Dietary factors and microRNA-binding site polymorphisms in the IL13 gene: risk and prognosis analysis of colorectal cancer

Yanming Yu1,*, Junde Zhou2,*, Chen Gong1, Zhiping Long1, Jingshen Tian1, Lin Zhu1, Jing Li1, Hongyuan Yu1, Fan Wang1 and Yashuang Zhao1

1Department of Epidemiology, School of Public Health, Harbin Medical University, Harbin, Heilongjiang Province, P. R. China
2Department of Colorectal Surgery, The Second Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang Province, P. R. China
*These authors have contributed equally to this work

Correspondence to: Fan Wang, email: yifan.701@163.com
Yashuang Zhao, email: zhao_yashuang@263.net

Keywords: dietary factors, miRNA-binding site, polymorphisms, IL13, colorectal cancer

Received: December 20, 2016 Accepted: April 21, 2017 Published: May 07, 2017

Copyright: Yu et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License 3.0 (CC BY 3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

ABSTRACT

Long-term dietary intake influences the structure and activity of microorganisms residing in the human gut. The immune response and gut microbiota have a mutual influence on the risk of colorectal cancer (CRC). This study examines the association of gut microbiota–related dietary factors and polymorphisms in the microRNA-binding site of the interleukin 13 gene (IL13) with the risk and prognosis of CRC. Three polymorphisms (rs847, rs848, and rs1295685) were selected for genotyping in a case–control study (513 cases, 572 controls), and 386 CRC patients were followed up. Two dietary factors closely related with gut microbiota (allium vegetables, overnight meal) were significantly associated with CRC development. Although the three SNPs showed no statistically significant associations with the risk and prognosis of CRC, a significant antagonistic interaction was found between rs848 (G–T) and allium vegetable intake (ORi (odds ratio of interaction), 0.92; 95% CI (confidence interval): 0.86, 0.99; P = 0.03); moreover, significant combined and synergistic interactions were observed for all three SNPs and overnight meal intake. This is the first report of significant combined and interactive effects between dietary factors and polymorphisms in the microRNA binding site of IL13 in CRC and may provide direct guidance on intake of allium vegetable and overnight meals for individuals with specific genetic variants of IL13 to modify their susceptibility to CRC.

INTRODUCTION

Colorectal cancer (CRC) is a major public health problem worldwide [1]. The World Health Organization reported that it is the third most common malignancy and the fourth most common cause of cancer mortality in the world in 2012. The incidence of CRC is higher in most developed countries but has been rapidly increasing in developing countries over recent years. There were 253,427 new cases and 139,416 deaths due to CRC in China in 2012 [2].

An increasing number of recent research studies have indicated that the gut microbiota is associated with a variety of diseases including obesity, inflammatory bowel disease, adenomas, and CRC [3–5]. Shen et al. [6] characterized the composition of adherent bacteria in normal rectal mucosal biopsies and observed that the gut bacterial composition of subjects with adenomas differed significantly from that of control subjects without adenomas. Brim et al. also noted a trend of altered microbial changes between adenoma patients and healthy controls by comparing the fecal microbiota [7]. Diet-induced changes to gut-associated microbial communities are now suspected to contribute to the growing epidemics of chronic illness [8–10]. Especially, food-borne microbes from the diet, including bacteria, fungi, and even viruses, transiently colonize the gut. In addition, high-throughput sequencing results revealed that inflammation modified the gut microbial composition only in colitis-susceptible interleukin-10-deficient (Il10−/−) mice [11]. Sears et al. [12] indicated that
antibody-mediated blockade of IL-17 and the receptor for IL-23, a key cytokine that amplifies T-helper 17 cell responses, inhibits enterotoxicogenic Bacteroides fragilis–induced colitis, colonic hyperplasia, and tumor formation. As dietary factors influence the structure and activity of the microorganisms residing in the human gut, inter-individual differences in colorectal cancer susceptibility may be mediated by the mutual influence of inflammatory gene expression and dysbiosis of gut microbiota. However, it is unclear how the human inflammatory genome interacts with dietary factors to affect colorectal carcinogenesis.

Interleukin 13 (IL-13) is an anti-inflammatory immunomodulatory cytokine that is produced by T and B cells, mast cells, and basophils. IL-13 inhibits the secretion of pro-inflammatory mediators such as prostaglandins, reactive oxygen species (ROS) and nitrogen species, tumor necrosis factor (TNF) alpha, and IL-1, -6, -8, and -12 [13]. Consequently, IL-13 exhibits anti-inflammatory and anti-tumor functions by eliciting the expression of activation-induced cytidine deaminase (AID), which can lead to the development of colitis and promote neoplastic transformation [14]. microRNAs (miRNAs) are endogenous non-coding RNAs of ~22 nucleotides (nts) that regulate gene expression in animals and plants by pairing with the 3′-untranslated regions (UTRs) of the messenger RNAs (mRNAs) of target genes and specifying mRNA cleavage or repression of protein synthesis [15]. Consistent with the important role of miRNAs in gene regulation, some 3′UTR polymorphisms in the vicinity of a miRNA binding site have been reported to interfere with miRNA function and lead to differential gene expression. Single nucleotide polymorphisms (SNPs) located within miRNA-binding sites could thus influence cancer risk and overall survival [16–18]. SNPs in the IL13 gene have been reported to contribute to abnormal expression of IL-13 and modify susceptibility to cancer development [19]. However, the influence of SNPs in microRNA-binding sites of the IL13 gene on the risk of colorectal cancer and overall survival has not been reported.

