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ORIGINAL ARTICLE

A gene-disease association study of IL18 in thyroid cancer: genotype and haplotype analyses

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Abstract Thyroid cancer is the most common malignancy of the endocrine system, and genetic factors have been shown to be associated with its risk. Interleukin-18 (IL-18) is a pleiotropic pro-inflammatory cytokine that induces IFN-γ production and is involved in T helper type 1 development. To determine the role of IL-18 gene in thyroid cancer susceptibility, we conducted a case–control study, and genotyped five single nucleotide polymorphisms (SNPs) in IL-18 gene (−656 G/T (rs1946519), −607 C/A (rs1946518), and −137 G/C (rs187238) in the promoter region and +113 T/G (rs360718) and +127 C/T (rs360717) in 5′-untranslated region) in 105 patients with thyroid cancer and 148 healthy controls from Iranian population. Polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) and allele-specific primer-PCR were used for genotyping. The association of different genotypes with thyroid cancer, tumor type, and the tumor stage was analyzed. Comparing all of the patient population with the controls, TT genotype at position −656 G/T was observed to be associated with a significantly increased risk of thyroid cancer [31/105 (30.1 %) vs 19/148 (13.1 %), $p = 0.002$, OR 2.90, CI 1.40–5.70]. No association with thyroid cancer was found at other positions (−607 C/A, −137 G/C, +113 T/G, and +127 C/T). Excluding the patients with medullary carcinoma, and including only the ones with thyroid cancer derived from the follicular epithelium, nearly the same results were observed regarding the genotypes at position −656 G/T. Furthermore, significantly decreased risk of thyroid cancer derived from the follicular epithelium was observed upon inheritance of the homozygote genotype (CC) at position +127 C/T (40/94 (42.5 %) versus 84/148 (56.8 %) in patients and controls, respectively (OR 0.56, 95 % CI for OR 0.32–0.98, $p = 0.04$). Haplotype analysis indicated that among 32 possible haplotypes, TAGTT haplotype frequency was significantly higher in patients than in controls [12/188 (6.4 %) vs 2/292 (0.7 %), $p = 0.0008$] and this difference resisted Bonferroni correction ($n = 19$) and significant level set at 0.003. Nearly the same results were observed after excluding the patients with medullary carcinoma. No association was found between the SNPs and the stage of tumor. Our results suggest the increased susceptibility to thyroid cancer in subjects with TT genotype at position −656 G/T of the promoter of IL-18 gene, as well as TAGTT haplotype emerged from five studied SNPs in IL-18 gene. The data also suggest that the inheritance of +127 CC genotype may protect individuals from thyroid cancer derived from follicular epithelium.

Keywords Thyroid cancer · IL-18 · Polymorphism · Haplotype

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Introduction

Thyroid cancer is the most common malignancy of the endocrine system. Worldwide annual incidence of thyroid cancer and the resulting mortality are estimated to be about

213,179 (3.1 % of all new cancer cases) and 35383 (0.5 % of all cancer deaths), respectively [1]. Although the only proved risk factors for thyroid cancer are exposure to high levels of ionizing radiation and inheritance of familial syndromes, first degree relatives of thyroid cancer patients show a 4–10 fold increase in their risk of developing thyroid cancer [2]. Several genetic alterations have been indicated to be associated with, and consequently used in the diagnosis of thyroid cancer [3, 4].

Interleukin-18 (IL-18), formerly known as interferon-gamma (IFN-γ) inducing factor (IGIF), was cloned for the first time in 1995 [5]. This pro-inflammatory cytokine is the member of IL-1 superfamily and is produced by a variety of cells particularly kupffer cells of the liver and activated macrophages [5]. Thyroid cells have also been revealed to produce IL-18 in response to thyroid stimulating hormone (TSH) in a dose-dependent manner [6]. Intra-thyroidal cooperation of IL-18 with IFN-γ has been suggested to contribute to the thyroid tissue degeneration in Hashimoto's thyroiditis [7].

