**Abstract.** – OBJECTIVE: Human papillomavirus (HPV) is associated with cervical cancer. For the infection to occur, most HPV types depend on interactions with heparan sulfate proteoglycans (HSPGs); however, non-HSPGs receptors are also involved. Laminin 332 is a crucial component of the epidermis’s base membrane. It has shown interactions with HPV that suggest its function as a transient viral receptor in the extracellular matrix (ECM). We provide new information about Laminin 332 and HPV by identifying LAMA3 gene allelic variants from exons 30 and 31 and their distribution among women with and without HPV infection.

**PATIENTS AND METHODS:** We included 192 cervical cancer scrape samples from two groups of patients, 96 samples from patients with a low-grade squamous intraepithelial lesion (LSIL) and 96 samples from HPV-negative samples without LSIL. Identification of the HPV type was performed using an LCD-Array kit. Exons 30 and 31 of LAMA3 were amplified by PCR and analyzed by Sanger’s sequencing.

**RESULTS:** We identified a wide range of HPV types. The most frequent low-risk (lrHPV) HPV types were 6, 42, 44, and 90. For high-risk (hrHPV) HPV were 16, 31, 56, and 66. Only the genetic variant rs1131521 was identified in both groups. However, no significant association was observed between rs1131521 and the study groups.

**CONCLUSIONS:** A single silent polymorphism was identified in both groups with similar frequency, whereas no mutations related to increased epithelial friability were identified.

**Key Words:** Human papillomavirus, Infection predisposition, LAMA3 gene, Sequencing.

**Introduction**

Human Papillomavirus (HPV) are small and non-enveloped double-stranded DNA viruses, with more than 150 types described. Some HPV types are associated with multiple human cancers, especially cervical (CC)2,3. The two main strategies for CC prevention are HPV vaccination and cervical cancer screening. RT-PCR from homogenized samples is the primary method for HPV detection in cervical cancer screenings4. However, if the cell morphology needs to be preserved, *in situ* hybridization using Loop RNA probes (LRPs) can be used.5,6

Most uterine cervix, anal, and vaginal carcinomas are caused by persistent HPV infections2,4. HPV infection begins with the viral particle entry to the cell through microtraumas on the mucosa and skin. The viral particles’ L1 and L2 capsid proteins mediate receptor binding and internalization into the cell. Once inside, the early proteins E5, E6, and E7 promote cell proliferation and immortality to allow the viral DNA to replicate using the replication machinery of the host cell.7 The manipulation of the cell cycle performed by these
Variants of the LAMA3 gene in HPV infection.

Variants of the LAMA3 gene in HPV infection. Entry to the cell might be the most crucial step in viral infection; it requires complex viral capsid interactions with cellular proteins and receptors, especially heparan sulfate proteoglycans (HSPGs)4-10. However, non-HSPG receptors or receptor complexes are also involved11,12. Some extracellular matrix (ECM) proteins have been proposed as viral receptors13-16. One of these is Laminin 33217.

Laminin 332 (formerly laminin-5 or LN-5) is a crucial component of the base membrane of the epidermis. It is composed of the subunits α3, β3, and γ218,19. This protein anchors the epidermis to the dermis, and its absence is linked to some skin disorders and epidermis friability, such as epidermolysis bullosa20,21.

The LAMA3 gene, located on 18q11.2, codes the α3 subunit of this protein; it is 256,624 bp in size and is composed of 78 exons22. The LAMA3 gene is expressed in all body tissues, and different expression rates have been associated with multiple types of cancers22-24, specifically by modifying the architectural environment of the ECM, favoring the migration, cell adhesion, and invasion of tumor cells19.

Exons 30 and 31 of the LAMA3 gene are of particular interest because they are part of the LG3 domain, which is crucial for the correct function of Laminin 332. Currently, at exon 30, there are around 32 single-nucleotide variants (SNVs) described, and two of them are labeled as pathogenic in the ClinVar database25 (rs1401574168 and another at c.8755A>T). While at exon 31, there are around 18 variants described, five are considered pathogenic or likely pathogenic (rs34754160, rs772038362, rs137852758, rs1057517211, and another at c.8911C>T).

A previous study17 observed that the L1 protein of HPV can interact with Laminin 332 and aid in the adhesion to the host cell. Based on this, some variants may be linked to an increased susceptibility to HPV infection, either as a possible transient receptor17 or by increasing the cervical epithelium friability20,21.

We sequence exons 30 and 31 of the LAMA3 gene in HPV-positive and HPV-negative patients in search of possible genetic variants associated with HPV infection.

