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Influence of single nucleotide polymorphism in IL-27 and IL-33 genes on breast cancer

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Breast cancer is a common disease in women, and is increasing in frequency in both developed and developing countries [1]. Although the main cause is, as yet, fully established, aetiology is likely to be multifactorial, one of the most significant being alternations in certain genes such as tumour suppressor genes, growth adjusting genes, and oncogenes. Some of these are single nucleotide polymorphisms (SNPs), most of which are detrimental and so are likely to play a role in susceptibility to cancer and deterioration of the disease [2]. Chronic inflammation, together with intrinsic properties of malignant cells and other pathophysiological processes can promote tumour formation and its further development. Different factors involved in chronic inflammation can stimulate cell growth, invasion, mutagenesis and angiogenesis [3].

The gene for interleukin 27 (IL-27) encodes a product with (depending on the particular situation) both anti-inflammatory and pro-inflammatory properties. It is composed of two subunits: IL-27 P28 and Epstein-Barr virus-induced gene 3 protein, which are produced after the stimulation of immune cells by microbial products or inflammatory mediators [4]. IL-27 can increase the differentiation of T helper 1 (Th1), inhibit T helper 2 (Th2) proliferation, stimulate the activity of cytotoxic T cells, and cooperate with IL-12 in the production of interferon gamma (IFN-γ) by T lymphocyte and natural killer cells [5]. The rs153109 A/G polymorphism is located on the promoter region of IL-27 gene, and accordingly may play an important role in modulating the transcription processes and protein expression. Zhang et al. reported this SNP to be protective of breast cancer in pre-menopausal Chinese women [6]. The gene for interleukin 33 (IL-33), at 9p24.1, encodes a protein of 270 amino acids with a helix-turn-helix domain and a IL-1 receptor-like 1 (IL-1RL1) domain [7]. Produced by endothelial cells, IL33 plays an important role in the regulation of immune system and inflammation processes, such as the activation of Th2 and mast cells [8]. As a member of the IL-1 family it also plays a fundamental role in various pathological and physiological processes, such as tissue homeostasis, autoimmune diseases and cancers [9]. Several *IL-33* SNPs have been reported, such that of rs1929992 A/G in intron 3. Jafarzadeh et al. failed to find a link between rs1929992 A/G and breast cancer [10]. We hypothesised links between SNPs rs153109 A/G (IL-27) and rs1929992 A/G (IL-33), and breast cancer.

We recruited 280 women from Tehran Hospitals during 2015 to 2017: 140 women with breast cancer (confirmed by histology, physical examination and imaging) and 140 healthy controls without any familial history were enrolled which referred to routine physical examination. Women with liver, metabolic, cardiovascular, kidney and any systemic disease were excluded. All participants were informed about the study and signed a consent form according to the Declaration of Helsinki. Genomic DNA extraction was performed using salting-out procedure from peripheral blood (5 ml). The polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method was used to genotyping. The primers for rs153109 A/G polymorphism were forward 5′-ACCAAGAAACCCCATCCTCT-3′ and reverse 5′-TCAGTCAGTGACCAGGATCG-3′. The primers

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for rs1929992 A/G polymorphism were forward 5′-GAAGTCATCATCAACTTGGAACC-3′ and reverse 5′-GGATTGGAATCCCATGGTC-3′. The amplicon size produced by used primers was 224 bp for rs153109 A/G and 217 bp for rs1929992 A/G. The amplicon produced were digested with the *PaeR7I* (rs153109 A/G) and *SspI* (rs1929992 A/G) restriction enzymes. The RFLP pattern for rs153109 A/G and rs1929992 A/G polymorphisms were as follows: (AA: 224bp; AG: 224bp+ 179bp+ 45bp; GG: 179bp+ 45bp) and (GG: 217bp; AG: 217bp+ 134bp+ 83bp; AA: 134bp+ 83bp), respectively. The PCR reaction was performed in volume of 25 µL as follows: each primer 2 µL, PCR master mix (Cinagen, Iran) 12.5 µL, sterile double distilled water 7.5 µL and extracted DNA 1 µL. The PCR conditions were 1 cycle initial denaturation (94 °C for 4 min), 40 cycles denaturation (94 °C for 40 s), 40 cycles annealing (62 °C and 61 °C for 30 s), 40 cycles extension (72 °C for 25 s) and 1 cycle final extension (72 °C for 5 min). Finally, digested PCR products were separated by 3% agarose gel stained with ethidium bromide and visualised by ultraviolet radiation. The association between polymorphisms and breast cancer risk was analysed by logistic regression. The chi-square (χ^2) test was used to analyse Hardy-Weinberg equilibrium (HWE) for case and control groups. The clinical features and demographic variables were analysed by *T*-test. $p < 0.05$ was considered statistically significant. All statistical analysis was performed by SPSS software (version 21.0).

