

# Complete Genome Sequence of the Human Herpesvirus 6A Strain AJ from Africa Resembles Strain GS from North America

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**The genome sequence of human herpesvirus 6A (HHV-6A) strain AJ was determined in a comparison of target enrichment and long-range PCR using next-generation sequencing methodologies. The analyses show 85 predicted open reading frames (ORFs), conservation with sequenced HHV-6A reference strain U1102, and closest identity to the recently determined GS strain, despite different geographic origins (United States and Gambia).**

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The *Roseolovirus* genus of the human *Betaherpesvirinae* subfamily comprises three members: human herpesvirus 6A (HHV-6A), the prototype, HHV-6B, and HHV-7. These viruses are common causes of infant fever, occasionally with rash (then termed exanthema subitum/roseola infantum) (1–3). The infections persist for life and are generally self-limiting, but they are also associated with central nervous system disease. HHV-6A has evidence for increased neurotropism, particularly in glial cells, and is identified in glioma and multiple sclerosis patient subsets (4–8). While HHV-6A infant infections are rare in Europe and North America, they have been observed in Africa (9) and are prevalent to a similar extent as HHV-6B in the recently defined germline chromosomally integrated form, ciHHV-6, in which virus genes might be expressed in every cell (10). Therefore, the drivers of HHV-6A distribution and evolution might be fundamentally different, and knowledge of the viral genomes is needed to understand these relationships.

To date, HHV-6A isolates and complete genome sequences are scarce, with those of only two strains identified, the NCBI reference 159-kbp strain U1102, from a Ugandan HIV/AIDS patient (11, 12), and the 157-kbp strain GS, from American patients with lymphoproliferative disease (13, 14) (GenBank/EBI accession no. X83413.1 [U1102] and KC465951.1 and KJ123690.1 [GS]). We report here the determination of a third HHV-6A strain, AJ, from an adult HIV/AIDS patient from Gambia (15).

We compared the amplification methods required to characterize strains directly from infected tissue, using both Agilent SureSelect target enrichment (16) and in-house long-range PCR methodologies to generate Illumina sequence libraries from infected-cell DNA. These were paired-end sequenced on an Illumina MiSeq, and a VelvetOptimiser, Velvet (17), and ABACAS (18) pipeline was used to optimize the *de novo* assembly. Both methodologies generated identical consensus sequences with similar variant-calling efficacy. Gaps, ambiguities, and repetitive regions were confirmed by PCR amplification and Sanger sequenc-

ing. Repetitive sequences at the genome ends were resolved utilizing the direct repeat structure of the termini and corresponding regions in the opposite termini for the first 715 and final 1,381 bp of direct repeat left (DR<sub>L</sub>) and right (DR<sub>R</sub>). Annotation was generated using the Rapid Annotation Transfer Tool (RATT) (19) with the reference HHV-6A strain U1102 (12) and updates based on GeneMark predictions (20) as well as other subsequently sequenced HHV-6A and HHV-7 strains (13, 21, 22).

The HHV-6A AJ genome is 156,714 bp in length, maintaining a typical class A herpesviral genomic organization consisting of a 140,401-bp unique long region flanked by 8,156-bp direct repeats (DR<sub>R</sub> and DR<sub>L</sub>). The DRs are bounded by DNA packaging, *pac1* and *pac2* sites, and human telomeric repeats, as shown previously for U1102 (23). Phylogenetic analyses showed the closest relationship to be with the North American isolate HHV-6A strain GS, at 99.1%, followed by HHV-6A strain U1102, at 98.4%. Single-nucleotide polymorphisms (SNPs) are found across the genome, and they are increased in DRs with small indels. Of note, both the AJ and GS strains show DNA polymerase gene (U38) sequence variation confounding the commonly used PCR-based methods for HHV-6A diagnostics (24). There are 85 genes, as shown previously, including analyses of HHV-6A GS, plus HHV-7, a distant roseolovirus (13, 21, 22). Even with distinct geographic origins, the HHV-6A strains AJ and GS are closely conserved, which may reflect highly evolved viral status or recent emergence.

