

ORIGINAL ARTICLE

Interleukin-18 promoter polymorphism is associated with lung cancer: A case-control study

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Abstract

Background. Interleukin-18 (IL-18) is a multifunctional cytokine that augments IFN- γ production and affects tumor immune response. In the present case-control study, we tested whether IL-18 promoter polymorphism contributes to lung cancer susceptibility in Iranian patients. **Material and methods.** The study groups were 73 patients with lung cancer, including 53 with squamous carcinoma (SC) and 20 with small cell lung carcinoma (SCLC), and 97 healthy regional age-matched individuals. The frequency of IL-18 promoter single nucleotide polymorphisms (SNPs) at positions -656 (G/T), -607 (C/A), and -137 (G/C) was determined by polymerase chain reaction analyses. **Results.** There were significant differences in the IL-18 -607 allele and genotype distributions between the 73 lung cancer patients and controls. A significantly higher A allele frequency at position -607, which is associated with lower IL-18 production, was observed in lung cancer patients (48.6% vs. 35%; OR = 1.75; 95% CI 1.13–2.72). Also, patients with the -607 CA and the AA genotypes had a 2.60-fold (95% CI 1.26–5.36) and 3.15-fold (95% CI 1.16–8.55) increase in risk of lung cancer. Subdivision of the patients according to histological type revealed that SC was significantly associated with IL-18 -607 SNPs. Although the percentages of -607 alleles and genotypes in SCLC patients were similar to the results in SC patients, the differences compared to control individuals did not reach statistical significance. Analysis with Arlequin software identified eight haplotypes from three SNPs analyzed here. The distributions of IL-18 gene haplotypes were not significantly different between patients and controls after Bonferroni correction. **Discussion.** This is the first report to investigate the association between IL-18 polymorphism and lung cancer. Our results suggest that IL-18 polymorphism contributes to the lung cancer risk, particularly among SC patients. Further studies with larger numbers of patients are required to determine the possible association between IL-18 polymorphisms and different histological types of lung cancer.

Lung cancer is one of the most fatal malignant disorders in both genders [1]. Most patients are diagnosed at a late clinical stage, and despite significant improvements in conventional therapies for lung cancer, the overall 5-year survival remains poor [2].

Increasing knowledge of lung cancer at the molecular level can point the way to novel biomarkers for cancer susceptibility and diagnosis. Interleukin-18 (IL-18), initially called IFN- γ inducing factor, is a pro-inflammatory, systemic cytokine produced by activated macrophages, epithelial cells, osteoblasts, keratinocytes, and also cancer cells [3,4]. It has been shown that IL-18 administration resulted in a significant suppression of tumor growth

in animal models [4,5], suggesting a role for this cytokine in the host's defense against cancer. IL-18 exerts its anti-tumor activity by augmenting IFN- γ production particularly in the presence of IL-12, promoting Th1 differentiation, enhancing cytotoxic activities of NK cells and CD8+ lymphocytes [4], inducing cancer cell apoptosis [6] and inhibiting angiogenesis [4]. However, IL-18 activities are influenced by the microenvironmental milieu; for instance, IL-18 enhances Th2 differentiation in the presence of IL-4 [4]. Moreover, IL-18 has the ability to inhibit the recognition of cancer cells by immune cells, increase cancer cell adherence to the microvascular wall, induce the production of angiogenic and growth factors, and promote a prometastatic

microenvironment [7,8]. IL-18 concentrations were found to be increased in the blood of metastatic patients compared to patients without metastasis and healthy donors, leading to the suggestion that serum IL-18 concentrations can be used as a noninvasive marker for suspecting metastasis in breast cancer [9]. These findings support the recent suggestion that the pleiotropic cytokine IL-18 can exert both anticancerous and procancerous activities [8].

