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IL-1RN VNTR polymorphism and genetic susceptibility to cervical cancer in Portugal

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Abstract Human Papillomavirus infection is considered as the main etiological factor of cervical cancer (ICC), although, the role of host genetic factors in ICC susceptibility has been increasing. Immunological response is crucial for the prevention of viral associated diseases. Interleukin 1 receptor antagonist (IL-1RN) is considered to be an important regulator of host immunity and several studies have shown a potential role of a 86 bp VNTR polymorphism within intron 2 of the IL-1RN gene in host immune response variability. We investigated the role of this polymorphism in cervical cancer development in Portugal with a case–control study developed with peripheral blood samples from 196 healthy women and 340 women with cervical lesions from the Northern Region of Portugal. We observed that IL-1RN Allele 2 homozygosis was significantly higher in cases than in controls. In fact, IL-1RN A2*A2 homozygous revealed to be associated with an increased risk of HSIL + ICC (OR = 1.90; 95 % IC 1.13–3.21; $p = 0.015$). Furthermore, we also observed that median age of onset of HSIL + ICC was significantly different (46.0 vs 52.0) in *IL-1RN* A2*A2 homozygous comparing to non-A2*A2 ($p = 0.028$). Our results indicated that IL-1RN A2 allele is associated with an increased susceptibility to cervical cancer development, probably by increasing predisposition to shorter immune responses.

Keywords Cervical cancer · Human Papillomavirus (HPV) · Genetic polymorphism · Interleukin 1 · IL-1RN

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Introduction

Cervical cancer is the second most common cancer in women with almost 500,000 new cases each year and over 260,000 deaths [1, 2]. Moreover, it is estimated that over 1 million women worldwide have cervical cancer and the majority of them have not yet been diagnosed [2].

In the early 90's persistent infection by the oncogenic types of the Human Papillomavirus (HPV) was established as the etiological factor for the development of cervical cancer [3–8]. Over time, several studies have been focusing on the role of risk factors that influence either the acquisition of persistent HPV infection or by contributing for the

progression from pre-invasive lesions to cervical cancer. Literature has demonstrated that despite its oncogenic potential, HPV is not sufficient for cervical carcinogenesis [9–11]. Epidemiological studies have revealed a number of co-factors for cervical cancer development (number of sexual partners, age at first sexual intercourse, parity, tobacco and alcohol consumption, co-infection with other sexually transmitted agents and host genetic factors) [9–11]. Despite the role of some genetic factors is being emphasized in scientific community, there is still a great discussion on their potential inclusion clinically.

Immunologic factors are especially important in the prevention of pathogenic infections, and therefore they are expected to have great impact on viral associated diseases where they act as markers of individual susceptibility [12–14]. The interleukin 1 receptor antagonist (IL-1RN) is an important immunologic regulator that competes with other interleukin 1 family members (IL-1α and IL-1β) for the IL-1 receptor in target cells, acting as its negative regulator with anti-inflammatory effect [15–18]. Its expression blocks pro-inflammatory signals from unwanted cellular damages during extensive immunologic responses [15–22].

Literature refers the existence of several polymorphism on the *IL-1RN* gene sequence, and despite the majority are single nucleotide polymorphisms [23, 24], the majority of studies have focused on a common variable number of tandem repeats (VNTR) polymorphism characterized by a 86 bp variation within intron 2 [25–28]. Genetic studies have shown that the distribution of IL-1RN alleles is associated with ancestral distribution with increased distribution of alleles 1 and 2 and repression of all other alleles [29, 30]. The role of IL-1RN VNTR has being studied for many years in the development of inflammatory disorders [19, 22, 31], nevertheless great discussion has being held on the potential role of this polymorphism in cancer development [28, 30]. In fact, several studies showed different associations of IL-1RN VNTR alleles with the development of several cancers, such as of gastric [26–28, 32–39], oesophageal [40], bladder [41–43], breast [44], colorectal [45, 46], lung [47–49], brain [50, 51], nasopharyngeal [52] and gallbladder [41]. Moreover, and despite differences in ethnic distribution [29, 30], some authors have discussed a possible role of this polymorphism in cervical carcinoma [53–56].

Since literature is suggesting that this polymorphism may be correlated with increased susceptibility of viral diseases, including viral-associated neoplasias, we have developed a hospital-based case control study to characterize the role IL1RN VNTR polymorphism in cervical carcinoma development in the Northern region of Portugal.

Materials and methods

Cases and specimens

The group of women with cervical lesions consisted in a total of 340 women with median age of 46 years old (Table 1) attended at the Gynaecologic Oncology Department from the Portuguese Institute of Oncology of Porto. Patients were further classified according to histological data: 28 patients with histological confirmed low grade squamous intraepithelial lesions (LSIL); 84 with high grade SIL (HSIL); and 228 patients with invasive cervical cancer (ICC).

