doi:10.4149/neo_2009_01_22

Association of Interleukin-18 gene promoter polymorphisms with breast cancer

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Received April 24, 2008

Interleukin-18 [IL-18] gene promoter polymorphism is reported to be a genetic risk factor for several types of cancer. The aims of this investigation were to evaluate and compare the frequencies of IL-18 gene promoter polymorphisms at positions -137 [G/C] and -607 [C/A] in breast cancer patients and healthy controls as well as to study the contribution of these data with clinicopathological parameters at diagnosis. The studied populations comprised 250 cases with breast carcinoma and 206 healthy subjects. IL-18 gene promoter polymorphisms at positions -137 and -607 were amplified in patient and control groups using allele specific polymerase chain reaction [AS-PCR]. The frequencies of GG, GC and CC genotypes of –137 SNP were 141 [56.4%], 96 [38.4%] and 13 [5.2%] in patients *vs*. 110 [53.4%], 72 [34.9%] and 24 [11.7%] in controls, respectively. A significant decrease of the CC genotype was observed in patients [$p = 0.04$]. The frequency of the CC genotype at position -137 was also significantly higher in patients with metastasis than non-metastatic patients [21.4% *vs*. 4.3%] [$p = 0.02$]. There was no significant association between genotype frequencies at position -607 with breast cancer or its clinicopathological parameters at diagnosis. Moreover, allelic frequencies at these positions did not contribute to breast cancer incidence. The distribution of IL-18 gene haplotypes and genotype combinations were not significantly different between patients and normal control individuals. This is the first report investigating the contribution of IL-18 gene promoter polymorphisms to breast cancer. These results suggest contrast effects of IL-18 gene in cancer induction and progression.

Key words: Breast cancer, IL-18, polymorphism

Interleukin-18 [IL-18], initially was found to be an IFN-γ inducing factor, is a pro-inflammatory, systemic cytokine produced by activated macrophages, epithelial cells, osteoblasts, keratinocytes, and also cancer cells [1]. Stimulation with IL-18 alone induces low levels of IFN-γ, but IFN-γ production is markedly augmented in the presence of IL-12. IL-18 promotes differentiation of T cells to a Th1 phenotype and enhances cytotoxic activities of NK cells and CD8+ lymphocytes [2, 3].

IL-18 administration resulted in significant suppression of tumor growth [4, 5], suggesting a role of this cytokine in the host defense against cancer. A decreased or abolished IL-18 production was observed in colon adenocarcinoma compared to normal colonic mucosa [6]. Induction of cancer cell apoptosis [7] and inhibition of angiogenesis [8] are other mechanisms by which IL-18 can exert these antitumor effects. However, IL-18 functions are influenced by its microenvironment milieu; for instance, IL-18 enhances Th2 cell differentiation in the presence of IL-4 [2]. It was shown that IL-18 has the ability to inhibit recognition of cancer cells by immune cells, increase the adherence of cancer cells to microvascular wall, induce production of angiogenic and growth factors and promote a prometastatic microenvironment [9]. IL-18 levels have also been increased in the blood of metastatic patients compared to patients without metastasis and healthy donors, i.e., serum IL-18 levels are suggested to be used as a non-invasive marker for suspecting metstasis in certain types of cancer [10, 11].

The IL-18 protein expression seems to be regulated by two single nucleotide polymorphisms [SNPs] at positions -137 and -607 in the promoter region of the gene. These SNPs were suggested to change transcription factor binding sites and thus have an impact on IL-18 gene activity [12]. A change at position -137 from G to C abolishes the human histone H4 gene-specific transcription factor-1 [H4TF-1] nuclear factor

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binding site to a bining site for an unknown factor found in the GM-CSF promoter. A change at from C to A at position -607 disrupts a potential cAMP-responsive element-binding protein site [12]. The IL-18 gene polymorphisms, at these sites, are reported to be associated with susceptibility to or pathologic stage of several inflammatory conditions such as autoimmune diseases [13], post-injury sepsis [14] and cancers of ovary [15], nasopharynx [16] and esophagus [17]; its role in breast cancer, however, has not yet been fairly explained.

