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Human papillomavirus 13 *L1* gene polimorphism: first report

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RESUMEN

Polimorfismo del gen *L1* del virus de papilloma humano 13: primer reporte.

Introducción. El virus de papilloma humano 13 (VPH-13) pertenece a los *Alphapapillomavirus-10*. Es un virus con tropismo por la mucosa oral relacionado filogenéticamente con otros papilomavirus humanos (6, 11, 44, 55 y 74). Es responsable de la Hiperplasia Epitelial Multifocal, patología oral benigna poco común, reportada principalment en grupos étnicos de América.

Objetivo. Describir la variabilidad genética del gen *L1* del virus del VPH-13.

Material y Métodos. Se realizó un estudio retrospectivo que incluyó 50 muestras orales de pacientes con Hiperplasia Epitelial Multifocal, y 25 de portadores asintomáticos del VPH- 13, todos residentes del area Maya de México. Se amplificó y secuenció un segmento de 240 pb del gen *L1* del VPH-13, que codifica para la proteína mayor de la cápside. Las secuencias fueron comparadas con las únicas dos secuencias de referencias disponibles. Se construyó el árbol filogenético utilizando el software PAUP*

Resultados. Se identificaron ocho polimorfismos de un solo nucleótido, incluidos tres polimorfismos sinónimos y cinco no sinónimos. Según el árbol filogenético las variantes se agruparon en dos brazos.

Conclusión. Éste es el primer reporte de la presencia de polimorfismos genéticos del VPH 13. Estos resultados son relevantes para futuras investigaciones con el objetivo de identificar linajes y sublinajes. Nuestro análisis in silico sugiere que los cambios de aminoácidos involucran estructuras directamente relacionadas con el reconocimiento de antígenos, lo que requiere de mas investigaciones.

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ABSTRACT

Introduction. Human papillomavirus 13 (HPV-13) belonging to *Alphapapillomavirus-10* species, is an oral mucosotropic virus phylogenetically related to other human papillomavirus (6, 11, 44, 55 and 74). It causes Multifocal Epithelial Hyperplasia, an uncommon benign oral disease reported mainly amongst ethnic groups from America.

Objective. To describe the genetic variability of the HPV-13 *L1* gene.

Material and Methods. A retrospective study including 50 oral samples from patients with Multifocal Epithelial Hyperplasia, and 25 samples from asymptomatic carriers of HPV-13, all subjects living in Mayan area of Mexico. A fragment 240 bp corresponding to the HPV 13*L1* gene, which encodes the major capsid protein was amplified, sequenced, and compared with the only two reference sequences of HPV-13 available. A phylogenetic tree was constructed using PAUP* software.

Results. Eight single nucleotide polymorphisms were identified including three synonymous polymorphism and five non-synonymous. According to the phylogenetic tree, the variants were grouped in two branches.

Conclusion. This is the first report for the presence of genetic polymorphisms of HPV-13. These results are relevant for future research aiming to identify lineages and sub lineages. Our in-silico analysis suggests that the amino acid changes would be involving structures directly related to antigen recognition, which requires further investigations.

INTRODUCTION

HPV-13 is a mucosotropic type of human papillomavirus (HPV) phylogenetically related to the pygmy chimpanzee papillomavirus type 1 (PCPV-1) and other HPV types (HPV-6, HPV-11, HPV-44, HPV-55, and HPV-74), which all together integrates the species Alphapapillomavirus-10 (1, 2). In 1983, the HPV-13 was first characterized from Multifocal Epithelial Hyperplasia (MEH) lesions (3). HPV-13 has a genome of 7,880 bp which shares 80% of sequence homology with PCPV-1, and both produce a similar pathology (4).

MEH is an uncommon oral disease worldwide but reported in diverse ethnic groups of Latin America countries (5-7). In Mexico, this pathology has been poor reported, it occurs mainly in indigenous communities living in poverty with poor access to health services, however, it is endemic in habitants from Mayan areas of Yucatán (8, 9). MEH in the Mayan area of Mexico has a prevalence of 11.8% among subjects < 13 years living in rural areas, contrasting with the prevalence of 3.2% of children living in urban areas (10).

