CLINICAL SCIENCE

Interleukin-10 but not *interleukin-18* may be associated with the immune response against well-differentiated thyroid cancer

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OBJECTIVES: The aim of this study was to investigate the role of the *interleukin-18* +105A/C and *interleukin-10* -1082A/G germline polymorphisms in the development and outcome of differentiated thyroid carcinoma associated or not with concurrent thyroiditis.

METHODS: We studied 346 patients with differentiated thyroid carcinomas, comprising 292 papillary carcinomas and 54 follicular carcinomas, who were followed up for 12-298 months (mean 76.10 $\pm$ 68.23 months) according to a standard protocol. We genotyped 200 patients and 144 control individuals for the *interleukin-18* +105A/C polymorphism, and we genotyped 183 patients and 137 controls for the i*nterleukin-10* -1082A/G polymorphism.

RESULTS: *Interleukin-18* polymorphisms were not associated with chronic lymphocytic thyroiditis or any clinical or pathological feature of tumor aggressiveness. However, there was an association between the presence of *interleukin-10* variants and chronic lymphocytic thyroiditis. Chronic lymphocytic thyroiditis was present in 21.74% of differentiated thyroid carcinoma patients, most frequently affecting women previously diagnosed with Hashimoto's thyroiditis who had received a lower ^{131}I cumulative dose and did not present lymph node metastases.

CONCLUSIONS: We conclude that the inheritance of a G allele at the *interleukin-10* -1082A/G polymorphism may favor a concurrent thyroid autoimmunity in differentiated thyroid carcinoma patients, and this autoimmunity may favor a better prognosis for these patients.

KEYWORDS: Tumor immunity; *Interleukin-10*; *Interleukin-18*; Chronic lymphocytic thyroiditis; Immunogenetics.

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INTRODUCTION

An association between chronic lymphocytic thyroiditis (CLT), also known as Hashimoto's thyroiditis, and differentiated thyroid carcinoma (DTC), especially the papillary histotype, has long been recognized and supported by a series of epidemiological studies.[1,2] Chronic inflammatory infiltrates and chronic inflammatory thyroiditis are observed in 20-50% of papillary thyroid carcinoma (PTC) cases.[3,4] Moreover, autoimmune thyroid diseases and DTC share numerous morphological and molecular traits.[5,6] However, the relationship between autoimmune thyroid diseases and DTC remains controversial. On the one hand, chronic inflammation related to Hashimoto's thyroiditis might predispose individuals to neoplastic transformation and cancer development.[6] Several cytokines, including interleukin (IL)-1, IL-6 and tumor necrosis factor (which are produced by tumor cells), and tumor-associated leukocytes and platelets have been found to contribute to the invasive phenotype.[7] On the other hand, genetic alterations implicated in PTC, such as *RET/PTC, BRAFV600E* and *RASG12V*, have been demonstrated to help promote a proinflammatory environment characterized by high levels of proinflammatory cytokines that provide a protumorigenic condition.[8] Thyroid cancer cells also secrete several cytokines and chemokines necessary to sustain cancer cell growth and recruit leukocytes to tumor sites.[7,9]

Interleukin-18 has multiple functions, including the induction of interferon-gamma synthesis by T cells and natural killer cells,[10] the promotion of T-helper (Th) 1-type immune

responses, the augmentation of the proliferative response, and activated T-cell cytokine production.[11] The *IL-18* gene is located on chromosome 11q22.2-q22.3 and consists of six exons and five introns. The promoter region of the gene can harbor nucleotide variations that affect *IL-18* synthesis.[12] An *IL-18* gene polymorphism at position +105 has been linked to increased production of this cytokine.[13]

Interleukin-10 is a pleiotropic cytokine with both anti-inflammatory and anti-angiogenic properties that may be involved in the pathogenesis of autoimmune thyroid diseases.[14] It is normally produced by activated T cells, monocytes, B cells, and thymocytes, contributing to antigen- or mitogen-driven B cell differentiation, acting as a growth factor, and stimulating the humoral immune response.[15] The *IL-10* gene is located on chromosome 1q31-q32. Thyroid cancer cells of all histological variants produce considerable amounts of *IL-10*, which might be important in the pathological features of thyroid cancer and/or outcome.[16] A polymorphism at codon -1082 has been associated with increased *IL-10* protein production.[17]

The aim of this study was to investigate the roles of the *IL-18* +105A/C and *IL-10* -1082A/G germline polymorphisms in the development and outcome of DTC associated or not with concurrent thyroiditis.