The study included a control group constituted by 196 randomly selected healthy females without evidence of neoplastic disease with median age of 44 years old (Table 1) collected from a Healthy Donor's database at the *Institute of Molecular Pathology and Immunology of University of Porto* (IPATIMUP).

Informed consent according to the Declaration of Helsinki was obtained from each individual. Individuals from control group were submitted to a clinical evaluation and have no individual history of cancer.

Sample processing and genotyping

Freshly collected peripheral blood samples were collected following standard venipuncture technique in EDTA-containing tubes, and the DNA was extracted from the white blood cell fraction using a salting out protocol.

The *IL-1RN* VNTR polymorphism was genotyped by polymerase chain reaction (PCR) with 5′-CCCCTCAGCAACACTCC-3′ forward primer and 5′-GGTCAGAAGGGCAGAG-3′ reverse primer as previously described [26]. PCR products were analysed by electrophoresis in a 3 % Agarose gel (Cambrex Bio Science Rockland Inc, USA) stained with 5 % ethidium bromide for identification of the following fragments: 410 bp (allele 1), 240 bp (allele 2), 325 bp (allele 3), 500 bp (allele 4) and 595 bp (allele 5). All possible genotypes were considered: A1*A1, A1*A2, A1*A3, A1*A4, A1*A5, A2*A2, A2*A3, A2*A4, A2*A5, A3*A3, A3*A4, A3*A5, A4*A4, A4*A5 and A5*A5.

Statistical analysis

For genetic comparison alleles were combined according to the number of repeats: A2, the short allele that corresponds to two repeats; and L, the long alleles that corresponds to three or more repeats. Hardy–Weinberg equilibrium (HWE) was calculated considering the stratification of IL-1RN genotypes as A2*A2, A2*L and L*L with the public software available at http://www.oege.org/software/hwe-mr-calc.shtml [57, 58].

Genotypes were analysed with Statistical Package for Social Sciences for Windows (SPSS version 16.0) using Chi-square (χ^2) analysis to compare the categorical variables with a 5 % significance level. Statistical analysis was performed by considering the status of IL1-RN Allele homozygousy in a dichotomic variable (A2*A2 homozygous vs non-A2*A2) with odds ratio (OR) and its 95 % confidence interval (CI) to be used as a measure of the association between the genotype and the risk of developing disease. Logistic regression analysis was also performed by adjusting data for median age of onset. Kaplan–Meier (Log-rank and Breslow test) was used for calculation of cumulative hazard considering genotype-specific distributions according to age of disease onset.

The sample size and power of analysis was calculated with the OpenEpi: Open Source Epidemiologic Statistics for Public Health, Version 2.3. www.OpenEpi.com, updated 2009/20/05, accessed 2012/01/14.

Table 1 Characteristics of the study population

Population description	Mean age (± SD), y.o.	Median age (min–max), y.o.
Controls ($n = 196$)	43.7 ± 15.6	44.0 (18–81)
Cases ($n = 340$)	46.6 ± 12.6	46.0 (20–83)
LSIL ($n = 28$)	38.3 ± 12.2	34.5 (21–65)
HSIL ($n = 84$)	41.3 ± 12.9	38.5 (21–81)
ICC ($n = 228$)	49.6 ± 11.5	48.0 (20–83)

y.o. years old, *LSIL* low-grade squamous intraepithelial lesion, *HSIL* high-grade squamous intraepithelial lesion, *ICC* invasive cervical cancer

Results

In this study, it was not possible to identify the following genotypes: A2*A4, A2*A5, A3*A3, A3*A4, A3*A5, A4*A4, A4*A5 and A5*A5 (Table 2). HWE was tested and results showed that genotypes were according to the expected in the control group ($p = 0.363$) while patients with cervical lesions did not respect the HWE ($p < 0.001$). The genotype distribution showed that 19.7 % of the cases with cervical lesion/cancer were A2*A2 homozygous in comparison with 11.7 % in controls, which turn to be statistically different ($p = 0.017$).

