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Association between 11 genetic polymorphisms in folate-metabolising genes and head and neck cancer risk

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ABSTRACT

Genetic polymorphisms in folate metabolism may affect the risk of head and neck cancer (HNSCC) due to its involvement in DNA methylation and synthesis. We conducted a case-control study (265 HNSCC cases and 466 non-cancer controls) to investigate associations of *MTHFR* C677T and A1298C, *MTR* A2756G, *MTRR* A66G, *RFC1* A80G, *MTHFD1* G1958A, *CBS* 844ins68, *TC2* C776G and A67G, *SHMT* C1420T and *BHMT* G742A polymorphisms with HNSCC risk. Interactions between polymorphisms and survival time, tobacco and alcohol habits, age, gender and tumour staging (TNM classification) were evaluated by multiple logistic regression analysis. We found that age $\geqslant$49 years ($P < 0.001$), male gender ($P = 0.03$), tobacco habit ($P < 0.001$), *MTHFR* 1298AC/CC ($P = 0.028$), *MTR* 2756AG/GG ($P = 0.010$) and *RFC1* 80AG/GG ($P = 0.015$) genotypes were associated with an increased risk of HNSCC. There were interactions between lower survival and *CBS* 844ins68 ($P = 0.005$); age $\geqslant$49 years and *MTR* 2756 AG/GG ($P = 0.004$) and *RFC1* 80AG/GG ($P = 0.006$) genotypes; male gender and *MTHFR* 1298 AC/CC ($P = 0.030$), *MTR* 2756 AG/GG ($P = 0.006$) and *RFC1* 80 AG/GG ($P = 0.009$); tobacco non-habit and *MTHFD1* 1958GA/AA ($P = 0.040$); tobacco and *MTHFR* 1298 AC/CC ($P = 0.054$) and *MTR* 2756 AG/GG ($P = 0.010$); alcohol non-consume and *RFC1* 80 AG/GG ($P = 0.008$) with HNSCC increased risk. *MTHFR* C677CT/TT genotypes were less frequently in advanced tumours ($P = 0.04$). In conclusion, our data provide evidence that folate metabolism genetic polymorphisms associated with variables as advanced age, male gender, tobacco and alcohol increase HNSCC development; *CBS* 844ins68 and *MTHFR* C677T polymorphisms are associated with less survival time and advanced stage tumours, respectively.

1. Introduction

Head and neck squamous cell carcinoma (HNSCC) is the fifth most common cancer worldwide and only 40–50% of patients with the disease survive for 5 years.[1,2] In Brazil, it is the fifth most common cancer type among men and seventh type among women.[3] The predominant risk factors are tobacco and alcohol consumption and infection with high-risk types

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of human papillomavirus (HPV).[2] Moreover, associations with genetic polymorphisms in folate metabolising enzymes may support a causal relationship between folate and head and neck carcinogenesis.[4–11]

Biological mechanisms linking folate metabolism genetic polymorphisms to head and neck cancer include an altered provision of S-adenosylmethionine for methylation reactions, DNA methylation, and changes in the availability of nucleotides for DNA synthesis and repair.[6–8,12–14] Studies support the importance of folate pathway polymorphisms in head and neck carcinogenesis, but results are not always consistent.[4–7,9–11,15–18]

The *MTHFR* 677CT and 1298AC/CC genotypes were associated with decreased risk for head and neck cancer.[5,7] The 677CT polymorphism also was associated with increased risk for this disease[6] and other studies did not observe any significant association with the polymorphisms and the disease.[9,15–17] The study of Solomon et al. found that *MTHFR* 677CT genotype is more frequent in etilists patients.[8]

The *MTR* 2756AG or GG genotypes and G polymorphic allele were associated with increased risk for head and neck carcinogenesis in three case-control studies.[4,9,10] However, Suzuki et al. did not find association between the polymorphism and the disease.[15] For *MTRR* A66G polymorphism, Suzuki et al showed no association of *MTRR* 66AG or GG genotypes with head and neck cancer.[15] However, Zhang et al. showed that individuals with the homozygous wild type (*MTRR* 66AA) have a decreased risk for head and neck cancer and may have a joint effect on risk of HNSCC.[4] The *RFC1* A80G variant was evaluated only in one study. Preview study of our research group showed that 80AG or GG genotypes were associated with increased risk for head and neck cancer.[11] The *MTHFD1* and *CBS* 844ins68 polymorphisms also were evaluated and did not demonstrate any association with the disease.[9,18]