MATERIALS AND METHODS

Subjects

This retrospective case-control study was approved by the Research Ethics Committee of the Faculty of Medical Sciences – University of Campinas (FCM-Unicamp), and informed written consent was obtained from all individuals. The study was designed and conducted in accordance with good clinical practice guidelines, the relevant laws regarding the conduct of clinical studies, and the Declaration of Helsinki. The study population comprised 346 patients with DTC (292 PTC cases and 54 follicular carcinoma [FTC] cases) who underwent surgery between 1999 and 2007 in Hospital das Clínicas – Faculty of Medical Sciences – Unicamp. Thyroid carcinoma had been either diagnosed or suspected in these patients for clinical or epidemiological reasons, including the results of fine-needle aspiration cytology and/or histological analysis. All of the patients underwent total or near-total thyroidectomy. Patients with preoperatively or intraoperatively palpable neck node metastases underwent regional neck dissection. The stage and grade of tumor differentiation were obtained from surgical and pathological records. Experienced pathologists of the Hospital das Clínicas – Faculty of Medical Sciences – Unicamp confirmed all diagnoses. All the cases were managed according to a standard protocol. Total body ^{131}I scans were performed 4-6 weeks after the operations. A total of 27 patients who were classified as very low-risk – patients with unifocal microcarcinoma (T1$\leq$1 cm, according to the International Union Against Cancer [AJCC/UICC] staging system) and no extension beyond the thyroid capsule or distant metastases (N0M0) – did not receive any actinic dose after ablation; all other patients received at least 100 mCi of ^{131}I. Long-term levothyroxine therapy at suppressive doses was administered following total body scans to keep serum thyrotropin (TSH) at low-normal levels.

Data on lifetime occupational history, dietary habits, alcohol and drug consumption, medical history with an emphasis on previous and/or current thyroid diseases, family history of DTC, and other anamnestic data were obtained by interviews, using a structured questionnaire. Individuals with a history of accidental or medical radiation exposure or other antecedent malignancies were excluded. Skin color was determined by the interviewer in accordance with the Brazilian Institute of Geography and Statistics (http://www.ibge.gov.br/english/); however, due to the difficulty in classifying our highly heterogeneous population, we grouped it into Caucasian and non-Caucasian. Cigarette smoking habits were recorded; however, due to the limited reliable data obtained on smoking duration, the age at which smoking began, the quantity smoked, and the time elapsed since smoking cessation, the patients were grouped into never smokers and ever smokers. The latter group comprised individuals who had consumed at least 20 packs (at 20 cigarettes per pack) for 1 of the prior 5 years. All data, including nodule size, tumor histological features, and the results of laboratory examinations, were confirmed by the patients' records.

Follow-up

According to a routine protocol based on American Thyroid Association and Latin American Thyroid Society recommendations,[18,19] the follow-up of cancer patients included periodic total body scans, serum TSH and thyroglobulin (Tg) measurements and other eventual procedures for detecting distant metastases for a period of 1-336 months (76.10 ± 68.23 months). Patients presenting with or suspected of high non-stimulated serum Tg levels (>2 mg/dl) underwent total body scans. We defined tumors as recurrent and/or presenting long-distance metastasis according to the above parameters.

According to the total actinic ablation received prior to this study, the patients were divided into two groups: (a) patients with cumulative doses bellow 250 mCi and (b) patients with cumulative doses above 250 mCi.

Controls

We selected 416 healthy controls from the general population of our region; we considered these individuals to have a normal iodine intake, and they were matched to the patients on the basis of gender, age, ethnicity, and environmental exposure risk. Hence, the 200 DTC patients genotyped for the *IL-18* polymorphism were compared with 144 control individuals, and the 183 patients genotyped for the *IL-10* polymorphism were compared with 137 controls. The history obtained from these control subjects included demographic and ethnic background, diet, lifetime occupational history, smoking and drinking history, general health conditions, and disease history. Individuals with a history of thyroid disease, radiation exposure, specific environmental or occupational exposure risks, or antecedent malignancy were excluded.

