Polymorphisms in *MTHFR*, *MTR*, *RFC1* and *CβS* genes involved in folate metabolism and thyroid cancer: a case-control study

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Abstract

Introduction: Polymorphisms in genes coding enzymes involved in folate metabolism may cause alterations in this metabolic pathway and contribute to carcinogenesis, because folate is essential for DNA synthesis, methylation and repair. The objective of this study was to investigate the association of *MTHFR* 677C>T (rs1801133), *MTR* 2756A>G (rs1805087), *RFC1* 80A>G (rs1051266) and *CβS* 844ins(68) (no rs#) polymorphisms and thyroid cancer development. The association of these polymorphisms with demographic risk factors and clinical histopathological parameters was also evaluated.
Material and methods: The study is a case-control analysis with a total of 462 individuals (151 patients and 311 controls). Polymerase chain reaction-restriction fragment length polymorphism technique was used for genotyping. The χ^2 and multiple logistic regression were utilized for statistical analysis.
Results: The polymorphisms analysis revealed an association between the *MTHFR* 677C>T polymorphism (OR = 2.87, 95% CI: 1.50–5.48, $p < 0.01$, co-dominant model), (OR = 1.76, 95% CI: 1.18–2.64, $p < 0.01$, dominant model), (OR = 2.37, 95% CI: 1.28–4.39, $p < 0.01$, recessive model) and thyroid cancer. *RFC1* 80A>G polymorphism also was associated with thyroid cancer under recessive mode of inheritance (OR = 1.55; 95% CI: 1.02–2.38; $p = 0.04$); however, this polymorphism showed Hardy-Weinberg disequilibrium in the control group ($\chi^2 = 24.71$, $p < 0.001$). Furthermore, alcohol (OR = 1.56, 95% CI: 1.36–1.89, $p < 0.01$) and tobacco consumption (OR = 1.97, 95% CI: 1.28–3.04, $p < 0.01$) were associated with increased risk for thyroid cancer. The *MTR* 2756A>G polymorphism showed an association with tumor extent (OR = 2.69, 95% CI: 1.27–5.71, $p < 0.01$) and aggressiveness (OR = 4.51, 95% CI: 1.67–12.1, $p < 0.01$).
Conclusions: *MTHFR* 677C>T is significantly associated with increased risk for thyroid cancer and *MTR* 2756A>G is associated with tumor extent and aggressiveness. In addition, alcohol and tobacco consumption were associated with increased risk of thyroid cancer. These results may contribute to a better prognosis for thyroid cancer.

Key words: genes, genetic polymorphism, thyroid cancer.

Introduction

Thyroid cancer is the most common malignancy of the endocrine system. There are four main types: papillary, follicular, medullary and anaplastic. In Brazil in 2018 there were about 9610 new cases of thyroid cancer and this cancer is the eighth most common type in women [1].

Some risk factors were evaluated for thyroid cancer development, such as gender, age, hormonal factors, family history of cancer, alcohol and tobacco consumption and obesity [2, 3]. Moreover, genetic polymorphisms involved in folate metabolism are related to carcinogenesis, which leads to development of several types of cancer [4–6]. However, results in the literature are still controversial. Furthermore, research addressing the folate pathways is limited in thyroid cancer, and further studies are required in this area [7, 8].

Folate metabolism is involved in synthesis, methylation and DNA repair, and several genes including methylenetetrahydrofolate reductase (*MTHFR*), methionine synthase (*MTR*), reduced folate carrier 1 (*RFC1*) and cystathionine β-synthase (*CβS*) regulate this metabolism. The genetic polymorphisms change enzymatic activity, resulting in DNA hypomethylation and genomic instability [9–11].

The RFC1 enzyme is responsible for absorption and intracellular transport of folate, besides transporting 5-MTHFR to the interior of variety cells, being an important determinant of folate intracellularly concentrations. The gene is polymorphic in exon 2, with substitution of adenine for guanine at nucleotide 80A>G (rs 1051266), affecting plasma folate and homocysteine levels [12, 13]. The MTHFR enzyme, encoded by the *MTHFR* gene, is responsible for the catalysis of the irreversible reaction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which interferes in DNA synthesis and methylation. It supplies a methyl group for methylation of homocysteine, producing methionine [14]. The *MTHFR* gene presents a substitution of cytosine by thymine at nucleotide 677C>T (rs1801133) that may be associated with carcinogenesis.