Asymptomatic infections are higher in rural areas with 29.6%. Furthermore, within the same family group is common to find asymptomatic carriers of the virus as well as healthy subjects (8). MEH or Heck´s disease is a benign pathology of the oral cavity caused by HPV-13 or HPV-32 (11, 12). Papulonodular painless lesions with a smooth surface and rough in appearance characterize this pathology, additionally, resilience is an important feature of these injuries. Lips, tongue, cheeks, and hard palate are the anatomical sites more frequently affected (12). Histologically, HEM is characterized by thickening and elongation of the epithelial crests, acanthosis and parakeratosis (13).

HPV *L1* gene is the most conserved gene among Papillomavirus, functionally encodes the most abundant capsid protein. The relevance of the *L1* protein lies in the presence of inmune response epitopes and its interaction with the host cell receptor. Worldwide HPV 13 infection is uncommon, however, in endemic on rural area of Yucatan State, Mexico. The genetic variability of HPVs is being widely studied for several genotypes, mainly HPV-16 and HPV-18 (14- 16). However, information about the genetic variation of HPV-13 is practically non-existent; therefore, this study describes and analyzes the polymorphisms that occur in the HPV-13 *L1* gene from subjects living in a Mayan area of Mexico.

MATERIAL AND METHODS

Sample collections.

Oral swabs used in this study belong to the repository of the virology laboratory of Regional Research Center "Dr. Hideyo Noguchi," Autonomous Yucatan University stored at -70° C, and a previous Project with ethical committee registration 06-82-2015. Twenty-five samples

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were collected from patients without MEH lesions, but positives to HPV-13 by PCR (asymptomatic carriers) living in the rural area of Chemax, Yucatán, Mexico. Fifty samples were collected from patients with MEH and positive for HPV-13 living in the rural area of Yaxhachen, Yucatán, Mexico.

DNA extraction and molecular detection of HPV 13.

DNA was extracted from the oral samples using the DNeasy® Blood & Tissue kit (QIAGEN). A polymerase chain reaction (PCR) was performed using the set of primers HPV-13 forward (5'-AAATCCCAGCAGAATTATAT-3') and HPV-13 reverse (5'-AAAGAGATGATGTAGTGGC-3') to amplify a fragment (240 bp) of the *L1* gene, as previously described (16). PCR was performed in 50 µl mix reaction containing 1x PCR buffer, 50 µM MgCl2, 10 µM dNTPs, 25 pM each primer and 1.0 unit of Taq polymerase (Invitrogen). The thermal conditions started with a pre-heat of 94°C for 4 min, followed by 40 cycles according to the protocol: 30 sec denaturation at 94°C, 30 sec annealing at 50°C, 30 sec extensions at 72°C, and 10 min final elongation at 72°C. The amplicons were visualized on 2% agarose gel stained with SYBR® Safe DNA (Invitrogen).

Sequencing and analysis of polymorphisms.

Amplicons were purified using ExoSAP-IT® (Affymetrix). Sequencing reaction was performed using the BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) according to the manufacturer's protocol. This reaction was performed separately with forward and reverse primers. The excess of DyeDeoxy™ terminators was removed using Centri-SepTM spin columns (Princeton Separations). The samples were processed using an ABI PRISM® 310 genetic analyzer (Applied Biosystems). The multiple sequence alignment was performed using ClustalW available at Geneious package (17). Our sequences were aligned with the only two sequences HPV-13 prototypes: X62843 and DQ344807.

Prediction and secondary structure analysis.

In silico prediction of secondary structure from HPV-13 amino acid sequences was estimated using the I-TASSER server (18, 19). To identify the amino acid positions corresponding to hypervariable regions, the amino acid sequences from HPV-13 were aligned to HPV-16 reference map by using ClustalW. To avoid mistakes at positioning of residues (since our L1 proteins from HPV-13 are partial) an entire genome obtained from one of the samples (data not shown, available GenBank ID: MT068446) as well as the translated sequence of complete *L1* gene were included. The ESPript 3.0 server was used to make a scheme and visualize the protein alignment results (https://espript.ibcp.fr/ESPript/ESPript/).

Phylogenetic analysis.

The phylogenetic analysis included all unique sequences obtained in this work and two HPV-13 sequences obtained from the GenBank database: X62843 (Turkey) and DQ344807 (Central Amazon). The phylogenetic tree was generated by software package PAUP 4.0, using the Hasegawa-Kishino-Yano (HKY85) nucleotide substitution model, and maximum likelihood method. The phylogenetic tree topology was evaluated by bootstrap resampling with 1000 replicates. Annotations and the visualizations of the phylogenetic analysis were built using the online tool iTOL.