Histopathology

Experienced pathologists confirmed the diagnosis of PTC in 292 patients (159 with the classical form of the disease, 88 with follicular variants, 23 with tall-cell variants, and 22 with less-differentiated variants) and FTC in 54 cases (all clearly invasive tumors). CLT was investigated in the non-malignant thyroid parenchyma of all the DTC patients. The presence of thyroiditis was confirmed by extensive diffuse lymphocytic infiltration with the formation of lymphoid follicles or reactive germinal centers and scarring, as well as

follicular regenerative activity in the form of numerous small follicles frequently lined by Hurthle cells. The Hurthle cells had abundant eosinophilic cytoplasm, enlarged hyperchromatic nuclei, mildly to moderately pleomorphic nuclei, and prominent nucleoli. Peritumoral inflammatory response was not considered chronic lymphocytic thyroiditis.

Identification of Genotypic Profiles

Blood specimens were obtained from patients and controls. Genomic DNA was extracted from frozen specimens, and leukocytes were separated from whole blood using a standard proteinase K-phenol-chloroform protocol. Both the *IL-18* (+105A/C) and *IL-10* (-1082A/G) genotypes were analyzed by the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods. Genotyping was conducted with blinding to case/control status. The primers for *IL-18* +105A/C were forward 5′- AGA TTT AAT GTT TAT TGT AGA AAA CCT GGA CTC -3′ and reverse 5′- CAG TCA TAT CTT CAA ATA GAG GCC G-3′ , which produced a 141-bp fragment. The primers for *IL-10* -1082A/G were forward 5′- CTG GCT GCA ACC CAA CTG GC -3′ and reverse 5′- TCT TAC CTA TCC CTA CTT CC -3′, which generated a 139-bp fragment. These fragments were amplified separately under similar conditions in 25-μl volumes of a mixture containing 100 ng of DNA, 10 μM of each primer, 10 mM Tris-HCl (pH 8.0), 0.1 mM of each dNTP, 2.0 mM $MgCl_2$, and 0.5 U Taq DNA polymerase. The samples were amplified for 35 cycles at 94℃ for 55 sec followed by 72℃ for 1 min, with an initial denaturation step at 94℃ for 5 min and a final extension step at 72℃ for 10 min, using a Thermocycler MJ PTC-200 PCR System (Harlow Scientific, Arlington, USA). The PCR fragments were visualized in ethidium bromide-stained gels. The DdeI restriction enzyme was used to identify the *IL-18* polymorphism according to the manufacturer's protocol (Fermentas, Vilnius, Lithuania). The *IL-18* A allele generated a double band representing 109- and 32-bp fragments, and the *IL-18* C variant allele produced one fragment of 141 bp. The MnlI restriction enzyme was used to identify the *IL-10* polymorphism according to the manufacturer's protocol (Fermentas, Vilnius, Lithuania). The *IL-10* A allele generated a single band representing a 139-bp fragment, and the *IL-10* G variant allele produced two fragments of 106 and 33 bp. The restriction products were analyzed by electrophoresis in 3% agarose gels containing ethidium bromide.

Statistical Analysis

Statistical analyses were conducted using SAS statistical software (Statistical Analysis System, version 8.1, Cary, NC, USA, 1999-2000). Associations were assessed using 2X2 or 2Xn contingency table analysis; the chi-squared (χ^2) or Fisher's (F) exact test was used to examine homogeneity between cases and controls in terms of gender, race, previous thyroid disease, thyroid nodule size, medication use, cigarette smoking, disease extent, and genotypes. The Kruskal-Wallis (KW) test was used to compare age among the groups. The Mann-Whitney or Wilcoxon test was used to compare age among the different genotype groups. The observed genotype frequencies were compared with those calculated using Hardy-Weinberg equilibrium theory. The 95% CI odds ratio (OR) provided a measure of strength of association. To further explore the significance of the studied genotypes in different age ranges, we performed a univariate logistic regression analysis in patients under and over 45 years old after adjusting for gender. The 23 tall-cell variant cases and the 22 samples of less-differentiated variants were excluded from all comparisons concerning DTC. Logistic regression analysis was used to evaluate the effects of all genotypes after adjusting for potential confounders, such as age, gender, race, tobacco use, and alcohol use. A multivariate logistic regression model was applied using the presence/absence of CLT as the dependent variable and all genotypes and clinical risk factors, including gender, age, and cumulative radioiodine doses, as explicative variables. The Cox regression model was used to search for associations between risk factors and disease-free survival in thyroid cancer patients. All tests were conducted at a 5% significance level ($p<0.05$).