The homocysteine remethylation to methionine is catalyzed by MTR enzyme. This reaction is essential to adequately maintain normal methionine and intracellular homocysteine concentrations. The *MTR* gene presents a transition of adenine to guanine at position 2756 A>G (rs1805087). This polymorphism is related to increased homocysteine in the plasma and DNA hypomethylation, thus possibly influencing the risk of cancer [15].

The *CβS* gene encodes cystathionine β-synthase, which is the central enzyme in the transsulfuration pathway that irreversibly metabolizes homocysteine (removes homocysteine from the methionine) to cystathionine. It is polymorphic in exon 8 with an insertion of 68 base pairs at nucleotide 844ins(68). This polymorphism has been associated with reduction of homocysteine levels and changes in DNA methylation because of low availability of S-adenosylmethionine, the main methyl donor for methylation reactions, and consequently DNA hypomethylation and carcinogenesis may occur [16].

The aims of the present study were to investigate associations between *MTHFR* 677C>T, *MTR* 2756A>G, *RFC*1 80A>G and *CβS* 844ins(68) polymorphisms and thyroid cancer patients, to compare the results with subjects without cancer, and to evaluate the association of polymorphisms with risk factors (gender, age, alcohol and tobacco consumption, body mass index – BMI) and clinical histopathological parameters.

Material and methods

Subjects

This study protocol was approved by the Ethics Research Committee (20187413.8.0000.5415). All individuals who agreed to participate in the study signed an informed consent form. A total of 462 individuals (151 patients and 311 controls) were evaluated in this case-control study. The case group consisted of 151 patients who were diagnosed with thyroid cancer (125 papillary and 26 follicular) at Hospital de Base, São Jose do Rio Preto, Brazil. The definitive diagnosis was made through examining the results of imaging studies, histopathological analysis, and biopsies. The exclusion criteria were patients with other neoplasms. The tumors were classified based on three criteria of the Union of Cancer Control (UICC) 2010 (http: www.uicc.org – accessed in January 2016) – tumor extent (T), presence of regional lymph node involvement (N) and presence of distant metastasis (M). The clinical stage was used to analyze aggressiveness, being stage I and II (non-aggressive); stage III and IV (aggressive). The presence or absence of extrathyroid extension was also evaluated.

The control group included 311 healthy individuals from the Hospital de Base of the city of São Jose do Rio Preto. Individuals were excluded if they had a family history of cancer, other neoplasms, and chronic disease.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes by the method described by Miller *et al.* [17] with modifications. The genotyping of *CβS* 844ins(68) polymorphisms was determined by PCR. The PCR-RFLP assay was used to identify the *MTHFR* 677C>T (rs1801133), *MTR* 2756A>G (rs1805087) and RFC1 80A>G (rs1051266) polymorphisms with *Hinf I*, *Hae III* and *Hha I* en-

zymes, respectively. The resulting fragments were: *MTHFR* gene (C allele – 198 bp); T allele – 175 bp, 23 bp); *MTR* gene (A allele – 413 bp, 85 bp; G allele – 290 bp, 123 bp, 85 bp); *RFC1* gene (A allele – 162 bp, 68 bp; G allele – 125 bp, 68 bp, 37 bp); and *CβS* gene (Wild allele – 171 bp; polymorphic allele – 239 bp).

The genotyping confirmation was accomplished in 10% of random samples of each group, and we observed 100% concordance. The primer sequences used for amplification of the region presenting these polymorphisms are described in Table I.