In this study we explored the association of dietary factors and polymorphisms in the microRNA-binding site of IL13 with the risk and prognosis of CRC with the aim of providing meaningful instructions on dietary intake for individuals with specific genetic variants of IL13.

RESULTS

In Supplementary Table 1 we present all the SNPs with minor allele frequency (MAF) > 5% located at the miRNA binding sites of genes involved in inflammatory processes. The sum of all |ΔΔG| values for each SNP was listed as the basis for the selection of SNPs in our study. Among SNPs located in microRNA binding sites, two SNPs (rs847, rs848) in the IL13 3′UTR had the highest values of |ΔΔG tot|, therefore we decided to examine polymorphisms in IL13 in this study.

Table 1 shows the distribution of demographic characteristics for cases and controls and the baseline characteristics of cancer patients. A total of 513 CRC patients and 576 controls were recruited in this study. The mean age was 60.14 years for cases and 57.16 years for controls (P < 0.001). There was a higher proportion of workers with mental occupations and a lower proportion of physical workers among cases compared with controls (P = 0.003). The mean body mass index was 23.26 ± 3.35 and 24.27±4.14 in cases and controls, respectively (P < 0.001). No significant difference was found for the distribution of gender (P = 0.805), education level (P = 0.424), and family history of cancer (P = 0.168) between cases and controls.

Based on multivariate logistic regression analysis for the association of dietary factors and CRC risk, cereals, vegetables, and milk, had protective roles whereas excessive consumption of pork, soybean, and fish braised in soy sauce were risk factors for CRC (detailed results are shown in Supplementary Table 2). Within the multivariate model, two dietary factors (allium vegetables and overnight meal) were found to be significantly associated with CRC development.

Table 2 shows the genotype distributions of three SNPs in IL13 and their odds ratios (ORs) and 95% confidence intervals (CIs) for the risk of CRC. Age, BMI, and occupation were calculated as adjusted factors in the following analyses. Genotype distributions among controls were in agreement with the Hardy–Weinberg equilibrium. The frequencies of AA, AG, and GG genotypes of rs847 were 48.09%, 42.71%, and 8.51% in controls and 48.15%, 15.03%, and 6.04% in cases, respectively. The genotype frequencies for rs847 were 48.26% for GG, 41.67% for GT, and 8.50% for TT among controls and 48.15% for GG, 44.25% for GT, and 6.82% for TT among cases. None of the variant alleles was associated with the risk of CRC. Similarly, no significant association was observed between rs1295685 and the risk of CRC.

As shown in Table 3, for allium vegetables, among individuals carrying the GG genotype of rs847 those with intake of allium vegetables (including green onion, garlic, onion) 4–6 times per week showed a statistically reduced risk of CRC compared with those with intake less than once per week (ORd = 0.53; 95% CI: 0.31, 0.91; P = 0.04). Similar results were obtained among individuals carrying the CC
These concordant results indicate that overnight meal is a risk factor for CRC. Combined effects (altered susceptibility to CRC because of co-exposure to genetic variation and dietary intake) and interactive effects (how the two different genotypes respond to environmental variation in different ways) were analyzed for these three microRNA binding site polymorphisms and two dietary factors. For allium vegetables, we found a statistically significant antagonistic interaction for rs848 (G–T) and allium vegetable intake (OR_{interactive} [OR], 0.92; 95% CI: 0.86, 0.99; P = 0.03), and marginally significant interactive effects between rs847 (G–A) and overnight meal, indicating that overnight meal and rs847 jointly increase the risk of CRC. Similar results were found for the association of rs848 or rs1295685 and overnight meal with the risk of CRC; significant combined effects were observed for overnight meal and rs848 (OR_{dietary&genetic} [OR], 1.82; 95% CI: 1.12, 2.96; P = 0.02) and overnight meal and rs1295685 (OR_{dietary&genetic}, 1.82; 95% CI: 1.16, 2.98; P = 0.02). Corresponding synergistic effects were also significant for overnight meal and rs848 (OR_{interactive}, 1.24; 95% CI: 1.08, 1.42; P = 0.002) and overnight meal and rs1295685 (OR_{interactive}, 1.21; 95% CI: 1.05, 1.39; P = 0.008).

We analyzed the correlation between these three polymorphisms and clinical characteristics; however, no significant results were found (data are shown in Supplementary Table 3). Supplementary Table 4 shows the hazard ratio (HRs) and 95% CIs from univariate and multivariate Cox regression. Only general classification and Duke’s stage remained significant in multivariate analysis. Compared with patients with protruding type of CRC, those with invasive and ulcer types showed shorter survival times and an increased risk of death (HR, 1.73; 95% CI: 1.23, 2.44). In addition, Duke’s stage showed significance as a prognostic predictor; mean survival time among Duke’s stage I patients was 9.4 months, while that among Duke’s stage IV patients was 6.5 months.