RESULTS

Table 1 summarizes the demographics, pathological features, and follow-up characteristics of the 346 patients with DTC, including the 200 patients genotyped for the *IL-18* +105A/C polymorphism and the 183 patients genotyped for the *IL-10* -1082A/G polymorphism. As expected, the DTC patients were predominantly female (85%). There were no differences between the control individuals and the cancer patients in terms of ethnicity (78.06% Caucasian and 21.94% non-Caucasian versus 82.82% Caucasian and 17.18% non-Caucasian patients, respectively; $p = 0.117$) or smoking habits (70.95% non-smokers and 29.05% smokers versus 72.17% non-smokers and 27.82% smokers, respectively; $p = 0.729$). The mean age at the time of diagnosis was 41.62 ± 14.89 years (range of 5-87 years). According to the pathologic TNM staging system, 210 (60.58%) patients were stage I, 84 (24.36%) stage II, 30 (8.65%) stage III, and 22 (6.41%) stage IV. Most DTC cases were classified as differentiated tumors (88.26%), 42.28% were multicentric, and 86 (24.85%) presented lymph node metastasis at the time of diagnosis. Lymph node metastases were more frequent in patients with multifocal tumors (36.64%) than in patients with a single tumor focus (19.25%; $p = 0.0007$).

A total of 24 DTC patients had previously been diagnosed with autoimmune disease, including eight cases (8 PTC) of Hashimoto's thyroiditis and 16 cases (14 PTC and 2 FTC) of Basedow-Graves' disease.

The *IL-18* and *IL-10* genotype profiles were according to a Hardy-Weinberg equilibrium (*IL-18*: $\chi^2 = 0.210$, $gl = 2$, $p = 0.900$; *IL-10*: $\chi^2 = 1.616$, $gl = 2$, $p = 0.445$). We were unable to find any differences in *IL-18* or *IL-10* genotype distribution between the controls and patients ($p = 0.342$ and $p = 0.665$, respectively). Further investigation into possible associations among *IL-18* and *IL-10* polymorphism profiles, tumor features, and patient outcomes, as shown in Table 1, did not reveal any association between genotype and aggressive clinical characteristics at diagnosis or during follow-up. Moreover, there was no relationship between *IL-18* or *IL-10* genotype and previous diagnosis of autoimmune disease. There were no differences in *IL-18* polymorphism profiles between patients with or without CLT. However, a multivariate regression analysis showed that CLT was more frequent in patients with the *IL-10* variant allele than in patients with the wild-type AA genotype ($p = 0.039$).

Whereas CLT was present in 21.41% of the DTC samples, 257 (78.59%) patients did not present with CLT, and histological characteristics were ambiguous in the remaining

Table 1 - Demographics, pathological features and follow-up characteristics of the differentiated thyroid cancer patients according to their genotypic distribution of *interleukin-18* +105A/C (*IL-18* +105A/C) and *interleukin-10* -1082A/G (*IL-10* -1082A/G) polymorphic profiles. PTC = papillary thyroid carcinoma; N = absolute number of patients; pTNM = International Union Against Cancer (AJCC/UICC) staging system.

