ISID Small Grants Program Short Report

by Ines Badano, Licenciada en Genetica • Laboratorio de Biología Molecular Aplicada. FCEQyN Universidad Nacional de Misiones • Argentina

TNF-Alpha Promoter SNPs and Human Papillomavirus (HPV) Cervical Infection in Guarani Indian Women of Misiones, Argentina

1. Introduction

Infection with Human Papillomavirus (HPV) is known to play a central role in the development of cervical cancer. Although many infections are transient others are strongly associated with progression to cancer, suggesting that additional risk factors are involved [1, 2].

Host genetic factors are likely to play a role in this process, in particular immune system genes since they have a central function in the recognition and subsequent clearance of virally-infected cells [3-5].

The TNF-alpha is a multifunctional cytokine that has been implicated in the control of HPV infection by induction of apoptosis in HPV-infected cells and cervical cancer cells [6, 7]; stimulation of the inflammatory response [8, 9] and down-regulation of HPV gene transcription [10, 11]. Although its beneficial functions, an excessive production can also contribute to the disease, in particular high plasma TNF levels in cancer patients are associated with a poor disease outcome [12-15].

Single Nucleotide Polymorphisms (SNPs) located in the promoter region of the gene have been reported (-862, -856, -375, -307, -243, -237) [16-19] and some of them appear to influence its production [20-22]. According to this findings SNPs has been classified as a *¨High¨* (-307A) or *¨Low¨* (-237A) producers based on their effects on TNF transcription and as a *¨Risk¨* or *¨Protective¨* factors in the development of cervical cancer respectively [23, 24] but results are contradictory [25, 26].

The present study aims to determinate the frequencies of SNPs -237, -307, -243, -375 and its potential association with HPV infection, in native American women of the Guarani population settled in Misiones.

2. Materials and Methods

Samples

Genomic DNA from cervical cells (n 80) and information about HPV diagnosis were kindly provided by Tonon SA. [27].

Analysis of the TNF-alpha polymorphism

To genotype the population, we used PCR and sequencing. SNPs were verified manually and with Codon Code Aligner V1.6.3 Software. After analysis only 77 samples meet the quality criteria to be included in this report.

Statistical analysis

Odds ratios (95% confidence intervals) and Fisher's exact test were used to calculate statistical significance (Epi Info version 3.3.2).

3. Results

Genotype and allele frequencies for each SNPs are showed in Table 1.

TABLE 1: DISTRIBUTION OF TNF-ALPHA SNPS

SNPs TNF-alpha	Genotype Distribution			Allele Frequency	
	G/G	G/A	A/A	G	A
-375	0.987	0.013	0	0.994	0.006
-307	1	0	0	1	0
-243	1	0	0	1	0
-237	0. 948	0.052	0	0.974	0.026

Potential associations between genotypes and HPV infection were evaluated only for SNP -237, whereas no statistical analysis could be done for SNPs -307 and -243 (only the common G allele present in the population) and –375 (we found a single patient A/G which occurred in the HPV infected group).

Individuals carrying the SNP- 237A allele were less frequently in the HPV positive group (4%) compared to negative women (10%). In agreement with the potential protective effect of the -237A genotype [24], the OR analysis showed 0.35 (CI 95% 0.05-2.7), but was not statistically significant.

4. Discussion

The Guarani population settled in Misiones consists of around 5,000 people distributed in more than 40 small communities [28]. They still inhabit regions of difficult access deep into the rain forest, preserving their ancestral cultural patterns and customs [29, 30].

Epidemiological data have shown that Guarani women have a high prevalence of HPV infection (64%) compared to white-urban women (43%) of the region [27, 31]. These differences have been primarily attributed to socio/cultural, geographical and nutritional characteristics.

In order to explore potential host genetic factors, we analyzed 80 samples from the original study referred above [27] at the level of SNPs in the

continued on page 8

Ines Badano, Licenciada en Genetica

Ms. Badano has a degree in Genetics (Licenciatura en Genetica) and is currently finishing her PhD program at the University of Buenos Aires, Argentina. Her research focuses on the detection and characterization of Human Papilloma virus infection (the etiological agent of cervical cancer) in aboriginal and non-aboriginal women inhabiting Misiones. Along with epidemiology she is interested in the relationship between human genetic markers and viral infection, in particular the genetic history of human populations and viral co-evolution. Her work is performed in the Laboratorio de Biologia Molecular Aplicada, National University of Misiones.

ISID Small Grants Program Short Report *continued from page 7*

by Ines Badano, Licenciada en Genetica • Laboratorio de Biología Molecular Aplicada. FCEQyN Universidad Nacional de Misiones • Argentina

promoter region of the TNF-alpha gene (positions -375, -307, -243, -237) which appear to be biologically important in the pathogenesis of cervical cancer and HPV infection [4, 5, 23, 24].

Our results showed no statistically significant association between the presence of SNPs and the risk of HPV infection. However the low number of women analyzed has limited us to explore the relationships adequately.

Nevertheless, we found differences in the frequency of SNPs compared to other groups of different ethnic origin (data not showed), Guarani share with other American Indians the absence of allele -307A and this pattern has been associated (at least in part) with: *i*) a dramatic increase of the –856 SNPs and *ii*) with an Amerindian haplotype fragment defined by HLA-Cw*0102, -B*1522, -DR*0407 [32].

It may be interesting to explore if linkage disequilibrium with other HLA genes exist in this population, these differences may help to understand in the future the role of genetic factors in the development of disease. ❖

I wish to thank Maia Cabrera and Ivana Quintero for technical support, Javier Liotta for aid in the grant administration, Mariana Mampaey for coordinating the Guarani contact and Sergio Tonon for providing samples and mentoring my research career. This work is in the memory of Sergio Tonon. This research was supported by a Small Grant of the International Society for Infectious Disease. *E-mail: inesbadano@yahoo.com.ar*