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Complete Genome Sequence of a Novel Human Papillomavirus Isolated from Oral Rinse

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ABSTRACT A novel human papillomavirus (HPV TG550) isolated from the oral rinse of a Chinese male resident was fully characterized. The L1 open reading frame of HPV TG550 shares 82.5% nucleotide sequence similarity with its closest relative, HPV166, and clusters within the species group *Gammapapillomavirus 19*.

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Human papillomaviruses (HPVs) are a heterogeneous group of circular double-stranded DNA viruses with a size around 8 kb and infect the epithelial surfaces of genital and cutaneous sites of humans. A distinct HPV type is defined when the complete L1 open reading frame (ORF) is less than 90% similar to those of the characterized types (1, 2). Currently, nearly 200 types of human papillomaviruses have been fully characterized and are classified mainly in 5 genera, *Alphapapillomavirus*, *Betapapillomavirus*, *Gammapapillomavirus*, *Mupapillomavirus*, and *Nupapillomavirus*, within the family *Papillomaviridae*. The *Alpha-PV* genus contains all of the genital oncogenic HPV types associated with cancers (3, 4).

In this study, we characterized the complete genome of a novel HPV type isolated from the oral rinse of a 22-year-old Chinese male resident without visible oral lesions. Viral DNA was initially detected using a multiplexed next-generation sequencing (NGS) assay targeting a 125-bp fragment within the L1 ORF (5). A BLASTn search of the sequence against an HPV database showed less than 90% similarity to characterized PV types. The total DNA was further enriched using rolling-circle amplification (RCA) with random primers, and we conducted metagenomic sequencing on an Illumina HiSeq4000 instrument at Weill Cornell Medicine Genomics Resources Core Facility, New York, using paired-end reads. The short reads were filtered for human genome contamination and *de novo* assembled; a total of 11 valid contigs (243 to 1,420 bp) covered nearly 80% of the complete genome. Type-specific primers were designed based on NGS contigs to amplify the complete genome in three overlapping fragments. The complete genome sequence was assembled and validated using a proofreading polymerase, followed by Sanger sequencing using a primer-walking strategy.

This novel HPV genome (HPV TG550) is 7,216 bp in size, with a GC content of 37.4%. It contains five early genes (E6, E7, E1, E2, and E4) and two late genes (L1 and L2). The complete L1 ORF shows 82.5% nucleotide sequence similarity with its closest relative, HPV 166 (HPV166, GenBank accession number JX413104), meeting the criteria to be considered a distinct HPV type. Phylogenetic trees using either the L1 ORF or the complete genome sequence unambiguously cluster HPV TG550 into the species group *Gammapapillomavirus 19*, together with HPV161 (JX413109), HPV162 (JX413108), and HPV166. The nucleotide sequence of a small region of the HPV TG550 L1 ORF shares 97 to 99% similarities with three previously reported partial sequences, SIBX58 (KP768216; 360 bp), SE9 (JF906531; 235 bp), and SE71 (JQ250766; 222 bp).

The identification of novel HPVs reveals high genomic diversity of papillomaviruses. Further studies on the epidemiology, evolution, and pathogenesis of this novel virus are warranted.

Accession number(s). The complete genome sequence of HPV TG550 is available in GenBank under the accession number MF176072.

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REFERENCES

1. Bernard HU, Burk RD, Chen Z, van Doorslaer K, zur Hausen H, de Villiers EM. 2010. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology 401:70–79. https://doi.org/10.1016/j.virol.2010.02.002.
2. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. 2004. Classification of papillomaviruses. Virology 324:17–27. https://doi.org/10.1016/j.virol.2004.03.033.
3. Schiffman M, Herrero R, Desalle R, Hildesheim A, Wacholder S, Rodriguez AC, Bratti MC, Sherman ME, Morales J, Guillen D, Alfaro M, Hutchinson M, Wright TC, Solomon D, Chen Z, Schussler J, Castle PE, Burk RD. 2005. The carcinogenicity of human papillomavirus types reflects viral evolution. Virology 337:76–84. https://doi.org/10.1016/j.virol.2005.04.002.
4. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 2012. Biological agents, Volume 100 B. A review of human carcinogens. IARC Monogr Eval Carcinog Risks Hum 100:1–441.
5. Fonseca AJ, Taeko D, Chaves TA, Amorim LD, Murari RS, Miranda AE, Chen Z, Burk RD, Ferreira LC. 2015. HPV infection and cervical screening in socially isolated indigenous women inhabitants of the Amazonian rainforest. PLoS One 10:e0133635. https://doi.org/10.1371/journal.pone.0133635.