IL-18 encoding gene is located on chromosome 11q22.2–q22.3 and is composed of six exons and five introns. Human IL-18 gene has 5 well-known SNPs, three of which are located in the promoter region (−656 G/T or rs1946519, −607 C/A or rs1946518, −137 G/C or rs187238), and two are in 5′-untranslated region (UTR) (+113 T/G or rs360718, +127 C/T or rs360717) [8, 9]. It is proposed that −607 C/A and −137 G/C SNPs in the promoter region interfere with transcription factors (TFs)-binding sites [9]. These two SNPs have been reported, by our group, to be associated with susceptibility to stomach, colorectal, breast, and lung cancers in Iranian population [10–12].

The aim of this study was to investigate the association of five IL-18 genetic variations, including −656 G/T (rs1946519), −607 C/A (rs1946518), −137 G/C (rs187238), +113 T/G (rs360718), +127 C/T (rs360717), and the emerged haplotype with thyroid cancer. The associations of these genetic variations with clinical and pathological factors involved in thyroid cancer were also investigated.

Subjects and methods

One hundred and five patients with thyroid cancer and 148 age- and sex-matched healthy control subjects were enrolled in this study. Cases were patients who were surgically treated for their FNA-confirmed primary thyroid cancer in Shiraz University of Medical Sciences teaching hospitals, Shiraz, Iran, and were confirmed histopathologically to have thyroid cancer. Data regarding age, sex, cancer type (and subtype if applicable), and cancer stage (based on AJCC cancer staging manual [13] were collected. Control subjects were healthy blood donors with negative history of autoimmune diseases and cancer in their first degree relatives. All the cases and the controls had self-reported Iranian ethnicity and neither of the cases nor the controls were first or second degree relatives. Approval for the study was obtained from Shiraz University of Medical Sciences Ethics Committee. The study was described to each participant and informed consent was obtained from them.

Ten mL venous blood was collected in EDTA-containing tubes by venipuncture from 105 cases with thyroid cancer at the time of diagnosis, as well as 148 healthy controls. Leukocyte DNA extraction from the specimens was done by salting out method [14]. The concentration and the purity of extracted DNA were evaluated by spectrophotometry.

For three SNPs (positions −656 G/T, +113 T/G and +127 C/T), genotypes were determined by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP), and for the other two positions (−607 C/A and −137 G/C), genotyping was done by allele-specific primer (ASP)-PCR as previously mentioned by Folwaczny and Giedraitis with minor modifications [9, 15]. The primer sequences (Bioneer, Korea), annealing temperatures, and restriction enzymes (Fermentas, Lithuania) used for the genotyping of each SNP using either PCR-RFLP or ASP-PCR are summarized in Table 1.

Statistical analysis

Data were collected and analyzed by SPSS Statistics for Windows (version 11.5; SPSS Inc., Chicago, IL, USA). Test of Hardy–Weinberg equilibrium was done using Arlequin 3.1 software package [16]. The sex, genotype, and allele distribution comparison between the case and the control groups were also done by Chi-squared test (level of significance: 5 %). Mean age comparison between the groups was done using independent samples *T* test. In the first set of analyses, all the patient population (with different thyroid tumor types) was compared with the controls, while in the second set of analyses, patients with medullary thyroid tumor type were excluded, and the comparison was made between patients with thyroid cancer derived from the follicular epithelium and the control subjects. Risk assessment was calculated using logistic regression analysis. Odds ratio and 95 % confidence interval were also calculated. Haplotype analysis, linkage disequilibrium calculation, and haplotype frequency comparison was done using Haploview software version 4.2 [17].

