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Research Article

Interleukin-1 Receptor Antagonist VNTR Polymorphism and Ovarian Cancer Susceptibility in Tunisian Women

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Abstract

Several studies indicate that interleukin-1 receptor antagonist (IL-1ra) is an important regulator of host immunity and play a key role in cancers development. Some of these studies have shown a potential role of a variable number of tandem repeat (VNTR) of 86 bp polymorphism within IL-1ra gene (IL-1RN) in host immune response variability. We investigated what VNTR polymorphism involves in susceptibility to ovarian cancer in Tunisia with a case control study.

VNTR polymorphism within IL-1RN gene is genotyped by a simple PCR and agarose gel electrophoresis in 257 healthy women and 55 women with ovarian cancer.

Our results indicated that allele 1 is associated with an increased susceptibility to ovarian cancer development (p=0.009; OR: 1.95, 95% CI, 1.14-3.36) and in the histological grading in Tunisian women. However, allele 3 seems to play a protective role (p=0.018; OR: 1.95, 95% CI, 0, 00-0.73).

There were no significant differences between IL-1ra polymorphism alleles and tumor stage (International Federation of Gynecology and Obstetrics) but the allele 1 is significantly associated with histological grading in Tunisian women.

The allele 1 of the IL-1RN seems to play a role in histological grading and susceptibility to ovarian cancer but it's not associated with disease progression. Nevertheless, the risk for

developing ovarian cancer is decreased by the presence of allele 3.

Keywords: IL-1 receptor antagonist, ovarian cancer, polymorphism, Tunisia.

Introduction

Ovarian cancer occupies the third rank among cancer in women in Tunisia and its incidence is about 4,1 cases/100000 women [Ben Abdallah, 2012]. The majority of patients diagnosed with ovarian cancer present advanced-stage disease, indeed it is mostly symptomless in early stages and there are currently no effective screening methods. Although, hormonal factors, inflammation, and wound healing are thought to play important roles in its occurrence, the etiology of ovarian cancer remains unknown [Risch, 1998]. Many genetic association studies have been already performed in cancer and suggested that tumor predisposition may be due to the combination of low penetrance genetic variants [Balmain, 2003; Kotnis, 2005] including genes coding for pro-inflammatory cytokines and their receptors

[Balkwill, 2001; Martin Howell, 2007]. Interleukin-1 (IL-1), an inflammatory regulator, plays an important role in the development of several pathologies [Alrayes, 2003; Langdahl, 2000]. The IL-1 gene family is located on chromosome 2q and includes IL-1A, IL-1B, and IL-1RN genes, which encode IL-1α, IL-1 β, and IL-1 receptor antagonist (IL-1ra) [Hefler, 2002]. This receptor is in anti-inflammatory cytokine which acts as competitive inhibitor to control the inflammatory action of IL-1 by binding to the IL-1 receptor [Arend, 1998]. Within the IL-1RN gene, a variable number of tandem repeats (VNTR) of 86-bp length was found to correlate with many diseases [Alrayes, 2003; Langdahl, 2000; Al-Moundhri, 2006; Sehouli, 2002]. Nevertheless, there are limited reports on the influence of this variant with host susceptibility to ovarian cancer and the results are controversial [Sehouli, 2003; Hefler, 2002].

In the present study, we investigated the association between the IL-1RN VNTR polymorphism and susceptibility to ovarian cancer and its correlation with established clinical prognostic factors in Tunisian patients in North Africa (figure1).

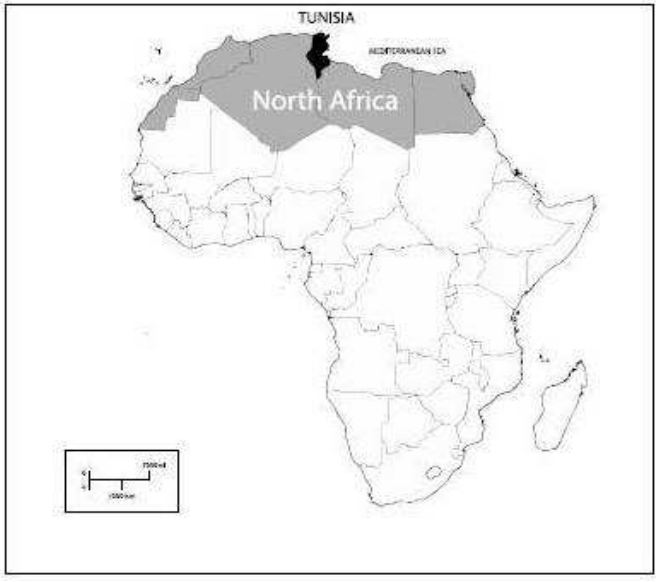

Figure 1: Geographic Localisation of Tunisia (The Study Population)

