

LUND UNIVERSITY

Characterization of human papillomavirus subtype 72b.

Johansson, Hanna K; Kravtchenko, Evgenia; Forslund, Ola

Published in: Genome Announcements

DOI: 10.1128/genomeA.01320-14

2014

Link to publication

Citation for published version (APA): Johansson, H. K., Kravtchenko, E., & Forslund, O. (2014). Characterization of human papillomavirus subtype 72b. Genome Announcements, 2(6). https://doi.org/10.1128/genomeA.01320-14

Total number of authors: 3

General rights

Unless other specific re-use rights are stated the following general rights apply: Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

· Users may download and print one copy of any publication from the public portal for the purpose of private study

or research.
You may not further distribute the material or use it for any profit-making activity or commercial gain

· You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: https://creativecommons.org/licenses/

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117 221 00 Lund +46 46-222 00 00





Characterization of Human Papillomavirus Subtype 72b

Hanna Johansson, Evgenia Kravtchenko, Ola Forslund

Department of Laboratory Medicine, Medical Microbiology, Lund University, Malmö, Sweden

We report the characterization of human papillomavirus (HPV) subtype 72b of the genus *Alphapapillomavirus* isolated from an oral rinse sample of a healthy woman. The HPV72b L1 open reading frame (ORF) was 90.2% identical to that of HPV72, indicating a subtype close to the border of a novel HPV type.

Received 8 November 2014 Accepted 11 November 2014 Published 18 December 2014

Citation Johansson H, Kravtchenko E, Forslund O. 2014. Characterization of human papillomavirus subtype 72b. Genome Announc. 2(6):e01320-14. doi:10.1128/genomeA.01320-14.

Copyright © 2014 Johansson et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license. Address correspondence to Ola Forslund, ola.forslund@med.lu.se.

n an oral rinse sample from a 44-year-old woman we identified a novel sub-genomic Forslund Antonsson primer (FAP)fragment, FA171, with closest sequence identity to HPV72 (1). After cloning of its complete genome we determined that the L1 open reading frame (ORF) was 90.2% identical to that of HPV72 (genus *Alphapapillomavirus*). Therefore, it was labeled as subtype HPV72b of HPV72, originally isolated from an oral papillomatous lesion of an HIV positive patient (2).

The complete genome of HPV72b (8,098 bp) was obtained using the PrimeSTAR GXL DNA polymerase kit (TaKaRa Bio, Shiga, Japan). Briefly, 2.5 μ L purified DNA (Magna LC, Roche) was amplified in 25 μ L containing a 1× PrimeSTAR GXL buffer, 200 μ M of each dNTP, 0.2 μ M of each primer (fwd 5'-TGA CTA CAA GCA AAC ACA GTT GCT T-3' and rev 5'-ACA GAT ATA TTG TCC CGG CTG TC-3' from DNA-Technology, Denmark), and 0.625 U PrimeSTAR GXL DNA polymerase. Amplification was performed for 45 cycles at 98°C for 10 s, 60°C for 15 s, and 68°C for 8 min in a thermocycler (Mastercycler Eppendorf, Germany). The amplicon was cloned using the TOPO TA cloning kit and the pCR 2.1-TOPO vector (Invitrogen, Carlsbad, CA) and sequenced using primer-walking (Eurofins, Germany).

A novel HPV type shares less than 90% similarity to the closest papillomavirus (PV) type in the L1 ORF (3). Pairwise comparisons between L1 ORFs of HPV72b and HPV72 (second ATG) (2) demonstrated 90.2% nucleotide (nt) identity, whereas the other ORFs showed identities less than 90% (E6: 85.0%, E7: 85.4%, E1: 89.2%, E2: 85.5%, E4: 80.6%, E5: 69.5%, L2: 87.2%) and URR 78.6%. The overall similarity between HPV72b and HPV72 was 85.3%. There was also a segment of 101 nt (3,874 to 3,974) downstream of the HPV72b E2 ORF that was not present in HPV72.

HPV72b had a G+C content of 45.3% and the typical genome organization of HPVs of the genus *Alphapapillomavirus* species-3 with an E5-beta ORF (nt 4,241 to 4,369) (4). In the upstream regulatory region (URR) of HPV72b (804 bp) we identified five consensus E2-binding sites (ACC-N₆-GGT), one putative TATA box (TATAA), and one putative polyadenylation site (ATATAA).

The putative E6 proteins contained two zinc-finger domains $(CxxC[x]_{29}CxxC)$ (5) separated by 39 amino acids. One zinc-finger domain was present in the E7 protein. The LxCxE-motif (binding site for the pRB) (6) was not observed in the E7 protein as

the cysteine was substituted for a serine in the corresponding site, LxSxE, identical to that of HPV72.

The putative E1 protein did not exhibit the conserved ATPbinding site (GPPDTGKS) (7, 8), instead GPSNTGKS was found. The initiation codon of the putative start of E4 ORF was absent, as the ATG codon was changed to ACG.

Nucleotide sequence accession number. The complete genomic sequence of HPV72b is available in GenBank under the accession no. KJ145795.

ACKNOWLEDGMENTS

This project was supported by BioCARE, a Strategic Research Program at Lund University, Sweden, and by the Swedish Cancer Society.

REFERENCES

- 1. Forslund O, Johansson H, Madsen KG, Kofoed K. 2013. The nasal mucosa contains a large spectrum of human papillomavirus types from the *Betapapillomavirus* and *Gammapapillomavirus* genera. J. Infect. Dis. 208: 1335–1341. http://dx.doi.org/10.1093/infdis/jit326.
- Völter C, He Y, Delius H, Roy-Burman A, Greenspan JS, Greenspan D, de Villiers EM. 1996. Novel HPV types present in oral papillomatous lesions from patients with HIV infection. Int. J. Cancer 66:453–456. http:// dx.doi.org/10.1002/(SICI)1097-0215(19960516)66:4<453::AID-IJC7 >3.3.CO;2-O.
- de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. 2004. Classification of papillomaviruses. Virology 324:17–27. http://dx.doi.org/ 10.1016/j.virol.2004.03.033.
- Bravo IG, Alonso A. 2004. Mucosal human papillomaviruses encode four different E5 proteins whose chemistry and phylogeny correlate with malignant or benign growth. J. Virol. 78:13613–13626. http://dx.doi.org/ 10.1128/JVI.78.24.13613-13626.2004.
- Ullman CG, Haris PI, Galloway DA, Emery VC, Perkins SJ. 1996. Predicted alpha-helix/beta-sheet secondary structures for the zinc-binding motifs of human papillomavirus E7 and E6 proteins by consensus prediction averaging and spectroscopic studies of E7. Biochem. J. 319:229–239.
- Dahiya A, Gavin MR, Luo RX, Dean DC. 2000. Role of the LXCXE binding site in Rb function. Mol. Cell. Biol. 20:6799-6805. http:// dx.doi.org/10.1128/MCB.20.18.6799-6805.2000.
- Iyer LM, Leipe DD, Koonin EV, Aravind L. 2004. Evolutionary history and higher order classification of AAA+ ATPases. J. Struct. Biol. 146: 11–31. http://dx.doi.org/10.1016/j.jsb.2003.10.010.
- Liu X, Schuck S, Stenlund A. 2010. Structure-based mutational analysis of the bovine papillomavirus E1 helicase domain identifies residues involved in the nonspecific DNA binding activity required for double trimer formation. J. Virol. 84:4264–4276. http://dx.doi.org/10.1128/JVI.02214-09.