Demographic/Pathological/ Follow-up Factors	*IL-18* genotype				*IL-10* genotype			
	AA	AC	CC		AA	AG	GG	
	N (%)	N (%)	N (%)	*p*-value	N (%)	N (%)	N (%)	*p*-value
Gender								
Female	69 (42.86)	75 (46.58)	17 (10.56)	0.279	63 (42.86)	61 (41.50)	23 (15.64)	0.330
Male	9 (28.12)	18 (56.25)	5 (15.63)		10 (32.26)	13 (41.93)	8 (25.81)	
Ethnicity								
White	77 (40.96)	90 (47.87)	21 (11.17)	0.684	54 (38.57)	60 (42.86)	26 (18.57)	0.453
Non-white	17 (44.74)	16 (42.10)	5 (13.16)		18 (50.00)	13 (36.11)	5 (13.89)	
Smoking								
Smoker	21 (35.00)	33 (55.00)	6 (10.00)	0.435	23 (41.81)	21 (38.18)	11 (20.01)	0.694
Non-smoker	56 (43.41)	58 (44.96)	15 (11.63)		48 (40.00)	53 (44.17)	19 (15.83)	
Age								
<40	33 (39.29)	43 (51.90)	8 (8.81)	0.745	35 (45.45)	32 (41.56)	10 (12.99)	0.336
≥40	45 (40.90)	51 (46.36)	14 (12.74)		38 (37.62)	42 (41.58)	21 (20.80)	
Tumor Type								
Papillary (PTC)	62 (38.99)	80 (50.31)	17 (10.70)	0.429	62 (40.00)	67 (43.23)	26 (16.77)	0.504
Follicular (FTC)	16 (47.06)	13 (38.23)	5 (14.71)		11 (47.83)	7 (30.43)	5 (21.74)	
PTC histological variants								
Classical	33 (38.82)	43 (50.58)	9 (10.60)	0.622	30 (36.14)	40 (48.19)	13 (15.67)	0.409
Follicular	14 (31.81)	23 (52.27)	7 (15.92)		22 (51.16)	14 (32.56)	7 (16.28)	
Others	25 (46.29)	23 (42.59)	6 (11.12)		19 (40.42)	18 (38.30)	10 (21.28)	
Concurrent thyroiditis								
Yes	18 (40.90)	24 (54.54)	2 (4.56)	0.241	13 (31.71)	22 (53.66)	6 (14.63)	0.208
No	57 (39.04)	69 (47.26)	20 (13.70)		60 (44.12)	52 (38.23)	24 (17.65)	
Multifocality								
Multinodular	26 (37.68)	36 (52.17)	7 (10.15)	0.740	30 (46.15)	27 (41.54)	8 (12.31)	0.447
Uninodular	46 (40.00)	54 (46.95)	15 (13.05)		41 (38.68)	45 (42.45)	20 (18.87)	
Extrathyroidal invasion								
Yes	33 (37.50)	47 (53.41)	8 (9.09)	0.527	31 (39.24)	34 (43.04)	14 (17.72)	0.725
No	39 (41.49)	43 (45.74)	12 (12.77)		40 (44.94)	36 (40.45)	13 (14.61)	
pTNM stage								
I	43 (41.35)	51 (49.04)	10 (9.61)	0.653	44 (44.90)	39 (39.80)	15 (15.30)	0.705
II	19 (39.58)	22 (45.83)	7 (14.59)		15 (34.09)	20 (45.45)	9 (20.46)	
III	6 (27.27)	12 (54.54)	4 (18.19)		9 (42.86)	8 (38.09)	4 (19.05)	
IV	5 (41.66)	7 (58.34)	0 (0.00)		2 (22.22)	6 (66.67)	1 (11.11)	
Microcarcinoma								
Yes	23 (48.93)	19 (40.42)	5 (10.65)	0.230	20 (43.48)	18 (39.13)	8 (17.44)	0.946
No	49 (35.00)	74 (52.86)	17 (12.14)		53 (41.08)	54 (41.86)	22 (17.06)	
Differentiation grade								
Well differentiated	64 (40.76)	72 (45.86)	21 (13.38)	0.128	64 (43.84)	57 (39.04)	25 (17.12)	0.283
Poorly differentiated	4 (28.57)	10 (71.43)	0 (0.00)		4 (26.67)	9 (60.00)	2 (13.33)	
Lymph node metastasis								
Yes	16 (31.37)	30 (58.82)	5 (9.80)	0.176	22 (43.14)	23 (45.10)	6 (11.76)	0.485
No	66 (44.59)	65 (43.92)	17 (11.49)		54 (41.22)	52 (39.69)	25 (19.09)	
Distant metastasis								
Yes	7 (30.43)	15 (65.22)	1 (4.35)	0.171	6 (30.00)	12 (60.00)	2 (10.00)	0.181
No	76 (42.94)	80 (45.20)	21 (11.86)		70 (42.94)	63 (38.65)	30 (18.41)	
Familial cases								
Yes	2 (40.00)	2 (40.00)	1 (20.00)	0.652	2 (50.00)	2 (50.00)	0 (0.00)	1.000
No	81 (41.54)	93 (47.69)	21 (10.77)		74 (41.34)	73 (40.78)	32 (17.88)	
Hashimoto's thyroiditis								
Yes	2 (50.00)	2 (50.00)	0 (0.00)	1.000	2 (50.00)	2 (50.00)	0 (0.00)	1.000
No	73 (39.67)	89 (48.37)	22 (11.96)		71 (41.52)	70 (40.94)	30 (17.54)	
Basedow-Graves' disease								
Yes	3 (42.86)	3 (42.86)	1 (14.28)	1.000	3 (37.50)	1 (12.50)	4 (50.00)	0.053
No	72 (39.78)	88 (48.62)	21 (11.60)		70 (41.92)	71 (42.51)	26 (15.57)	
Metastasis during follow-up								
Yes	6 (50.00)	5 (41.67)	1 (8.33)	0.909	7 (53.85)	5 (38.46)	1 (7.69)	0.522
No	77 (40.96)	90 (47.87)	21 (11.17)		69 (40.59)	70 (41.18)	31 (18.23)	
Cumulative ^{131}I dose								
<250 mCi	16 (41.03)	20 (51.28)	3 (7.69)	0.753	13 (37.14)	16 (45.71)	6 (17.15)	0.353
≥250 mCi	10 (32.26)	19 (61.29)	2 (6.45)		15 (50.00)	13 (43.33)	2 (6.67)	