Materials and Methods

Sample Collection

All blood samples were collected from histopathological confirmed Tunisian patients with ovarian cancer (n=55) and unrelated, healthy female controls of similar ethnicity (n=257). Clinical diagnosis/staging of ovarian cancer patients was performed by trained medical personnel as per the guidelines outlined by the International Federation of Gynecology and Obstetrics (FIGO). Written informed consent was taken from all subjects and the study was approved by local ethics committees of the Salah Azeiz Institute of oncology.

Blood Collection and DNA Extraction

Five milliliters of venous blood with EDTA, as an anticoagulant, were collected from each subject. The blood was obtained from patients prior to radiation therapy or chemotherapy. Genomic DNA was extracted using QIAamp® DNA blood Mini Kit (QiagenGmbH, Hilden).

IL-1RN Gene Polymorphism

The polymorphic region within the second intron of IL-1RN, which contains a VNTR of 86-pb, was amplified by polymerase chain reaction with specific primers: forward, 5' CTCCAGCAACACTCCTAT 3'; reverse, 5' TCCTGGTCTGCAGGTAA 3'. For each sample, the PCR was performed in a total reaction

volume of 10µl containing 1µl (100 ng) genomic DNA, 25mM of dNTP, 1µl of buffer 10X and 0,25U of Taq polymerase (Fermentas). The PCR cycling program comprised an initial denaturation at 95 ◦C for 10 minutes, followed by 30 cycles of 95 ◦C for 20 seconds, annealing of primers at 51 ◦C for 30 seconds and extension at 72 ◦C for 50 seconds and a final extension at 72°C for 10 minutes. Amplified DNA fragments were separated on 1.5% ethidium bromide agarose gel, and visualized under ultraviolet light.

Statistical Analysis

Allele and genotype frequencies were calculated by direct counting and compared with the $\chi 2$ or Fisher's exact test when appropriate (e.g., the number of subjects in a cell was less than 5)

using the EPI INFO 6 package program (http://wwwn.cdc.gov/epiinfo/html/downloads.htm) and SPSS 17.0 statistic software system (SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc.).

The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated in order to measure the strength of the association of individual alleles or genotypes with risk of ovarian cancer, in general, or their FIGO stage and histological type, in particular. A value of $P<0.05$ was considered statistically significant.

Results

The characteristics of women with ovarian cancer are summarized in Table 1. The median age of patients was 51.80 years (range 25-78). Of 55 patients, 40% have a premenopausal statute and 60% are postmenopausal. The most common histological type was Adenocarcinoma (27.28%). Only 20% were diagnosed in FIGO stage I, 21.83% were in FIGO stage II and 45.45% were in FIGO stage III-IV.

Table 1: Clinicopathological Characteristics of Tunisian Ovarian Cancer Woman Patients

Variable	*Patients*	
	N=55	*%*
Age at diagnosis (yr)		
25-40	9	16.36
41-50	17	30.93
51-60	18	32.72
61-70	6	10.90
71-80	5	9.09
Menopausal status		
Premenopausal	22	40.00
Postmenopausal	33	60.00
Histological type		
Serious-papillary	13	23.64
Adenocarcinoma	15	27.28
Endometrioid	12	21.81
Mucinous	1	1.81
Mixed/others	5	9.09
W I	9	16.37
FIGO staging		
Stage I	11	20.00
Stage II	12	21.83
Stage III	17	30.91
Stage IV	8	14.54
W I	7	12.72

FIGO. Federation International of Gynecology and Obstetris: W I: Without Indication

Allelic frequencies of IL-1RN for patients with ovarian cancer and healthy women are given in Table 2. Five different alleles of 86-base pair repeat were detected (allele 1= 4 repeats, allele 2= 2 repeats, allele 3= 5 repeats, allele 4= 3 repeats and allele 5= 6 repeats). Allele 1 was the most common allele in cases and healthy controls with respectively 80.92 and 68.49 % frequencies. The comparison of the allelic distribution of IL-1RN VNTR polymorphism has revealed a significant positive association between allele 1 and the occurrence of ovarian cancer ($p=0.009$; OR: 1.95, 95% CI, 1.14-3.36) and a negative association between allele 3 ($p=0.018$. OR: 1.95, 95% CI, 0.00-0.73) and this pathology (table 2).