### Table 1: Demographics and baseline characteristics of study subjects

| Characteristics                  | Cases (513) | Controls (576) | P- value |
|----------------------------------|-------------|----------------|----------|
| Age(years)\(a\) mean ± s.d.     | 60.14 ± 11.29 | 57.16 ± 11.25 | < 0.001  |
| Gender                           |             |                | 0.805    |
| Male                             | 302 (58.87) | 334 (57.99)    |          |
| Female                           | 211 (41.13) | 242 (42.01)    |          |
| BMI(kg/m\(2\))\(a\) mean ± s.d. | 23.26 ± 3.35 | 24.27 ± 4.14   | < 0.001  |
| Occupation                       |             |                | < 0.001  |
| Mental worker                    | 199 (38.79) | 156 (27.08)    |          |
| Physical worker                  | 224 (43.66) | 266 (46.18)    | 0.003    |
| Combined                         | 86 (16.76)  | 154 (26.74)    |          |
| Education                        |             |                | 0.424    |
| Illiterate                       | 134 (26.12) | 168 (29.17)    |          |
| Primary school                   | 151 (29.43) | 174 (30.21)    |          |
| high school or above             | 204 (39.77) | 210 (36.46)    |          |
| Family history of cancer         |             |                | 0.168    |
| No                               | 408 (79.53) | 484 (84.03)    |          |
| Yes                              | 94 (18.32)  | 89 (15.45)     |          |
| Tumor site                       |             |                |          |
| Colon                            | 168 (32.75) |               |          |
| Rectal                           | 312 (60.82) |               |          |
| Cecum                            | 30 (5.85)   |               |          |

\(^a\)Age and BMI are continuous variables, the others are categorical variables.

Missing data: tumor site, 3.
times of the patients decreased with an increase in Dukes’ stage. Compared with patients with Dukes’ stage I, the HR increased significantly for the other stages (Dukes’ stage II, HR, 1.60; 95% CI, 0.71, 3.61; Dukes’ stage III, HR, 4.29; 95% CI, 1.95, 9.42; Dukes’ stage IV, HR, 14.79; 95% CI, 5.97, 36.66).

DISCUSSION

We first explored the associations between gut microbiota–related dietary factors (allium vegetables, overnight meal), polymorphisms in miRNA-binding sites of the *IL13* gene, and the risk of CRC. A key novel finding of this study was evidence for combined and interactive effects of SNPs in *IL13* and dietary factors in CRC development. The lack of association between *IL13* polymorphisms and overall survival or clinical pathological characteristics is unsurprising given that the altered gut microbiota caused by dietary factors and genetic variants in *IL13* may play a lead role in carcinogenesis rather than prognosis of CRC.

Several studies have reported the influence of SNPs in *IL13* on the risk of cancer, and a meta-analysis concluded that *IL13* rs20541 polymorphisms contribute to susceptibility to cancer [19]. In addition, growing evidence has indicated that genetic variants in the sequences of miRNA-binding sites could affect miRNA regulation to target gene expression and consequently modify susceptibility and the prognosis of several cancers [20–23]. Based on bioinformatics analysis, Mark et al. found an increased risk in both bladder and breast cancer for the homozygote variant of the *PARP-1* SNP rs8679 [24]. Landi et al. examined the association between SNPs in miRNA-binding regions and sporadic colorectal cancer risk and showed statistical significance of variant alleles of *CD86* [25]. Another study conducted by Pan et al. reported that the let-7–targeted KRAS rs712 polymorphism was associated with an increased risk of colorectal cancer and may play crucial roles in the etiology of CRC [26].

| Genotypes    | Cases No.(%) | Controls No.(%) | OR<sub>adjusted</sub> (95% CI) | P-value |
|--------------|-------------|----------------|--------------------------------|---------|
| rs847 A>G    |             |                |                                |         |
| GG           | 247 (48.15) | 277 (48.09)    | 1.00                           |         |
| AG           | 231 (45.03) | 246 (42.71)    | 0.69 (0.42–1.14)               | 0.15    |
| AA           | 31 (6.04)   | 49 (8.51)      | 1.03 (0.80–1.33)               | 0.84    |
| Dominant model |         |                | 0.97 (0.76–1.25)               | 0.83    |
| Recessive model |       |                | 0.68 (0.42–1.11)               | 0.12    |
| rs848 T>G    |             |                |                                |         |
| GG           | 247 (48.15) | 278 (48.26)    | 1.00                           |         |
| GT           | 227 (44.25) | 240 (41.67)    | 1.03 (0.79–1.33)               | 0.84    |
| TT           | 35 (6.82)   | 49 (8.50)      | 0.78 (0.48–1.26)               | 0.31    |
| Dominant model |         |                | 0.99 (0.77–1.27)               | 0.93    |
| Recessive model |       |                | 0.77 (0.48–1.23)               | 0.27    |
| rs1295685 C>T|             |                |                                |         |
| CC           | 245 (47.76) | 272 (47.22)    | 1.00                           |         |
| CT           | 225 (43.86) | 238 (41.32)    | 1.02 (0.79–1.32)               | 0.87    |
| TT           | 31 (6.04)   | 53 (9.20)      | 0.63 (0.39–1.04)               | 0.07    |
| Dominant model |         |                | 0.96 (0.75–1.22)               | 0.72    |
| Recessive model |       |                | 0.63 (0.39–1.01)               | 0.06    |