Table 1 Reaction conditions for SNP amplification and genotyping

Locus	Primer	Primer sequence	References	Annealing temperature (°C)	RE	Reaction product
−607 C/A	FI	5′-GTTGCAGAAAGTGTAAAAATTATTAC -3′	[9]	62.5 and 56.5	–	Main band: 196-bp
	FII	5′-GTTGCAGAAAGTGTAAAAATTATTAA -3′				
	R	5′-TAACCTCATTCAGGACTTCC -3′				Control band: 301-bp
	IC	5′-CTTTGCTATCATTCCAGGAA -3				
−137 G/C	FI	5′-CCCCAACTTTTACGGAAGAAAAG -3′	[9]	68 and 62	–	Main band:261-bp
	FII	5′- CCCCAACTTTTACGGAAGAAAAC -3′				
	R	5′- AGGAGGGCAAAATGCACTGG -3′				Control band: 446-bp
	IC	5′-CCAATAGGACTGATTATTCCGCA -3′				
−656	F	5′-AGGTCAGTCTTTGCTATCATTCCAGG-3′	[15]	60	*Mwo* I	G:96 and 24-bp
	R	5′- TGCAACAGAAAGTAAGCTTGCGGAGAGG -3′				T:120-bp
+127	F	5′-CCAGCTTGCTGAGCCCTTTGCTCC -3′	[15]	63	*Eag* I	C:113 and 21-bp
	R	5′-CTGTGTAGACTGCAGCAGGTGGCGGCC -3′				T:134-bp
+113	F	5′-CCAGCTTGCTGAGCCCTTTGCTCC -3′	[15]	58	*Nhe* I	G:93 and 27-bp
	R	5′-GCAGGTGGCAGCCGCTTTAGCAGCTAG -3′				T:120-bp

F forward, *R* reveres, *IC* internal control, *RE* restriction enzyme

Results

In this case–control study, we genotyped five SNPs in the promoter and 5′-untranslated region of IL-18 gene in 105 cases with thyroid cancer and 148 healthy controls. There were 31 males (29.5 %) and 74 females (70.5 %) with a total mean age of 41.16 ± 14.42 in the case group, and 40 males (27.0 %) and 108 females (73.0 %) with a mean age of 42.22 ± 14.09 in the control group. Groups were matched regarding sex and age (Table 2). Genotype distribution in the control group did not deviate from Hardy–Weinberg equilibrium ($p > 0.05$).

Allele and genotype frequencies in patients with thyroid cancer and healthy control subjects are presented in Table 3. As indicated in the table, genotype distribution at position −656 G/T was observed to be significantly different between cases and controls ($p = 0.003$). Risk estimate analysis showed a significantly increased risk of thyroid cancer upon inheritance of the homozygous genotype (TT) at this position [31/105 (30.1 %) vs 19/148 (13.1 %), OR 2.90, 95 % CI for OR 1.40–5.70, $p = 0.002$, Table 3]. Inversely, inheritance of GT heterozygote at this position was observed to be associated with a decreased risk of thyroid cancer [41/105 (39.8 %) vs 81/148 (55.9 %), $p = 0.02$, OR 0.52, 95 %CI for OR 0.30–0.90, Table 3].

As indicated in Table 3, no association was found between the genotype or allele frequencies and thyroid cancer at other positions. No association was also found between genotype distribution and the stage of thyroid cancer ($p > 0.05$). SNPs at all positions were in linkage disequilibrium but LD was strongest between positions +113/+127, and −137/+113, with $|D'|$ of 0.93 and 0.92, respectively (Fig. 1).

Table 2 Characteristics of 105 thyroid cancer and 148 control subjects enrolled in the study

	Patients	Controls
Age (mean ± SD)	41.16 ± 14.42	42.22 ± 14.09
Sex		
Male	31 (29.5 %)	40 (27.0 %)
Female	74 (70.5 %)	108 (73.0 %)
Tumor type		
Papillary	88 (83.8 %)	–
Follicular	5 (4.8 %)	–
Medullary	11 (10.5 %)	–
Undifferentiated	1 (1 %)	–
Papillary tumor subtype		
Classic	59 (67.0 %)	–
Follicular variant	18 (20.5 %)	–
Columnar	2 (2.3 %)	–
Missing	9 (10.2 %)	–
Tumor stage		
I	50 (47.6 %)	–
II	20 (19.0 %)	–
III	14 (13.3 %)	–
IV	20 (19.0 %)	–
Missing	1 (1 %)	–

Table 3 Genotype distribution and allele frequencies of five IL-18 SNPs (−656 G/T (rs1946519), −607 C/A (rs1946518), −137 G/C (rs187238), +113 T/G (rs360718), +127 C/T (rs360717)) in patients with thyroid cancer and healthy controls