Expression of IL-18 seems to be regulated by two single nucleotide polymorphisms (SNPs) at positions of -607 (C/A) and -137 (G/C) in the promoter region of the gene. A change from C to A at position -607 disrupts a potential cAMP-responsive element-binding protein site. A change at position -137 from G to C abolishes the human histone H4 gene-specific transcription factor-1 (H4TF-1) nuclear factor binding site [10]. These differences in transcription factor binding are suggestive of mechanisms by which the two promoter polymorphisms affect IL-18 gene activity. Another SNP in the IL-18 promoter region, whose function is unknown, is -656 (G/T). This promoter polymorphism has been investigated thus far in autoimmune diseases [10], but not in malignant diseases.

A number of studies have investigated IL-18, -607 and -137 polymorphisms in malignant processes. Some [11–14], but not all of them [15,16], showed a positive association between malignancy and IL-18 gene polymorphism. We previously studied -607 and -137 polymorphisms in breast cancer [17] and head and neck squamous cell carcinoma (HNSCC) [16] in Iranian patients. We found that these functional SNPs were a genetic risk factor for breast cancer [17], but not for HNSCC [16]. The role of IL-18 promoter polymorphism in lung cancer has not been tested so far. In the present study, -656 (G/T), -607 (C/A) and -137 (G/C) SNPs in the promoter region of the IL-18 gene were analyzed in patients with lung cancer.

Material and methods

Subjects

The study was approved by the Ethics Committee of Shiraz University of Medical Sciences. All participants were informed that blood samples would be used for genotyping, and their consent was obtained.

A total of 73 nonrelated patients (65 men and 8 women; mean age 65.3 ± 9.7 years) and 97 healthy controls (71 men and 26 women; mean age 62.8 ± 9.7 years) were enrolled in this case-control study. The patients were admitted to Faghihi hospital in Shiraz, Iran. The mean age at onset of the disease was

64.5 ± 10.3 years, ranging from 36 to 80 years. Diagnosis of lung cancer was confirmed histopathologically. Fifty-three patients had squamous carcinoma (SC) and 20 had small cell lung carcinoma (SCLC).

Control participants were apparently healthy nonsmokers with no history of malignant, metabolic or autoimmune diseases, who came to Motahari Clinic in Shiraz, Iran for routine blood tests.

DNA preparation

Peripheral blood samples were collected from patients and healthy volunteers in 5 ml volumes in tubes containing EDTA, and genomic DNA was extracted from leukocytes by the salting out method [18].

IL-18 promoter amplification

Polymorphisms at positions -607 (C/A) and -137 (G/C) were detected by allele specific-polymerase chain reaction (AS-PCR) as described previously [16]. Amplification products of 261 bp and 196 bp were detected in -137 and -607 SNPs, respectively.

For genotyping of the -656 (G/T) polymorphism, PCR-restriction fragment length polymorphism (PCR-RFLP) was used with the primers described by Flowaczny et al. [19]. The forward primer 5' AGGTCAGTCTTTGCTATCATTCAGG 3' and reverse primer 5' CTGCAACAGAAAGTAAGCTTGCGGAGAGG 3' amplified a 120-bp fragment of the IL-18 gene. Approximately 100 ng genomic DNA was amplified in each 25 μ l PCR reaction containing 300 μ M dNTP (CinnaGen, Tehran, Iran), 2 U of Taq DNA polymerase (CinaGen), 1 $\times$ PCR buffer, 1.5 mM $MgCl_2$ and 0.8 μ M of each primer (CinaGen). The reaction mixture was first heated to 94°C for 4 min and amplification was done for 30 cycles in a PCR thermocycler (Eppendorf Mastercycler, Bochum, Germany) by denaturation at 94°C for 60 s, annealing at 60°C for 60 s, extension at 72°C for 60 s at each cycle and a 5 min final extension step at 72°C. The volume of the restriction assay was 12.5 μ l, containing 8 μ l PCR products, 2 U *Mwo* I restriction enzyme (Fermentas, Vilnius, Lithuania) and 1 $\times$ buffer. The reaction mixture was incubated at 60°C for 16 h. DNA fragments were resolved in 3.0% agarose gel at 80 V. Ethidium bromide staining was used to reveal the fragments under UV (260 nm) light. For the T allele no digestion of the 120-bp PCR fragment was occurred, and for the G allele two 96/24-bp fragments were obtained.