OR<sub>adjusted</sub>: adjusted for BMI, occupation, and age

Table 2: Association between microRNA-binding site polymorphisms in *IL13* gene and the risk of colorectal cancer
Table 3: Combined and interactive effects of microRNA–binding site polymorphisms in IL13 and dietary factors on the risk of colorectal cancer

| SNPs         | Dietary factors | Cases (No.) | Controls (No.) | Adjusted OR (95% CI) | P-value | Adjusted ORi (95% CI) | P-value* |
|--------------|-----------------|-------------|----------------|----------------------|---------|-----------------------|----------|
| rs847        | Allium vegetables (times/week) |             |                | 0.92 (0.84–1.01)     | 0.08    |                       |          |
| GG           | < 1             | 83          | 69             | 1                    |         |                       |          |
| GG           | 1–3             | 59          | 68             | 0.85 (0.52–1.39)     | 0.53    |                       |          |
| GG           | 4–6             | 34          | 59             | 0.51 (0.30–0.88)     | 0.02    |                       |          |
| GG           | > 7             | 71          | 79             | 0.81 (0.51–1.29)     | 0.37    |                       |          |
| AG+AA        | < 1             | 70          | 64             | 0.92 (0.57–1.49)     | 0.74    |                       |          |
| AG+AA        | 1–3             | 61          | 75             | 0.68 (0.42–1.10)     | 0.11    |                       |          |
| AG+AA        | 4–6             | 50          | 68             | 0.65 (0.39–1.08)     | 0.09    |                       |          |
| AG+AA        | > 7             | 80          | 88             | 0.83 (0.53–1.32)     | 0.44    |                       |          |
| rs847        | Overnight meal (times/week) | 1.25 (1.08–1.43) | 0.002 |
| GG           | < 1             | 47          | 64             | 1                    |         |                       |          |
| GG           | 1–3             | 96          | 121            | 1.11 (0.69–1.79)     | 0.68    |                       |          |
| GG           | > 3             | 104         | 92             | 1.66 (1.02–2.70)     | 0.04    |                       |          |
| AG+AA        | < 1             | 54          | 63             | 1.14 (0.67–1.97)     | 0.63    |                       |          |
| AG+AA        | 1–3             | 96          | 142            | 0.90 (0.56–1.45)     | 0.67    |                       |          |
| AG+AA        | > 3             | 111         | 88             | 1.84 (1.13–3.01)     | 0.01    |                       |          |
| rs848        | Allium vegetables (times/week) | 0.92 (0.86–0.99) | 0.03 |
| GG           | < 1             | 84          | 69             | 1                    |         |                       |          |
| GG           | 1–3             | 57          | 70             | 0.79 (0.48–1.29)     | 0.34    |                       |          |
| GG           | 4–6             | 35          | 57             | 0.53 (0.31–0.91)     | 0.02    |                       |          |
| GG           | > 7             | 71          | 80             | 0.78 (0.49–1.24)     | 0.29    |                       |          |
| GT+TT        | < 1             | 68          | 63             | 0.89 (0.55–1.44)     | 0.63    |                       |          |
| GT+TT        | 1–3             | 63          | 70             | 0.72 (0.44–1.17)     | 0.18    |                       |          |
| GT+TT        | 4–6             | 49          | 67             | 0.63 (0.38–1.05)     | 0.07    |                       |          |
| GT+TT        | > 7             | 81          | 89             | 0.81 (0.52–1.27)     | 0.36    |                       |          |
| rs848        | Overnight meal (times/week) | 1.24 (1.08–1.42) | 0.002 |
| GG           | < 1             | 49          | 63             | 1                    |         |                       |          |
| GG           | 1–3             | 97          | 120            | 1.07 (0.66–1.72)     | 0.79    |                       |          |
| GG           | > 3             | 101         | 95             | 1.41 (0.91–2.39)     | 0.12    |                       |          |
| GT+TT        | < 1             | 54          | 63             | 1.07 (0.62–1.83)     | 0.81    |                       |          |
| GT+TT        | 1–3             | 93          | 139            | 0.84 (0.52–1.34)     | 0.45    |                       |          |
| GT+TT        | > 3             | 114         | 85             | 1.82 (1.12–2.96)     | 0.02    |                       |          |
| rs1295685    | Allium vegetables (times/week) | 0.92 (0.84–1.00) | 0.06 |
| CC           | < 1             | 84          | 67             | 1                    |         |                       |          |
| CC           | 1–3             | 58          | 66             | 0.82 (0.50–1.34)     | 0.42    |                       |          |
| CC           | 4–6             | 34          | 58             | 0.50 (0.29–0.86)     | 0.01    |                       |          |
| CC           | > 7             | 69          | 79             | 0.76 (0.41–1.21)     | 0.24    |                       |          |
| CT+TT        | < 1             | 67          | 65             | 0.84 (0.52–1.36)     | 0.47    |                       |          |
| SNP       | Overnight meal (times/week) | OR          | 95% CI      | P     |
|-----------|-----------------------------|-------------|-------------|-------|
| rs1295685 | 1–3                         | 0.64 (0.40–1.05) | 0.08       |
|           | 4–6                         | 0.65 (0.39–1.07) | 0.09       |
|           | > 7                         | 0.78 (0.50–1.24) | 0.29       |

*P value for interaction analysis conducted by multivariate logistic regression.