Table 2: Interleukin-IRN VNTR Alleles in Ovarian Cancer Patients and Controls: Frequencies and Association Analysis

Alleles	Alleles frequencies				Association analysis	
	Patients		Controls		Patients vs controls	
	2n=110	(%)	2n= 514	(%)	OR (95% CI)	*P*-value
1 (4 repeats of 86pb)	89	(80.92)	352	(68.49)	**1.95 (1.14-3.36)**	**0.009**
2 (2 repeats of 86pb)	16	(14.54)	88	(17.12)	0.82 (0.44-1.51)	0.510
3 (5 repeats of 86pb)	0	(0.00)	30	(5.83)	**0.00 (0.00-0.73)**	**0.018**
4 (3 repeats of 86pb)	5	(4.54)	43	(8.36)	0.52 (0.18-1.42)	0.172
5 (6 repeats of 86pb)	0	(0.00)	1	(0.20)	0.00 (0.00-81.50)	0.823

OR, odds ratio; nominal value of comparison; $P>0.05$, no significant association; degree of freedom = 1. Values in bold are statistically significant at the 5% level; vs, versus.

The different combination between the five identified alleles gives 15 genotypes for VNTR IL-1RN polymorphism. But in the present study only 11 genotypes were observed (figure 2).

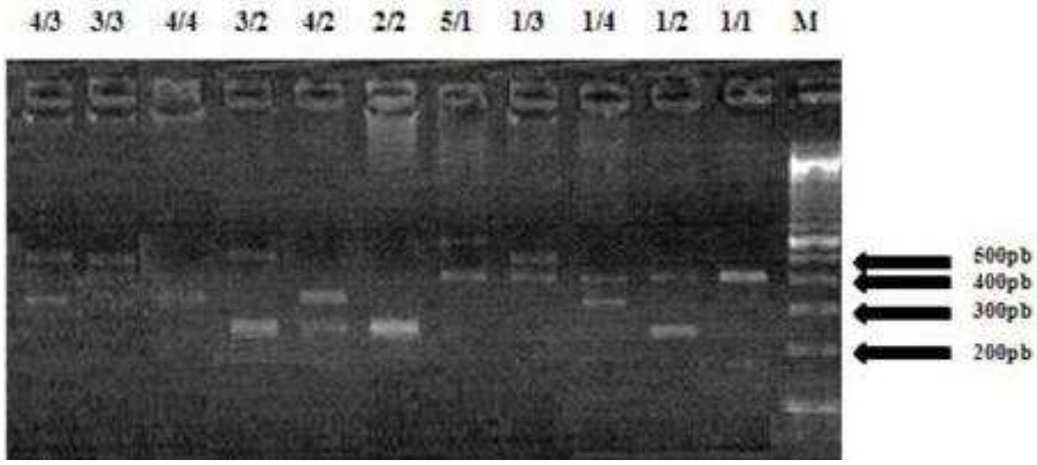

Figure 2: Electrophoresie of PCR Products of VNTR IL-1RN

Lanes 1 to 11: The 11 Different Genotypes Present in the Case Controls Population of this Study. Lane 12: M= The Marker 100 pb.

Allele 1 (4 Repeats of 86pb = 410pb): Allele 2 (2 Repeats of 86pb = 240pb): Allele 3 (5 Repeats of 86pb=500pb): Allele 4 (3 Repeats of 86pb= 325pb): Allele 5 (6 Repeats of 86pb= 595pb)

The distribution of pooled genotypes between carriers and non carriers of allele 1 shows a p value<0,005 however the confidence interval at 95% for odds ratio includes 1, so this association is not statistically significant (table 3).

Tabl 3: Interleukin-IRN VNTR Genotypes in Ovarian Cancer Patients and Controls: Frequencies and Association Analysis

Genotypes	Genotypes frequencies				Association analysis	
	Patients		Controls		Patients vs controls	
	n=55	(%)	n= 257	(%)	OR (95% CI)	*P*-value
1/1	39	(70.91)	148	(57.60)	1.80 (0.92-3.55)	0.067
2/2	3	(5.50)	15	(5.83)	0.93 (0.21-3.60)	0.912
1/2	9	(16.36)	40	(15.60)	1.06 (0.44-2.47)	0.882
4/4	1	(1.80)	9	(3.50)	0.51 (0.02-4.07)	0.448
1/4	2	(3.63)	10	(3.90)	-	0.643
2/4	1	(1.80)	10	(3.90)	0.46 (0.02-3.59)	0.393
3/3	0	(0.00)	6	(2.33)	0.00 (0.00-4.42)	0.309
1/3	0	(0.00)	5	(1.92)	0.00 (0.00-5.51)	0.376
2/3	0	(0.00)	8	(3.10)	0.00 (0.00-3.16)	0.207
3/4	0	(0.00)	5	(1.92)	0.00 (0.00-5.51)	0.376
5/1	0	(0.00)	1	(0.40)	0.00 (0.00-82.16)	0.823
1/12345	50	(20.00)	204	(21.78)	2.60 (0.93-7.80)	0.046

