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Cancer Detection and Prevention 29 (2005) 146–154

Cancer Detection and Prevention

www.elsevier.com/locate/cdp

Diets, polymorphisms of methylenetetrahydrofolate reductase, and the susceptibility of colon cancer and rectal cancer

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Accepted 16 November 2004

Abstract

The aim of this study was to investigate the association of environmental factors (dietary folate, methionine and drinking status) and polymorphisms in the methylenetetrahydrofolate reductase (*MTHFR* C677T and A1298C) gene, as well as the combination of these factors, with the risk of colon cancer and rectal cancer. A case-control study of 53 colon cancer patients, 73 rectal cancer patients and 343 healthy controls was conducted. Genotypes of C677T and A1298C polymorphisms were analyzed by PCR-RFLP. The dietary folate and methionine intakes were assessed using food-frequency questionnaires and food consumption tables. Unconditional logistic regression was applied to estimate the odds ratios (ORs) and their 95% confidence intervals (CIs). The frequency of *MTHFR* 677T and 1298C alleles in healthy population were 39.4 and 17.2%, respectively. After adjustment for specific variants, the *MTHFR* 677TT genotype showed a significantly reduced risk of colon cancer compared with the wild type (OR = 0.22, 95% CI: 0.50–0.98), and 1298C allele-carrier showed an inverse association with the risk of rectal cancer compared to the wild type (OR = 0.52, 95% CI: 0.28–0.98). Adequate intake of folate was a protective factor from colon cancer (OR = 0.32, 95% CI: 0.12–0.88) and *MTHFR* C677T polymorphism showed a statistically significant effect (OR = 0.25, 95% CI: 0.06–0.93), reducing the risk of colon cancer in groups that have an intake of folate exceeding 115.64 ng per 1000 kcal per day. This study suggests that *MTHFR* C677T and A1298C polymorphisms are associated with the reduced risk of colon and rectal cancers, respectively. Adequate folate intake shows an inverse association with the risk of colon cancer. There is a significant interaction between *MTHFR* C677T polymorphism and folate intake in reducing the risk of colon cancer.

Keywords: Colon and rectal cancer; Methylenetetrahydrofolate reductase; Genetic polymorphism(s); Folate; Methionine

1. Introduction

Folate, the main methyl donor in vivo, plays a central role in methylation of homocysteine and the synthesis of nucleotide. Animal and cell models suggest a role for folate in reducing colon carcinogenesis [1]. Epidemiological studies indicated high vegetable intake was related to a decrease in the risk of colorectal cancer (CRC) [2]. Vegetables, particularly green, leafy vegetables, are a major source of folate. Previous studies reported dietary folate intake would reduce risk of colon cancer [3–5]; however, no consistent association was reported between rectal cancer and folate intake [6,7]. Some studies indicated a positive association between alcohol intake, which could adversely affect folate metabolism, and colorectal neoplasia [8]. Diets low in methyl donors, such as folate and methionine, particularly in combination with

Abbreviations: MTHFR, 5,10-methylenetetrahydrofolate reductase; CRC, colorectal cancer; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; ANOVA, analysis of variance; ors, odds ratios; CIs, confidence intervals; SD, standard deviation

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0361-090X/$30.00
doi:10.1016/j.cdp.2004.11.004

alcohol intake, were associated with increased risk of colon cancer [9].

5,10-Methylenetetrahydrofolate reductase (*MTHFR*) is a central enzyme in folate metabolism [10] in vivo, irreversibly converting 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulating form of folate. This product provides methyl group for synthesis of methionine, a decreased pool of which may affect DNA synthesis [8]. Two common mutations, the C677T (Alanine–Valine) and A1298C (Glutamic–Alanine) in the *MTHFR* gene, have been found to be associated with the risk of colon and rectal cancer [3,4,11]. These mutations cause reduced enzyme activity, leading a decline in plasma folate level [3,12]. Alternatively, alterations in the levels of 5-methyltetrahydrofolate may change *S*-adenosylamethionine levels and may possibly influence DNA methylation patterns: both hypomethylation and hypermethylation of DNA have been shown to be of importance in carcinogenesis [13,14]. A growing body of evidence suggests that *MTHFR* maybe at the ''crossroads'' between methyl-group transfer and the manufacture of nucleotides ensuring that risk of carcinogenesis increases only when there is both a deficiency of folate and a variant genotype present [4,11,15].