IL-18 genetic variation	Patients, $N = 105$ (valid %)	Controls, $N = 148$ (valid %)	p value	Odds ratio (OR)	95 % CI for OR
−656 G/T (rs1946519)					
Genotype					
GG	31 (30.1)	45 (31.0)	0.98	0.096	0.53–1.72
GT	41 (39.8)	81 (55.9)	0.02	0.52	0.30–0.90
TT	31 (30.1)	19 (13.1)	0.002	2.9	1.40–5.70
Missing	2	3	–	–	–
Allele					
G	103 (50.0)	171 (58.9)	0.06	0.70	0.48–1.01
T	103 (50.0)	119 (41.1)			
Missing	4	6	–	–	–
−607 C/A (rs1946518)					
Genotype					
CC	31 (30.7)	54 (36.7)	0.40	0.76	0.43–1.35
CA	55 (54.5)	76 (51.7)	0.76	1.12	0.65–1.92
AA	15 (14.8)	17 (11.6)	0.57	1.33	0.59–2.99
Missing	4	1	–	–	–
Allele					
C	117 (58.0)	184 (62.5)	0.34	0.82	0.55–1.21
A	85 (42.0)	110 (37.5)			
Missing	8	2	–	–	–
−137 G/C (rs187238)					
Genotype					
GG	59 (60.2)	85 (57.5)	0.76	1.12	0.65–1.95
GC	33 (33.7)	56 (37.8)	0.6	0.83	0.47–1.47
CC	6 (6.1)	7 (4.7)	0.85	1.31	0.38–4.52
Missing	7	–	–	–	–
Allele					
G	151 (77.0)	226 (76.4)	0.94	1.04	0.66–1.63
C	45 (23.0)	70 (23.6)			
Missing	14	–	–	–	–
+113 T/G (rs360718)					
Genotype					
TT	70 (66.7)	86 (58.5)	0.23	1.42	0.81–2.47
TG	33 (31.4)	57 (38.8)	0.23	0.72	0.41–1.27
GG	2 (1.9)	4 (2.7)	1	0.69	0.09–4.51
Missing	–	1	–	–	–
Allele					
T	173 (82.4)	229 (77.8)	0.26	1.33	0.83–2.13
G	37 (17.6)	65 (22.2)			
Missing	–	2	–	–	–
+127 C/T (rs360717)					
Genotype					
CC	47 (44.8)	84 (56.8)	0.08	0.62	0.36–1.05
CT	51 (48.6)	58 (39.2)	0.17	1.47	0.86–2.51
TT	7 (6.6)	6 (4.0)	0.52	1.69	0.49–5.88
Missing	–		–	–	–
Allele					

Table 3 continued

IL-18 genetic variation	Patients, $N = 105$ (valid %)	Controls, $N = 148$ (valid %)	p value	Odds ratio (OR)	95 % CI for OR
C	145 (69.0)	226 (76.4)	0.07	0.68	0.45–1.03
T	65 (31)	70 (23.6)			
Missing	–	–	–	–	–

In a second set of analysis, upon excluding the patients with medullary thyroid cancer ($n = 11$), a comparison was made between patients with thyroid cancer derived from follicular epithelium and the control subjects. As indicated in Table 4, genotype distribution at position −656 G/T was, again, observed to be significantly different between cases and controls with the same pattern as the whole patient population mentioned above. Despite this similarity, genotype distribution at position +127 C/T was also observed to be significantly different between the patients and the controls only after excluding the data of patients with medullary thyroid cancer from our analysis. Risk estimate analysis showed a significantly decreased risk of thyroid cancer upon inheritance of the homozygous genotype (CC) at this position (40/94 (42.5 %) in patients compared to 84/148 (56.8 %) in the controls (OR 0.56, 95 % CI for OR 0.32–0.98, $p = 0.04$, Table 4). No association was found between genotypes or allele frequencies and the thyroid cancer at other positions (Table 4).

Haplotype analysis of the whole patient population showed 21 haplotypes out of 32 possible ones (Table 5, in which only subjects who had no missing genotype are included and haplotypes with more than 1 % frequency are shown). After Bonferroni correction ($n = 19$), significant level was set at 0.003 for haplotype analysis. Accordingly, haplotype TAGTT frequency was significantly higher [12/188 (6.4 %) in patients compared to 2/292 (0.7 %), $p = 0.0008$] in controls. Other significant differences did not resist Bonferroni correction. Nearly the same results were observed after excluding the patients with medullary thyroid cancer from our analysis (Table 6).

