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ORIGINAL ARTICLE

Relationships between Common and Novel Interleukin-6 Gene Polymorphisms and Risk of Cervical Cancer: a Case-Control Study

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Abstract We investigated the association between six common and novel interleukin-6 (IL-6) polymorphisms with the risk of cervical cancer (CC) among Tunisians. Study subjects comprised 112 CC cases and 164 control women. Genotyping of IL-6 rs2069845, rs2069840, rs1474348, rs1800795, rs1800797, rs2069827 variants was done by real-time PCR, with defined clusters. The allelic and genotypic distributions of the tested IL-6 SNPs were comparable between CC patients and control women. Stratification according to FIGO staging revealed that rs1800795 homozygous major allele genotype (P = 0.033; OR =0.49(0.25–0.95)) and major allele (P = 0.037; OR = 0.57 (0.33–0.97)) were protective of CC. Moreover, carriage of rs1474348 major allele was also protective of CC (P = 0.014; OR = 0.53(0.32–0.88)), while higher rs1474348 minor allele frequency was seen in CC patients with early FIGO stage (P = 0.044; OR = 0.39 (0.15–1.00)), thus implicating rs1474348 in CC evolution and progression of angiogenesis. Haploview analysis demonstrated high linkage disequilibrium (LD) between rs2069845, rs2069840, rs1474348 and rs1800795, and 6-locus haplotype analysis identified GACCCA haplotype to be positively associated with increased CC, while GAGGGG haplotype was negatively associated with CC, thus suggesting a protective role for this haplotype in CC. Furthermore, there was a significant association between the incidence of CC and the use hormonal contraception (P = 0.047; OR = 1.97 (0.94–4.13)) and smoking (P < 0.001; OR = 7.12 (2.97–17.04)). The IL-6 variants rs1800795 and rs1474348, and haplotypes GACCCA and GAGGGG, along with use of hormonal contraceptives and smoking, are major risk factors of CC susceptibility and evolution among Tunisian women.

Keywords Interleukin-6 · Cervical cancer · FIGO stages · Polymorphisms · Haplotypes · Tunisians

Introduction

Cervical cancer (CC) is a serious public health concern, and is the fourth leading cause of cancer-related deaths in women worldwide [1]. Persistent infection with oncogenic human papilloma virus (HPV) and chronic inflammation are well-established causes of CC [2, 3]. Other (modifiable) co-factors were also implicated in the development of CC, including smoking, use of oral contraceptives, and the number of sexual partners [4–6]. While their exact contribution is not fully understood, it is believed that they enhance carcinogenesis, precipitate a state of immunosuppression, which in turn accelerate the state of inflammation frequently linked with CC. In this regard, several reports documented that tumor angiogenesis of CC is controlled by numerous growth factors and different cytokines, including interleukin (IL)-6.

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IL-6 is a pro-inflammatory cytokine, with a critical role in inhibition of apoptosis, stimulation of cellular proliferation and angiogenesis, and to enhance the invasive and metastatic potential of several cancers [7]. The expression IL-6 is controlled by the transcription factor NF-κB, which is constitutively active in most tumors, and induced by carcinogens, tumor promoters, and carcinogenic viral proteins (including HPV), chemotherapeutic agents, and γ-irradiation. This assigns a central role for IL-6 in the prevention, and possibly treatment of CC. Previous studies dmonstrated over-expression of IL-6 mRNA expression and protein secretion in CC cells, and indicated that IL-6 exerts its effects in promoting cervical tumor cell growth both in autocrine and paracrine fashions [8–11]. Moreover, IL-6 levels were reportedly increased in cervicovaginal secretions of patients with uterine CC; its levels being directly related to the severity of cervical neoplasia.

