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The methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and cancer risk: The Croatian case–control study

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Abstract

Objectives: Methylation abnormalities appear to be important for the pathogenesis of many cancer types. Since methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in the methylation process catalyzing reduction of 5,10-methylenetetrahydrofolate to 5-methyl-tetrahydrofolate, C677T polymorphism, which decreases enzyme activity, may be associated with cancer susceptibility. The aim of this work was to investigate the distribution of MTHFR C677T polymorphism between various types of cancer and cancer-free controls and to assess if there is a difference in frequency.

Materials and methods: 269 Cancer cases (95 prostate cancer, PC; 81 head and neck, HN; and 93 breast cancers, BC) and 102 healthy controls, free of cancer, were genotyped for C677T MTHFR polymorphism using the PCR-RFLP method.

Results: There was no overall difference in C677T genotype distribution between total cancer cohort and controls ($p=0.064$). However, a significant difference and protective OR was found for the C/T genotype (OR = 0.574, 95% CI = 0.352–0.935). In a comparison of different cancer types and respective controls, genotype frequencies were significantly different between head and neck carcinoma and controls ($p=0.004$), again with protective role of C/T genotype (OR = 0.356, 95% CI = 0.189–0.671). Moderate overrepresentation of C/T was found in respective male controls when compared with prostate cancer patients (p value was 0.074 for C/T vs. C/C comparison). The OR for heterozygous C/T genotype in prostate cancer group was 0.404, pointing to its putative protective role. Genotype and allelic frequencies did not differ significantly between 93 breast cancer patients and their 65 age-matched female controls.

Conclusion: Our data indicate that the C677T MTHFR polymorphism does not significantly contribute to the inherited genetic susceptibility to breast and prostate cancer, while we show some evidence for possible genetic contribution of this polymorphism to the development of head and neck carcinoma.

Keywords: MTHFR; Genetic predisposition to disease; Genetic polymorphism; Prostate cancer; Breast cancer; Head and neck cancer

Introduction

It is well known that hereditary factors contribute to cancer susceptibility. There are a vast number of genes already being discovered that may predispose a person to develop cancer. For some of those genetic markers testing is available and recommended to individuals with personal or family history features suggestive of hereditary risk for cancer [1]. Genetic polymorphism of methylenetetrahydrofolate reductase (MTHFR) has during the last decade received an increased attention as a potential genetic marker associated with the cancer risk [2,3]. MTHFR plays a key role in the metabolism of folates, which are nutrients of utmost importance for DNA biosynthesis, methylation and genomic integrity. MTHFR catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyl-tetrahydrofolate, which is the predominant form of folate in circulation and serves as a carbon donor for the remethylation of homocysteine to methionine. Two common polymorphisms in the *MTHFR* gene have been described: C677T and A1298C. The C677T polymorphism has been proven to affect the enzymatic activity and homocysteine level. Homozygous C677T carriers have ap-

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Table 1
Baseline characteristics of cases and controls

	All cases (PC+HN+B, N=269)	PC (N=95)	HN (N=81)	BC (N=93)	Controls (N=102)
Mean age, years (mean±SD)	65±10	69±7	61±13	65±5	69±7
Female (N, %)	99 (37)	0 (0)	8 (10)	91 (98)	65 (64)

proximately 30% and heterozygous 60% of the wild-type enzymatic activity [4]. However, the exact biological relevance of the A1298C polymorphism is still unclear. Individuals carrying the A1298C variant mostly have plasma homocysteine and folate concentrations within normal range.

Considering that methylation abnormalities appear to be important for the pathogenesis of many cancer types, many authors have examined the association between the genotype of the MTHFR C677T polymorphism with various cancers. However, the results of those studies are inconsistent [5–7]. There are several meta-analyses indicating that MTHFR C677T polymorphism could moderately modify the susceptibility to various cancer types [8].