RESULTS

Eight unique sequences or molecular variants were identified from the SNPs combinations. These sequences are available from the GenBank database under accession numbers: KY690157 - KY690164. Particularly, KY690157 and KY690161 share the same sequence, however, because the first are from asymptomatic carriers and the second from patients with MEH lesions these sequences were considered independent.

Genetic variation

Compared to the HPV-13 reference sequence X62843 a total of eight SNPs were identified, of which 5/8 (62.5%) were non-synonymous, and

3/8 (37.5%) were synonymous (Table 1). All non-synonymous SNPs were missenses mutations. Among all sequences, 89.3% (67/75) had only one polymorphism, 8 % (6/75) had two polymorphisms, and 2.6% (2/75) had three polymorphisms. Of these, three SNPs were unique among asymptomatic carriers (A6554G, A6557G, and A6574C), and four SNPs occurred exclusively among patients with MEH lesions (A6541T, G6561T, A6595C, and C6705A). The T6629C polymorphism was the most common, since it was present among all samples. The rate of variability in the nucleotides of the analyzed *L1* gene fragments was 3.3% (8/249), while 96.7% (232/240) of nucleotides remained conserved. Moreover, the maximum pairwise nucleotide difference was 1.2%.

Table 1. Polymorphisms in *L1* gene of HPV-13

Nucleotide position	6541	6554	6557	6561	6574	6595	6629	6705	n
X62843: prototype	A	A	A	G	A	A	T	C	
DQ344807	T	A	A	G	A	A	C	C	
KY690157*	-	-	-	-	-	-	C	-	23
KY690158*	-	-	-	-	C	-	C	-	1
KY690159*	-	G	G	-	-	-	C	-	1
KY690160**	-	-	-	-	-	C	C	-	1
KY690161**	-	-	-	-	-	-	C	-	44
KY690162**	T	-	-	T	-	-	C	-	1
KY690163**	-	-	-	-	-	-	C	A	1
KY690164**	T	-	-	-	-	-	C	-	3
Amino acid position	267	271	272	274	278	285	296	322	
Amino acid change	Q - L	E	L	V - F	N - T	Y – S	S	H - N	

n Number of samples included within each variant.
* Variants isolates from asymptomatic carriers.
** Variants isolates from patients with MEH lesions

Secondary structure analysis.

Based on L1 protein analysis, we identified five amino acid changes: glutamine to leucine (Q267L), valine to phenylalanine (V274F), asparagine to threonine (N278T), tyrosine to serine (Y285S) and histidine to asparagine (H322N). The *in- silico* analysis showed that 80% (4/5) of the mutations occurred in coil structures, and only 20% (1/5) occurred in a β-sheet structure. On the other hand, the FG loop was affected by four mutations, while the HI loop domain remained unchanged (Figure 1). According to our comparison between the

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hypervariable regions of our results and HPV-16, the non- synonymous mutations found in this study occur on exposed loops of the L1 protein (20). In this way, amino acid changes Q267L, V274F, N278T, and Y285S were located within FG loop, while the H322N mutation did not occur within some loop structure but is close to HI loop (21).