We previously reported on the association of gene variants in the vascular endothelial growth factor (*VEGFA*) gene with the presence of CC [12]. Insofar as IL-6 was reported to induce VEGF gene expression [13, 14], this raised the possibility that IL-6 may also be involved in tumor angiogenesis in CC. Previous studies that investigated the association of IL-6 SNPs in CC, revealed an inconclusive findings [15–18]: IL-6 rs1800797 was associated with decreased CC risk in Sweden [15] and rs1800795 confer a high risk of CC [16, 17] which is further modulated in patients who are tobacco users [16]. However, among chines women, IL-6 rs2069837 and rs2069840 increased CC risk but rs2069840 showed a lack of association with CC [18].

Here we investigate the contribution of rs2069845, rs2069840, rs1474348, rs1800795, rs1800797, rs2069827 nucleotide polymorphisms (SNPs) in the *IL-6* gene to CC susceptibility and evolution among Tunisian population.

Subjects and Methods

Subjects

This retrospective case–control study was performed between October 2010 and August 2012 at Salah Azeiz Oncology Institute (SAI), Tunis, Tunisia. Study subjects comprised 112 patients with histological confirmed CC, and 164 age-matched healthy women who were free of malignancy, drug allergy, hypertension, diabetes, or cardiovascular disease. Clinical data were collected through self-reported questionnaires, and tumor staging was according to International Federation of Gynecology and Obstetrics (FIGO) classification (www.figo.org). Study subjects were from different regions of Tunisia, and were asked to sign a consent form agreeing to participate in the study; all institutional ethics requirements were met. Blood samples were taken from all participants in EDTA-containing tube for total genomic DNA extraction shortly before to radiation therapy or chemotherapy. Genomic DNA was extracted using QIAamp DNA blood Mini Kit, according to manufacturer's instruction (Qiagen GmbH, Hilden, Germany).

IL-6 Genotyping

Six SNPs in the *IL-6* gene were selected, based on minor allele frequency (MAF) of >5 % in Caucasians. *IL-6* genotyping was performed by the allelic (VIC- and FAM-labeled) discrimination method. TaqMan assays, as assay-on-demand, were ordered from Applied Biosystems (Foster City, CA). The reaction was performed in 6 μl volume on StepOne/StepOne Plus real-time PCR systems, according to manufacturer's instructions (Applied Biosystems). Replicate blinded quality control samples were included to assess reproducibility of the genotyping reaction and concordance was >99 %. Additional quality control measures comprised direct DNA re-sequencing of patient (n = 40) and control (n = 40) specimens (ABI 3130_l Genetic Analyzer; Applied Biosystems). Genotyping call rate exceeded 99 %, with no significant differences between cases and control samples.

Statistical Analysis

Statistical analysis was performed on SPSS v. 21.0 (SPSS Inc., Chicago, IL) and Epi info 7. Data were expressed as percentages of total (*categorical variables*) or as mean ± SD (*continuous variables*). Student's *t*-test was used to determine differences in means, and Pearson χ^2 or Fisher's exact test were used to assess inter-group significance. Allele frequencies were calculated by the gene-counting method, genotypes were tested for departures from Hardy–Weinberg equilibrium (HWE) in the control population using Haploview version 4.2 (http://www.broad.mit.edu/mpg/haploview).

All analyses were conducted under additive genetic effect, as it is the conservative model, using SNPStats software (bioinfo.iconcologia.net/snpstats/). Linkage disequilibrium analysis was performed using Haploview 4.2, and haplotype reconstruction was performed by the expectation maximization method (Haploview 4.2). Logistic regression analysis was performed in order to determine the odds ratios (OR) and 95 % confidence intervals (95%CI) associated with the cervical cancer risk, taking the control as the reference group. Statistical significance was set at $P < 0.05$; statistically significant differences being designated as boldface in the tables.

