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IL-1RN VNTR polymorphism as a susceptibility marker for nasopharyngeal carcinoma in Portugal

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ABSTRACT

Background: Nasopharyngeal carcinoma (NPC) is a rare malignancy in Western countries that is widely associated with the infection by Epstein–Barr virus (EBV). Several studies have showed that a common allele (allele 2) of the 86-bp variable number of tandem repeats (VNTR) polymorphism within intron 2 of the interleukin 1 receptor antagonist (*IL-1RN*) gene is associated with several disorders, including viral-associated cancers.
Methods: We have developed a hospital-based case–control study to characterise the role of the *IL-1RN* 86-bp VNTR polymorphism in the development of NPC with 112 patients with the disease and 433 healthy individuals from the northern region of Portugal. *IL-1RN* genotypes were combined according to the number of repeats: allele 2 (A2), the short allele that corresponds to two repeats, and L, the long allele that corresponds to three or more repeats.
Results: Our study revealed that 31.2% of NPC patients were *IL-1RN* A2*A2, compared with 9.7% observed in the control group. The statistical analysis revealed that *IL-1RN**A2 homozygosity for the A2 allele was associated with a fourfold increased risk for NPC development ($p < 0.001$). Additionally, cumulative hazard analysis revealed that estimated median age of onset of NPC is significantly ($p < 0.001$) different for A2*A2 homozygous versus non-A2*A2 (57.0 vs. 74.0, respectively).
Conclusions: This is the first study to evaluate the role of the *IL-1RN* VNTR in NPC development in Portugal. Our study indicates *IL-1RN**A2 homozygosity as a significant risk marker in our population and that it should be further investigated for the potential role in the definition of a susceptibility profile for NPC onset.

1. Introduction

Nasopharyngeal carcinoma (NPC) is a frequent malignancy in Southeast Asia, particularly in Southern China, while in Western countries its incidence rates are below 1 per 100,000.[1–3] NPC is considered to be a multifactorial disease, where environmental factors such as tobacco smoking, alcohol consumption, exposure to wood dust and consumption of salted fish and foods containing

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nitrosamine or its precursors have been associated with its development.[3–5]

In addition, epidemiological evidence suggests that undifferentiated NPCs (UNPCs) are associated up to 100% with infection by the Epstein–Barr virus (EBV).[6–10] Recently it has been discussed that deficiencies in the immune response to viral infection can play a role in the different individual susceptibility to the development of viral-associated neoplasias.[11–16]

Upon viral infection the host immune system activates a response that is mediated mainly by the interleukin 1 (IL-1) family. This family combines potent pro-inflammatory cytokines, IL-1α and IL-1β, and its negative regulator with anti-inflammatory effect, the IL-1 receptor antagonist (*IL-1RN*).[17–22] The last-named competes with IL-1α and IL-1β for the IL-1 cell receptor, preventing the transmission of pro-inflammatory signals.[17,23–25] Moreover, *IL-1RN* avoids cellular damages in the case of a severe and prolonged inflammatory response and can also be used to follow the immune response, as its levels increase during the final steps of inflammatory response.[18–27]

Genetic polymorphisms have been considered to be important regarding the definition of a susceptibility profile for the development of several cancers.[28] Several genetic studies have indicated that polymorphisms of the *IL-1RN* gene sequence could influence its expression and therefore have an important role in the development of several disorders.[27–30] In fact, several authors have been referring that a 86-bp variable number of tandem repeats (VNTR) polymorphism within intron 2 is associated with the development of several cancers: gastric,[31–41] oesophageal,[42] bladder,[43–45] breast,[46] colorectal,[47,48] lung,[49–51] brain,[52,53] gallbladder,[43] cervical[54] and nasopharyngeal.[55]

A previous study from our group has demonstrated that that *IL-1RN* VNTR polymorphism is associated with the development of cervical cancer.[54] Hence, with this hospital-based case–control study we aimed to characterise the role of the *IL-1RN* VNTR polymorphism in the development of NPC within a southern European population from the northern region of Portugal.

2. Materials and methods

2.1. Type of study and population

We have performed a hospital-based case–control study considering unrelated individuals from a Caucasian population from the northern region of Portugal (Table 1).

The group of patients consisted of 112 individuals with mean age of 47.2 years (standard deviation 14.3) who attended consecutively at the Otorhinolaryngology Department of the Portuguese Institute of Oncology, of Porto. All cases were histologically confirmed by a senior pathologist from our institution according to the World Health Organisation (WHO) classification: 11 type I (keratinising squamous cell carcinoma) or type II (non-keratinising squamous cell carcinoma), combined as well or moderated differentiated (WMDNPC) and 101 type III characterised as undifferentiated carcinoma (UNPC).

The control group consisted of 433 (237 males and 196 females) randomly selected healthy individuals with mean age of 42.2 years (standard deviation 15.3) recruited from a community-based Healthy Blood Donors Database at the Institute of Molecular Pathology and Immunology of University of Porto (IPATIMUP). All individuals were submitted to a previous clinical evaluation and have no individual history of cancer.

Table 1 – Distribution of clinical data in cases and controls included in the study.

	Gender, no. (%)		Age, y (mean ± SD)
	Female	Male	
Controls	196 (45.3)	237 (54.7)	42.2 ± 15.2
NPC patients	33 (29.0)	79 (71.0)	47.2 ± 14.3
WMDNPC (n = 11)	2 (18.2)	9 (81.8)	49.5 ± 12.7
UNPC (n = 101)	31 (30.7)	70 (69.3)	47.0 ± 14.5

WMDNPC, well or moderated differentiated NPC; UNPC, undifferentiated nasopharyngeal cancer; SD, standard deviation.

2.2. Ethical questions

This study does not include any clinical procedure other than collection of blood samples and it has not interfered with the routine procedures decided upon by clinicians. Clinico-pathological data were collected from individual clinical records and entered into a database with unique codification to assure confidentiality. Informed consent according to the Declaration of Helsinki was obtained from each individual.

2.3. Sample collection and DNA extraction

Peripheral blood samples were collected following a standard venipuncture technique in ethylene diamine tetraacetic acid (EDTA)-containing tubes and the DNA was extracted from the white blood cell fraction using a salting-out protocol.

2.4. Analysis of IL-1RN VNTR polymorphism

The analysis of the *IL-1RN* VNTR polymorphism located within intron 2 was performed by polymerase chain reaction (PCR) with the primers described previously[37] (forward: 5′-CCCCTCAGCAACACTCC-3′; and reverse: 5′-GGTCAGAAGGGCAGAG-3′). Briefly, the PCR reaction was performed in a total volume of 50 μl including: 1× Taq buffer, 2.0 mM of $MgCl_2$, 0.4 mM of deoxynucleotide triphosphates (dNTPs), 0.30 μM of each primer and 1 U Taq DNA polymerase. Amplification was performed with an initial denaturation step of 4 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 57 °C and 60 s at 72 °C and a final extension step of 5 min at 72 °C. PCR amplification products (allele 1 – 410 bp; allele 2 – 240 bp; allele 3 – 325 bp; allele 4 – 500 bp; and allele 5 – 595 bp) were separated by electrophoresis in a 3% agarose gel (Cambrex Bio Science Rockland Inc., Rockland, ME, USA) stained with 5% ethidium bromide – Fig. 1. Possible genotype combinations include: 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.