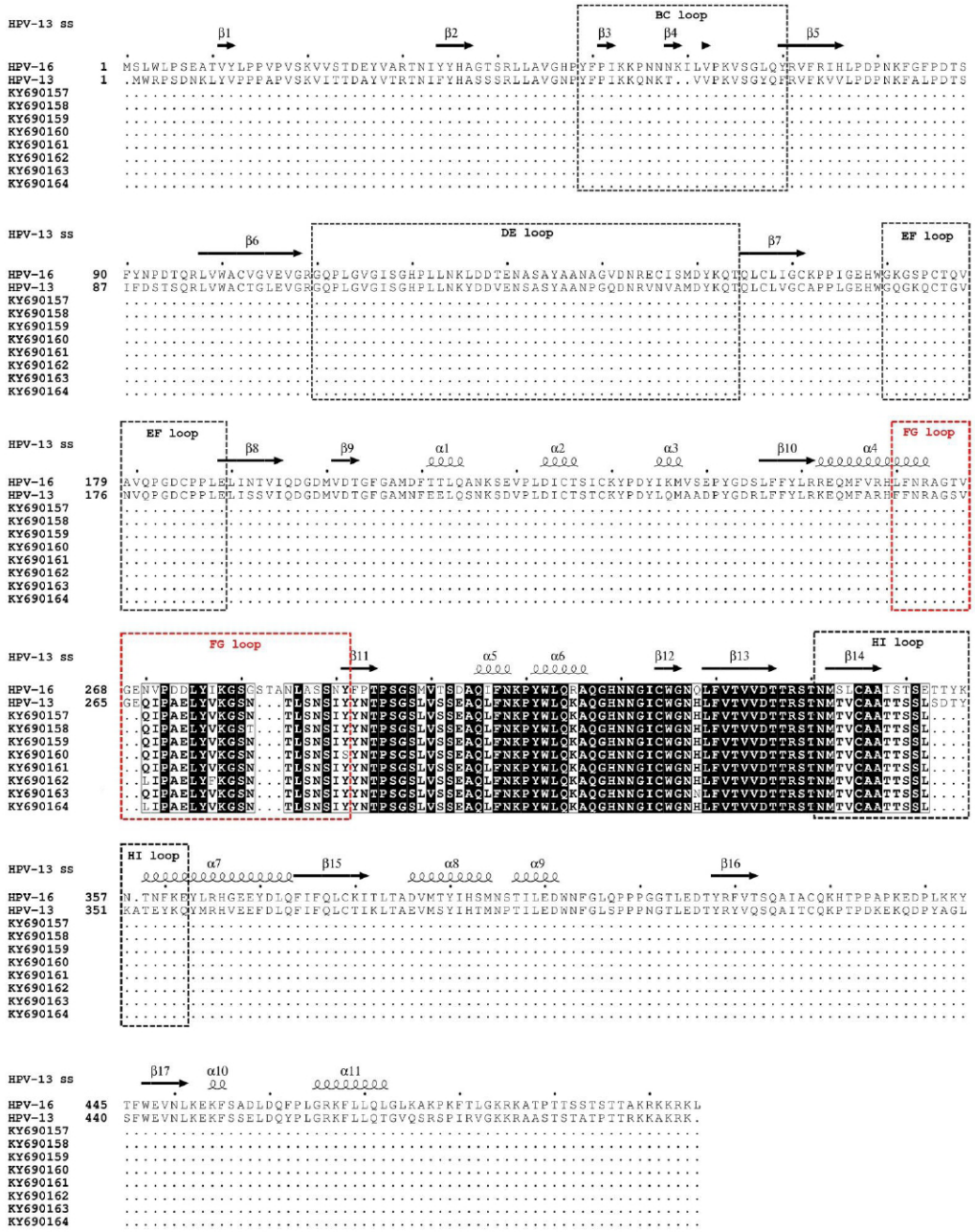

Figure 1. *In silico* prediction of the secondary structure of the HPV-13 L1 protein. Highlighted boxes with dotted lines correspond to the exposed-surface loops on HPV-13 L1 amino acid sequence: BC, DE, EF, FG, and HI. Highlighted box with red dotted lines concentrates four amino acid changes on the FG loop. The HPV-16 data (PDB: 1DZL, https://www.rcsb.org/structure/1DZL) was used as a reference to identify sites corresponding to surface-exposed loops of the HPV-13 L1 protein.

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Phylogenetic analysis.

The phylogenetic analyses clustered our variants (representing 75 sequences) in two main groups (Figure 2). A major branch A was formed by three variants and the HPV-13 isolated from the Central Amazon (DQ344807). Specifically, the sub-A1 included only one variant the KY690159, whereas the sub-A2 contained the variants KY690162 and KY690164 closely related to DQ344807. On the other side, a major branch B had five variants, where the sub-B1 showed a close relationship between the variants KY690158 and KY690163. Likewise, the sub-B2 included the variants KY690157, KY690160, and KY690161. Interestingly, the prototype X62843 is the most ancestral.