Statistical analysis

All genotype frequencies were tested for the Hardy-Weinberg equilibrium. The fit to the equilibrium was checked with the χ^2 test. Haplotype frequencies were calculated with Arlequin version 2.000 software.

The data were analyzed with SPSS software (version 11.5). Odds ratios (OR) and 95% confidence intervals (CI) were calculated by the Mantel-Haenszel method to estimate the relative risk associated with each genotype and allele. Pearson's χ^2 test was used to evaluate the differences in the distribution of haplotypes between groups. The p-values for the haplotypes were corrected with the Bonferroni method (p_c) for the number of comparisons tested ($n=8$). Two-sided p-values less than 0.05 were deemed statistically significant.

Results

Polymorphisms at positions -656 (G/T), -607 (C/A) and -137 (G/C) were determined in 73 patients with lung cancer and 97 healthy controls. The frequencies of genotypes in both patient and control groups were in agreement with the Hardy-Weinberg equilibrium (data not shown).

Of 73 patients, 53 had SC and 20 had SCLC. Tables I and II indicate the genotype and allele frequencies and percentages in the 73 patients and in SC and SCLC subgroups.

Neither the genotypes nor alleles at positions -656 and -137 showed any correlation with lung cancer in any of the groups. However, the -607 SNP was significantly associated with lung cancer. The

frequencies of CC, CA and AA genotypes were respectively 15 (20.5%), 45 (61.7%) and 13 (17.8%) in patients with lung cancer, and 40 (41.2%), 46 (47.5%) and 11 (11.3%) in control individuals.

A significant increase in the CA and AA genotypes was observed in lung cancer patients (OR=2.60; 95% CI 1.26–5.36 for CA, and OR=3.15; 95% CI 1.16–8.55 for AA). Moreover, the frequency of the -607A allele was significantly higher in patients (48.6%) than in control individuals (35%) (OR=1.75; 95% CI 1.13–2.72). In 53 SC patients, the CA genotype showed a significant increase (OR=3.04; 95% CI 1.34–6.9), and the AA genotype showed a nonsignificant increase (OR=2.90; 95% CI 0.92–9.13) compared to control individuals. The frequency of the -607A allele was also statistically higher in SC patients (48.1%) compared to control individuals (35%) (OR=1.71; 95% CI 1.06–2.78). In 20 SCLC patients, a nearly statistical significant increase in the disease risk was observed in carriers of the AA genotype (OR=3.63; 95% CI 0.89–14.86) and the -607A allele (OR=1.85; 95% CI 0.93–3.68) (Tables I and II).

Eight possible haplotypes deduced from these SNPs were detected (Table III). Haplotype analysis indicated that none of the haplotype frequencies were significantly different between patients and control individuals after correction by the Bonferroni method. All the possible haplotypes were detected in both patient groups and the control group except the -656T/-607C/-137C haplotype (haplotype 8), which was not found in patients (Table III).

Table I. IL-18 promoter genotypes in Iranian lung cancer patients and controls.