Table 1 Characteristics of study participants

	Cases (n = 112)	Controls (n = 164)	P [a]
Environnemental characteristics			
Age (mean ± SD)	52.0 ± 1.2	52.2 ± 0.9	0.992
≥50 years	60 (53.58 %) [b]	66 (40.24 %)	**0.036**
Married status	108 (96.40 %)	150 (91.50 %)	0.163
Sexual partner: 0	2 (1.80 %)	9 (5.50 %)	0.218
≥1	110 (98.20 %)	155 (94.50 %)	
Post-menopausal	82 (73.20 %)	112 (68.30 %)	0.422
Oral contraceptive users	101(90.10 %)	135 (82.30 %)	0.081
Smokers	27 (24.00 %)	7 (4.30 %)	**0.000**
Family history of cancer	25 (22.30 %)	10 (6.09 %)	**0.000**
FIGO staging: Stage I	39 (34.80 %)	NA	
Stage II	41 (36.60 %)	NA	
Stage III	26 (23.20 %)	NA	
Stage IV	6 (5.40 %)	NA	
Histology: Squamous cell carcinoma	93 (83.03 %)	NA	
Adenocarcinoma	16 (14.27 %)	NA	
Sarcoma	3 (2.70 %)	NA	

FIGO International Federation of Gynecology and Obstetrics, *NA* not applicable

[a] Student's t-test (continuous variables), Pearson χ^2 test (categorical variables)

[b] Number of subjects (percent total within group)

Results

Study Subjects

Demographic and clinical characteristics of cases and control groups are described in Table 1. The median age was 52 years for patients and healthy controls (range: 30–70 years), with most CC cases being in the 51–60 years category. Among CC patients; 108(96.40 %) were married, 110 (98.20 %) reported one or more sex partners, 82 (73.20 %) were post-menopausal. In addition, 101 (90.10 %) used oral contraceptives, 27 (24.00 %) were smokers, and 25 (22.30 %) reported positive family history of cancer. Diagnoses of squamous cell carcinoma confirmed by histology as per FIGO revealed 39 (34.80 %) with stage I, 41 (36.60 %) with stage II, 26 (32.20 %) with stage III, and the remaining 6 (5.40 %) with stage IV. The majority of the histological types identified were squamous cell carcinoma 93 (83.03 %), followed by adenocarcinoma 16 (14.27 %), and sarcoma 3 (2.70 %).

Association Studies of IL-6 Alleles and Genotypes

Six IL-6 SNPs were selected for this study based on their minor allele frequency (MAF) of >5 % in Tunisians. The allelic distribution of the *IL-6* SNPs rs2069845, rs2069840, rs1474348, rs1800795, rs1800797, rs2069827 between CC patients and controls are summarized in Table 2. MAF frequencies of all tested SNPs were comparable between CC patients and healthy women. Taking homozygous wild-type genotype as reference (OR =1.00), the genotype distribution of the tested IL-6 SNPs was similar between both CC cases and control groups (Table 3).

Association Studies of IL-6 Alleles and Genotypes According FIGO Stages, and CC Evolution

The observed genotype and allele frequency distribution of IL-6 gene polymorphisms between cases with a tumor in stage (I + II) and (III + IV) versus controls are depicted in Table 4. Taking homozygous wild-type genotype as reference (OR =1.00), decreased CC risk with early tumor stages was seen with rs1474348 and rs1800795. The minor allele of rs1474348 is more frequent among CC patients with a tumor in early stages, while the major allele appeared as protective of CC (P = 0.014; OR =0.53 (0.32–0.88)). In addition, both major allele (P = 0.037; OR =0.57 (0.33–0.97)), and major allele homozygous genotype (P = 0.033; OR =0.49 (0.25–0.95)) of rs1800795 are also protective. No significant association was seen in the remaining SNPs. The *IL-6* allele genotype frequencies between CC cases with advanced tumor stages and healthy controls were comparable, and no significant association was observed for CC development in stage (III + IV).

