Sequence analysis of exons 30 and 31 of *LAMA3* gene variants and its association with human papillomavirus infection predisposition: no evidence was found

M.E. VILLANUEVA-AGUILAR[1,2], L.C. RIZO-DE LA TORRE[1], A.E. BENCOMO-ÁLVAREZ[1], M.P. GALLEGOS-ARREOLA[3,4], H. MONTOYA-FUENTES[1,3]

[1]Division of Molecular Medicine, Western Biomedical Research Center (CIBO), Mexican Social Security Institute (IMSS), Sierra Mojada 800, Independencia Oriente, Guadalajara, Jalisco, México
[2]Human Genetics (DGH), University Center for Health Science (CUCS), University of Guadalajara (UDG), Sierra Mojada 950, Independencia Oriente, Guadalajara, Jalisco, México
[3]Epidemiologic Surveillance and Research Support Laboratory (LAVIE), Western Biomedical Research Center (CIBO), Mexican Social Security Institute (IMSS), Sierra Mojada 800, Independencia Oriente, Guadalajara, Jalisco, México
[4]Division of Genetics, Western Biomedical Research Center (CIBO), Mexican Social Security Institute (IMSS), Sierra Mojada 800, Independencia Oriente, Guadalajara, Jalisco, México.

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.

Introduction

Human Papillomavirus (HPV) are small and non-enveloped double-stranded DNA viruses, with more than 150 types described[1]. 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 screenings[4]. 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 infections[2,4]. 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

Corresponding Author: H. Montoya-Fuentes, MD; e-mail: schlagzeugger@hotmail.com

proteins increases genetic instability and favors the malignant transformation of the cell[4].

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)[8-10]. However, non-HSPG receptors or receptor complexes are also involved[11,12]. Some extracellular matrix (ECM) proteins have been proposed as viral receptors[11,13-16]. One of these is Laminin 332[17].

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 γ2[18,19]. This protein anchors the epidermis to the dermis, and its absence is linked to some skin disorders and epidermis friability, such as epidermolysis bulbosa[20,21].

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 exons[22]. The *LAMA3* gene is expressed in all body tissues, and different expression rates have been associated with multiple types of cancers[22-24], specifically by modifying the architectural environment of the ECM, favoring the migration, cell adhesion, and invasion of tumor cells[19].

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 database[25] (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 study[17] 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 receptor[17] or by increasing the cervical epithelium friability[20,21].

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 study[26]. 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-ACCAACCCCTCTTCATCC 3' and 5' TTC-CCCAATATCTCCCACAA 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 (χ^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-infec-

Table I. Types of high and low-risk HPV identified in the case samples.

HPV presence	N (%)
lrHPV simple infection	20 (20.8)
hrHPV simple infection	12 (12.5)
lrHPV coinfection	8 (8.3)
hrHPV coinfection	6 (6.3)
lrHPV and hrHPV	50 (52.1)
Total	96 (100.0)

lrHPV: Low-Risk Human Papillomavirus. hrHPV: High-Risk Human Papillomavirus.

tions, 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[27-29] on the distribution of HPV types in the Mexican population.

In these studies[27-29], HPV16 is one of the most frequent hrHPV types. As for lrHPVs, HPV6 is frequent in the Mexican population[28,30], especially in men[31,32]. HPV31[30] is also frequent and associated with tonsillar and nasopharyngeal carcinoma in Mexicans[31].

In our study, 66.6% of cases were co-infections, mainly lrHPVs with hrHPVs. Compared to other studies[27-30] 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[32]. Types 16 and 18 were more prevalent in coinfections[28,29].

We find these results relevant because most HPV studies are focused on the United States and Europe[33]. 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.[34,35].