Allium vegetables, vegetables in the *Allium* genus include onions, shallots, leeks and scallions, as well as herbs like garlic and chives.

Overnight meal, the vegetables, egg, meat that have been cooked and left overnight.

SNP, Single Nucleotide Polymorphism

OR, OR for combined effects of dietary factors and genetic factors

OR*, OR for interactive effects of dietary factors and genetic factors

A recent study demonstrated that bacterial diversity is remarkably decreased in the gut microbiota of mice models of sporadic colorectal cancer and colitis-associated cancer [42]. Bacterial toxins in overnight meals could cause destruction of the normal gut microbial ecosystem and induce chronic gastroenteritis [3]. Moreover, a high level of nitrite is generated when bacteria multiply rapidly if the food storage method is incorrect. Nitrate and nitrite are precursors in the endogenous formation of potentially carcinogenic N-nitroso compounds (NOC). The Shanghai Women's Health study suggested that high dietary nitrate and nitrite intake results in increased exposure to endogenously formed NOCs and increased risk of CRC [43]. In this study, we found a significantly increased risk of CRC for individuals carrying genetic variants of all three SNPs combined with a high frequency of intake of overnight meals. The statistically significant synergistic interactions among the SNPs and overnight meal intake indicated that genetic variants and overnight meal collectively increased the susceptibility to CRC. These results highlighted the carcinogenic effects of overnight meals especially in individuals carrying variant alleles of these three SNPs, and suggest that long-term consumption of overnight meals should be avoided in such cases.

The role of polymorphisms in miRNA-binding sites as prognostic biomarkers and their correlations with the response to chemotherapy has been researched. Chae et al. [44] found that CRC patients carrying a variant allele of rs1044129 (miRNA-367 binding site) showed poor recurrence-free-survival compared with those with the AA or AG genotype. However, Kjersem et al. did not find a significant effect of SNPs in the let-7 microRNA binding site in KRAS (rs61764370) on progression-free survival and overall survival in patients receiving Nordic FLOX+cetuximab in the NORDIC-VII trial.
In the present study, no significant correlations between the different genotypes of the three SNPs of *IL13* and clinical pathological characteristics were observed. Similarly, there was no statistically significant effect of the three SNPs on overall survival of CRC patients.

There were some limitations in this study. First, recall bias may be inevitable in the collection of information on dietary factors, although we did our best to minimize this bias. Second, we only investigated three microRNA binding site polymorphisms in *IL13* rather than all inflammatory genes. Third, the bioinformatics strategy that we used for screening of microRNA-binding site polymorphisms may not be powerful enough to find genetic variants with the greatest biological impact. Although our study has a relatively large sample size, the number of individuals in some subgroups with variant homozygotes is still too small to obtain sufficient statistical power.

In summary, this is the first study using population epidemiological methods to elucidate the role of gut microbiota–related dietary factors and polymorphisms in miRNA-binding site in *IL13* in CRC. Although the three SNPs selected by screening using bioinformatics tools did not show significant independent associations with the development and prognosis of CRC, we observed significant combined and interactive effects between these three SNPs and dietary intake of allium vegetables and overnight meal. Future guidelines for dietary intake based on individual genetic background should be addressed.

**MATERIALS AND METHODS**

**Study subjects**

We performed this study after obtaining informed written consent from study subjects and approval from the Human Research and Ethics Committee of Harbin Medical University. All experiments were performed in accordance with relevant guidelines and regulations.

A case–control study was designed to assess the role of genetic polymorphisms and dietary factors on the risk of CRC. Cases were incident patients who underwent surgery at the Cancer Hospital and the Second Affiliated Hospital of Harbin Medical University from June 2004 to January 2008. Patients with neuroendocrine carcinoma, malignant melanoma, non-Hodgkin’s lymphoma, gastrointestinal stromal tumors, and Lynch syndrome colorectal cancer were excluded. A total of 513 CRC patients with pathologic diagnosis were recruited. Controls were enrolled from patients in the orthopedic and ophthalmology departments who were admitted to the Second Affiliated Hospital of Harbin Medical University and volunteers from the Xiangfang community of Harbin city within the same time period. Any individual with a history of polyps, adenomas, or other disease related to cancer was excluded from controls. In total, 576 controls (77 community-based and 499 hospital-based) were recruited.

A patient cohort study was proposed to explore the potential factors associated with the prognosis of CRC. Among the 513 CRC patients, 386 were followed up from November 2004 to March 2014 with telephone interview.

All subjects in this study were informed and gave written consent to participate in the study. All procedures, including participant recruitment, questionnaire information collection, and all experimental protocols, were approved by the Human Research and Ethics Committee of Harbin Medical University.

**Data collection**

The questionnaire is structured to collect information on demographic characteristics (age, gender, height and weight, education, marital status, occupation, and race) and dietary factors relevant to CRC development. For each subject, history of smoking and drinking, detailed disease history and family history of cancer, and dietary status during the past 1 year before cancer diagnosis were recalled. Peripheral venous blood was obtained and stored at −80°C immediately after separation of plasma. DNA was extracted from blood samples of 513 cases and 576 controls using the classic phenol–chloroform procedure [46] and QIAamp DNA Blood mini kits (Hilden, Germany).