The clinical features and demographic characteristics of the patients and controls are shown in Table 1. The only significant difference was in family history of breast cancer. The genotype and allele frequencies for rs153109 A/G and rs1929992 A/G polymorphisms hold the HWE according to $\chi 2$ tests ($p > 0.05$). The frequencies of *IL27* rs153109 A/G polymorphism AA, AG and GG codominant genotypes in patients were 37.9%, 47.9% and 14.3%, respectively; whereas in the controls these were 42.1%, 47.1% and 10.7%, respectively ($p = 0.594$) (Table 2). There were no significant differences between case and controls in the rs153109 A/G genotypes frequencies in the codominant, dominant, recessive and overdominant inheritance models. Also, evaluation of alleles' frequencies of rs153109 A/G polymorphism showed no significant differences between case and controls ($p > 0.05$). The frequencies of the *IL33* rs1929992 A/G polymorphism GG, AG and AA codominant genotypes in patients were 17.1%, 43.6% and 39.3%, respectively; whereas in the controls these were 20%, 47.1% and 32.9%, respectively ($p = 0.520$) (Table 2). There were no significant differences between case and controls in the rs1929992 A/G genotypes frequencies in the codominant, dominant and overdominant inheritance models, but the differences in the recessive inheritance model was significant. Also, evaluation of alleles' frequencies of rs1929992 A/G polymorphism showed no significant differences between case and controls ($p > 0.05$).

Table 1. The clinical features and demographic variables of participants.

Variables	Patients ($n = 140$)	Controls ($n = 140$)	*p*-Value
Age, years	53.2 ± 9.1	52.9 ± 10.0	0.455
BMI, kg/m^2	23.3 ± 2.8	22.5 ± 3.2	0.295
Age at menarche, years	12.5 ± 2.1	12.6 ± 2.1	0.395
Menopausal status			
Premenopausal	104 (74.3%)	113 (80.7%)	
Postmenopausal	36 (25.7%)	27 (19.3%)	0.121
Tobacco smoking			
Never	131 (93.6%)	133 (95.0%)	
Ever	9 (6.4%)	7 (5.0%)	0.566
Alcohol drinking			
Never	126 (90.0%)	131 (93.6%)	
Ever	14 (10.0%)	9 (6.4%)	0.449
Age at first delivery, year	25.1 ± 3.5	23.8 ± 3.2	0.288
Family history			
Positive	11 (7.8%)	3 (2.1%)	
Negative	129 (92.1%)	137 (97.96%)	0.023

Data mean (SD) or *n* (%). BMI: body mass index.

Cytokines involved in the inflammation process play important role in altering epithelial tissues in many types of cancers. Inflammation precedes and enhances tumour development and progression, and promotes angiogenesis and suppresses the innate anti-cancer immune response [11]. Cytokines are a protein family of regulatory factors derived from leukocytes, fibroblasts, endothelial cells and tumours that contribute to the growth, invasion and metastasis of breast cancer [12]. IL-27 is likely to play a pivotal role cancer progression as tumour oncogenes or tumour suppressors, being involved in differentiation, apoptosis and proliferation [4–6]. Yu et al. showed that rs153109 A/G polymorphism is associated with susceptibility to endometrial cancer in Chinese women [13]. Zhang et al. demonstrated no significant relationship between the *IL27* rs153109 A/G SNP and risk of breast cancer in a Chinese population [6], a finding with which we concur, in a Iranian population. However, due to small sample size and limited studies, we cannot confirm that this SNP is associated with breast cancer in women.