Patients and Methods

Study Design and Subjects

This study was a comparative cross-sectional study. We used data from two different and randomized groups in this analysis. The case group included 96 HPV-positive samples obtained from patients with low-grade squamous intraepithelial lesions (LSIL), and the control group had 96 HPV-negative samples without LSIL. We paired cases and controls by age. Cervical scrape samples were collected from the sample library of the Molecular Microbiology Laboratory, Molecular Medicine Division of CIBO-IMSS.

DNA Isolation

The cervical scrapes (squamocolumnar epithelium of the cervix) were collected with a sterile isotonic saline pre-wet cytobrush. The samples were preserved in a viral transport medium (Digene HC2 DNA Collection Device, QIAGEN GmbH, Hilden, Germany) at 4°C until analysis.

DNA isolation was performed with the High Pure Viral Nucleic Acid kit (Roche Molecular Systems Inc. Pleasanton, CA, USA), under the manufacturer’s recommended conditions. The quality and quantity of the isolated DNA were evaluated in a NanoDrop 2000 instrument (Thermo Fisher Scientific, Wilmington, DE, USA). The DNA concentration of each sample was adjusted to 100 ng/µL.

HPV Detection and Typing

Identification of HPV was performed with HPV Type 3.5 LCD-Array kit (Chipron Technologies, Berlin, Germany). This array identifies 32 clinically relevant alpha-human papillomavirus types. Two primer sets for PCR amplification were provided in the kit. The first was based on the published and commonly used My11/My09 system (primer mix HPV My11/09). The second primer set produces shorter amplicons of 125 bp in length (primer mix HPV 125).

The obtained amplicons were hybridized in the LCD chips for typing, using the Prime film 3650u scanner (Pacific Image Electronic Inc. Torrance, CA, USA) and the SlideReader V9 software (Chipron Technologies GmbH, Berlin, Germany).

Molecular Analysis

To identify LAMA3 gene variants in exons 30 and 31, we used the primers described in a previous study26. Amplification was performed according to the reaction conditions and PCR amplification programs standardized in the Molecular Microbiology Laboratory.

For exon 30, the primers used were 5’ TTA-ACCAACCCTTCTCATC 3’ and 5’ TTC-CCCAATATCTTCCCAAA 3’, whose product
is a 354 bp amplicon. PCR conditions were as follows: initial denaturing at 94°C for 5 min, followed by 45 cycles (94°C for 30 s, 55.7°C for 30 s, and 72°C for 30 s), and a final extension at 72°C for 5 min.

For exon 31, we used the primers 5’ GG-CCTTCTATTGCCCTACTG 3’ 5’ ATGGTGT-CATGGCAGTCAGA 3’, whose product is a 375 bp amplicon. PCR conditions were as follows: initial denaturing at 94°C for 5 min, followed by 42 cycles (94°C for 45 s, 55.7°C for 30 s, and 72°C for 30 s), and a final extension at 72°C for 5 min.

PCR products from exons 30 and 31 of LAMA3 were purified with QIAquick (QIAGEN, Hilden, Germany), and then analyzed with Sanger sequencing using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, USA) following the manufacturer’s recommendations.

**Statistical Analysis**

Descriptive statistics for clustering data were used for reporting the frequency and distribution of the HPV types. The qualitative data analysis was performed using nonparametric statistical tests such as Chi-square ($\chi^2$) from the obtained allelic and genotypic frequencies. IBM SPSS Statistics Version 24 (IBM Corp., Armonk, NY, USA) was used for data analysis. Statistical significance is considered when the $p$-value was <0.05.

**Results**

**HPV Typification**

We found a wide range of low-risk (lrHPV) and high-risk (hrHPV) HPV types in the 96 HPV-positive samples (Table I). The most frequent lrHPV were types 6, 41, 44, 90, and 91, while the most frequent hrHPV were 16, 31, 66, and 56 (Figure 1).

Of all HPV-positive cases, 64 were co-infections, where the most frequent HPV types found together were 16/6 and 16/61 (5 cases each), followed by 16/81, 16/90, and 16/44 (4 cases each). The case with the highest number of HPV types in a single co-infection contained HPV16, 61, 81, 83, 84, 91, 45, 68, and 82.

**LAMA3 exons 30 and 31 sequencing.**

Of the 192 samples, nine samples (5 HPV-positive and 4 HPV-negative) were excluded from the analysis due to insufficient DNA sequencing data. Only variant rs1131521, located on exon 30, was identified in both study groups. We found 150 cases with the C/C genotype, 33 with the C/T, and none with the T/T genotype. Chi-square showed no significant differences in the distribution of the genotypes and alleles between the two groups ($p=0.587$) (Table II).