Clinical information including tumor size, Duke’s stage, chemotherapy, histological and pathological types, and serum levels of carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) before surgery were extracted from medical records. Overall survival (OS) was calculated from the first day of cancer diagnosis to death. Patients who suffered from recurrence and were still alive at the end of follow-up were measured as censored data.

**SNP selection and genotyping**

We initially analyzed 49 candidate genes involved in inflammatory processes. All SNPs residing on miRNA binding sites within the 3′UTRs were captured by an extensive search in dbSMR [https://omictools.com/dbsmr-tool](https://omictools.com/dbsmr-tool). Of these, 21 genes were selected according to minor allele frequency of the Chinese population > 5% in Pubmed (Supplementary Table 1). Using RNA hybrid (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/submission.html), the Gibbs free energy (DG, expressed in kilojoules per mole [kJ/mol]) for both wild-type and variant alleles of each identified SNP was determined and calculated from the first day of cancer diagnosis to death. Patients who suffered from recurrence and were still alive at the end of follow-up were measured as censored data.
The fluorogenic 5’-nuclease assay (TaqMan SNP Genotyping Assay, Applied Biosystems, Foster City, CA, USA) was used to analyze genomic DNA samples for IL13 polymorphisms. Analysis was performed using the Roche Lightcycler 480II Sequence Detection System. The 25-μl reaction mix contained at least 10 ng DNA, 12.5 μl Universal PCR Master Mix, and 0.625 μl Probe/Primer mix. The assay ID numbers of IL13 were as follows: rs847: C_8932046_10; rs848: C_8932051_20; rs1295685: C_8932052_10. PCR amplification conditions were an initial step of 95°C for 10 min followed by 40 cycles of 92°C for 15 s and 60°C for 1 min. The genotyping experiment was conducted according to the protocol of the TaqMan® Assay.

Statistical analysis

Each polymorphism was tested to confirm fit with Hardy–Weinberg equilibrium with alpha threshold of 0.05 for controls. Categorical and continuous variables were tested by chi-square test and two-sample t test respectively. Univariate and multivariate logistic-regression analyses were used to calculate crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for the association of IL13 microRNA binding site SNPs and CRC risk. In multivariate analysis, significant variables from univariate analysis were selected and manually entered into the model step by step. The combined and interactive effects between genetic variants and dietary factors were estimated by crossover study and multivariate logistic regression. The cutoff of \( P \) -values was 0.05 in both univariate and multivariate analyses.

Kaplan–Meier curves and log-rank test were used to assess the influence of IL13 variants on overall survival. Hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) were computed using univariate and multivariate Cox proportional hazard models. Data were analyzed using SPSS 17.0 software, and all \( P \)-values represent two-sided statistical tests.

Abbreviations

CRC (colorectal cancer); IL-17 (interleukin-17); IL-13 (interleukin 13); TNF (tumor necrosis factor); AID (activation-induced cytidine deaminase); miRNAs (microRNAs); mRNAs (messenger RNAs); Nts (nucleotides); UTRs (untranslated regions); SNPs (Single Nucleotide Polymorphisms); OR (OR\textsubscript{univariate}); OR\textsuperscript{dg} (OR\textsuperscript{dualgene}); NOC (N-nitroso compounds); RFS (recurrence-free-survival); CEA (carcinoembryonic antigen); CA19-9 (carbohydrate antigen 19-9); OS (overall survival); ORs (odds ratios); CIs (confidence intervals); HRs (Hazard Ratios).

Authors’ contributions

Fan Wang and Yashuang Zhao contributed to the study design, data interpretation, study supervision, drafting the manuscript, and the acquisition of funding. Yanming Yu and Chen Gong contributed to DNA extraction, genotyping, data analysis. Junde Zhou, Zhiping Long, and Jingshen Tian contributed to data analysis and manuscript draft. Lin Zhu, Jing Li, and Hongyuan Yu contributed to questionnaire, sample collection, and DNA extraction. All authors contributed to review and revision of the manuscript.

CONFLICTS OF INTEREST

None of the authors declared a conflicts of interest.

FINANCIAL SUPPORT

This study was supported by grants from Postdoctoral Science Foundation of China (No. 20100481026), Doctoral Program Foundation of State Education Ministry (20122307120005) and Dr. Wu Lien-teh Science Foundation of Harbin Medical University (WLD-QN1106).

REFERENCES

1. Abuli A, Fernandez-Rozadilla C, Giraldez MD, Munoz J, Gonzalo V, Bessa X, Bujanda L, Rene JM, Lanas A, Garcia AM, Salo J, Arguello L, Villela A, et al. A two-phase case-control study for colorectal cancer genetic susceptibility: candidate genes from chromosomal regions 9q22 and 3q22. Br J Cancer. 2011; 105:870–875.

2. Acrar E, Lawaetz AJ, Rasmussen MA, Bro R. Structure-revealing data fusion model with applications in metabolomics. Conf Proc IEEE Eng Med Biol Soc. 2013; 2013:6023–6026.

3. Candela M, Turroni S, Biagi E, Carbonero F, Rampelli S, Fiorentini C, Brigidi P. Inflammation and colorectal cancer, when microbiota-host mutualism breaks. World journal of gastroenterology. 2014; 20:908–922.