There are few, but contradictory, reports in role of IL-33 in tumour formation. Some suggest that IL-33 may have pro-tumour activities, and in contrast others suggest anti-tumour activities [14,15]. Jafarzadeh et al. reported no significant association between the *IL33* rs1929992 A/G SNP and risk of breast cancer in a Iranian cohort [10]. However, with more power and complex analyses we find that the recessive GG+ GA genotype protects against breast cancer compared to the AA genotype. The reasons for this difference in mentioned studies is unclear but could be due to the presence of other related genes and environmental factors, the clinical status of the cases and controls (stage of the cancer, age, family history, menopause,

Table 2. Genotype and allele distribution of rs153109 and rs1929992 SNPs.

Polymorphisms	Inheritance model	Genotype and allele	Patients (n = 140)	Controls (n = 140)	p-Value	OR (95% CI)
IL-27	Codominant	AA	53 (37.9%)	59 (42.1%)	Ref	Ref = 1
rs153109 A/G		AG	67 (47.9%)	66 (47.1%)	0.666	1.09 (0.64–2.11)
		GG	20 (14.3%)	15 (10.7%)	0.475	0.85 (0.50–1.43)
	Dominant	AA	53 (37.9%)	59 (42.1%)	Ref	Ref = 1
		AG + GG	87 (62.1%)	81 (57.8%)	0.258	0.77 (0.49–1.39)
	Recessive	GG	20 (14.3%)	15 (10.7%)	Ref	Ref = 1
		AA + AG	120 (85.7%)	125 (89.3%)	0.287	1.49 (0.88–2.71)
	Overdominant	AG	67 (47.9%)	66 (47.1%)	Ref	Ref = 1
		AA + GG	73 (52.1%)	74 (52.8%)	1.000	0.78 (0.11–4.00)
		A normal	173 (61.8%)	184 (65.7%)	Ref	Ref = 1
		G minor	107 (38.2%)	96 (34.3%)	0.345	0.63 (0.41–1.69)
IL-33	Codominant	GG	24 (17.1%)	28 (20.0%)	Ref	Ref = 1
rs1929992 A/G		GA	61 (43.6%)	66 (47.1%)	0.576	1.24 (0.35–2.15)
		AA	55 (39.3%)	46 (32.9%)	0.230	1.15 (0.69–1.95)
	Dominant	GG	24 (17.1%)	28 (20.0%)	Ref	Ref = 1
		GA + AA	116 (82.9%)	112 (80.0%)	0.708	1.27 (0.65–1.99)
	Recessive	AA	55 (39.3%)	46 (32.9%)	Ref	Ref = 1
		GG + GA	85 (60.7%)	94 (67.1%)	0.035	4.75 (0.89–23.78)
	Overdominant	GA	61 (43.6%)	66 (47.1%)	Ref	Ref = 1
		GG + AA	79 (56.4%)	74 (52.9%)	0.455	0.90 (0.57–1.33)
		G normal	109 (38.9%)	122 (43.6%)	Ref	Ref = 1
		A minor	171 (61.1%)	158 (56.4%)	0.454	0.99 (0.44–2.21)

OR: odds ratio; CI: confidence interval.

etc.), and in differing sample sizes. Although our data add to our understanding of breast cancer as a multifactorial disease, we recognise our sample size is not large, and so further studies are recommended on other populations and races with larger sample sizes.

This work represents an advance in biomedical science because it excludes the rs153109 A/G (IL-27) SNP, but points to the value of the rs1929992 A/G (IL-33) SNP, in determining susceptibility to breast cancer, so that the latter SNP may be used as a marker to identify individuals at high risk for this disease.

Disclosure statement

No potential conflict of interest was reported by the authors.

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