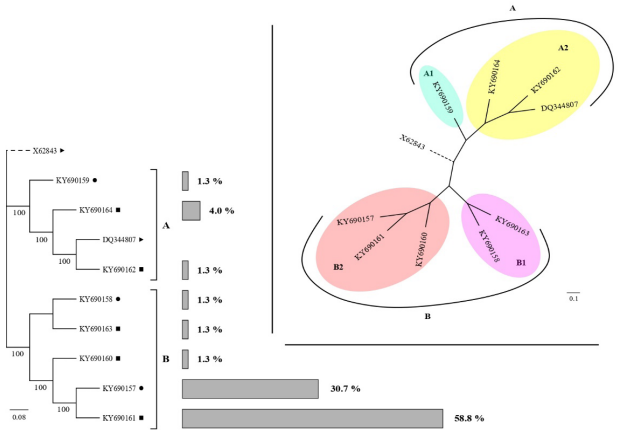

Figure 2. Phylogenetic analysis based on nucleotide sequences of HPV-13 *L1* gene Phylogenetic analysis performed using the Maximum Likelihood method. The first view (A) includes the percentage of sequences in each HPV-13 variant. Variants identified from asymptomatic carriers (n = 25) are indicated by black circles, and variants identified from patients with MEH lesions (n = 50) are indicated by black squares. The second view (B) shows the distribution of HPV-13 variants.

DISCUSSION

We report novel information concerning the genetic variability in the HPV-13 *L1* gene. To the best of our knowledge, this is the first report of the existence of polymorphisms in the HPV-13. Worldwide, HEM is considered rare, and Mexico is not the excepcition cases have been reported in the states of Mexico and Puebla (22, 23), but in southeast Mexico is very frequent and endemic in some Mayan rural areas HEM and only HPV-13 has been identified (8-10).

The study of HPV genetic variability dates from the 1990s, and most of the reports have focused on HPV-16 and HPV-18. More recently, the genetic variation also has been reported on HPV-58, HPV-31, and others high-risk HPV (24-26). The intratypic variability of HPV-16 has demonstrated that HPVs have co-evolved with humans. This means that the distribution of HPVs is consistent with the geographical distribution of the major human groups, therefore, HPV-16 is classified according to the geographic region of origin in following lineages: European (E), Asian-American (AA), African 1 (Af1), African 2 (Af2), and North American (NA1) (27). Genetic variability has clinical relevance, for example, has been demonstrated that women infected with AA and Af variants of HPV-16 have a higher risk to develop HSIL (high-grade squamous intraepithelial lesions); conversely, women affected with E variants present a lower risk to produce HSIL (28).

Here, the polymorphisms identified in HPV-13 *L1* sequences represent the 3,3% (8/240) of the nucleotides studied, which is relatively high, considering that only 16% (240/1500) of the *L1* gene was analyzed. Similar studies of other genotypes identified variations of 1, 2% (19/1595) and 2,9% (13/450) for *L1* gene (29, 30). Our results identified that the genetic variation of the HPV sequences is congruent with HPVs rates of nucleotide mutations, occurring at rates of 10-8 – 10-7 nucleotides/year (31). Although, the *L1* gene is the most conserved within the HPVs genome, polymorphisms can occur, and one fixed in the population, they cause genetic variability (32). We found that amino acid changes in the L1 protein of the HPV-13 occurred on well documented (for other types of HPV) immunodominant epitopes such as FG and HI loops. Studies with virus-like particles (VPLs) shown that amino acid changes within FG and HI loops are biologically important.

Such changes can modify the structure of those loops, decreasing the reactivity of the specific

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monoclonal antibodies (MAbs) against the epitopes of viral capsid (33-35). The above suggests that conformational alterations in the protein structure induced by amino acid changes might decrease the antigen-antibody affinity of the virus in vivo. Nevertheless, the biological implications previously mentioned should be examined exhaustively by in vitro assays that have been developed to analyze other genotypes (36, 37).

The phylogenetic distribution of the HPV-13 variants does not correspond to their pathological characteristics such as symptomatic or healthy carrier; therefore, the clustering occurred regarding their genetic identity. Interestingly, in branch A, three HPV-13 variants from Yucatan cluster with the HPV-13 strain from the Central Amazon, whereas branch B formed a closed separated cluster that can be considered endemic of the Yucatan Peninsula, and possibly suggesting two independent introduction events. The dichotomy of the phylogenetic tree suggests the existence of different lineages for HPV-13; however, we are limited to establish this taxonomic level since this study just considered partial *L1* sequences. Although it will be indispensable a complete genome analysis to establish lineages of HPV-13 (1). We report show the first time an overview of the phylogenetic distribution of an HPV genotype not studied at the genetic level. Furthermore, the phylogenetic analysis data provides information that can be used in future research to determine lineages and sublineages for HPV-13. In fact, the complete *L1* gene obtained from the sample labeled KY690157, corresponds to a sequence present in 89% of the studied population (67 samples), and therefore can be cautiously considered as "representative".