4. Hold GL, Smith M, Grange C, Watt ER, El-Omar EM, Mukhopadhya I. Role of the gut microbiota in inflammatory bowel disease pathogenesis: what have we learnt in the past 10 years? World journal of gastroenterology. 2014; 20:1192–1210.

5. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarell BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, et al. A core gut microbiome in obese and lean twins. Nature. 2009; 457:480–484.

6. Shen XJ, Rawls JF, Randall T, Burcal L, Mpanel CN, Jenkins N, Jovov B, Abdo Z, Sandler RS, Keku TO. Molecular characterization of mucosal adherent bacteria and associations with colorectal adenomas. Gut microbes. 2010; 1:138–147.

7. Brim H, Yooseph S, Zoetendal EG, Lee E, Tortalbo M, Laiyemo AO, Shokrani B, Nelson K, Ashktorab H.
Microbiome analysis of stool samples from African Americans with colon polyps. PloS one. 2013; 8:e81352.
8. Walker AW, Ince J, Duncan SH, Webster LM, Holotrop G, Ze X, Brown D, Stares MD, Scott P, Bergerat A, Louis P, McIntosh F, Johnstone AM, et al. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. ISME J. 2011; 5:220–230.
9. Devkota S, Wang Y, Musch MW, Leone V, Fehlner-Pech H, Nadimpalli A, Antonopoulos DA, Jabri B, Chang EB. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in IL10−/− mice. Nature. 2012; 487:104–108.
10. Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JJ. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Science translational medicine. 2009; 1:6ra14.
11. Arthur JC, Perez-Chanona E, Muhlbauer M, Tomkovich S, Uronis JM, Fan TJ, Campbell BJ, Abujamel T, Dogan B, Rogers AB, Rhodes JM, Stintzi A, Simpson KW, et al. Intestinal inflammation targets cancer-inducing activity of the microbiota. Science. 2012; 338:120–123.
12. Wu S, Rhee KJ, Albesiano E, Rabizadeh S, Wu X, Yen HR, Huso DL, Brancati FL, Wick E, McCallister F, Housseau F, Pardoll DM, Sears CL. A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. Nat Med. 2009; 15:1016–1022.
13. Minty A, Ferrara P, Caput D. Interleukin-13 effects on activated monocytes lead to novel cytokine secretion profiles intermediate between those induced by interleukin-10 and by interferon-gamma. Eur Cytokine Netw. 1997; 8:189–201.
14. Endo Y, Marusawa H, Kout T, Nakase H, Fujii S, Fujimori T, Kinoshita K, Honjo T, Chiba T. Activation-induced cytidine deaminase links between inflammation and the development of colitis-associated colorectal cancers. Gastroenterology. 2008; 135:889–898.e3.
15. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004; 116:281–297.
16. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, et al. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A. 2002; 99:15524–15529.
17. Calin GA, Liu CG, Sevignani C, Ferracin M, Felli N, Dumitru CD, Shimizu M, Cimmino A, Zupo S, Dono M, Dell’Aquila ML, Alder H, Rassenti L, et al. MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukaemias. Proc Natl Acad Sci U S A. 2004; 101:11755–11760.
18. Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer. 2006; 6:259–269.
19. Su T, Mi Y, Zhang L, Wang S, Lu H, Shi L, Sun H, Wu X, Zhang W, Zuo L, Zou J. Association between IL13 gene polymorphisms and susceptibility to cancer: a meta-analysis. Gene. 2013; 515:56–61.
20. Jiang Y, Qin Z, Hu Z, Guan X, Wang Y, He Y, Xue J, Liu X, Chen J, Dai J, Jin G, Ma H, Wang S, Shen H. Genetic variation in the hsa-let-7 binding site in RAD52 is associated with breast cancer susceptibility. Carcinogenesis. 2013; 34:689–693.
21. Pauling JK, Christensen AG, Batra R, Alcaraz N, Barbosa E, Larsen MR, Beck HC, Leth-Larsen R, Azevedo V, Ditzel HJ, Baumbach J. Elucidation of epithelial-mesenchymal transition-related pathways in a triple-negative breast cancer cell line model by multi-omics interactome analysis. Integrative biology. 2014; 6:1058–1068.
22. Mi Y, Wang L, Zong L, Pei M, Lu Q, Huang P. Genetic variants in microRNA target sites of 37 selected cancer-related genes and the risk of cervical cancer. PloS one. 2014; 9:e86061.
23. Wang C, Guo Z, Wu C, Li Y, Kang S. A polymorphism at the miR-502 binding site in the 3′ untranslated region of the SET8 gene is associated with the risk of epithelial ovarian cancer. Cancer Genet. 2012; 205:373–376.
24. Teo MT, Landi D, Taylor CF, Elliott F, Vasilin L, Cox DG, Hall J, Landi S, Bishop DT, Kiltie AE. The role of microRNA-binding site polymorphisms in DNA repair genes as risk factors for bladder cancer and breast cancer and their impact on radiotherapy outcomes. Carcinogenesis. 2012; 33:581–586.
25. Landi D, Gemignani F, Naccarati A, Pardini B, Vodicka P, Vodickova L, Novotny J, Forsti A, Hemminki K, Canzian F, Landi S. Polymorphisms within micro-RNA-binding sites and risk of sporadic colorectal cancer. Carcinogenesis. 2008; 29:579–584.
26. Pan XM, Sun RF, Li ZH, Guo XM, Zhang Z, Qin HJ, Xu GH, Gao LB. A let-7 KRAS rs712 polymorphism increases colorectal cancer risk. Tumour Biol. 2014; 35:831–835.
27. Brennan CA, Garrett WS. Gut Microbiota, Inflammation, and Colorectal Cancer. Annual review of microbiology. 2016; 70:395–411.
28. Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. Nature reviews Microbiology. 2014; 12:661–672.
29. Bultman SJ. Interplay between diet, gut microbiota, epigenetic events, and colorectal cancer. Molecular nutrition & food research. 2016; 61.
30. Chang HS, Yamato O, Yamasaki M, Ko M, Maede Y. Growth inhibitory effect of alk(en)yl thiosulfates derived from onion and garlic in human immortalized and tumor cell lines. Cancer Lett. 2005; 223:47–55.
31. Bibbo S, Ianiro G, Giorgio V, Scaldaferri F, Masucci L, Gasbarrini A, Cammarota G. The role of diet on gut microbiota composition. European review for medical and pharmacological sciences. 2016; 20:4742–4749.
32. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R, Sinha R, Gilroy E, Gupta K, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science. 2011; 334:105–108.