Statistical analysis

The Hardy-Weinberg equilibrium (HWE) was assessed using the χ^2 test (BioEstat 5.4 program) to evaluate the distribution of genotypes in case and control groups. The multiple regression logistic test was performed by the Minitab/Version 14.0 computer program, adjusting for gender (reference: male), age (reference: < 50 years), alcohol consumption (reference: no alcohol consumption), tobacco consumption (reference: nonsmoking), BMI (reference: < 24.9), *MTHFR* 677C>T (reference: genotype CC), *MTR* 2756A>G (reference: genotype AA), *RFC1* 80A>G (reference: genotype AA) and *CβS* 844ins(68) (reference: homozygous without insertion). In this study, we considered smokers those who smoked > 100 cigarettes in their lifetime and drinkers those who have at least 4 drinks per week. One drink is equivalent to 30 ml of liquor; 102 ml of wine, and 340 ml of beer [18]. Subjects with BMI ≥ 25.0 kg/m³ were considered obese. The clinical histopathological parameters also were evaluated by multiple logistic regression.

SNPstat online computer program (available: (<http://bioinfo.iconcologia.net/SNPstats>) was used to analyze the polymorphisms' effect in models (1) codominant (heterozygous versus homozygous wild type and polymorphic homozygous versus homozygous wild type), (2) dominant (heterozygous and polymorphic homozygous versus homozygous wild type), (3) recessive (polymorphic homozygous versus homozygous wild type and heterozygous), (4) overdominant (heterozygous versus homozygous wild type and polymorphic homozygous), (5) additive (weight polymorphic homozygote and heterozygote versus homozygous wild-type).

The results were presented as odds ratio (OR), 95% confidence interval (95% CI) and a value of $p < 0.05$ was considered significant.

Results

Table II shows the association of *MTHFR* 677C>T, *MTR* 2756A>G, *RFC1* 80A>G and *CβS* 844ins(68) polymorphisms with thyroid cancer according to genetic models. For *MTHFR* 677C>T polymorphism the association with increased risk was observed for codominant, dominant and recessive models ($p < 0.01$). *RFC1* 80A>G polymorphism was associated with thyroid cancer in recessive model (OR = 1.55; 95% CI: 1.02–2.38; $p = 0.04$). The *MTR* 2756A>G and *CβS* 844ins(68) polymorphisms were not associated with thyroid cancer.

The Hardy-Weinberg equilibrium analysis showed that the genotypic frequencies of *MTHFR* 677C>T, *MTR* 2756A>G, *RFC1* 80A>G and *CβS* 844ins(68) polymorphisms are in equilibrium in the patients (*MTHFR* 677C>T: $\chi^2 = 1.79$, $p = 0.17$;

Table I. List of primer sequences

Polymorphism	Sequence of primers
MTHFR 677C>T:	
Sense	5'- TGA AGG AGA AGG TGT CTG CGG GA 3'
Antisense	5'- AGG ACG GTG CGG TGA GAG TG 3'
MTR 2756A>G:	
Sense	5'- CCA GGG TGC CAG GTA TAC AG 3'
Antisense	5'- GCC TTT TAC ACT CCT CAA AAC 3'
RFC1 80A>G:	
Sense	5'- AGT GTC ACC TTC GTC CC 3'
Antisense	5'- TCC CGC GTG AAG TTC TTG 3'
CβS 844ins(68):	
Sense	5'- GTT GTT AAC GGC GGT ATT GG 3'
Antisense	5'- GTT GTC TGC TCC GTC TGG TT 3'

Table II. Association between *MTHFR* 677C>T, *MTR* 2756A>G, *RFC1* 80A>G and *CβS* 844ins(68) polymorphisms and thyroid cancer