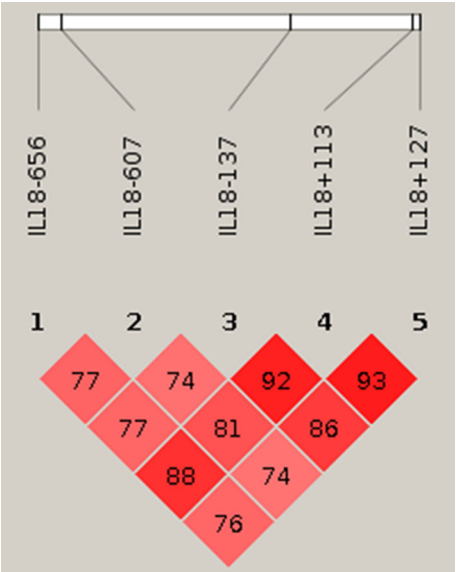

Fig. 1 Linkage disequilibrium between five IL18 gene polymorphisms investigated in this study (−656 G/T (rs1946519), −607 C/A (rs1946518), −137 G/C (rs187238), +113 T/G (rs360718), +127 C/T (rs360717)). SNPs at all positions were in linkage disequilibrium but LD was strongest between positions +113/+127, and −137/+113, with $|D'|$ of 0.93 and 0.92, respectively

Discussion

Thyroid cancer is the most common neoplasm of the endocrine system and both environmental and genetic factors have been reported to be associated with this type of cancer (2). Genetic alterations in the genes of MAPK pathway including BRAF, RET/PTC, and RAS [3, 18], as well as genetic alteration in activating Killer Cell Immunoglobulin-Like Receptors (KIRs) gene, especially KIR2DS5 receptor [4], have been reported to be associated with the risk of thyroid cancer. IL-18 is a pleiotropic pro-inflammatory cytokine with the ability to stimulate innate immunity as well as both Th1 and Th2 arms of specific immunity depending on the host genetic background as well as local cytokine micro environment [19].

Here we report, for the first time, the genotype distribution and allele frequencies of five SNPs of IL-18 gene as new candidate loci for susceptibility to thyroid cancer in a population residing in the south of Iran. The distribution of −607 C/A, −137 G/C, and −656 G/T SNPs in the promoter region as well as +127 C/T and +113 T/G in the 5′-untranslated region of IL-18 was investigated in 105 thyroid cancer patients compared to 148 healthy controls. Our results suggest that IL-18 polymorphism at position −656 G/T (rs1946519) is significantly associated with the risk of thyroid cancer. Our analysis indicated that −656 homozygous minor genotype TT shows 2.9-fold increased risk of thyroid cancer with a 95 % CI of 1.4–5.7 in comparison with the other two possible genotypes. Genotype and allele analysis did not show any association between the two more common SNPs in IL-18 gene promoter, −607

Table 4 Genotype distribution and allele frequencies of five IL-18 SNPs (−656 G/T (rs1946519), −607 C/A (rs1946518), −137 G/C (rs187238), +113 T/G (rs360718), +127 C/T (rs360717)) in patients with thyroid cancer derived from the follicular epithelium (all patients except those with medullary carcinoma) and healthy controls