Allele 1 (4 repeats of 86pb); Allele 2 (2 repeats of 86pb); Allele 3 (5 repeats of 86pb); Allele 4 (3 repeats of 86pb); Allele 5 (6 repeats of 86pb). OR, odds ratio; nominal value of comparison; $P > 0.05$, no significant association; degree of freedom = 1. vs,versus; Values in bold are statistically significant at the 5% level.

Furthermore, when stratified the cases by histological type, we detect a positive association between allele 1 (p= 0.002; OR: 11.51, 95% CI, 1.65-230.19) and genotype 1/1 (p= 0.012; OR: 8.84, 95% CI, 1.17-184.66) and the susceptibility to Serious-papillary type of ovarian cancer (table 4). In contrast, IL-1RN VNTR alleles and genotypes did not correlate with tumour stage (table 5).

Table 4: Interleukin-IRN VNTR Genotypes in Patients Stratified by Histological Type of Ovarian Cancer and Controls: Frequencies and Association Analysis

	Serious-papillary vs Controls (n=13) (n=257)		Adenocarcinoma vs Controls (n=15) (n=257)		Endometrioid vs Controls (n=12) (n=257)		Mucinous, Mixed/others vs Controls (n=6) (n=257)	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Alleles								
1	**11.51 (1.65-230.19)**	**0.002**	2.30 (0.82-98)	0.086	0.92 (0.36-2.40)	0.851	0.64 (0.18-2.37)	0.322
2	0.19 (0.01-1.37)	0.052	0.54 (0.13-1.91)	0.309	1.27 (0.40-3.74)	0.402	1.62 (0.34-6.67)	0.344
4	-	-	0.78 (0.12-3.55)	0.540	1.56 (0.36-5.84)	0.337	-	0.273
Genotypes								
1/1	**8.84 (1.17-184.66)**	**0.012**	2.95 (0.75-13.49)	0.086	0.53 (0.14-1.91)	0.276	0.75 (0.12-4.67)	0.509
1/2	0.45 (0.02-3.50)	0.385	0.39 (0.02-2.95)	0.307	2.71 (0.65-10.59)	0.113	-	0.642
2/2	-	-	-	0.607	-	-	-	0.316
4/4	-	-	-	0.438	-	-	-	0.209
1/4	-	-	-	-	4.94 (0.65-29.50)	0.093	-	-
2/4	-	-	-	-	-	0.400	-	-

Allele 1 (4 repeats of 86pb); Allele 2 (2 repeats of 86pb); Allele 3 (5 repeats of 86pb); Allele 4 (3 repeats of 86pb); Allele 5 (6 repeats of 86pb). OR, odds ratio; nominal value of comparison; $P > 0.05$, no significant association; degree of freedom = 1. Values in bold are statistically significant at the 5% level.

Table 5: Interleukin-IRN VNTR Alleles and Genotypes in Overian Cencer Patients Stratified by Tumour Stage: Frequencies and Assciation Analysis

	I (n=11) vs II+III+IV (n=37)			I+II(n=23) vs III+IV (n= 25)			I+II+III (n=40) vs IV (n=8)		
	OR	IC (95%)	P-value	OR	IC (95%)	P-value	OR	IC (95%)	P-value
Alleles									
1	0.52	(0.15-1.83)	0.193	1.57	(0.50-5.08)	0.395	1.00	(0.20-4.49)	0.618
2	1.60	(0.36-6.69)	0.341	0.64	(0.16-2.41)	0.463	0.62	(0.13-3.29)	0.371
4	1.13	(0.12-9.46)	0.622	0.71	(0.08-5.60)	0.539	-		0.613
Genotypes									
1/1	0.25	(0.05-1.30)	0.062	0.89	(0.22-3.67)	0.852	-		0.572
1/2	-		0.068	-		0.325	-		0.599
2/2	-		0.590	-		0.265	-		0.308
1/4	-		0.048	-		0.224	-		0.691
2/4	0.00	(0.00-63.20)	0.770	0.00	(0.00-19.49)	0.520	-		0.833
4/4	0.00	(0.00-64.92)	0.775	0.00	(0.00-19.49)	0.520	-		0.830

Allele 1 (4 repeats of 86pb); Allele 2 (2 repeats of 86pb); Allele 3 (5 repeats of 86pb); Allele 4 (3 repeats of 86pb); Allele 5 (6 repeats of 86pb). OR, odds ratio; nominal value of comparison; $P > 0.05$, no significant association; degree of freedom = 1. Values in bold are statistically significant at the 5% level.