Although only *L1* partial sequences were analyzed, the complete genome from one of the samples was recovered (data not shown), which supported the structural implications inferred for the complete HPV-13 *L1* gene respect to HPV-16 *L1* gene. Likewise, this provides the basis for deeper studies. On the other hand, the disproportionate knowledge about other HPV genotypes in comparison to the scarce information on HPV13, reflects the fact that it is responsible of a neglected disease affecting predominantly Latin American populations and minority ethnic groups, and urges to generate more knowledge on its biology, physiopathology, and epidemiology.

Finally, the limitations of this study were that samples represent merely HPV13 viruses circulating in two communities of the Mayan area on Yucatan and that the *L1* sequences analyzed are partial. The strengths are that a non-depreciable number of samples were included in the analyses, and this is an important contribution on the molecular characteristics of an understudied viral genotype, considering that most presently available reports are medicales cases.

CONCLUSIONS

We have reported a significant knowledge about the genetic variation of HPV-13 from asymptomatic carriers and patients with MEH lesions. Likewise, to the best of our knowledge, this is the first report for the presence of genetic polymorphisms of this genotype. These results are relevant for future research aiming to identify lineages and sub lineages for HPV-13. Our in-silico analysis suggests that the amino acid changes would be involving structures directly related to antigen recognition, which requires further investigations.

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REFERENCES

1. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. Virology. 2004 Jun; 20;324(1):17-7. doi: 10.1016/j.virol.2004.03.033.
2. Bernard HU, Burk RD, Chen Z, van Doorslaer K, zur Hausen H, de Villiers EM. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology. 2010 May; 401(1):70-79. doi: 10.1016/j.virol.2010.02.002.