IL-18 genetic variation	Patients, $N = 94$ (valid %)	Controls, $N = 148$ (valid %)	p value	Odds ratio (OR)	95 % CI for OR
−656 G/T (rs1946519)					
Genotype					
GG	29 (31.5)	45 (31.0)	0.94	1.02	0.56–1.87
GT	35 (38)	81 (55.9)	0.01	0.49	0.27–0.86
TT	28 (30.5)	19 (13.1)	0.002	2.9	1.44–5.89
Missing	2	3	–	–	–
Allele					
G	93 (50.5)	171 (58.9)	0.09	0.71	0.48–1.05
T	91 (49.5)	119 (41.1)			
Missing	4	6	–	–	–
−607 C/A (rs1946518)					
Genotype					
CC	27 (30.0)	54 (36.7)	0.36	0.76	0.40–1.34
CA	48 (53.3)	76 (51.7)	0.91	1.07	0.61–1.87
AA	15 (16.7)	17 (11.6)	0.36	1.53	0.68–3.44
Missing	4	1	–	–	–
Allele					
C	102 (56.7)	184 (62.5)	0.24	0.78	0.53–1.16
A	78 (43.3)	110 (37.5)			
Missing	8	2	–	–	–
−137 G/C (rs187238)					
Genotype					
GG	50 (57.5)	85 (57.5)	0.89	1.00	0.57–1.77
GC	31 (35.6)	56 (37.8)	0.84	0.91	0.51–1.63
CC	6 (6.9)	7 (4.7)	0.55	1.49	0.43–5.16
Missing	7	–	–	–	–
Allele					
G	131 (75.3)	226 (76.4)	0.88	0.94	0.60–1.49
C	43 (24.7)	70 (23.6)			
Missing	14	–	–	–	–
+113 T/G (rs360718)					
Genotype					
TT	61 (64.9)	86 (58.5)	0.39	1.31	0.74–2.32
TG	31 (33)	57 (38.8)	0.43	0.78	0.44–1.39
GG	2 (2.1)	4 (2.7)	1.00	0.77	0.10–5.02
Missing	–	1	–	–	–
Allele					
T	153 (81.4)	229 (77.8)	0.41	1.24	0.72–2.02
G	35 (18.6)	65 (22.2)			
Missing	–	2	–	–	–
+127 C/T (rs360717)					
Genotype					
CC	40 (42.5)	84 (56.8)	0.04	0.56	0.32–0.98
CT	48 (51)	58 (39.2)	0.09	1.62	0.93–2.82
TT	6 (6.5)	6 (4.0)	0.54	1.61	0.44–5.87
Missing	–		–	–	–

Table 4 continued

IL-18 genetic variation	Patients, $N = 94$ (valid %)	Controls, $N = 148$ (valid %)	p value	Odds ratio (OR)	95 % CI for OR
Allele					
C	128 (68.0)	226 (76.4)	0.06	0.66	0.43–1.01
T	60 (32.0)	70 (23.6)			
Missing	–	–	–	–	–

Table 5 Haplotype analysis of five IL-18 gene polymorphisms (−656 G/T (rs1946519), −607 C/A (rs1946518), −137 G/C (rs187238), +113 T/G (rs360718), +127 C/T (rs360717)) in patients with thyroid cancer and healthy controls

Haplotype	Cases 2 N = 188 (%),	Controls 2 N = 292 (%),	P value[a]	Odds ratio	95 % CI for OR
GCGTC	77 (41)	157 (53.8)	0.008	0.60	0.40–0.88
TACGT	26 (13.8)	51 (17.5)	0.35	0.76	0.44–1.30
TAGTC	23 (12.2)	34 (11.6)	0.96	1.10	0.58–1.92
TCGTC	23 (12.2)	15 (5.1)	0.008	2.57	1.25–5.35
TAGTT	12 (6.4)	2 (0.7)	0.0008	9.90	2.10–64.7
GAGTC	6 (3.2)	6 (2.0)	0.55	1.57	0.44–0.59
GCGTT	5 (2.7)	2 (0.7)	NA[c]	NA[c]	NA[c]
TACTT	4 (2.1)	4 (1.4)	NA[c]	NA[c]	NA[c]
TCCGT	2 (1.1)	4 (1.4)	NA[c]	NA[c]	NA[c]
Others[b]	10 (5.3)	17 (5.8)	NA[c]	NA[c]	NA[c]

[a] Significant level set to 0.003 based on Bonferroni correction (n = 19)

[b] Others: Haplotypes with frequencies lower than 1 %

[c] NA: Not applicable

Table 6 Haplotype analysis of five IL-18 gene polymorphisms (−656 G/T (rs1946519), −607 C/A (rs1946518), −137 G/C (rs187238), +113 T/G (rs360718), +127 C/T (rs360717)) in patients with thyroid cancer derived from the follicular epithelium (all patients except those with medullary carcinoma) and healthy controls