When stratifying patients according to the histological data for A2*A2 genotype, we observed that there were no statistical significant differences between controls and patients with LSIL or HSIL independently ($p > 0.050$). Nevertheless, results have shown an almost twofold increased risk for the development of ICC (OR = 1.85;

Table 2 Distribution of IL-1RN VNTR genotypes

	A1*A1	A1*A2	A1*A3	A1*A4	A1*A5	A2*A2	A2*A3
Controls ($n = 196$)	96 (49.0)	69 (35.2)	6 (3.1)	–	1 (0.5)	23 (11.7)	1 (0.5)
Cervical lesions ($n = 340$)	159 (46.8)	106 (31.2)	6 (1.8)	1 (0.3)	–	67 (19.7)	1 (0.3)
LSIL ($n = 28$)	14 (50.0)	8 (28.6)	1 (3.6)	–	–	5 (17.9)	–
HSIL ($n = 84$)	36 (42.9)	30 (35.7)	1 (1.2)	–	–	17 (20.2)	–
ICC ($n = 228$)	109 (47.8)	68 (29.8)	4 (1.8)	1 (0.4)	–	45 (19.7)	1 (0.4)

LSIL low-grade squamous intraepithelial lesion, *HSIL* high-grade squamous intraepithelial lesion, *ICC* invasive cervical cancer

Values in parenthesis indicate percentage

Table 3 Association of IL-1RN VNTR genotypes and cervical cancer development

	Non A2*A2 n (%)	A2*A2 n (%)	*p*	OR	95 % CI	p^a	OR[a]	95 % CIa
Controls ($n = 196$)	173 (88.3)	23 (11.7)		1.00	Reference		1.00	Reference
Cervical lesions ($n = 340$)	273 (80.3)	67 (19.7)	**0.017**	**1.85**	1.11–3.08	**0.017**	**1.87**	1.12–3.11
LSIL ($n = 28$)	23 (82.1)	5 (17.9)	0.359	1.64	0.57–4.72	0.275	1.82	0.62–5.36
HSIL ($n = 84$)	67 (79.8)	17 (20.2)	0.062	1.91	0.96–3.80	0.067	1.91	0.96–3.80
ICC ($n = 228$)	183 (80.3)	45 (19.7)	**0.025**	**1.85**	1.07–3.19	**0.030**	**1.85**	1.06–3.21
HSIL + ICC ($n = 312$)	250 (80.1)	62 (19.9)	**0.017**	**1.87**	1.11–3.13	**0.015**	**1.91**	1.13–3.21

LSIL low-grade squamous intraepithelial lesion, *HSIL* high-grade squamous intraepithelial lesion, *ICC* invasive cervical cancer, χ^2 Chi-square, *P* Pearson χ^2, *OR* odds ratio, *CI* confidence interval

[a] Logistic regression adjusted for age

Bold values denote the statistical significance

95 % IC 1.07–3.19; $p = 0.025$). Statistical analysis also revealed an almost twofold increased risk for IL1-RN A2*A2 homozygous of developing lesions more severe than HSIL (OR = 1.87; 95 % IC 1.11–3.13; $p = 0.017$). Data from multivariate logistic regression (Table 3), adjusting the OR for age, revealed no significant differences from the predicted ORs either for ICC only (OR = 1.85; 95 % IC 1.06–3.21; $p = 0.030$) or for HSIL + ICC (OR = 1.90; 95 % IC 1.13–3.21; $p = 0.015$).

Kaplan–Meier revealed that the median age of onset of cases with cervical lesion/cancer with *IL-1RN* A2*A2 homozygosis was of 46.0 years old comparing to 52.0 in non-A2*A2 ($p = 0.042$)—Fig. 1a. Although we have not found statistical significant data considered the different types of lesion isolated (data not shown), results were similar if considering HSIL and ICC together (48.0 vs 52.0; $p = 0.028$)—Fig. 1b.

Discussion

For decades, cervical cancer has been one of the most studied cancer with thousands of publications from all over the world. Actually, the infection by oncogenic HPVs is established as the necessary cause for cervical cancer development, nevertheless, not all infected women will develop cancer. The establishment of a persistent infection by oncogenic HPVs is the key point for the progression from normal epithelium to pre-invasive lesions and ultimately to ICC.

In the past decade, our group has been developing several studies regarding the role of genetic factors in the development of several cancers, including cervical cancer [59–62]. Literature has been revealing that HPV infection is influenced by environmental and host conditions, such as diet and host immune status, which can lead to immunosuppressed conditions [63, 64]. Hence, the study of genetic factors involved in immune response regulation is expected to reveal important data on HPV-associated carcinogenesis [61, 65].

The interleukin 1 superfamily combines some of the most important cytokines involved in host-pathogen immune response [20, 66, 67]. While IL-1α and IL-1β have potent pro-inflammatory activity, the antagonist of the receptor for IL-1 (IL-1RN) avoids the transmission of pro-inflammatory signals abolishing the immune response [14, 17, 18]. Many studies have reported that IL-1RN levels are useful predictors of host immune response, since its levels increase during the final steps of inflammatory response [22]. Moreover, several studies have shown that this molecule is also altered in cancer development [30, 68] either by promoting the suppression of immune response to pathogens [69–73] that are able to promote cancer development or by promoting chronic inflammation states that induce cell lesions and tissue differentiation [33, 35, 74].