Table 2 IL-6 SNPs allelic distribution in patient and control groups

IL-6 SNPs			MAF		HW[c]	χ^2	p_{value}	OR (95 % CI)
rs number	Location [b]	MA	Cases[a]	Controls[a]				
rs2069827	22,725,837	T	0.089	0.054	0.548	1.185	0.276	1.65 (0.66–4.14)
rs1800797	22,726,602	G	1.625	1.615	0.001	0.018	0.893	1.03 (0.66–1.58)
rs1800795	22,727,026	C	0.330	0.225	0.001	3.145	0.076	1.55 (0.95–2.54)
rs1474348	22,728,289	C	0.348	0.243	0.006	2.952	0.085	1.51 (0.94–2.44)
rs2069840	22,728,953	C	1.535	1.426	1.000	2.028	0.154	1.32 (0.89–1.96)
rs2069845	22,730,530	G	1.571	1.567	0.184	0.004	0.951	1.01 (0.67–1.53)

[a] Study subjects included 112 CC cases and 164 control women

[b] Location on chromosome

[c] HWE, Hardy-Weinberg equilibrium; MA: minor allele; MAF: minor allele frequency

A case-only analysis (early vs. advanced FIGO stages) was carried out to investigate the implication of *IL-6* gene polymorphisms in CC evolution. Our data demonstrated a statistically significant relationship between the incidence of rs1474348 and the clinical progression of CC according to FIGO classification. The minor allele of rs1474348 is more common among patients with an early FIGO stage (P = 0.044; OR =0.39 (0.15–1.00)), thus prompting the conclusion that this SNP is implicated in tumor evolution and

Table 3 *IL-6* genotype distribution in cases and controls

SNPs	Genotypes	Cases [a] (n,%)	Controls [a](n,%)	P_{value}	OR (95 % CI)
rs2069845					
	G/G	69 (61.60 %)	105 (64.00 %)	-	**1.00 (Reference)**
	G/A	38 (33.90 %)	47(28.65 %)	0.260	0.81(0.48–1.37)
	A/A	5 (5.00 %)	12 (7.35 %)	0.290	1.577 (0.53–4.67)
rs2069840					
	C/C	63 (56.30 %)	85 (51.90 %)	-	**1.00 (Reference)**
	C/G	45 (40.00 %)	64 (39.00 %)	0.469	1.05 (0.63–1.74)
	G/G	4 (3.70 %)	15 (9.10 %)	0.120	2.77 (0.88–8.77)
rs1474348					
	G/G	80 (71.42 %)	129 (78.65 %)	-	**1.00 (Reference)**
	G/C	25 (22.33 %)	30 (18.30 %)	0.207	0.744 (0.40–1.35)
	C/C	7 (6.25 %)	5 (3.05 %)	0.140	0.44 (0.13–1.44)
rs1800795					
	G/G	81 (72.30 %)	133 (81.10 %)	-	**1.00 (Reference)**
	G/C	25 (22.35 %)	25 (15.25 %)	0.078	0.60 (0.32–1.13)
	C/C	6 (5.35 %)	6 (3.65 %)	0.291	0.60 (0.19–1.95)
rs1800797					
	G/G	74 (66.07 %)	116 (70.75 %)	-	**1.00 (Reference)**
	G/A	34 (30.36 %)	33 (20.12 %)	0.062	0.61 (0.35–1.08)
	A/A	4 (3.57 %)	15 (9.15 %)	0.197	2.39 (0.76–7.48)
rs2069827					
	G/G	103 (92.00 %)	155 (94.50 %)		**1.00 (Reference)**
	G/T	8 (7.14 %)	9 (5.50 %)	0.367	0.747 (0.279–2.00)
	T/T	1 (0.86 %)	0 (0.00 %)	0.840	ND

Values in bold are statistically significant at the 5 % level; nd: not defined

[a] Study subjects comprised 112 CC patients and 164 control women; n number of women, OR: odds ratio; nominal value of comparison; P > 0.05 no significant association, degree of freedom = 1