The present study attempts to elucidate the question of whether the polymorphisms of IL-18 promoter gene in the regulatory regions of -137 [G/C] and -607 [C/A] afford genetic risk for breast cancer, and to evaluate the possible correlation of these SNPs with prognostic factors.

Patients and methods

Subject. After obtaining informed consent, a total of 250 female Iranian patients with breast cancer from the city of Shiraz, in the southern part of Iran, and 206 healthy female regional volunteers were enrolled in this case-control study. Patients were the individuals admitted in different wards of Shiraz University of Medical Sciences hospitals or attended the outpatient clinics. They all had proven breast carcinoma on the basis of surgical and histopathological confirmation.

Neither the cases nor the control individuals were first-degree relatives. Cases and control individuals were matched in the case of age. None of the study participants had any evidence or positive history of a previous malignancy or an autoimmune disease.

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

IL-18 gene amplification. Polymorphisms were detected by allele specific-polymerase chain reactions [AS-PCR] as described previously [19]. Amplification products of 261-bp and 196-bp were detected in the case of -137 and –607 SNPs, respectively.

Statistical analysis. All genotype frequencies were tested for Hardy-Weinberg equilibrium. The fit to the equilibrium was tested by calculating the x^2 test. Haplotype frequencies were calculated by ARLEQUIN version 2.000 [http://www.anthro.unige.ch/arlequin]. The data were analyzed using SPSS software [version 11.5]. Pearson's Chi square test and Fisher's exact probability test [two-sided] were used, when appropriate, to estimate the differences in the distribution of alleles, genotypes, haplotypes and genotype combinations between the studied groups. Findings were deemed to be statistically significant at a p value < 0.05.

Results

Demographic data. The mean and median ages were 49.11 and 48 years [Std. Deviation = 11.57, ranging from 25 to 81], in the patients, and 57.8 and 56 years [Std. Deviation = 14.8, ranging from 40 to 85], in the control subjects. Age of participants was not available in 33 of cases.

The information on clinicopathological parameters at diagnosis including tumor histological type, estrogen receptor, progesterone receptor, menopausal status, lymph node involvement, tumor size, distant metastasis, and breast cancer stage were collected and shown in Table 1.

Genotype distribution. Neither patient nor control genotype frequencies significantly differed from those expected according to the Hardy-Weinberg equilibrium [data not shown].

As it is given in Table 2, the frequency of IL-18 gene polymorphism at position –137 in patients and control individuals revealed a significant difference [$p = 0.04$]. The frequencies of GG, GC and CC genotypes were respectively 141 [56.4%], 96 [38.4%] and 13 [5.2%] in patients with breast cancer, and 110 [53.4%], 72 [34.9%] and 24 [11.7%] in control individuals. The difference denoted a decrease in the CC genotype in breast cancer patients. The distribution of allele frequency at this site, between patient and control groups, did not reach the statistical significant difference [$p > 0.05$] [Table 2].

SNPs at position -607 were determined in 200 of the recruited patients. In this case, the A allele and A allele-bearing genotypes in patients were more frequent than that of the con-

Table 1. Clinicopathological characteristics of breast cancer patients

Features	n [%]
Histological type	220 [100][a]
Infiltrative ductal carcinoma	188 [85.5]
Non-iInfiltrative ductal carcinoma	32 [14.5]
Distant metastasis	247 [100][a]
Positive	14 [5.7]
Negative	233 [94.3]
Estrogen receptor	175 [100][a]
Positive	95 [54.3]
Negative	80 [45.7]
Progesterone receptor	175 [100][a]
Positive	104 [59.4]
Negative	71 [40.6]
Lymphatic involvement	212 [100][a]
Positive	170 [80.2]
Negative	42 [19.8]
Menopausal status	179 [100][a]
Pre-Menopause	104 [58.1]
Post-Menopause	75 [41.9]
Tumor size [cm]	193 [100][a]
≤ 2	76 [39.4]
2 – 5	97 [50.3]
> 5	20 [10.3]
Stage	242 [100][a]
0	6 [2.5]
I	37 [15.3]
II	164 [67.7]
III	21 [8.7]
IV	14 [5.8]

Data are n [%]. [a] Number of patients with the corresponding available data.

