et al., 2012). Genotyping was performed using Taqman allelic discrimination assays (Xiao et al., 2012). Owing to DNA quantity or quality issues, genotyping of the polymorphisms failed (none of the four polymorphisms were determined) for 17 GC cases (4.0%) and for 10 controls (2.2%).

2.3. Helicobacter pylori antibody assays

Antecedent of *H. pylori* (Hp) infection was evaluated with anti-Hp antibodies measured in plasma using ELISA. An individual was considered Hp positive when the corresponding adjusted absorbance value was >0.99.

2.4. Microsatellite instability analysis

Genomic DNA was extracted from 314 tumor samples. The single fluorescent multiplex PCR was used to evaluate MSI status based on five quasimonomorphic mononucleotide repeat markers (BAT-25, BAT-26, NR-21, NR-24 and NR-27) (Buhard et al., 2004). Tumors showing two or more instable markers were classified as MSI, otherwise were classified as microsatellite stable (MSS) tumors.

2.5. Statistical analysis

Subjects who had smoked 200 cigarettes or more during their life were classified as smokers; those who had not were considered nonsmokers. Pack-years were calculated according to the number of packs (20 cigarettes per pack) smoked per day multiplied by the number of smoking years. Subjects who drunk alcohol at least one time per week for more than one year were classified as alcohol drinkers; those who had not were considered nondrinkers of alcohol. Subjects who drunk at least one cup of green tea per day for more than half a year were classified as drinkers of green tea; those who had not were considered nondrinkers of green tea. Both alcohol drinking and green tea drinking levels were calculated by multiplying average frequency of drinking, average amounts, and duration of drinking in years. The monthly amounts of pickled vegetables, soy foods, milk/dairy products, and fruit consumed were estimated by multiplying the monthly frequency of consumption and the usual portion size. We used the median values in controls as potential group cut-point for these variables.

The Hardy–Weinberg equilibrium for each polymorphism was tested for the controls. Lewontin's standardized coefficient D′ (|D′|) was used to evaluate the linkage disequilibrium (LD) among the polymorphisms. The statistical significance of the differences between the demographic characteristics of the study population was assessed with χ^2 tests and Student's t-tests. Odds ratios (OR) and 95% confidence intervals (CI) were calculated from conditional logistic regression models to estimate the main effect of each polymorphism with GC while adjusting for continuous age, Hp infection, and life exposures. Trend tests were computed using the number of variant alleles as a continuous variable (http://www.stata.com/support/faqs/statistics/ test-for-trend/). The association between combined genotypes and GC risk was then evaluated according to the number of at-risk genotypes of the subjects. We further stratified the data, based on tumor MSI and life exposures, to investigate the potential heterogeneous effects of the genotypes, and unconditional logistic regression was used. Interactions between genotypes and exposures were assessed by a multiplicative scale. Effect modification was determined by evaluating the improvement in fit (i.e., difference in −2 log likelihood values) of a model that included a multiplicative interaction term compared, with a restricted model with no interaction term (Campbell et al., 2009). Statistical analyses were carried out using Stata/SE version 11.0 software (Stata Corp., College Station, TX).

3. Results

3.1. Characteristics of the studied subjects

There were no significant differences between the cases and controls for mean age or gender distribution, suggesting that the matching based on these two variables was adequate. Hp infection, smoking, alcohol drinking, and high intake of pickled vegetables were found to be risk factors, while green tea drinking, high intake of milk/dairy products, and high fruit consumption were found to be protective factors for GC (Table 1).

3.2. Associations between MMR genotypes and GC risk

All genotype distributions among the controls were in Hardy–Weinberg equilibrium. The rs1800734 *G*-allele was associated with a decreased risk of GC compared with the *A*-allele (*GA* or *GG* vs *AA*, OR = 0.72; 95% CI: 0.50–1.05; P_{trend} for the *G*-allele = 0.029, Table 2). In an additional recessive model, a similar result was observed for the rs1800734 *GG* genotype (*GG* vs *AA* or *GA*, OR = 0.60; 95% CI: 0.38–0.95; *P* for OR = 0.030). No additional insight based on the recessive model was obtained from the following other analyses, thus data were not shown.