Genotypes	Lung cancer patients			Controls n = 97 (%)	Odds ratio (95% confidence interval)
	Total n = 73 (%)	SC* n = 53 (%)	SCLC** n = 20 (%)		
Position -656					
GG	25 (34.2)	18 (34.0)	7 (35.0)	31 (32.0)	Reference [¶]
GT	31 (42.5)	22 (41.5)	9 (45.0)	39 (40.2)	0.98 (0.48–1.99) [†] , 0.97 (0.44–2.12) [‡] , 1.02 (0.34–3.05) [§]
TT	17 (23.3)	13 (24.5)	4 (20.0)	27 (27.8)	0.78 (0.35–1.74) [†] , 0.82 (0.34–2.00) [‡] , 0.65 (0.17–2.48) [§]
Position -607					
CC	15 (20.5)	10 (18.9)	5 (25.0)	40 (41.2)	Reference [¶]
CA	45 (61.7)	35 (66.0)	10 (50.0)	46 (47.5)	2.60 (1.26–5.36) [†] , 3.04 (1.34–6.91) [‡] , 1.73 (0.54–5.51) [§]
AA	13 (17.8)	8 (15.1)	5 (25.0)	11 (11.3)	3.15 (1.16–8.55) [†] , 2.90 (0.92–9.13) [‡] , 3.63 (0.89–14.86) [§]
Position -137					
GG	33 (45.2)	25 (47.2)	8 (40.0)	53 (54.6)	Reference [¶]
GC	33 (45.2)	24 (45.3)	9 (45.0)	35 (36.1)	1.54 (0.79–2.88) [†] , 1.45 (0.71–2.94) [‡] , 1.70 (0.60–4.83) [§]
CC	7 (9.6)	4 (7.5)	3 (15.0)	9 (9.3)	1.24 (0.42–3.67) [†] , 0.94 (0.26–3.35) [‡] , 2.20 (0.49–9.93) [§]

*Squamous carcinoma. **Small cell lung carcinoma. [¶]Homozygotes for the common allele were used as a reference. [†]Odds ratio and 95% confidence interval for all lung cancer patients (Total). [‡]Odds ratio and 95% confidence interval for SC. [§]Odds ratio and 95% confidence interval for SCLC.

Table II. Allele frequencies of the IL-18 promoter gene in lung cancer patients and controls.

Alleles	Lung cancer patients			Controls 2n = 194
	Total 2n = 146 (%)	Squamous carcinoma 2n = 106 (%)	Small cell lung carcinoma 2n = 40 (%)	
Position -656				
G	81 (55.5)	58 (54.7)	23 (57.5)	101 (52.1)
T	65 (44.5)	48 (45.3)	17 (42.5)	93 (47.9)
OR (95% CI) [†]	0.87 (0.54–1.34)	0.89 (0.55–1.44)	0.80 (0.40–1.59)	
Position -607				
C	75 (51.4)	55 (51.9)	20 (50.0)	126 (65.0)
A	71 (48.6)	51 (48.1)	20 (50.0)	68 (35.0)
OR (95% CI)	1.75 (1.13–2.72)	1.71 (1.06–2.78)	1.85 (0.93–3.68)	
Position -137				
G	99 (67.8)	74 (69.8)	25 (62.5)	141 (72.6)
C	47 (32.2)	32 (30.2)	15 (37.5)	53 (27.4)
OR (95% CI)	1.26 (0.79–2.01)	1.15 (0.68–1.93)	1.59 (0.78–3.25)	

[†]Odds ratios with 95% confidence intervals were calculated with the Mantel-Haenszel method.

Discussion

Cytokines play a complex role in malignant transformation [20]. The proinflammatory cytokine IL-18 showed protective effects against cancer, including lung cancer, in several murine models [8,21], and recombinant human IL-18 is in preclinical trials for the treatment of cancer [22]. Despite the conventional view of IL-18 as an anticancer agent, recent data suggest a procancerous activity for this multifunctional cytokine under some conditions [8].