Table 2. Allele, genotype and haplotype frequencies of IL-18 promoter polymorphisms in breast cancer patients and controls

	Patients n [frequency]	Healthy Controls n [frequency]	*p* Value
Alleles			
Position -137			
G	378/500 [75.6]	292/412 [70.9]	NS
C	122/500 [24.4]	120/412 [29.1]	
Position -607			
C	231/400 [57.7]	249/412 [60.4]	NS
A	169/400 [42.3]	163/412 [39.6]	
Genotypes			
Position -137			
GG	141/250 [56.4]	110/206 [53.4]	0.04
GC	96/250 [38.4]	72/206 [34.9]	
CC	13/250 [5.2]	24/206 [11.7]	
Position -607			
CC	64/200 [32]	76/206 [36.9]	NS
AC	103/200 [51.5]	97/206 [47.1]	
AA	33/200 [16.5]	33/206 [16]	
Haplotyp*es*			
type I [C/A +]	44 [22]	54 [26.2]	NS
type I [C/A -]	156 [78]	152 [73.8]	
type II [G/A +]	41 [20.5]	29 [14.1]	NS
type II [G/A -]	159 [79.5]	177 [85.9]	
type III [C/C +]	3 [1.5]	7 [3.4]	NS
type III [C/C -]	197 [98.5]	199 [96.6]	
type IV [G/C +]	112 [56]	118 [57.3]	NS
type IV [G/C -]	88 [44]	88 [42.7]	

"+" or "-" indicates the presence or absence of a particular haplotype; n = number of subjects; Data are *n* [%]; NS: Not Significant.

trols, but the genotype and allele frequencies at position -607 did not differ significantly between patients and healthy control group [Table 2].

All four possible haplotypes subsequent to these SNPs were detected in studied groups. The haplotype analysis indicated that none of the haplotype frequencies were significantly different between patients and control individuals [Table 2].

In addition, combinations of IL-18 SNPs were also compared in breast cancer patients and controls. No statistical significance was found. All the possible genotype combinations were detected in both patient and control groups excluding -137CC/-607CC and -137CC/-607AC which were not found in patients [Table 3].

The frequency of IL-18 alleles and genotypes were also compared to the prognostic factors at diagnosis including histological type, tumor size, estrogen and progesterone receptors [ER and PR] expression, lymph node involvement and metastasis. Of all these, only the SNP at position -137 showed an association with metastasis. The frequencies of GG, GC and CC genotypes were respectively 132 [56.6%], 91 [39.1%] and 10 [4.3%] in non-metastatic patients, and 6 [42.9%], 5 [35.7%] and 3 [21.4%] in patients with metastasis. The difference indicated an increase in the CC genotype in patients with breast cancer [$p = 0.02$].

Table 3. Genotype combination frequencies of IL-18 gene polymorphisms in breast cancer patients and controls

Genotype combination	Patients [n = 200] n [%]	Controls [n = 206] n [%]	*P* Value
-137GG/-607CC	60 [30]	71 [34.5]	NS
-137GG/-607AC	49 [24.5]	36 [17.5]	NS
-137GG/-607AA	9 [4.5]	3 [1.5]	NS
-137GC/-607CC	4 [2]	4 [1.9]	NS
-137GC/-607AC	54 [27]	55 [26.7]	NS
-137GC/-607AA	13 [6.5]	13 [6.3]	NS
-137CC/-607CC	0 [0]	1 [0.5]	*
-137CC/-607AC	0 [0]	6 [2.9]	*
-137CC/-607AA	11 [5.5]	17 [8.2]	NS

n = number of subjects; Data are *n* [%]. NS: Not Significant.
* The Number of subjects was not frequent enough to permit statistical analysis.