Haplotype	Cases 2 $N = 166$ (%),	Controls 2 $N = 292$ (%),	p value[a]	Odds ratio	95 % CI for OR
GCGTC	68 (41)	157 (53.8)	0.01	0.60	0.40–0.89
TACGT	24 (14.5)	51 (17.5)	0.48	0.80	0.46–1.39
TAGTC	19 (11.4)	34 (11.6)	0.92	0.98	0.52–1.85
TCGTC	18 (10.8)	15 (5.1)	0.04	2.25	1.04–4.85
TAGTT	11 (6.6)	2 (0.7)	0.0004	10.29	2.12–68.09
GAGTC	6 (3.6)	6 (2.0)	0.36	1.79	0.50–6.37
GCGTT	4 (2.4)	2 (0.7)	NA[c]	NA[c]	NA[c]
TACTT	4 (2.4)	4 (1.4)	NA[c]	NA[c]	NA[c]
GACGT	3 (1.8)	1 (0.3)	NA[c]	NA[c]	NA[c]
TCCGT	2 (1.2)	4 (1.4)	NA[c]	NA[c]	NA[c]
TCCTT	2 (1.2)	1 (0.3)	NA[c]	NA[c]	NA[c]
GACTT	2 (1.2)	1 (0.3)	NA[c]	NA[c]	NA[c]
Others[b]	3 (1.8)	14 (4.9)	NA[c]	NA[c]	NA[c]

[a] Significant level set to 0.003 based on Bonferroni correction (n = 19)

[b] Others: Haplotypes with frequencies lower than 1 %

[c] NA: Not applicable

C/A and −137 G/C with thyroid cancer. Since the pathogenesis and molecular pathology of follicular and papillary thyroid cancers are quite different from those of medullary thyroid cancer, we excluded the patients with medullary thyroid cancer form the second set of analyses. After excluding these patients, and including only the patients with thyroid cancer derived from the follicular epithelium, nearly the same results were observed regarding the genotypes at positions −656 G/T, −607 C/A, and −137G/C.

Polymorphisms at positions −607 C/A and −137G/C have been already reported to influence the binding sites for c-AMP responsive element-binding protein and the H4TF-1 nuclear factors and consequently affect protein production [9, 20]. The functional consequence of −656 G/T SNP in IL-18 gene is not, however, yet fully clarified in the pathogenesis of diseases, particularly in cancer. Despite of this, a recent study specified that T allele at −656 G/T SNP may be considered as a genetic marker associated with resistance to visceral leishmaniasis among Iranian subjects [21]. On the contrary, other studies indicated no significant association between this SNP and lung cancer in Iranians [12], Alzheimer's disease in an Italian population [22], inflammatory bowel disease (IBD) in Japanese patients [23], and destructive periodontal disease in a Caucasian population from Germany [15].

In contrast to −656 G/T SNP, which has been rarely investigated in cancer patients, two other SNPS in IL-18 gene promoter polymorphisms (−607 C/A and −137G/C) have been studied more frequently, and have been suggested to be associated with the risk of cancer in different ethnicities including Iranians [10–12, 24–26, and review in Ref. 27]. In consistent with the findings of this study, our group was not also able to find an association between −607 C/A with other immune-related diseases including stomach and colorectal cancer [10], breast cancer [11], ovarian cancer [28], head and neck squamous cell carcinoma [29], coronary artery disease (CAD) [30], gestational trophoblastic disease (GTD) [31], and recurrent spontaneous abortion (RSA) [32] in Iranian population. We did not also observe any association between −137 G/C SNP and a predisposition to lung cancer [12], ovarian cancer [28], head and neck squamous cell carcinoma [29], CAD [30], GTD [31], and RSA [32] in our previous investigations. Notably, the LD between positions −607 C/A and −137 G/C reported in this study was less than the ones reported previously ($|D'| = 0.74$).