19 cases, which were therefore excluded from further analysis. We also evaluated the influence of CLT on DTC presentation and outcome, as shown in Table 2. CLT was more frequent in women ($p = 0.020$) previously diagnosed with Hashimoto's thyroiditis ($p<0.001$) and who had received a lower ^{131}I cumulative dose ($p = 0.005$). A multivariate regression analysis confirmed significant associations among the presence of CLT, female gender ($p = 0.013$) and the absence of lymph node metastasis at diagnosis ($p = 0.012$).

To further explore the influence of autoimmunity on DTC prognosis, we grouped all the DTC patients with lymphocytic infiltration surrounding neoplastic tissue and/or circulating autoantibodies and/or preoperative diagnosis of Graves' disease or Hashimoto's thyroiditis. These 89 patients (83 women and 6 men, mean age ± SD 41.45 ± 14.84) were then compared with 239 DTC patients (198 women and 41 men, mean age ± SD 41.62 ± 14.89) without any histological or serological evidence of thyroid autoimmunity. Eighteen patients with ambiguous data were excluded from further analysis. Again, the presence of autoimmunity was correlated with female gender ($p = 0.020$), absence of lymph node metastasis at diagnosis ($p = 0.017$), and lower ^{131}I cumulative dose ($p = 0.025$).

Table 2 - Demographics, pathological features and follow-up characteristics of 327 differentiated thyroid carcinoma patients presenting concurrent lymphocytic thyroiditis (N = 70 patients) or not (N = 257 patients). The data were obtained using a multivariate regression analysis. IL = interleukin; N = absolute number of patients.