We hypothesized that common C677T polymorphism in the *MTHFR* gene is associated with increased risk of various cancers. To evaluate our hypothesis, we undertook this case–control study of various cancer cases and age-matched controls free of cancer.

Materials and methods

Subjects

269 Cases with various malignancies referred to Sestre milosrdnice University hospital between 2001 and 2005 were included in the study. Cases were diagnosed with following cancer types: prostate cancer, PC (N=95); head and neck, HN (N=81); and breast cancer, BC (N=93). Cases were histologically diagnosed according to the WHO criteria for carcinoma of the prostate [9], breast [10] and head and neck [11].

Controls (N=102) were recruited in the study from the Health Center "Zagreb Zapad," as a part of the community health screening program during the period from 2004 to 2005, and were closely age-matched to cases. Total control cohort served as control group for head and neck cancer, while only male (N=37) and female (N=65) controls served for prostate and breast carcinoma groups, respectively. Control individuals had no personal or family history of cancer. Female controls were women regularly undergoing mammography screening for early breast cancer detection. Male controls were men annually visiting urologist for their PSA laboratory test along with a digital rectal exam (DRE) for early prostate cancer detection. Health status was confirmed by checking their medical records by experienced family physician.

The study was approved by the University hospital ethical committee. A written informed consent was obtained from all individuals included in the study.

All cases and controls were Caucasians of Croatian nationality, residents of the narrow geographic region in the surroundings of Zagreb. Baseline characteristics of the studied groups are presented in Table 1.

Genotyping

Genomic DNA was extracted from EDTA-treated blood using QIAamp DNA Blood Mini Kit (Qiagen, Germany). MTHFR genotyping was performed using a modified method of Skibola et al. [12]. Briefly, primer sequences were: forward: 5′-TGAAGGAGAAGGTGTCTGCGGGA-3′ and reverse: 5′-AGGACGGTGCGGTGAGAGTG-3′. These primers amplified 198-bp fragment with restriction site for *Hin*fI if C677T polymorphism is present. Cycling conditions were as follows: initial preheat 95 °C for 5 min, 30 cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 60 s, and final amplification of 72 °C for 10 min. 13 μL of PCR product was digested with *Hin*fI, and fragments separated on 4% NuSieve® 3:1 Agarose gel (Cambrex, USA): 175-bp and 23-bp fragments for polymorphic T and 198-bp fragment for wild-type C allele. Both negative and positive controls were included for each reaction. Our laboratory participates in external DGKL quality control scheme and has valid DGKL certificate for C677T MTHFR polymorphism genotyping.

Statistical analysis

Distributions were tested for normality using Kolmogorov–Smirnov test. Results are presented as mean, standard deviation. Differences in genotype and allelic frequencies were tested using Chi-squared test (p value, odds ratio and confidence interval was calculated and presented). p Values <0.05 were considered significant. Statistical analysis was performed using the MedCalc® 9.0.1.0 (F. Schoonjans, Belgium).

Results

Both groups (cases and controls) were in Hardy–Weinberg equilibrium (Chi-squared test, p values were 0.981 and 0.212 for cases and controls, respectively). Differences in allelic and genotype distribution for MTHFR C677T polymorphism were tested between a total cohort of cancer patients and controls, as well as across the different cancer type groups and their respective controls. Observed differences are presented in Tables 2 and 3 for a total cohort vs. controls and different cancer types vs. controls respectively.

Table 2
Allelic and genotype differences for MTHFR C677T polymorphism for total cohort vs. controls comparison

MTHFR C677T genotype	Cases (N, %) N=269	Controls (N, %) N=102	OR	95% CI	p value
C/C	123 (45.7)	35 (34.3)	1		p=0.064
C/T	119 (44.2)	59 (57.8)	0.574	0.352–0.935	
T/T	27 (10.1)	8 (7.9)	0.960	0.401–2.301	
C allele	365 (67.8)	129 (63.2)	1		p=0.271
T allele	173 (32.2)	75 (36.8)	0.815	0.582–1.142	