33. Wu GD, Bushman FD, Lewis JD. Diet, the human gut microbiota, and IBD. Anaerobe. 2013; 24:117–120.

34. de Wouters T, Dore J, Lepage P. Does our food (environment) change our gut microbiome (‘in-vironment’): a potential role for inflammatory bowel disease? Dig Dis. 2012; 30:33–39.

35. Block E. The chemistry of garlic and onions. Sci Am. 1985; 252:114–119.

36. Seki T, Hosono T, Hosono-Fukao T, Inada K, Tanaka R, Ogihara J, Ariga T. Anticancer effects of diallyl trisulfide derived from garlic. Asia Pac J Clin Nutr. 2008; 17: 249–252.

37. O’Gara EA, Hill DJ, Maslin DJ. Activities of garlic oil, garlic powder, and their diallyl constituents against Helicobacter pylori. Applied and environmental microbiology. 2000; 66:2269–2273.

38. Lu X, Rasco BA, Jabal JM, Aston DE, Lin M, Konkel ME. Investigating antibacterial effects of garlic (Allium sativum) concentrate and garlic-derived organosulfur compounds on Campylobacter jejuni by using Fourier transform infrared spectroscopy, Raman spectroscopy, and electron microscopy. Applied and environmental microbiology. 2011; 77:5257–5269.

39. Lu X, Rasco BA, Kang DH, Jabal JM, Aston DE, Konkel ME. Infrared and Raman spectroscopic studies of the antimicrobial effects of garlic concentrates and diallyl constituents on foodborne pathogens. Anal Chem. 2011; 83:4137–4146.

40. Galeone C, Pelucchi C, Dal Maso L, Negri E, Montella M, Zucchetto A, Talamini R, La Vecchia C. Allium vegetables intake and endometrial cancer risk. Public Health Nutr. 2009; 12:1576–1579.

41. Galeone C, Pelucchi C, Levi F, Negri E, Franceschi S, Talamini R, Giacosa A, La Vecchia C. Onion and garlic use and human cancer. Am J Clin Nutr. 2006; 84:1027–1032.

42. Zackular JP, Baxter NT, Iverson KD, Sadler WD, Petrosino JF, Chen GY, Schloss PD. The gut microbiome modulates colon tumorigenesis. mBio. 2013; 4:e00692–00613.

43. Dellavalle CT, Xiao Q, Yang G, Shu XO, Aschebrook-Kilfoy B, Zheng W, Lan Li H, Ji BT, Rothman N, Chow WH, Gao YT, Ward MH. Dietary nitrate and nitrite intake and risk of colorectal cancer in the Shanghai Women’s Health Study. Int J Cancer. 2014; 134:2917–2926.

44. Chae YS, Kim JG, Kang BW, Lee SJ, Lee YJ, Park JS, Choi GS, Lee WK, Jeon HS. Functional polymorphism in the MicroRNA-367 binding site as a prognostic factor for colon cancer. Anticancer Res. 2013; 33:513–519.

45. Kjersem JB, Ikdahl T, Guren T, Skovlund E, Sorbye H, Hamfjord J, Pfeiffer P, Glimelius B, Kersten C, Solvang H, Tveit KM, Kure EH. Let-7 miRNA-binding site polymorphism in the KRAS 3’UTR; colorectal cancer screening population prevalence and influence on clinical outcome in patients with metastatic colorectal cancer treated with 5-fluorouracil and oxaliplatin +/- cetuximab. BMC Cancer. 2012; 12:534.

46. Miyaki M, Seki M, Okamoto M, Yamanaka A, Maeda Y, Tanaka K, Kikuchi R, Iwama T, Ikeuchi T, Tonomura A, Nakamura Y, White R, Miki Y, et al. Genetic changes and histopathological types in colorectal tumors from patients with familial adenomatous polyposis. Cancer Res. 1990; 50:7166–7173.