3. Pfister H, Hettich I, Runne U, Gissmann L, Chilf GN. Characterization of human papillomavirus type 13 from focal epithelial hyperplasia Heck lesions. J. Virol. 1983 Aug; 47(2): 363-6. doi:10.1128/JVI.47.2.363-366.
4. Van Ranst M, Fuse A, Fiten P, Beuken E, Pfister H, Burk RD, Opdenakker G. Human papillomavirus type 13 and pygmy chimpanzee papillomavirus type 1: comparison of the genome organizations. Virology. 1992 Oct;190(2):587-96. doi: 10.1016/0042- 6822(92)90896-w.
5. Santos PP, Vasconcelos MG, Pereira KMA, de Souza LB, de A. Freitas R, de L.L. Costa A, *et al*. Hiperplasia epitelial focal (doença de Heck) em descendente de indios brasileiros: relato de caso. J. Bras. Patol. e Med. Lab. 2007 Dec; 43 (6): 431–4. available: https://www.scielo.br/j/jbpml/a/Xg7FFxxkhr9wkd8xHrnN9hN/
6. Cuberos V, Perez J, Lopez CJ, Castro F, Gonzalez LV, Correa LA, *et al.* Molecular and serological evidence of the epidemiological association of HPV 13 with focal epithelial hyperplasia: A case-control study. J. Clin. Virol. 2066 Sep;37(1):21-6. doi: 10.1016/j.jcv.2006.04.003.
7. Roman CB, Sedano HO. Multifocal papilloma virus epithelial hiperplasia. Oral Surg Oral Med Oral Pathol 1994 Jun;77(6):631-5. doi: 10.1016/0030-4220(94)90325-5.
8. Lopez-Villanueva ME, Conde-Ferráez L, Ayora-Talavera G, Cerón-Espinosa JD, González-Losa MR. Human papillomavirus 13 in a Mexican Mayan community with multifocal epithelial hyperplasia: ¿Could saliva be involved in household transmission? Eur. J. Dermatology. 2011 May-Jun;21(3):396-400. doi: 10.1684/ejd.2011.1347.
9. González-Losa M del R, Suarez-Allén RE, Canul-Canche J, Conde-Ferráez L, Eljure Lopez N. Multifocal epithelial hyperplasia in a community in the Mayan area of Mexico. Int. J. Dermato. 2011 March; 50 (3): 304–309. doi: 10.1111/j.1365 4632.2010.04718.x.
10. Zavala-García A, Briceño-Mena R, Romero-Beltran L, Kelly GA, Cerón-Espinosa JD, González-Losa MR. Epithelial Focal Hyplerplasia Prevalence in an endemic population. Molecular association of hpv 13 to asymptomatic patients and comparison between three elementary school of different income levels. J Immuno Biol 2017, 2:4 doi:10.4172/2476- 1966.1000133
11. Ozden B, Gunduz K, Gunhan O, Ozden FO. A Case Report of Focal Epithelial Hyperplasia (Heck's disease) with PCR Detection of Human Papillomavirus. J Maxillofac Oral Surg. 2011 Dec;10(4):357-60. doi: 10.1007/s12663-011-0184-2.
12. Ledesma-Montes C, Garcés-Ortíz M, Edmundo B. Multifocal Epithelial Hyperplasia. An Unusual Lesion. Case Report. Webmed Cent. Pathol. 2012 Feb; 3 (2):1 https://www.researchgate.net/publication/272564352_
13. Sarabadani J, Heydari S, Mashreghi A, Poornazari N. A Case Report of Multifocal Epithelial Hyperplasia (Heck's Disease) Treated with CO 2 Laser. J Dent Mater Tech. 2017 June; 6(3): 138–41. doi:10.22038/JDMT.2017.8992
14. King AJ, Sonsma JA, Vriend HJ, van der Sande MAB, Feltkamp MC, Boot HJ, *et al*. Genetic Diversity in the Major Capsid L1 Protein of HPV-16 and HPV-18 in the Netherlands. PLoS One. 2016 Apr 12;11(4): e0152782. doi: 10.1371/journal.pone.0152782
15. Fang L, Lin X, Yang Y, Song Z, Ding X, Tan L, Gao P. Genetic variability, phylogeny and functional implication of the long control region in human papillomavirus ype 16, 18 and 58 in Chengdu, China. Virol J. 2020 Jul 16;17(1):106. doi: 10.1186/s12985-020-01349-3.
16. Williamson A-L, Dennis SJ. The use of the polymerase chain reaction for the detection of human papillomavirus type 13. J Virol Methods. 1991 Jan;31(1):57-65. doi: 10.1016/0166-0934(91)90144-o.
17. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, *et al*. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012 Jun ;28(12):1647-9. doi: 10.1093/bioinformatics/bts199.
18. Yang J, Zhang Y. I-TASSER server: New development for protein structure and function predictions. Nucleic Acids Research. 2015 Jul; 43(W1): W174-81. doi:org/10.1093/nar/gkv342
19. Zhang C, Freddolino PL, Zhang Y. COFACTOR: Improved protein function prediction by combining structure, sequence and protein-protein interaction information. Nucleic Acids Res. 2017 Jul 3;45(W1): W291-W299. doi:10.1093/nar/gkx366.
20. Faus H & Dillner J. Mutations in human papillomavirus type 16 L1 hypervariable surface exposed loops affect L2 binding and DNA encapsidation. J Gen Virol. 2013 Aug;94(8):1841-1849. doi: 10.1099/vir.0.054205-0.
21. Olcese VA, Chen Y, Schlegel R, Yuan H. Characterization of HPV16 L1 loop domains in the formation of a type-specific, conformational epitope. BMC Microbiol. 2004 Jul 19; 4:29. doi: 10.1186/1471-2180-4-29.
22. Gonzalez-Lopez BS. Hiperplasia epitelial focal en la cavida bucal. Reporte de casos en dos comunidades del Estado de México. CIENCIA ergo-sum. 1999 Nov ;6(3):253-256.
23. Morales Palacios MG, PazBuezo R, Tamayo Pérez R, Hernánez Jáuregui P. Estudio comparativo de prevalencia de hiperplasia epitelial focal en tres grupos poblacionales del estado de Puebla. Rev. ADM. 1989 Enero; 46(1): 15-17.
24. Calleja-Macias IE, Villa LL, Prado JC, Allan B, Williamson A, Collins RJ, *et al*. Worldwide genomic diversity of the high-risk human papillomavirus types 31, 35, 52, and 58, four close relatives of human papillomavirus type 16. J Virol. 2005 Nov;79(21):13630-40. doi: 10.1128/JVI.79.21.13630-13640.2005.