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
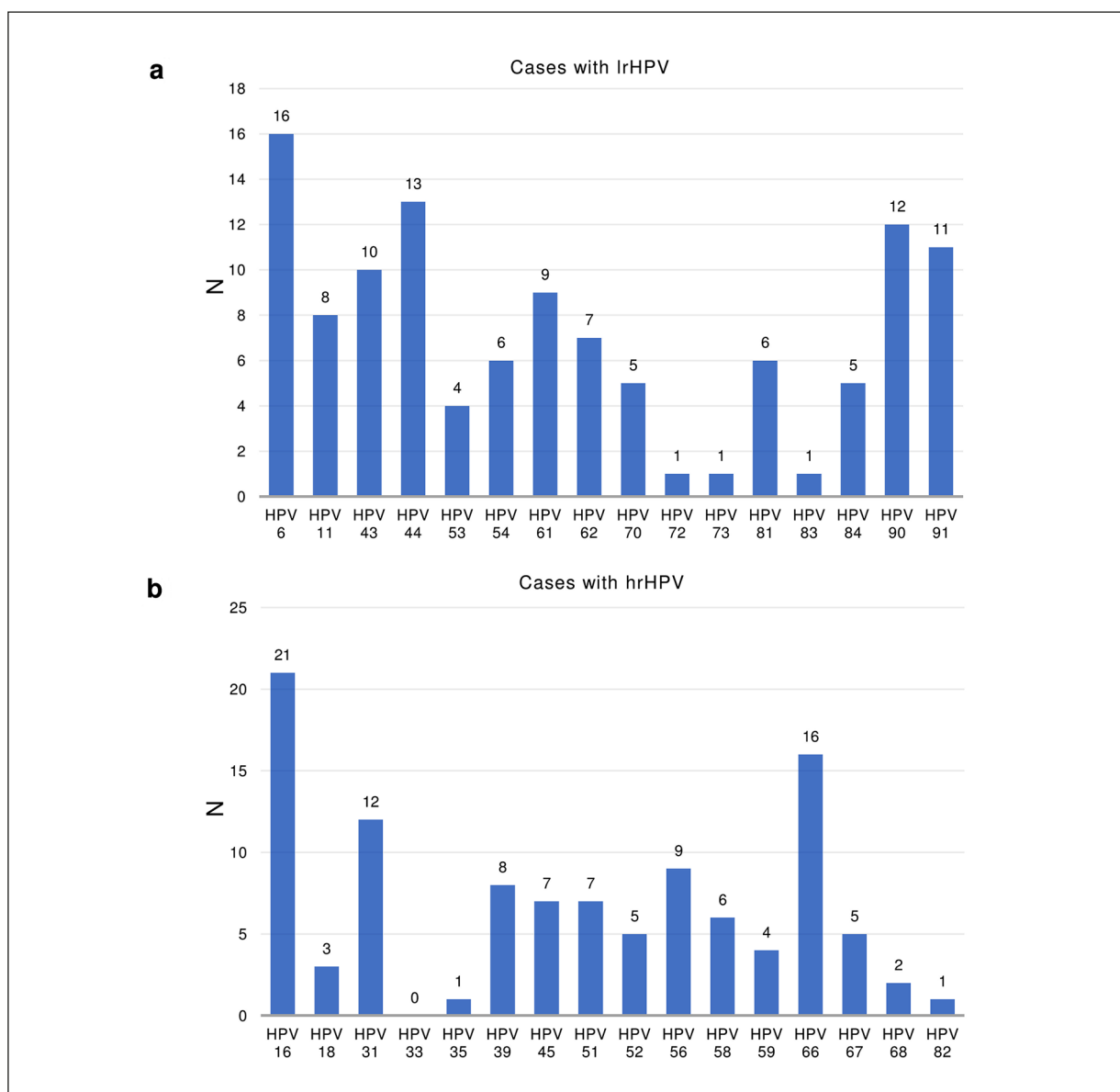
Figure 1. Number of cases with (**a**) low-risk (lrHPV) or (**b**) high-risk (hrHPV) human papillomavirus in the 96 study samples.

Table II. Genotypic and allelic frequencies of variant rs1131521.

Groups	N	Genotypic frequencies[†]			Allele frequencies[‡]	
		C/C	C/T	T/T	C	T
HPV (+)	91	76 (0.835)	15 (0.165)	-	167 (0.918)	15 (0.082)
HPV (-)	92	74 (0.804)	18 (0.196)	-	166 (0.902)	18 (0.098)
Total	183					

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 χ^2).

essential to mention that rs1131521 is a silent mutation, both the wild CTA codon and the mutant TTA codon code for leucine[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[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[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.

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

ORCID ID
Monica Edith Villanueva-Aguilar: 0000-0001-6279-0771
Lourdes del Carmen Rizo-delaTorre: 0000-0001-5482-1611
Alfonso Enrique Bencomo-Alvarez: 0000-0002-1771-8532
Martha Patricia Gallegos-Arreola: 0000-0003-4539-1693
Hector Montoya-Fuentes: 000-0002-8922-8225.

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

References

1) Doorbar J, Egawa N, Griffin H, Kranjec C, Murakami I. Human papillomavirus molecular biology and disease association. Rev Med Virol 2015; 25: 2-23.

2) Origoni M, Cristoforoni P, Mariani L, Costa S, Preti M, Sandri MT, Preti EP, Ghelardi A, Perino A. Extending Human Papillomavirus (HPV) vaccination beyond female adolescents and after treatment for high grade CIN: The Italian HPV Study Group (IHSG) review and position paper. Eur Rev Med Pharmacol Sci 2020; 24: 8510-8528.

3) Broniarczyk J, Massimi P, Bergant M, Banks L. Human Papillomavirus Infectious Entry and Trafficking Is a Rapid Process. J Virol 2015; 89: 8727-8732.