Model	Genotype	Control *n* (%)	Case *n* (%)	OR+ (95% CI)	*P*-value*	Genotype	Control *n* (%)	Case *n* (%)	OR+ (95% CI)	*P*-value*
			MTHFR 677C>T					*MTR* 2756A>G		
Codominant	C/C	174 (56)	63 (41.7)	1.00		A/A	197 (63.3)	89 (58.9)	1.00	
	C/T	114 (36.7)	63 (41.7)	1.53 (0.99–2.35)	< 0.01*	A/G	100 (32.1)	50 (33.1)	1.13 (0.74–1.74)	0.4
	T/T	23 (7.4)	25 (16.6)	2.87 (1.50–5.48)		G/G	14 (4.5)	12 (8)	1.75 (0.77–4.02)	
Dominant	C/C	174 (56)	63 (41.7)	1.00		A/A	197 (63.3)	89 (58.9)	1.00	
	C/T-T/T	137 (44)	88 (58.3)	1.76 (1.18–2.64)	< 0.01*	A/G-G/G	114 (36.7)	62 (41.1)	1.21 (0.81–1.82)	0.35
Recessive	C/C-C/T	288 (92.6)	126 (83.4)	1.00		A/A-A/G	297 (95.5)	139 (92)	1.00	
	T/T	23 (7.4)	25 (16.6)	2.37 (1.28–4.39)	< 0.01*	G/G	14 (4.5)	12 (8)	1.68 (0.74–3.80)	0.22
Overdominant	C/C-T/T	197 (63.3)	88 (58.3)	1.00		A/A-G/G	211 (67.8)	101 (66.9)	1.00	
	C/T	114 (36.7)	63 (41.7)	1.24 (0.83–1.87)	0.3	A/G	100 (32.1)	50 (33.1)	1.08 (0.70–1.64)	0.74
Additive	–	–	–	1.64 (0.88–1.90)	0.3	–	–	–	1.23 (0.89–1.70)	0.22
			RFC1 80A>G					*CβS* 844ins(68)		
Codominant	A/A	125 (40.2)	45 (29.8)	1.00		W/W	251 (80.7)	119 (78.8)	1.00	
	A/G	109 (35)	65 (43)	1.71 (1.07–2.74)	0.07	W/Ins	56 (18)	30 (19.9)	1.17 (0.71–1.94)	0.83
	G/G	77 (24.8)	41 (27.1)	1.35 (0.80–2.27)		Ins/Ins	4 (1.3)	2 (1.3)	1.07 (0.19–6.11)	
Dominant	A/A	125 (40.2)	45 (29.8)	1.00		W/W	251 (80.7)	119 (78.8)	1.00	
	A/G-G/G	186 (59.8)	106 (70.2)	1.02 (0.65–1.60)	0.94	W/Ins - Ins/Ins	60 (19.3)	32 (21.2)	1.16 (0.71–1.90)	0.55
Recessive	A/A-A/G	234 (75.2)	110 (72.8)	1.00		W/W - W/Ins	307 (98.7)	149 (98.7)	1.00	
	G/G	77 (24.8)	41 (27.1)	1.55 (1.02–2.38)	0.04*	Ins/Ins	4 (1.3)	2 (1.3)	1.04 (0.18–5.92)	0.97
Overdominant	A/A-G/G	202 (65)	86 (57)	1.00		W/W - Ins/Ins	255 (82)	121 (80.1)	1.00	
	A/G	109 (35)	65 (43)	1.51 (1.00–2.27)	0.05	W/Ins	56 (18)	30 (19.9)	1.17 (0.71–1.93)	0.55
Additive	–	–	–	1.18 (0.92–1.53)	0.19	–	–	–	1.13 (0.73–1.77)	0.58

*+Odds ratio (OR) adjusted for gender, age, alcohol and tobacco consumption and body mass index (BMI). W/W (homozygous wild type without 68 base pairs (bp) insertion; W/Ins (heterozygous genotype); Ins/Ins (homozygous polymorphic with 68 base pairs (bp) insertion). *Significant at p < 0.05.*

Table III. Distribution of demographic risk factors in patients with thyroid cancer and control individuals

Variable	Patients (n = 151) n (%)	Controls (n = 311) n (%)	OR+ (95% CI)	P-value*
Gender:				
Male	15 (9.94)	37 (11.90)	1.00 (ref.)	
Female	136 (90.06)	274 (88.10)	1.07 (0.55–2.10)	0.84
Age [years]:				
< 50	73 (48.34)	174 (55.94)	1.00 (ref.)	
≥ 50	78 (51.66)	137 (44.66)	1.21 (0.80–1.81)	0.36
Alcohol consumption:				
No	107 (70.87)	191 (61.42)	1.00 (ref.)	
Yes	44 (29.13)	120 (38.58)	0.56 (0.36–0.89)	< 0.001*
Tobacco consumption:				
No	89 (58.95)	222 (71.38)	1.00 (ref.)	
Yes	62 (41.05)	89 (28.62)	1.97 (1.28–3.04)	< 0.001*
BMI [kg/m²]:				
< 25.0	40 (26.49)	99 (31.83)	1.00 (ref.)	
≥ 25.0	111 (73.51)	212 (68.17)	1.24 (0.79–1.94)	0.35