In the current study, the polymorphisms observed in the 3′-untranslated region of IL-18 (+127 C/T and +113 T/G) had no association with the risk of thyroid cancer when all tumor types were included in the analysis. Despite of this, a significantly decreased risk of thyroid cancer derived from the follicular epithelium was observed upon the inheritance of the homozygous genotype (CC) at position +127 C/T. Observing different results before and after excluding thyroid medullary carcinoma may be due to probable differences in the carcinogenesis processes involved in the progression of follicular and papillary thyroid cancer and those of medullary thyroid cancer. However, this observation requires more investigation. The power of the study for the position +127 C/T was calculated to be 0.89 before, but 0.96 after excluding the thyroid medullary carcinoma, suggesting that this observation may also be due to the difference in the power of the study between two set of analyses.

Although the functional consequences of the polymorphisms in the 3′-untranslated region of IL-18 (+127 C/T and +113 T/G) are not yet fully understood, there are two different studies suggesting that +127 C/T and +113 T/G may be considered as genetic risk factors for IBD [23], but not for periodontitis [15].

The discrepancies regarding the association of IL-18 gene polymorphisms and diseases might be due to the nature of IL-18 as a multi-functional cytokine with both protective and pro-cancerous properties. The controversy might also come from the difference in the molecular pathology associated with different cancers, as well as the difference in the ethnicity of investigated populations. The presence of environmental factors associated with thyroid cancer should also not be ignored.

Haplotype analysis indicated a strong association of TAGTT haplotype (−656 T, −607 A, −137 G, +113 T, +127 T) with susceptibility to thyroid cancer in Iranians ($p = 0.0008$, OR 9.9). The significance of this association resisted Bonferroni correction for multiple comparison error. Although the frequency of this haplotype was not high in the population [12/188 (6.4 %) in patients and 2/292 (0.7 %) in controls], this finding suggests that studying the genetic markers at the haplotype level may resolve new aspects of genetic alterations. The functional consequence of TAGTT haplotype merits more investigation.

A trend toward higher frequency of TCGTC haplotype was also observed in patients in comparison to controls ($p = 0.008$), but the difference was not significant after applying the significant level introduced by Bonferroni correction ($p < 0.003$). A closer look at the haplotype data indicated that two haplotypes with trends toward higher risk of the disease, have in common, −656 T allele at the first position. Considering that −656 G/T polymorphism was the one with the strongest association with thyroid cancer susceptibility (Table 3), it might be suggested that the differences observed in haplotype analysis are, in fact, the trace of difference in the frequency of T allele at this position. The data presented in Table 4, however, indicated that for the other two haplotypes with T allele at their first positions (TAGTC and TACGT), no such trend was

observed. In fact, the opposite was true for TACGT, although it did not reach statistical significance. Accordingly, it is hard to conclude that the trend of the first two haplotypes i.e., association with an increased cancer risk is absolutely the trace of −656 T allele in haplotype analysis. From molecular genetic point of view, what finally determines the expression level of a gene is the combination of different *cis*- and *trans*-acting elements. Accordingly, the net effect of two different haplotypes, both sharing one or more alleles at the same position(s), may be absolutely different. The observation could, however, be potentially due to the fact that both heterozygotes and homozygotes are included in the haplotype frequency count (Table 5), whereas in genotype analysis (Table 3) the major findings were based on the separation of heterozygotes (GT) from homozygotes (TT). Regarding the haplotype analysis, nearly the same results were observed after excluding the patients with medullary carcinoma.

In conclusion, our results show an increased risk of thyroid cancer upon inheritance of the homozygous genotype (TT) at −656 G/T position as well as TAGTT haplotype (−656 T, −607 A, −137 G, +113 T, +127 T) in IL-18 gene. A decreased risk of thyroid cancer seems to be associated with inheriting GT heterozygote at this position. The data also suggest that inheriting +127 CC genotype may protect individuals from thyroid cancer derived from follicular epithelium. Our results, however, does not exclude the possibility of other polymorphism(s) to be in strong linkage disequilibrium (LD) with IL-18 −656 G/T SNP. Furthermore, our sample size might affect the power of the study, making it possible to ignore smaller differences in genotype distribution especially at positions −607, −137, and +113 (for which the calculated power was less than 50 %), as well as in haplotype distribution between cases and controls.

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