Up to date, there are controversial data regarding the potential role of this polymorphism in the development of cervical cancer. While Qian et al., showed no association for cervical cancer development, Tamandani et al., showed protection and Singh et al., and Mustea et al., have proved that the allele 2 is associated with increased risk [53–56]. Hence, in this study we attempted to characterize the role of the VNTR polymorphism within the IL-1RN gene in the development of cervical cancer in Portugal.

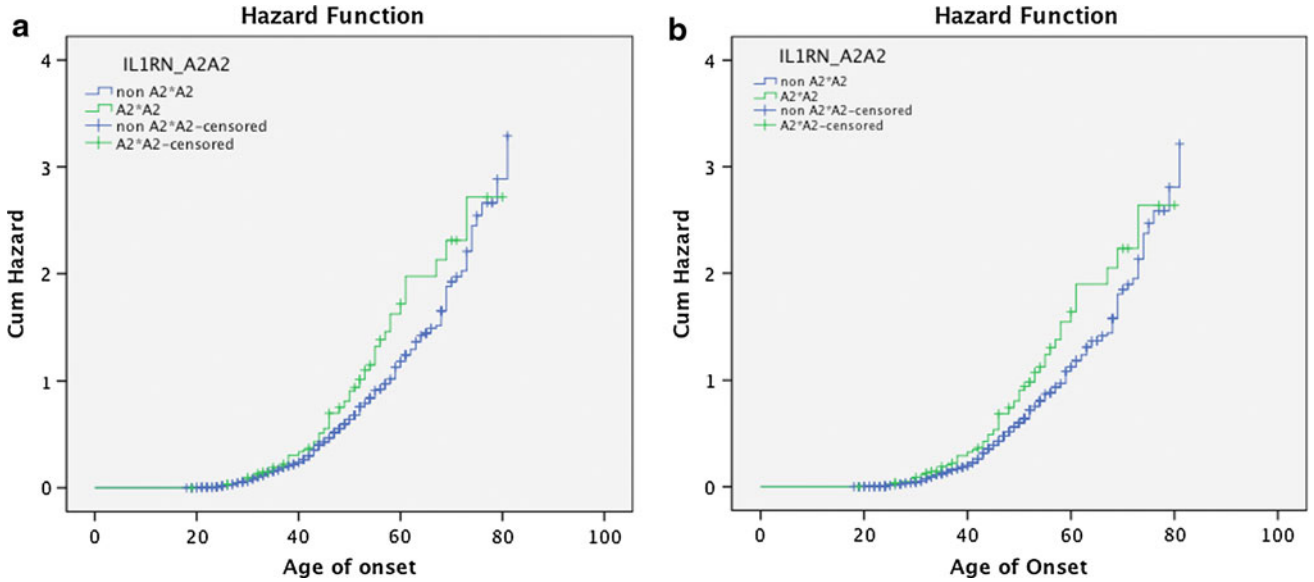

Fig. 1 Association between IL-1RN A2*A2 genotypes and the age of onset by Kaplan–Meier methodology with log-rank test for: **a** cases with cervical lesion/cancer; and **b** HSIL + ICC

Despite our study may reveal a small sample size, nevertheless, the power of the analysis was of 69.2 % (the sample size required to a power of analysis of 80 % with 95 % IC is of 529 cases and 265 controls). Our study suggests that IL-1RN Allele 2 homozygosis was significantly associated with lesions severe than HSIL with an almost twofold increased risk. This data is in accordance with literature suggestions, since (1) it has been established that in LSILs HPV is frequently transient as some of these lesions regress spontaneously and therefore immune surveillance is active; and (2) in lesions severe than HSIL HPV is supposed to be integrated more often, and with a shorter immune response, it will favour the establishment of persistent infections and the development of high-grade lesions that can progress to cancer [18].

This study characterizes the role of *IL-1RN* VNTR polymorphism in cervical cancer susceptibility in Portugal. IL-1RN Allele 2 homozygosis may be determinant for reduced immune responses in our population contributing for increased susceptibility for HPV infection and development of cervical cancer. Therefore, *IL-1RN* VNTR polymorphism may be a useful marker of host immune response and viral associated cancers susceptibility. Although the evidences pointed in our study, we consider that it would be of extreme importance to obtain data regarding the HPV status and Il-1Ra levels to corroborate our hypothesis, and therefore further studies are required.

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