As far as we know, there was no investigations of the *TC2* A67G and C776G, *SHMT* C1420T and *BHMT* G742A variants in head and neck cancer risk. Indeed *BHMT* G742A, it was associated with reduced breast cancer-specific mortality and colorectal cancer,[19,20] *TC2* C776G was associated with an increased risk of colorectal adenoma[21] and *SHMT* C1420T was associated with oesophageal squamous cell carcinoma.[22]

Based on the above evidence, in the present study we examined the association between 11 genetic polymorphisms in nine folate-metabolising genes (*MTHFR* C677T and A1298C, *MTR* A2756G, *MTRR* A66G, *RFC1* A80G, *MTHFD1* G1958A, *CBS* 844ins68, *TC2* C776G and A67G, *SHMT* C1420T and *BHMT* G742A) and head and neck cancer risk and explore the potential effect modification of these polymorphisms with variables associated with head and neck cancer risk such as age, gender, tobacco and alcohol habits.

2. Patients and methods

2.1. Subjects

Briefly, 731 individuals were recruited into the study between January 2008 and December 2010 (case group – 233 males; 32 females and 466 controls – 334 males; 132 females). The study protocol was approved by the National Ethics Committee (CONEP-5566/2005; SISNEP 0976.0.140.000-05).

All the cases were recruited from The Hospital of Base, São José do Rio Preto, São Paulo, Brazil. Diagnosis was made from pathological specimens either after total excision or biopsy. Patients with squamous cell carcinoma tumour cell types were included and patients previously treated for this tumour were excluded from this study. Information on lifestyle factors was collected from medical records.

The tumours were staged according to TNM classification following three criteria: extension of the tumour (T), presence of regional lymph node involvement (N) and presence of metastasis at a distance (M).[23] Tumour classification was divided into low T (T1, T2) and high T (T3, T4) classification categories. The N classification was dichotomised into no lymph node involvement (N0) and involvement (N1, N2, N3). The clinical stage (TNM) was used to analyse aggressiveness with tumours being grouped as non-aggressive (low T and no involvement lymphnode) and aggressive (high T and involvement lymphnode).

The control group included Brazilian blood donors without cancer diagnosis according to government guidelines for donated blood that tests for 20 related diseases (http://www.hemonline.com.br/portarias/rdc153/indexframe.htm). Individuals with family history of cancer were excluded and individuals with age higher than 40 years were included in this study. Each eligible subject was interviewed to obtain data on demographic and lifestyle factors.

2.2. Genotyping

DNA was isolated from blood using the methods previously described to Miller et al.[24] The PCR-RFLP assay was used to identify the *MTHFR* C677T (rs1801133) and A1298C (rs1801131), *MTR* A2756G (rs1805087), *RFC1* A80G (rs1051266), *TC2* C776G (rs1801198) and *MTHFD1* G1958A (rs2236225) polymorphisms with *Hinf* I, *Mbo* II, *Hae* III, *HhA*1, *ScrF* I and *Msp I* enzymes, respectively. The allelic discrimination for Real-Time PCR – SNP Genotyping Assay was used to identify the *MTRR* A66G, *BHMT* G742A, *SHMT* C1420T and *TC2* A67G polymorphisms using TaqMan probes in Step One Plus™ Real-Time PCR System equipment (Applied Biosystems). Briefly, for the *MTRR* A66G (rs1801394), as well as *BHMT* G742A (rs3733890) and *TC2* A67G (rs 9606756) polymorphisms extracted DNA was amplified with validated probes (assay ID: C__3068176_10, C__11646606_20 and C__25967461_10, respectively; Applied Biosystems). For *SHMT* C1420T (rs1979277) polymorphism was used probes for wild-type allele (5′-FAM-CGC CTC TCT CTT C-MGB-3′) and polymorphic allele (5′-VIC-CGC CTC TTT CTT C-MGB-3′).[25] The *CBS* 844ins68-bp polymorphism (no rs#) was determined by PCR and 2% agarose gel electrophoresis as described previously.[26]

2.3. Statistical analysis

The distribution of genotypes in case and control groups was tested for deviation from Hardy–Weinberg equilibrium (HWE). Multiple logistic regression analysis was used for comparison between the groups and to obtain the adjusted odds ratio (OR) and 95% confidence interval (95% CI). The multiple adjustment included age (reference: ⩾49 years; median), gender (reference: female), smoking status (reference: non-smokers), alcohol use (reference: non-drinkers) and genotypes