4) Tatullo M, Rengo S, Mortellaro C, Riva Cavalletti De Rossi G, Riva F. Translational and transdisciplinary approach to the human papilloma virus - Preliminary evidence from the Italian 'HPV board: a future without papilloma virus' project. Eur Rev Med Pharmacol Sci 2022; 26: 115-122.

5) La Rocca G, Coleman J, Rabbani E, Palermo G, Palermo F, Mauro M. Recent improvements in in situ hybridization for the detection of HPV infections in clinical samples. WCRJ 2020; 7: e1542.

6) Sabetta R, La Mantia E, Franco R, Zito Marino F. Detection of hpv infections in clinical samples: recent improvements in In Situ Hybridization (ISH) with Loop RNA Probes (LRPs) technology. WCRJ 2020; 7: e1570.

7) Aksoy P, Gottschalk EY, Meneses PI. HPV entry into cells. Mutat Res Rev Mutat Res 2017; 772: 13-22.

8) Joyce JG, Tung JS, Przysiecki CT, Cook JC, Lehman ED, Sands JA, Jansen KU, Keller PM. The L1 major capsid protein of human papillomavirus type 11 recombinant virus-like particles interacts with heparin and cell-surface glycosaminoglycans on human keratinocytes. J Biol Chem 1999; 274: 5810-5822.

9) Giroglou T, Florin L, Schäfer F, Streeck RE, Sapp M. Human papillomavirus infection requires cell surface heparan sulfate. J Virol 2001; 75: 1565-1570.

10) Surviladze Z, Dziduszko A, Ozbun MA. Essential roles for soluble virion-associated heparan sulfonated proteoglycans and growth factors in human papillomavirus infections. PLoS Pathog 2012; 8: e1002519.

11) Aksoy P, Abban CY, Kiyashka E, Qiang W, Meneses PI. HPV16 infection of HaCaTs is dependent on β4 integrin, and α6 integrin processing. Virology 2014; 449: 45-52.

12) Letian T, Tianyu Z. Cellular receptor binding and entry of human papillomavirus. Virol J 2010; 7: 2.

13) Evander M, Frazer IH, Payne E, Qi YM, Hengst K, McMillan NA. Identification of the alpha6 integrin as a candidate receptor for papillomaviruses. J Virol 1997; 71: 2449-2456.

14) Abban CY, Meneses PI. Usage of heparan sulfate, integrins, and FAK in HPV16 infection. Virology 2010; 403: 1-16.

15) Woodham AW, Da Silva DM, Skeate JG, Raff AB, Ambroso MR, Brand HE, Isas JM, Langen R, Kast WM. The S100A10 Subunit of the Annexin A2 Heterotetramer Facilitates L2-Mediated Human Papillomavirus Infection. PLoS One 2012; 7: e43519.

16) Scheffer KD, Gawlitza A, Spoden GA, Zhang XA, Lambert C, Berditchevski F, Florin L. Tetraspanin CD151 Mediates Papillomavirus Type 16 Endocytosis. J Virol 2013; 87: 3435-3446.

17) Culp TD, Budgeon LR, Marinkovich MP, Meneguzzi G, Christensen ND. Keratinocyte-secreted laminin 5 can function as a transient receptor for human papillomaviruses by binding virions and transferring them to adjacent cells. J Virol 2006; 80: 8940-8950.

18) Caley MP, Martins VL, Moore K, Lashari M, Nissinen L, Kähäri V -M., Alexander S, Jones E, Harwood CA, Jones J, Donaldson M, Marshall JF, O'Toole EA. Loss of the laminin subunit alpha-3 induces cell invasion and macrophage infiltration in cutaneous squamous cell carcinoma*. Br J Dermatol 2021; 184: 923-934.

19) Rousselle P, Scoazec JY. Laminin 332 in cancer: When the extracellular matrix turns signals from cell anchorage to cell movement. Seminars in Cancer Biology 2020; 62: 149-165.

20) Tayem R, Niemann C, Pesch M, Morgner J, Niessen CM, Wickström SA, Aumailley M. Laminin 332 Is Indispensable for Homeostatic Epidermal Differentiation Programs. J Invest Dermatol 2021; 141: 2602-2610.e3.

21) Pesch M, König S, Aumailley M. Targeted Disruption of the Lama3 Gene in Adult Mice Is Sufficient to Induce Skin Inflammation and Fibrosis. J Invest Dermatol 2017; 137: 332-340.

22) Tang L, Wang P, Wang Q, Zhong L. Correlation of LAMA3 with onset and prognosis of ovarian cancer. Oncol Lett 2019; 18: 2813-2818.

23) Pinatti LM, Walline HM, Carey TE, Klussmann JP, Huebbers CU. Viral Integration Analysis Reveals Likely Common Clonal Origin of Bilateral HPV16-Positive, p16-Positive Tonsil Tumors. Arch Clin Med Case Reports 2020; 4: 680.