*+Odds ratio (OR) adjusted for gender, age, alcohol and tobacco consumption, body mass index (BMI) and polymorphisms. *Significant at $p < 0.05$.*

MTR 2756A>G: $\chi^2 = 1.66$, $p = 1.19$; *RFC1* 80A>G: $\chi^2 = 2.89$, $p = 0.08$; *CβS* 844ins(68): $\chi^2 = 0.49$, $p = 0.94$). In the control group, the *MTHFR* 677C>T ($\chi^2 = 0.51$, $p = 0.47$), *MTR* 2756A>G ($\chi^2 = 0.08$, $p = 0.77$) and *CβS* 844ins(68) polymorphisms ($\chi^2 = 0.18$, $p = 0.66$) were in equilibrium. For the *RFC1* 80A>G polymorphisms the control group showed disequilibrium ($\chi^2 = 24.71$, $p < 0.001$).

The multiple logistic regression analysis (adjusted for gender, age, alcohol and tobacco consumption, BMI and polymorphisms) showed that alcohol consumption (OR = 1.56; 95% CI: 1.36–1.89; $p < 0.001$) and tobacco consumption (OR = 1.97; 95% CI: 1.28–3.04; $p < 0.001$) were associated with the disease. However, there was no association between gender (OR = 1.07; 95% CI: 0.55–2.10; $p = 0.84$), age ≥ 50 years (OR = 1.21; 95% CI: 0.80–1.81; $p = 0.36$), BMI (OR = 1.24; 95% CI: 0.79–1.94; $p = 0.35$) and thyroid cancer (Table III).

Regarding clinical histopathological parameters of thyroid cancer, Table IV shows the results for polymorphisms' association analysis with these parameters. The polymorphism *MTR* 2756A>G is associated with tumor extension (OR = 2.69; 95% CI: 1.27–5.71; $p = 0.01$) and aggressiveness (OR = 4.51; 95% CI: 1.67–12.1; $p = 0.01$). The other polymorphisms showed no association with tumor extent (T), regional lymph node involvement (N) and aggressiveness (Table IV). There was no association between extrathyroid extension and analyzed polymorphisms (Table V). Analyses of the clinical histopathological parameters were performed in the dominant model.

Discussion

In the present study, we evaluated the association of *MTHFR* 677C>T, *MTR* 2756A>G, *RFC1* 80A>G and *CβS* 844ins(68) polymorphisms involved in folate metabolism in thyroid cancer. The association of these polymorphisms with demographic risk factors and clinical histopathological parameters was also assessed. We found an association of the *MTHFR* 677C>T and *RFC1* 80A>G polymorphisms and increased risk for thyroid cancer. Alcohol and tobacco consumption were associated with development of this disease.

In addition, we did not observe the HWE in the control group for *RFC1* 80A>G polymorphism; thus these results should be interpreted with caution, because case-control studies assume the existence of HWE equilibrium at least in controls. The observed HWE disequilibrium may be due to random selection samples, model, and disease complexity [19].

Alterations in folate can be associated with polymorphisms. The synthesis of purines and pyrimidine, DNA methylation and repair is directly affected, because folate is a relevant precursor substance for normal cell metabolism. Methylation, in turn, is responsible for gene expression

Table IV. Distribution of clinical histopathological parameters in relation to *MTHFR* 677C>T, *MTR* 2756A>G, *RFC1* 80A>G and *CβS* 844ins(68) polymorphisms in patients with thyroid cancer