Table 4 Genotype and allele frequency distribution of IL-6 polymorphisms according FIGO stages

IL-6 SNPs		Controls	Early stages (n = 80)(%)	p_{value}	OR (CI 95 %)	Advanced stages (n = 32)(%)	p_{value}	OR (CI 95 %)
rs2069845	G/G	105 (64.00 %)	47(58.75 %)	-	**1.00 (Reference)**	23(71.85 %)	-	**1.00 (Reference)**
	G/A	47(28.65 %)	30(37.50 %)	0.223	0.7(0.39–1.24)	7(21.85 %)	0.056	0.37(0.13–1.05)
	A/A	12 (7.35 %)	3(3.75 %)	0.558	1.7(0.48–6.64)	2(6.30 %)	0.979	0.97(0.27–6.27)
	A	71(21.65 %)	36(55.50 %)	0.830	0.95(0.60–1.49)	11(17.20 %)	0.422	1.33(0.66–2.68)
rs2069840	C/C	85 (51.90 %)	46(57.50 %)	-	**1.00 (Reference)**	18(56.25 %)	-	**1.00 (Reference)**
	C/G	64 (39.00 %)	31(38.75 %)	0.697	1.1(0.63–1.95)	13(40.50 %)	0.917	1.04(0.47–2.28)
	G/G	15 (9.10 %)	3(3.75 %)	0.195	2.7(0.74–9.83)	1(3.25 %)	0.439	3.17(0.39–25.6)
	G	94(28.65 %)	37(23.12 %)	0.235	1.33(0.86–2.07)	15(23.45 %)	0.393	1.31(0.70–2.45)
rs1474348	G/G	129 (78.65 %)	53(66.25 %)	-	**1.00 (Reference)**	27(84.88 %)	-	**1.00 (Reference)**
	G/C	30 (18.30 %)	21(26.25 %)	0.125	0.58(0.30–1.11)	4(12.50 %)	0.591	1.56(0.51–4.82)
	C/C	5 (3.05 %)	6(7.50 %)	0.075	0.34(0.10–1.17)	1(3.12 %)	0.610	1.04(0.11–9.32)
	C	40(12.20 %)	33(20.60 %)	**0.014**	**0.53(0.32–0.88)**	6(9.40 %)	0.521	1.34(0.54–3.31)
rs1800795	G/G	133 (81.10 %)	55(68.75 %)	-	**1.00 (Reference)**	25(78.13 %)	-	**1.00 (Reference)**
	G/C	25 (15.25 %)	21(26.25 %)	**0.033**	**0.49(0.25–0.95)**	5(15.62 %)	0.907	0.93(0.32–2.68)
	C/C	6 (3.65 %)	4(5.00 %)	0.712	0.62(0.16–2.28)	2(6.25 %)	0.845	0.56(0.10–2.95)
	C	37(11.30 %)	29(18.12 %)	**0.037**	**0.57(0.33–0.97)**	9(14.00 %)	0.527	0.77(0.35–1.70)
rs1800797	G/G	116 (70.75 %)	51(63.75 %)	-	**1.00 (Reference)**	23(71.87 %)	-	**1.00 (Reference)**
	G/A	33 (20.12 %)	26(32.50 %)	0.056	0.55(0.30–1.02)	8(25.00 %)	0.198	0.81(0.33–1.99)
	A/A	15 (9.15 %)	3(3.75 %)	0.338	2.19(0.60–7.92)	1(3.13 %)	0.475	2.95(0.37–23.64)
	A	63(19.20 %)	32(20.00 %)	0.835	0.95(0.59–1.52)	10(15.62 %)	0.500	1.28(0.61–2.66)
rs2069827	G/G	155 (94.50 %)	74(92.50 %)	-	**1.00 (Reference)**	29(90.62 %)	-	**1.00 (Reference)**
	G/T	9 (5.50 %)	5(6.25 %)	0.972	0.85(0.27–2.64)	3(9.38 %)	0.662	0.56(0.14–2.19)
	T/T	0 (0.00 %)	1(1.25 %)	0.710	ND	0(0.00 %)	0.415	ND
	T	9(2.75 %)	7(4.40 %)	0.342	0.61(0.22–1.68)	3(4.70 %)	0.667	0.57(0.15–2.18)