Discussion

IL-18 is a systemic proinflammatory cytokine with both potential anti-cancerous and pro-cancerous effects [9]. In the present study we evaluated the two single nucleotide polymorphisms at positions -137 [G/C] and -607 [C/A] of the IL-18 gene promoter in breast cancer patients and healthy control individuals in a group of Iranian population. Both SNPs can change transcription factor binding sites and are suggested to have impact on IL-18 levels. However, this impact can vary in different cell types and depend on the local cytokine milieu [12]. We observed a significant increase in the CC genotype at position –137 of the IL-18 gene in metastatic patients compared to non-metastatic patients. In agreement with our results, investigation of IL-18 polymorphisms at positions -137 and -607 has revealed an association between -137 polymorphism and cancer progression in Caucasians, Japanese and native Hawaiians patients with ovarian cancer [15] and in Italian patients with undifferentiated carcinoma of nasopharyngeal type [UCNT] [16].

In our study, while the risk of metastasis has contributed to the -137 CC genotype of IL-18, lower susceptibility to breast cancer has also been associated with this genotype. These results indicate distinct effects of a pro-inflammatory gene on susceptibility and progression of breast cancer. Consistent with our results, this distinct effect of -137 polymorphism of the IL-18 gene has been reported in Hawaiian patients with ovarian cancer [15]. IL-18 and its receptor are expressed by many cell types [2]. There is a possibility that the multifunctional cytokine of IL-18 can act variably in different stages of cancer processes depending on the main cell types involved. Florian *et al*. investigated the effect of a potential inflammatory mediator, IKKß, on each stage of development of cancer [initiation and progression] by its tissue specific-inactivation in either intestinal epithelial cells or in myeloid cells. They suggested that the function of IKKß, during cancer initiation may be distinct to its function during cancer progression. They showed that during very early tumor promotion, IKKß acted within intestinal epithelial cells

and affected the incidence of the tumor through apoptosis of tumor cells without influencing inflammation, but once tumor cells were established, IKKß through secretion of proinflammatory cytokines and paracrine factors from myeloid cells encouraged tumor growth [20]. Moreover, evidence suggests that the local and systemic inflammation may have distinct effects on the immune system. It has been shown that local expression of TNF-α, a pro-inflammatory cytokine, by immune cells has therapeutic effects. However, when it is secreted in circulation can mediate a variety of diseases including cancer [21]. IL-18 serum levels also increase in cancer as the pathologic stage progresses [10, 11]. One can argue that local effects of -137 CC genotype during very early tumor formation may be different from effects of this genotype in an established cancer, due to a change in systemic levels of inflammatory cytokine of IL-18.

Alternative to the above conclusions, the association of -137 CC genotype of IL-18 with breast cancer could be due to a linkage disequilibrium between this position and a potential breast cancer susceptibility site in the IL-18 gene or another gene nearby.

When summarizing, in the present study a moderate significant association was observed between IL-18 gene promoter polymorphism and breast cancer. Our findings indicate that an Iranian individual with the CC genotype at position -137 of the promoter of IL-18 gene has a lower susceptibility to breast cancer, but when one becomes afflicted, the patient's prognosis would be worse than one not carrying the CC genotype. Contrast effects of a proinflammatory cytokine gene in tumor initiation and progression reflect double sword nature of inflammation.

Acknowledgement. The authors thank to Dr Shirin Farjadian for her assistance in statistical analysis. This work was funded by a grant from Shiraz University of Medical Sciences [Grant No. 82-1750] and by Shiraz Institute for Cancer Research [ICR-82-94].

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