Variable	Tumor extent				Regional lymph node involvement				Aggressiveness			
	T1/T2	T3/T4	OR+ (95% CI)	*P*-value*	*N* = 0	*N* ≥ 1	OR+ (95% CI)	*P*-value*	Non-aggressive	Aggressive	OR+ (95% CI)	*P*-value*
	n (%)	*n* (%)			*n* (%)	*n* (%)			*n* (%)	*n* (%)		
	108 (71.52)	**43 (28.48)**			**125 (82.78)**	**26 (17.22)**			**118 (78.14)**	**33 (21.86)**		
MTHFR:												
C/C	44 (40.74)	19 (44.18)	1.00		50 (40.00)	13 (50.00)	1.00		52 (44.06)	11 (33.34)	1.00	
C/T – T/T	64 (59.26)	24 (55.82)	0.73 (0.34–1.56)	0.41	75 (60.00)	13 (50.00)	0.72 (0.29–1.75)	0.46	66 (55.94)	22 (66.66)	0.98 (0.37–2.63)	0.97
MTR:												
A/A	69 (63.88)	20 (46.51)	1.00		74 (59.20)	15 (57.69)	1.00		76 (64.40)	13 (39.40)	1.00	
A/G – G/G	39 (36.12)	23 (53.49)	2.69 (1.27–5.71)	0.01*	51 (40.80)	11 (42.31)	1.07 (0.44–2.65)	0.87	42 (35.60)	20 (60.60)	4.51 (1.67–12.1)	< 0.01*
RFC1:												
A/A	33 (30.55)	12 (27.90)	1.00		38 (30.40)	07 (26.92)	1.00		38 (32.20)	07 (21.22)	1.00	
A/G – G/G	75 (69.45)	31 (72.10)	0.83 (0.36–1.90)	0.65	87(69.60)	19 (73.08)	1.63 (0.58–4.56)	0.35	80 (67.80)	26 (78.78)	1.08 (0.36–3.26)	0.89
CβS:												
W/W	83 (76.85)	36 (83.72)	1.00		101(80.80)	18 (69.23)	1.00		91 (77.11)	28 (84.84)	1.00	
W/Ins – Ins/Ins	25 (23.15)	07 (16.28)	0.68 (0.26–1.75)	0.42	24 (19.20)	08 (30.77)	1.95 (0.72–5.28)	0.19	27 (22.89)	05 (15.16)	0.64 (0.19–2.19)	0.18

*+Odds ratio (OR) adjusted for gender, age, alcohol and tobacco consumption. W/W (homozygous wild type without 68 base pairs (bp) insertion); W/Ins (heterozygous genotype); Ins/Ins (homozygous polymorphic with 68 base pairs (bp) insertion). *Significant at p < 0.05.*

Table V. Association between *MTHFR* 677C>T, *MTR* 2756A>G, *RFC1* 80A>G and C*ßS* 844ins(68) polymorphisms and extrathyroid extension

Variable	Absence *n* (%)	Presence *n* (%)	OR+ (95% CI)	*P*-value*
MTHFR 677C>T:				
C/C	49 (41.88)	14 (41.17)	1.00	
C/T – T/T	68 (58.12)	20 (58.83)	0.85 (0.37–1.94)	0.69
MTR 2756A>G:				
A/A	72 (61.53)	17 (50.00)	1.00	
A/G – G/G	45 (38.47)	17 (50.00)	1.72 (0.77–3.87)	0.18
RFC1 80A>G:				
A/A	37 (31.62)	08 (23.52)	1.00	
A/G – G/G	80 (68.38)	26 (76.48)	1.43 (0.56–3.68)	0.45
CßS 844ins(68):				
W/W	91 (77.77)	28 (82.35)	1.00	
W/Ins – Ins/Ins	26 (22.23)	06 (17.65)	0.77 (0.28–2.15)	0.62

*+Odds ratio (OR) adjusted for gender, age, alcohol and tobacco consumption, BMI and polymorphisms. *Significant at p < 0.05.*

control, chromatin structure and genomic stability [10, 20, 21].

In the present study, we found an association with *MTHFR* 677C>T and increased risk for thyroid cancer. The studies in the literature are controversial; a study in a Saudi Arabian population reported no association of this genetic variant [22]. Another study performed with papillary carcinoma also no found evidence supporting an association with this polymorphism [21]. On the other hand, a study performed in Turkey involving 60 cases and 50 controls also found increased risk for thyroid cancer [23], as well as the study by Fard-Esfahani *et al.* [24] conducted with the Iranian population. A meta-analysis on thyroid cancer involving four studies showed a significant association between this cancer and *MTHFR* 677C>T polymorphism [8]. A Brazilian study involving 100 patients with thyroid cancer and 100 with breast cancer found an increased risk for the 677TT genotype of the *MTHFR* gene in both types of cancer, which is very common in women worldwide [25].