Discussion

Many studies have indicated that IL-1 plays a key role in cancer's development [Dinarello, 1996; Fujiwaki, 1997; Viet, 2005]. The role of this cytokine as a mediator of malignant tumour growth has been reported for cervical [Fujiwaki, 1997; Zeisler, 1998], ovarian [Li, 1992], gastric [El Omar, 2001], and colorectal carcinogenesis [Viet, 2005].

Several studies have investigated the role of a common IL-1 RN VNTR polymorphism in the development of inflammatory disorders [Viet, 2005; Fujiwaki, 2003]. The majority of these studies have been enrolled on cancers, such as gastric [Al-Moundhri, 2006; Geon Shin, 2008], bladder [Bid, 2006], breast [Lee, 2007], colorectal [Viet, 2005], cervical [Sousa, 2012;

Fujiwaki, 2003], vulvar [Grimm, 2004], and ovarian [Sehouli, 2003; Hefler, 2002]. Nevertheless, there are controversial results regarding the potential role of this polymorphism in the development of cancer. In the present study, we attempted to establish an association between the IL-1RN VNTR polymorphism and susceptibility to ovarian cancer and its correlation with established clinical prognostic parameters in Tunisian women.

In our study, carriers of allele 1 have an almost twofold increased risk of developing ovarian cancer specifically it's associated to Serous-papillary type susceptibility. This allele seems to be the critical point in the molecular pathway of some diseases. In fact, it has recently been correlated with a higher risk of Multidrug-resistant Acinetobacter baumannii associated pneumonia [Hsu,

2012]. In literature, the presence of allele 1 is associated in general with a restricted immune reaction and the level of IL-1ra production in human endothelial cells with genotype 1/1 is three times higher than with 2/2 genotypes. However, the presence of allele 2 is correlated with a prolonged immune reaction [Dewberry, 2000]. The other alleles are rarely detected and positive or negative effect in inflammatory reaction is not elsewhere described [Witkin, 2002]. Contrary to our results, however, Hefler *et al*. [2002] have published a case-control study where IL1-RN VNTR polymorphism was genotyped in 94 ovarian cancer patients and 134 healthy women in the Austrian population and found no differences in the prevalence of the VNTR polymorphism in the IL-1RN between cases and controls. However, in Germany, Sehouli *et al*. [2003] have analysed this polymorphism in 108 women with ovarian cancer compared to

112 patients with benign gynaecological diseases and obtain results suggesting that the allele 2 seems to play a role in the occurrence of ovarian cancer.

Otherwise, our results have revealed, for the first time, a negative association between allele 3 and the occurrence of ovarian cancer in Tunisian women. This allele is rarely detected and his biological meaning on immune reaction is not elsewhere elucidated [Witkin, 2002].

Therefore, the VNTR polymorphism in the IL-1RN should be analyzed in further case-control studies in order to better evaluate this marker as a risk factor for ovarian cancer. Otherwise it needs to be pointed out that there were some limitations in this study. First, the limited sample size may

restrict us to identify other genotype or allele associations. Second, our study lacked the measurement of IL-1ra and IL-1β levels in the local environment of ovarian cancer and the representative of circulating IL-1ra and IL-1β levels need to be investigated in further studies. Furthermore, power analysis showed that to achieve a power of 80% at an alpha of 0,05 another 81729 patients would have to be genotyped, in light of the observed allelic frequencies.

In addition, we didn't observe any association between specific alleles and clinical features such as FIGO stage and histological types. Similar results were obtained by Hefler *et al.* [2002] and Sehouli *et al*. [2003]. It can thus speculate that IL-1 RN VNTR polymorphism doesn't influence ovarian cancer biology.

In conclusion, we observed a significant association of the allele 1 with Serous-papillary type and in the susceptibility to ovarian cancer in Tunisian women. However, the allele 3 is a protective factor for this pathology in Tunisia.

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