24) Deng G, Sun D, Zhou Q, Lv Y, Yan H, Han Q, Dai G. Identification of DNA methylation-driven genes and construction of a nomogram to predict overall survival in pancreatic cancer. BMC Genomics 2021; 22: 791.

25) Landrum MJ, Lee JM, Benson M, Brown GR, Chao C, Chitipiralla S, Gu B, Hart J, Hoffman D, Jang W, Karapetyan K, Katz K, Liu C, Maddipatla Z, Malheiro A, McDaniel K, Ovetsky M, Riley G, Zhou G, et al. ClinVar: Improving access to variant interpretations and supporting evidence. Nucleic Acids Res 2018; 46: D1062-D1067.

26) Mühle C, Jiang QJ, Charlesworth A, Bruckner-Tuderman L, Meneguzzi G, Schneider H. Novel and recurrent mutations in the laminin-5 genes causing lethal junctional epidermolysis bullosa: molecular basis and clinical course of Herlitz disease. Hum Genet 2005; 116: 33-42.

27) Aguilar-Lemarroy A, Vallejo-Ruiz V, Cortés-Gutiérrez EI, Salgado-Bernabé ME, Ramos-González NP, Ortega-Cervantes L, Arias-Flores R, Medina-Díaz IM, Hernández-Garza F, Santos-López G, Piña-Sánchez P. Human papillomavirus infections in Mexican women with normal cytology, precancerous lesions, and cervical cancer: type-specific prevalence and HPV co-infections. J Med Virol 2015; 87: 871-884.

28) Gallegos-Bolaños J, Rivera-Domínguez JA, Presno-Bernal JM, Cervantes-Villagrana RD. High prevalence of co-infection between human papillomavirus (HPV) 51 and 52 in Mexican population. BMC Cancer 2017; 17: 531.

29) Oyervides-Muñoz MA, Pérez-Maya AA, Sánchez-Domínguez CN, Berlanga-Garza A, Antonio-Macedo M, Valdéz-Chapa LD, Cerda-Flores RM, Trevino V, Barrera-Saldaña HA, Garza-Rodríguez ML. Multiple HPV Infections and Viral Load Association in Persistent Cervical Lesions in Mexican Women. Viruses; 12. Epub ahead of print March 2020. DOI: 10.3390/v12040380.

30) Hernández-Rosas F, Orozco-Hernández E, Maza-Sánchez L, Salgado-García PC, Navarro-Vidal

E, León-Bautista MP de. Prevalence and correlation of human papillomavirus genotypes with clinical factors in cervical samples from Mexican women. Exp Biol Med (Maywood) 2021; 246: 48-56.

31) Lopez-Lizarraga E, Sanchez-Corona J, Montoya-Fuentes H, Bravo-Cuellar A, Campollo-Rivas O, Lopez-Demerutis E, Morgan-Villela G, Arcaute-Velazquez F, Monreal-Martinez JA, Troyo R. Human papillomavirus in tonsillar and nasopharyngeal carcinoma: Isolation of HPV subtype 31. Ear, Nose Throat J 2000; 79: 942-944.

32) Montoya-Fuentes H, Rodriguez-Martin A, Pena-Iniguez DI, Gonzalez-Bonilla CR, Rosales-Gomez RC, Gallegos-Arreola MP, Santoscoy-Ascencio G, Gutierrez-Rubio SA. Molecular Detection and Typing of Human Papillomavirus in Men from Northwestern Mexico. Arch Med Res 2020; 51: 675-682.

33) Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, Clifford GM. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. Int J Cancer 2007; 121: 621-632.

34) Alshammari FD, Alharbi SA, Humaida MI, Abdalhabib EK, Bealy MAB, Elkhalifa AEO, Agabeldour AA. Human Papillomavirus genotypes associated with cervical intraepithelial lesions among Saudi women. Eur Rev Med Pharmacol Sci 2022; 26: 6367-6373.

35) Flores-Miramontes MG, Torres-Reyes LA, Aguilar-Lemarroy A, Vallejo-Ruíz V, Piña-Sánchez P, Cortés-Gutiérrez E, Reyes-Leyva J, Jave-Suárez LF. HPV genotypes prevalence in México and worldwide detected by Linear Array. Rev Med Inst Mex Seguro Soc 2015; 53 Suppl 2: S122-S130.

36) National Center for Biotechnology Information. Reference SNP (rs) Report: rs1131521. Available at: https://www.ncbi.nlm.nih.gov/snp/rs1131521#-frequency_tab (accessed June 3, 2021).

37) The 1000 Genomes Project Consortium. A global reference for human genetic variation. Nature 2015; 526: 68-74.