CRC is one of the most common cancers causing death in humans. It has been estimated that 945,000 cases of CRC occurred worldwide in 2000 and 492,000 persons died from the disease [16]. China is a nation with a relatively low incidence of CRC compared with the US and Canada [17]; however, in the eastern part of China, there has been a higher reported incidence of CRC. In particular, Jiashan County has been shown to have the highest incidence of CRC in China, with around mortality rates of 26.3/100,000 in males and 18.6/100,000 in females due to CRC [18]. Some epidemiological studies in Jiashan County reported the association between environmental factors and colon and rectal cancer [19,20], however, few studies have indicated an association between gene–gene and gene–environment interactions and the risk of colon and rectal cancer in China.

In this study, we investigated the association of *MTHFR* genetic polymorphisms and the risk of colon cancer and rectal cancer, as well as the interaction of the genetic polymorphisms with folate, methionine intake and drinking status. A case-control study was designed within a cohort study population in Jiashan Country.

2. Materials and methods

2.1. Subjects

Both case and control groups were originated from a CRC cohort-study population. This population was set up in 1989 and was described elsewhere [19]. All subjects aged 40 years or older, and were ethnic Han Chinese and residents in Jiashan County, Zhejiang Province, China. The follow-up for incidents of cancer in the entire cohort was established by the cancer registration system and CRC report system in Jiashan County. After about 12 years of follow-up, 163 surviving individuals had been diagnosed with CRC before 1 March 2002. One hundred and twenty-six subjects, including 53 colon cancer patients and 73 rectal cancer patients, completed the investigation and composed the case group in this study. Three hundred and eighty participants were randomly selected from the whole cohort population. There were 343 healthy individuals, except cancer cases, death and refused men, were absorbed into this study. All subjects were face-to-face interviewed by trained personnel using a structured questionnaire, related to general situations, and drinking status, food frequency, etc. In the section on food-frequency, eight categories (46 items) were investigated on the consumption of main local foods and the consumption frequency during the preceding year prior to the interview day. Some food-models, measuring cups and bowls were shown during the interview to facilitate and improve the quantification of food consumption.

Peripheral blood was collected into a vacuum tube containing sodium citrate solution at the time of interview or shortly after, frozen within 4–6 h, and stored at −20 °C.

2.2. Genotyping

Genomic DNA was extracted from whole frozen blood samples using a proteinase K treatment, followed by NaCl extractions and ethanol precipitation [21,22].

MTHFR polymorphism genotypes of C677T and A1298C were analyzed using polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) methods described elsewhere [23–25]. Briefly, the primer sequences were referred to by Miao et al. [25] and generated at the 198 bp fragment for the C677T site mutation and at 163 bp for the A1298C site. The primers for the 677 site were F (5′-TGAAGGAGAAGGTGTCTGCGGGA-3′) and R (5′-AGG ACG GTG CGG TGA GAG TG-3′), and for the 1298 site were F (5′-AGG ACG GTG CGG TGA GAG TG-3′) and R (5′-CAC TTT GTG ACC ATT CCG GTT TG-3′). These fragments were amplified separately under the same conditions as follows: 25 l reaction mixture consisted of ∼100 ng template DNA, 0.5 μM primer, 0.2 mM dNTP, 1.5 mM MgCl2, and 1. units of *Taq* DNA polymerase with 1× reaction buffer (Takara Biotechnology, Inc., Japan). Amplication was performed using an initial denaturation at 94 °C for 2 min, followed by 35 cycles at 94 °C for 30 s, 61 for 30 s, and 72 for 30 s, and a final elongation step at 72 °C for 7 min. The restriction enzymes *HinfI* and *MboII* (MBI Fermentas, Germany) were used to distinguish the C677T and A1298C polymorphisms, respectively. The *677CC* wild-type homozygote was identified by the presence of a 198 bp fragment; 677CT heterozygote by 198, 175, and 23 bp fragments; and 677TT mutant homozygote by 175 and 23 bp fragments. The 1298AA wild-type homozygote produced 5 fragments: 56, 31, 30, 28, and 18 bp; 1298AC heterozygote produced 6 fragments of 84, 56, 31, 30, 28 and 18 bp; and