25. Liu JH, Lu ZT, Wang GL, Zhou WQ, Liu C, Yang LX. Variations of human papillomavirus type 58 E6, E7, L1 genes and long control region in strains from women with cervical lesions in Liaoning province, China. Infect Genet Evol. 2012 Oct;12(7):1466-72. doi: 10.1016/j.meegid.2012.05.004.
26. Chen Z, Schiffman M, Herrero R, DeSalle R, Anastos K, Segondy M, *et al*. Evolution and taxonomic classification of human papillomavirus 16 (HPV16)-related variant genomes: HPV31, HPV33, HPV35, HPV52, HPV58 and HPV67. PLoS One. 2011;6(5): e20183. doi: 10.1371/journal.pone.0020183
27. Yamada T, Manos MM, Peto J, Greer CE, Munoz NN, Bosch FX, *et al*. Human papillomavirus type 16 sequence variation in cervical cancers: a worldwide perspective. J Virol. 1997 Mar;71(3):2463-72. doi: 10.1128/JVI.71.3.2463-2472.1997.
28. Xi LF, Koutsky LA, Hildesheim A, Galloway DA, Wheeler CM, Winer RL. Risk for High-Grade Cervical Intraepithelial Neoplasia Associated with Variants of Human Papillomavirus Types 16 and 18. Cancer Epidemiol Biomarkers Prev. 2007 Jan;16(1):4- 10. doi: 10.1158/1055-9965.EPI-06-0670.
29. Pande S, Jain N, Prusty BK, Bhambhani S, Gupta S, Sharma R, *et al*. Human papillomavirus type 16 variant analysis of E6, E7, and L1 genes and long control region in biopsy samples from cervical cancer patients in North India. J Clin Microbiol. 2008 Mar;46(3):1060-6. doi: 10.1128/JCM.02202-07.
30. Sun ZR, Ren G, Cui X, Zhou WQ, Liu C, Ruan Q. Genetic diversity of HPV-16 E6, E7, and L1 genes in women with cervical lesions in Liaoning Province, China. Int J Gynecol Cancer. 2011 Apr;21(3):551-8. doi: 10.1097/IGC.0b013e3182112023.
31. Harari A, Chen Z, Burk RD. Human Papillomavirus Genomics: Past, Present and Future. In: Hum. Papillomavirus Bench to Bedside. Germany: Karger;2012. P. 1–18.
32. Villiers E-M de. Cross-roads in the classification of papillomaviruses. Virology. 2013 Oct;445(1-2):2-10. doi: 10.1016/j.virol.2013.04.023
33. Bishop B, Dasgupta J, Klein M, Garcea RL, Christensen ND, Zhao R, Chen XS. Crystal structures of four types of human papillomavirus L1 capsid proteins: Understanding the specificity of neutralizing monoclonal antibodies. J Biol Chem. 2007 Oct 26;282(43):31803-11. doi: 10.1074/jbc.M706380200.
34. Xia L, Xian Y, Wang D, Chen Y, Huang X, Bi X, *et al*. A human monoclonal antibody against HPV16 recognizes an immunodominant and neutralizing epitope partially overlapping with that of H16.V5. Sci Rep. 2016 Jan 11; 6:19042. doi: 10.1038/srep19042.
35. Culp TD, Spatz CM, Reed CA, Christensen ND. Binding and neutralization efficiencies of monoclonal antibodies, Fab fragments, and scFv specific for L1 epitopes on the capsid of infectious HPV particles. Virology. 2007 May 10;361(2):435-46. doi: 10.1016/j.virol.2006.12.002.
36. Thönes N, Herreiner A, Schädlich L, Piuko K, Muller M. A Direct Comparison of Human Papillomavirus Type 16 L1 Particles Reveals a Lower Immunogenicity of Capsomeres than Viruslike Particles with Respect to the Induced Antibody Response. J Virol. 2008 Jun;82(11):5472-85. doi: 10.1128/JVI.02482-07.
37. El-Aliani A, El-Alaoui MA, Chaoui I, Ennaji MM, Attaleb M, El-Mzibri M. Naturally occurring capsid protein variants L1 of human papillomavirus genotype 16 in Morocco. Bioinformation 2017 Aug 31;13(8):241-248. doi: 10.6026/97320630013