**Nucleotide sequence accession number.** The whole HHV-6A strain AJ genome sequence has been deposited in GenBank under the accession no. [KP257584](https://www.ncbi.nlm.nih.gov/nuccore/KP257584).

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## REFERENCES

- Hall CB, Caserta MT, Schnabel KC, McDermott MP, Lofthus GK, Carnahan JA, Gilbert LM, Dewhurst S. 2006. Characteristics and acquisition of human herpesvirus (HHV) 7 infections in relation to infection with HHV-6. *J Infect Dis* 193:1063–1069. <http://dx.doi.org/10.1086/503434>.
- Hall CB, Long CE, Schnabel KC, Caserta MT, McIntyre KM, Costanzo MA, Knott A, Dewhurst S, Insel RA, Epstein LG. 1994. Human herpesvirus-6 infection in children. A prospective study of complications and reactivation. *N Engl J Med* 331:432–438. <http://dx.doi.org/10.1056/NEJM199408183310703>.
- Zerr DM, Meier AS, Selke SS, Frenkel LM, Huang ML, Wald A, Rhoads MP, Nguy L, Bornemann R, Morrow RA, Corey L. 2005. A population-based study of primary human herpesvirus 6 infection. *N Engl J Med* 352:768–776. <http://dx.doi.org/10.1056/NEJMoa042207>.
- Ahlqvist J, Donati D, Martinelli E, Akhyani N, Hou J, Major EO, Jacobson S, Fogdell-Hahn A. 2006. Complete replication cycle and acquisition of tegument in nucleus of human herpesvirus 6A in astrocytes and in T-cells. *J Med Virol* 78:1542–1553. <http://dx.doi.org/10.1002/jmv.20737>.
- Crawford JR, Santi MR, Thorarinsdottir HK, Cornelison R, Rushing EJ, Zhang H, Yao K, Jacobson S, Macdonald TJ. 2009. Detection of human herpesvirus-6 variants in pediatric brain tumors: association of viral antigen in low grade gliomas. *J Clin Virol* 46:37–42. <http://dx.doi.org/10.1016/j.jcv.2009.05.011>.
- Donati D, Martinelli E, Cassiani-Ingoni R, Ahlqvist J, Hou J, Major EO, Jacobson S. 2005. Variant-specific tropism of human herpesvirus 6 in human astrocytes. *J Virol* 79:9439–9448. <http://dx.doi.org/10.1128/JVI.79.15.9439-9448.2005>.
- Hall CB, Caserta MT, Schnabel KC, Long C, Epstein LG, Insel RA, Dewhurst S. 1998. Persistence of human herpesvirus 6 according to site and variant: possible greater neurotropism of variant A. *Clin Infect Dis* 26:132–137. <http://dx.doi.org/10.1086/516280>.
- Yao K, Crawford JR, Komaroff AL, Ablashi DV, Jacobson S. 2010. Review part 2: human herpesvirus-6 in central nervous system diseases. *J Med Virol* 82:1669–1678. <http://dx.doi.org/10.1002/jmv.21861>.
- Bates M, Monze M, Bima H, Kapambwe M, Clark D, Kasolo FC, Gompels UA. 2009. Predominant human herpesvirus 6 variant A infant infections in an HIV-1 endemic region of sub-Saharan Africa. *J Med Virol* 81:779–789. <http://dx.doi.org/10.1002/jmv.21455>.
- Huang Y, Hidalgo-Bravo A, Zhang E, Cotton VE, Mendez-Bermudez A, Wig G, Medina-Calzada Z, Neumann R, Jeffreys AJ, Winney B, Wilson JF, Clark DA, Dyer MJ, Royle NJ. 2014. Human telomeres that carry an integrated copy of human herpesvirus 6 are often short and unstable, facilitating release of the viral genome from the chromosome. *Nucleic Acids Res* 42:315–327. <http://dx.doi.org/10.1093/nar/gkt840>.