	Thyroiditis		
	Present	Absent	*p*-value
Demographic/Pathological/ Follow-up Factors	**N (%)**	**N (%)**	
Gender			
Female	66 (23.57)	214 (76.43)	**0.013**
Male	4 (8.51)	43 (91.49)	
Ethnicity			
White	60 (22.56)	206 (77.44)	0.475
Non-white	10 (18.18)	45 (81.82)	
Smoking			
smoker	15 (16.67)	75 (83.33)	0.175
non-smoker	55 (23.61)	178 (76.39)	
Age			
<40	32 (21.48)	117 (78.52)	0.978
≥40	38 (21.35)	140 (78.65)	
Extrathyroidal invasion			
Yes	28 (19.31)	117 (80.69)	0.377
No	38 (23.46)	124 (76.54)	
pTNM stage			
I	42 (22.46)	145 (77.54)	0.678
II	20 (26.32)	56 (73.68)	
III	5 (18.52)	22 (81.48)	
IV	3 (15.00)	17 (85.00)	
Microcarcinoma			
Yes	20 (27.03)	54 (72.97)	0.209
No	50 (20.16)	198 (79.84)	
Differentiation grade			
Well differentiated	54 (20.61)	208 (79.39)	0.282
Poorly differentiated	10 (28.57)	25 (71.43)	
Lymph node metastasis			
Yes	12 (14.12)	73 (85.88)	**0.012**
No	58 (24.17)	182 (75.83)	
Distant metastasis			
Yes	5 (14.71)	29 (85.29)	0.310
No	65 (22.26)	227 (77.74)	
Familial cases of thyroid cancer			
Yes	3 (33.33)	6 (66.67)	0.409
No	67 (21.07)	251 (78.93)	
Preoperative Hashimoto's thyroiditis			
Yes	7 (87.5)	1 (12.50)	**<0.001**
No	63 (20.00)	252 (80.00)	
Metastasis during follow-up			
Yes	2 (10.00)	18 (90.00)	0.267
No	68 (22.15)	239 (77.85)	
Cumulative radioiodine dose			
<250 mCi	18 (36.73)	31 (63.27)	**0.005**
≥250 mCi	6 (12.24)	43 (87.76)	
***IL-10* genotype**			
AA	13 (17.81)	60 (82.19)	**0.039**
AG/GG	28 (26.92)	76 (73.08)	
***IL-18* genotype**			
AA	18 (24.00)	57 (76.00)	0.969
AC/CC	26 (22.61)	89 (77.39)	

DISCUSSION

We were unable to find any association between the *IL-18* +105A/C polymorphism and DTC with CLT or previous autoimmune thyroid diseases. Neither the *IL-18* nor the *IL-10* genotype was differentially distributed between patients and controls, indicating that these genotypes are not a risk factor for DTC development. However, there was an association between the presence of *IL-10* variants and CLT. In addition, we demonstrated that CLT accompanied by DTC is associated with good prognostic features, including female gender, lower ^{131}I cumulative dose, and absence of lymph node metastasis at diagnosis.

Because *IL-10* may be involved in leukocyte recruitment,[14,20] and the studied polymorphism increased *IL-10* protein levels,[17] we could speculate that individuals with the inherited polymorphic variant would be more efficient at increasing their *IL-10* levels, leading to thyroid autoimmunity. The *IL-10* -1082A/G polymorphism has been associated with the development of various types of cancer, including thyroid cancer.[21-24] In a recent work, Erdogan and colleagues[21] studied the *IL-10* -1082A/G polymorphism in patients with papillary thyroid carcinoma and control individuals using the same genotyping technique as in our study but a smaller cohort. They obtained a genotype profile similar to that reported here. However, patients with a heterozygous genotype were more frequent in the Erdogan cohort (61.9%) than in our cohort (44.53%), which suggests that the genetic backgrounds of different populations may influence DTC development.

Although we confirmed a previously reported association between thyroid autoimmunity and tumor features related to a good prognosis,[3,25-30] this issue is still being debated. Although a worse prognosis has been reported for patients with thyroid autoimmunity in a few series,[31,32] most studies have shown either a protective effect[3,13,14,27,33] or no effect of thyroid autoimmunity on DTC behavior.[34]

Hashimoto's thyroiditis is characterized by follicular cell depletion due to an immune system reaction against a series of thyroid epithelial cell antigens. DTC cells might also be recognized and destroyed by the immune system, which would explain why DTC patients with concomitant autoimmune thyroid diseases may present a better prognosis.[25,35] The immune response is modulated by a complex network of cytokines, and the final output of this

interaction can be either the stimulation or the suppression of immunity. Therefore, merely analyzing *IL-10* and *IL-18* is not sufficient to understand the immune response against thyroid cancer. More studies are warranted to confirm the hypothesis of tumor destruction by immune reaction and to elucidate the mechanisms engaged in this process.

CONCLUSIONS

In conclusion, we suggest that neither the *IL-18* +105A/C nor the *IL-10* -1082A/G polymorphisms represent risk factors for the development or outcome of DTC. However, the inheritance of the G allele at *IL-10* -1082 may favor concurrent autoimmunity in DTC with lymphocyte recruitment and hence modulate an antitumor immune reaction. Further studies are needed to confirm the hypothesis of tumor cell depletion by immune cells and to elucidate the mechanisms engaged in this process.

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