Values in bold are statistically significant at the 5 % level; ND: not defined

n number of women, *OR* odds ratio; nominal value of comparison; P > 0.05 no significant association, degree of freedom = 1

angiogenesis. No significant association of the remaining *IL-6* SNPs was identified with clinical stages of CC (Table 5).

Haploview Analysis

We evaluated the interaction between the tested *IL-6* SNPs and by analyzing the distribution of 6-locus (rs2069827-rs1800797-rs1800795-rs1474348-rs2069840-rs2069845) haplotypes in CC cases and healthy controls by Haploview (Fig. 1). IL-6 haplotypes were constructed based on the prevalence of individual SNPs and LD between them (Fig. 1). Haploview analysis demonstrated strong linkage disequilibrium (LD) between rs2069845, rs2069840, rs1474348 and rs1800795 but moderate between rs1800797 and rs2069827 and the other SNPs (Fig.1). Of the possible 64 haplotypes, only 9 were common (frequency > 1 %), and thus were considered "common". Results from Table 6 demonstrated enrichment of GACCCA haplotype, and reduction in GAGGGG haplotype among CC cases than controls, thus conferring disease protection and protection to these haplotypes, respectively. The distribution of the other haplotypes was comparable between CC cases and control subjects.

Discussion

IL-6 is a pro-inflammatory cytokine, and plays an important role in the pathogenesis of several types of cancers, including CC. *IL-6* variants, associated with variations in immune response that contribute to an increased risk of cancer and considered as a prognostic indicator of CC [9, 19]. Six IL-6 SNPs were chosen for this study, which was based on their minor allele frequency (MAF) of >5 % in Caucasians, and their association with altered IL-6 secretion and link with altered immunity. This is the first study that evaluated the involvement of *IL-6* SNPs in CC evolution, and more studies are required for confirming, or alternatively ruling out the association of these variants with CC. In addition, haploview analysis revealed differential LD between the tested variants, but identified both CC-susceptible (GACCCA) and CC-protective (GAGGGG) haplotypes. To the best of our

Table 5 Implication of IL-6 polymorphisms in cervical cancer evolution

IL-6 SNPs		Early stages (n = 80)(%)	Advanced stages (n = 32)(%)	p_{value}	OR (CI95%)
rs2069845	G/G	47(58.75 %)	23(71.85 %)	-	**1.00(Reference)**
	G/A	30(37.50 %)	7(21.85 %)	0.126	0.47(0.18–1.24)
	A/A	3(3.75 %)	2(6.30 %)	0.869	1.36(0.21–8.72)
	A	36(55.50 %)	11(17.20 %)	0.377	0.71(0.33–1.51)
rs2069840	C/C	46(57.50 %)	18(56.25 %)	-	**1.00(Reference)**
	C/G	31(38.75 %)	13(40.50 %)	0.872	1.07(0.45–2.49)
	G/G	3(3.75 %)	1(3.25 %)	0.660	0.85(0.08–8.73)
	G	37(23.12 %)	15(23.45 %)	0.960	1.01(0.51–2.01)
rs1474348	G/G	53(66.25 %)	27(84.88 %)	-	**1.00(Reference)**
	G/C	21(26.25 %)	4(12.50 %)	0.147	0.37(0.11–1.09)
	C/C	6(7.50 %)	1(3.12 %)	0.525	0.32(0.03–2.85)
	C	33(20.60 %)	6(9.40 %)	**0.044**	**0.39(0.15–1.00)**
rs1800795	G/G	55(68.75 %)	25(78.13 %)	-	**1.00(Reference)**
	G/C	21(26.25 %)	5(15.62 %)	0.237	0.52(0.17–1.54)
	C/C	4(5.00 %)	2(6.25 %)	0.726	1.1(0.18–6.40)
	C	29(18.12 %)	9(14.00 %)	0.464	0.73(0.32–1.66)
rs1800797	G/G	51(63.75 %)	23(71.87 %)	-	**1.00(Reference)**
	G/A	26(32.50 %)	8(25.00 %)	0.420	0.68(0.26–1.73)
	A/A	3(3.75 %)	1(3.13 %)	0.764	0.73(0.07–7.49)
	A	32(20.00 %)	10(15.62 %)	0.448	0.74(0.34–1.61)
rs2069827	G/G	74(92.50 %)	29(90.62 %)	-	**1.00(Reference)**
	G/T	5(6.25 %)	3(9.38 %)	0.575	1.5(0.34–6.82)
	T/T	1(1.25 %)	0(0.00 %)	0.620	ND
	T	7(4.40 %)	3(4.70 %)	0.798	1.07(0.26–4.29)