- Downing RG, Sewankambo N, Serwadda D, Honess R, Crawford D, Jarrett R, Griffin BE. 1987. Isolation of human lymphotropic herpesviruses from Uganda. *Lancet* 2:390. [http://dx.doi.org/10.1016/S0140-6736\(87\)92403-2](http://dx.doi.org/10.1016/S0140-6736(87)92403-2).
- Gompels UA, Nicholas J, Lawrence G, Jones M, Thomson BJ, Martin ME, Efsthathiou S, Craxton M, Macaulay HA. 1995. The DNA sequence of human herpesvirus-6: structure, coding content, and genome evolution. *Virology* 209:29–51. <http://dx.doi.org/10.1006/viro.1995.1228>.
- Gravel A, Ablashi D, Flamand L. 2013. Complete genome sequence of early passaged human herpesvirus 6A (GS strain) isolated from North America. *Genome Announc* 1(3):e00012–13. <http://dx.doi.org/10.1128/genomeA.00012-13>.
- Salahuddin SZ, Ablashi DV, Markham PD, Josephs SF, Sturzenegger S, Kaplan M, Halligan G, Biberfeld P, Wong-Staal F, Kramarsky B, Gallo RC. 1986. Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. *Science* 234:596–601. <http://dx.doi.org/10.1126/science.2876520>.
- Tedder RS, Briggs M, Cameron CH, Honess R, Robertson D, Whittle H. 1987. A novel lymphotropic herpesvirus. *Lancet* 2:390–392. [http://dx.doi.org/10.1016/S0140-6736\(87\)92404-4](http://dx.doi.org/10.1016/S0140-6736(87)92404-4).
- Depledge DP, Palser AL, Watson SJ, Lai IY, Gray ER, Grant P, Kanda RK, Leproust E, Kellam P, Breuer J. 2011. Specific capture and whole-genome sequencing of viruses from clinical samples. *PLoS One* 6:e27805. <http://dx.doi.org/10.1371/journal.pone.0027805>.
- Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. *Genome Res* 18:821–829. <http://dx.doi.org/10.1101/gr.074492.107>.
- Assefa S, Keane TM, Otto TD, Newbold C, Berriman M. 2009. ABACAS: algorithm-based automatic contiguation of assembled sequences. *Bioinformatics* 25:1968–1969. <http://dx.doi.org/10.1093/bioinformatics/btp347>.
- Otto TD, Dillon GP, Degraeve WS, Berriman M. 2011. RATT: Rapid Annotation Transfer Tool. *Nucleic Acids Res* 39:e57. <http://dx.doi.org/10.1093/nar/gkq1268>.
- Besemer J, Lomsadze A, Borodovsky M. 2001. GeneMarkS: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. *Nucleic Acids Res* 29:2607–2618. <http://dx.doi.org/10.1093/nar/29.12.2607>.
- Donaldson CD, Clark DA, Kidd IM, Breuer J, Depledge DD. 2013. Genome sequence of human herpesvirus 7 strain UCL-1. *Genome Announc* 1(5):. <http://dx.doi.org/10.1128/genomeA.00830-13>
- Megaw AG, Rapaport D, Avidor B, Frenkel N, Davison AJ. 1998. The DNA sequence of the RK strain of human herpesvirus 7. *Virology* 244:119–132. <http://dx.doi.org/10.1006/viro.1998.9105>.
- Gompels UA, Macaulay HA. 1995. Characterization of human telomeric repeat sequences from human herpesvirus 6 and relationship to replication. *J Gen Virol* 76:451–458. <http://dx.doi.org/10.1099/0022-1317-76-2-451>.
- Tweedy J, Spyrou MA, Hubacek P, Kuhl U, Lassner D, Gompels UA. 29 October 2014. Analyses of germline, chromosomally integrated human herpesvirus 6A and B genomes indicate emergent infection and new inflammatory mediators. *J Gen Virol*. <http://dx.doi.org/10.1099/vir.0.068536-0>.