**Discussion**

**HPV Typification**

Although this is a small sample of the Mexican population, our results show a great diversity of HPV types that infect the cervical epithelium. The most common types are 16, 6/66, 44, 90/31, 91, 42, and 56. These results are consistent with previous studies on the distribution of HPV types in the Mexican population.

In these studies, HPV16 is one of the most frequent lrHPV types. As for hrHPVs, HPV6 is frequent in the Mexican population, especially in men. HPV31 and HPV42 are also frequent and associated with tonsillar and nasopharyngeal carcinoma in Mexicans.

In our study, 66.6% of cases were co-infections, mainly lrHPVs with hrHPVs. Compared to other studies with HPV-positive subjects, the co-infection percentage varies between 32% to 80%. The combination HPV6/16 was the most common in our study, as well as in a previous one. Types 16 and 18 were more prevalent in coinfections.

We find these results relevant because most HPV studies are focused on the United States and Europe. These populations have the most influence on the types considered for the HPV vaccines. As a consequence, the HPV vaccine may not be as efficient for people from developing countries such as Mexico, South Africa, Brazil, Saudi Arabia, etc.

**LAMA3 Exons 30 and 32 Sequencing**

In this study, we only identified one variant on exon 30 (rs1131521) and none on exon 31. It is...
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Figure 1. Number of cases with (a) low-risk (lrHPV) or (b) high-risk (hrHPV) human papillomavirus in the 96 study samples.

Table II. Genotypic and allelic frequencies of variant rs1131521.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Genotypic frequencies†</th>
<th>Allele frequencies‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C/C</td>
<td>C/T</td>
</tr>
<tr>
<td>HPV (+)</td>
<td>91</td>
<td>76 (0.835)</td>
<td>15 (0.165)</td>
</tr>
<tr>
<td>HPV (-)</td>
<td>92</td>
<td>74 (0.804)</td>
<td>18 (0.196)</td>
</tr>
<tr>
<td>Total</td>
<td>183</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HPV (+) Samples infected with HPV; HPV (-) Samples without HPV infection. †No T/T genotypes were found. ‡Comparison of allelic frequencies between the HPV (+)/HPV (-) groups did not show significant differences (p > 0.05, according to χ²).
essential to mention that rs1131521 is a silent mutation, both the wild CTA codon and the mutant TTA codon code for leucine\textsuperscript{36}. No significant differences were observed in the distribution of the allelic frequencies of variant rs1131521 between the study groups, or with a particular HPV type.

In our study, the observed frequencies were C=0.9176 and T=0.0824 for HPV-positive samples; C=0.9022 and T=0.0978 for the HPV-negative samples. These frequencies differ from those reported by the 1,000 genome project\textsuperscript{37} in a population with Mexican ancestry (C=0.8828 and T=0.1172).

Regarding other populations, Americans (C=0.8371 and T=0.1628) were the most similar to our frequencies, followed by Europeans (C=0.7942 and T=0.2058), Asians (C=0.7699 and T=0.2301), and African population (C=0.9886 and T=0.0113).

**Conclusions**

We only identified variant rs1131521 in both study groups. This is a silent mutation and thus does not contribute to the increased friability of the epithelium in the presence of any lrHPV or hrHPV. The frequency of rs1131521 polymorphism in our study population differed from the previously reported in the North American population\textsuperscript{37}. No other variants were found in the LAMA3 gene. Although this is a discrete sample, the distribution of low-risk and high-risk HPVs in the Mexican population differs from what is reported worldwide.

**Conflict of Interest**
The Authors declare that they have no conflict of interests.

**Acknowledgements**
We thank everyone who contributed directly or indirectly to this study.

**Informed Consent**
Informed consent was obtained from all individual participants included in the study. All samples were anonymized to protect the personal information that may identify the patients involved in this study.

**Ethics Approval**
This study was conducted according to the ethical principles of the Declaration of Helsinki and the principles of Good Clinical Practice. It was approved by the corresponding Institutional Review Boards and Regulatory Committees and registered with the National Health Committee under the registration code 2011-785-028.

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**Authors’ Contribution**
Monica Edith Villanueva-Aguilar: writing - review and editing; visualization. Lourdes del Carmen Rizo-de-la-Torre: writing - review and editing. Alfonso Enrique Bencomo-Alvarez: investigation, Martha Patricia Gallegos-Arreola: formal analysis; writing - review and editing. Héctor Montoya-Fuentes: conceptualization; supervision and main writing.

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**Data Availability**
The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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