Our results also revealed increased risk of thyroid cancer for genotype GG of *RFC1* polymorphism. However, the association of *RFC1* 80A>G polymorphism in thyroid cancer has not been analyzed, but was studied in others kinds of cancers. This is the first molecular epidemiological study of this polymorphism in thyroid cancer. In cervical cancer, Di *et al.* [26] found increased risk for variant 80GG in a Chinese population. Galbiatti *et al.* [13] found an association with head and neck cancer in males with age > 50 years. Wang *et al.* [27] and De Jonge *et al.* [28] also founded an association between this polymorphism in gastroesophageal cancer and pediatric acute lymphoblastic leukemia, respectively. On the other hand, studies performed with breast cancer [29] and colorectal cancer [12] did not find an association with this polymorphism.

In this study, the *MTR* 2756A>G and *CßS* 844ins(68) polymorphisms were not statistically significant; both had never been studied in thyroid cancer. In breast cancer, two studies did not find an association with this polymorphism [11, 20]. Zhou *et al.* [30] also did not find increased risk for colorectal cancer. In a meta-analysis Zhao *et al.* [31] showed no association with *MTR* 2756A>G polymorphism and digestive system cancer development, unlike a case-control study performed with a Brazilian population that found an association of *MTR* 2756A>G polymorphism and head and neck cancer [32]. The *MTR* 2756A>G polymorphism is with the elevation of homocysteine level and DNA hypomethylation due to decreased MTR enzyme [33]. Regarding *CßS* 844ins(68), a study with a Mexican population showed increased risk for breast cancer [16], unlike the study with head and neck cancer that did not find an association with this polymorphism [34].

In the present study, alcohol consumption (OR = 1.56; 95% CI: 1.36–1.89; $p < 0.001$) was associated with thyroid cancer development, unlike the study performed by Kabat *et al.* [35], who found no association between alcohol consumption and thyroid cancer development. Tobacco consumption (OR = 1.97; 95% CI: 1.28–3.04; $p < 0.001$) also was significant in this study. The meta-analysis performed by Jie Ma *et al.* [36]

showed a increase of thyroid cancer risk in obesity, regardless of smoking status. Cho *et al.* [37] observed that smoking may influence susceptibility to thyroid cancer, as our study.

Gender, age ≥ 50 years and BMI ≥ 25.0 kg/m³ were not associated with thyroid cancer. A study performed with a European population showed an association with obesity and thyroid cancer risk only in women [38]. Several others studies also showed increased risk for thyroid cancer in subjects with increased weight [36, 39].

Regarding clinical histopathological parameters, the *MTR* 2756A>G polymorphism is associated with tumor extent (T) (OR = 2.69; 95% CI: 1.27–5.71; $p < 0.01$) and aggressiveness (OR = 4.51; 95% CI: 1.67–12.1; $p < 0.01$). In addition, there was not a significant result for regional lymph node involvement (N), extrathyroid extension. Moreover, there are no previous studies in thyroid cancer evaluating these clinical variables and polymorphisms in genes involved in folate metabolism.

Our study may be limited by sample size, and the time of sample collection was relatively short. Studies with polymorphisms involved in the folate pathway and thyroid cancer are still sparse in the literature. *MTR* 2756A>G, *CβS* 844ins(68) and *RFC1* 80A>G polymorphisms have not yet been analyzed in thyroid cancer; hence the analysis of these molecular biomarkers may be important for a better understanding of thyroid cancer risk.

In conclusion, our data demonstrate the influence of *MTHFR* 677C>T polymorphism in thyroid cancer development in the population studied. The results concerning *RFC1* polymorphism should be interpreted with caution due to Hardy-Weinberg disequilibrium in this study. The tumor extent and aggressiveness may be influenced by *MTR* 2756A>G polymorphism. In addition, alcohol and tobacco consumption are associated with increased risk of this disease. These results may contribute to a better prognosis of thyroid cancer.

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Conflict of interest

The authors declare no conflict of interest.

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