Several studies have shown that polymorphisms of cytokine genes influence cytokine production, which may or may not be associated with susceptibility to certain diseases [10–16]. Three polymorphisms are present in the promoter region of the IL-18 gene: -656 (G/T), -607 (C/A), and -137 (G/C). Although the functional consequence of -656 polymorphism is not clear, -607 and -137 SNPs can cause differences in transcription factor binding, and may have an impact on IL-18 expression [10]. IL-18 promoter polymorphisms (-607 or -137) were previously shown to be statistically associated with esophageal squamous cell carcinoma [11] and prostate cancer [12] in

Chinese populations, colorectal cancer in a Greek population [13], ovarian cancer in native Hawaiians [14], and breast cancer in Iranians [17]. In contrast, there was no significant association between IL-18 polymorphism and squamous cell carcinoma of the head and neck in Iran [16], or with squamous oral carcinoma in Greece [15]. These discrepant results may be explained by the dual effects of IL-18 on tumor immune response [8] or different tumor sites [16].

In the present study, we evaluated three SNPs at positions -656 (G/T), -607 (C/A), and -137 (G/C) of the IL-18 promoter in lung cancer patients and healthy control individuals in an Iranian population. We observed a significant increase in the CA and AA genotypes and A allele at position -607 of the IL-18 gene in lung cancer patients. The association we found between -607 polymorphisms and lung cancer may be due to the disruption of the potential cAMP-responsive element-binding protein site and subsequent reduction in IL-18 production, as a consequence of the A allele [10]. In this regard, decreased or abolished expression of IL-18 was observed in

Table III. Haplotype frequencies of the IL-18 promoter in lung cancer patients and controls.

Haplotypes	-656	-607	-137	Patients (2n = 146)	Controls (2n = 194)	p-value	p _c [*]
1	G	C	G	50 (34.2%)	75 (38.7%)	0.40	NS [†]
2	T	C	G	22 (15.1%)	44 (22.6%)	0.07	NS [†]
3	T	A	G	23 (15.7%)	15 (7.7%)	0.02	NS [†]
4	T	A	C	20 (13.7%)	30 (15.5%)	0.64	NS [†]
5	G	A	C	22 (15.1%)	16 (8.2%)	0.04	NS [†]
6	G	A	G	6 (4.2%)	7 (3.6%)	0.81	NS [†]
7	G	C	C	3 (2.0%)	3 (1.6%)	0.71	NS [†]
8	T	C	C	0 (0%)	4 (2.1%)	‡	‡

^{*}Corrected p value. [†]Nonsignificant. [‡]The number of subjects was too small to permit statistical analysis.

p-values represent differences in the frequency of a given haplotype between patient and control groups, calculated with the χ^2 test.

human colon carcinoma samples compared to normal colonic mucosa, and the degree of differentiation correlated with the level of IL-18 expression in the colon epithelium [23]. Reduced production of IL-18 can result in decreased IFN- γ synthesis, imbalanced Th1/Th2 differentiation, insufficient activation of NK cells and CD8+ lymphocytes [4], impairment of cancer cell apoptosis [6], and efficient angiogenesis [4].

Subdivision of our lung cancer patients according to histological type revealed that squamous but not small cell disease was significantly associated with IL-18 -607 SNP. The percentage of -607 genotypes in SCLC patients was similar to SC patients, reflecting that the absence of association in the small cell group may be due to the limited number of cases (only 20) analyzed here.

We found no association between IL-18 promoter haplotypes and lung cancer. Similarly, Khalili-Azad et al. found no such association with breast cancer in a population of the same ethnic background [17], despite a positive association with IL-18 promoter polymorphism. In contrast, Wei et al. [11] and Liu et al. [12] found an association between IL-18 promoter haplotype and esophageal squamous cell carcinoma and prostate cancer in Chinese populations. These conflicting data may be due to differences in the linkage disequilibrium in different ethnic backgrounds.

This is the first attempt to investigate the association between IL-18 promoter polymorphism and lung cancer. Also, to the best of our knowledge, -656 SNP has not previously been tested in malignant processes. Our results showed a significant association between IL-18 promoter polymorphism and lung cancer, particularly in patients with squamous disease. More data from a larger number of patients are needed to determine the possible association between IL-18 promoter polymorphism and other histological types of lung cancer.

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