Values in bold are statistically significant at the 5 % level; ND: not defined

n number of women, *OR* odds ratio; nominal value of comparison; P > 0.05 no significant association, degree of freedom = 1

knowledge, no previous study has evaluated the association of those six SNPs in CC incidence and evolution.

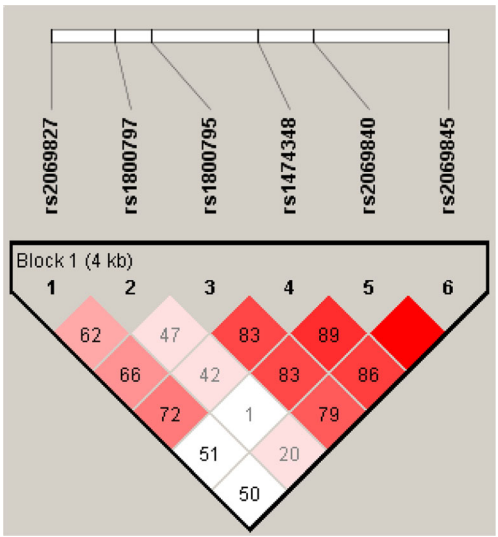

Fig. 1 Linkage disequilibrium (LD) map of the six IL-6 SNPs genotyped using haploview

HPV infection precipitates local chronic inflammation, which is enhanced in severe lesions, thus enhancing tumor cell growth in autocrine and/or paracrine fashions. This was

Table 6 Distribution of 6-locus IL-6 haplotypes in CC cases and controls

Haplotype[a]	Cases	Controls	X^2	p_{value}
GGGGCG	0.477	0.416	2.021	0.155
GGGGGG	0.192	0.228	0.994	0.318
GGGGCA	0.069	0.081	0.285	0.593
GAGGCG	0.039	0.069	2.27	0.131
GACCCA	**0.077**	**0.028**	**6.893**	**0.008**
GGCCCA	0.026	0.058	3.121	0.077
GAGGGG	**0.017**	**0.051**	**4.295**	**0.038**
TACCCA	0.021	0.014	0.366	0.545
GGGCCA	0.007	0.015	0.685	0.407

P > 0.05 no significant association, degree of freedom = 1; boldface indicates statistically significant differences

[a] rs2069840, rs2069827, rs1800797, rs1800796, rs1800795, rs1474348, rs1474347 and rs2069845 haplotypes frequency; Fisher's exact test

demonstrated for CC [8, 22]. Since some of the tested genetic variants in *IL-6* gene, including rs1800795, were associated with the plasma levels of the protein [23], the apparent contradiction of these findings may be attributed to ethnic or regional differences between different studies. The association of rs1800795 with reported for several cancer types, and a systematic review and meta-analysis reported its association with bladder [24] and prostate cancer [25]. Several case-control studies confirmed its association with heightened risk of squamous cell esophageal cancer [26], breast cancer [27], colorectal cancer [28], and oral cancer [29]. Furthermore, it was suggested that rs1800795 might serve a prognostic role in patients with vulvar cancer [29]. CC cases were also evaluated according to CC staging (early and advanced). Both major allele and homozygous major allele of rs1474348 were generally protective of CC in its early stages, in contrast to advanced stages. In addition, there was a significant relationship between rs1474348 and the clinical progression of CC. This protective association might be explained by transcriptional changes or in situ tissue effect, in which there is decrease in the local levels of IL-6, which in turn attenuate its participation in inflammation.

Allele and genotype analysis revealed comparable distribution of the *IL-6* polymorphisms in CC cases and control women. This raises the speculation of a role (if any) for these *IL-6* variants in CC. Mixed findings on the association of the tested *IL-6* variants with CC were reported, highlighted by the lack of association of CC with rs1800795 in Brazil and Australia [20, 21], and rs1800796 in China [17]. An earlier study on Indian CC patients suggested that rs1800797 minor allele-carrying genotype is at higher risk of developing CC [16], and among Chinese populations carriage of rs2069840 minor allele increased CC risk [17].

Most CC patients were married, with one sexual partner, and were post-menopausal. During menopause, estrogen levels falls considerably and the vagina loses some of its elasticity. Since estrogen is responsible for tissue elasticity round the vagina and for moisturizing the cervix, this explains the increased prevalence of CC among post-menopausal women. The involvement of altered genes involved in estrogen biosynthesis among pre- and post-menopausal women in CC remain to be seen. Previously it was reported that CC relative risk increases in oral contraceptives users, but of declines after cessation of treatment. It was demonstrated that 10 years' of oral contraceptives use in the 20–30 year age category increase the incidence of invasive cervical cancer by the age of 50 years from 7.3 to 8.3 per 1000 in less developed countries, and from 3.8 to 4.5 per 1000 in more developed countries [30].

Significant association was noted between the incidence of CC incidence and use of oral contraceptives and smoking, in agreement with a study on Korean women [31]. While not addressed here, it is likely that smoking and oral contraceptive use may synergize in suppressing local cervical immunity, thus facilitating the development and progression of cancerous lesions [31]. It is now known that long-term oral contraceptive use is associated with precancerous cervical lesions, and hence the combined smoking and oral contraceptive use use constitute major risk for severe cervical dysplasia. This strongly recommends cessation of smoking and oral contraceptive use in at-risk women, in order to prevent progression of cervical lesions. Future controlled studies involving larger sample size and molecular approaches are required to clarify the exact contribution of modifiable and non-modifiable (including genetic) factors to the development and/or evolution of CC.

The present study has some strengths, namely that CC cases and control women were of similar ethnicity, thus minimizing the possibility of admixture, and that only new CC cases prior to chemo−/radio-therapy were recruited. In addition, histological typing and staging of CC cases with was done according to FIGO, thus allowing for comparison with studies that utilized similar staging. Furthermore, we controlled for potential covariates which may influence uterine inflammation and thus cervical neoplasia, in our analysis. However, our studies had also some limitations, mostly due to the relatively limited sample size, which lowered the power of the study. Given that IL-6, as well as interferon-γ and TNF-α, levels increase in HPV infection [32], lack of information about HPV (genotype) status limited the interpretation of the findings. This was reminiscent of a previous study on 152 HPV-positive cases and 238 HPV-negative controls, which concluded that the variability of rs1800795 can be discriminatory only if analyzed in large population [20]. Despite these shortcomings, our results suggest a role for altered IL-6 expression stemming from specific variants and haplotypes in *IL-6* gene in the pathogenesis of CC among Tunisians.

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Compliance with Ethical Standards

Conflict of Interest None of the authors have any conflict of interest to declare.

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