Virologica Sinica 39 (2024) 705–707

Contents lists available at ScienceDirect

Virologica Sinica

journal homepage: www.keaipublishing.com/en/journals/virologica-sinica
www.virosin.org

Letter

Coexistence of adeno-associated virus 2 with adenovirus 18 or herpesvirus may be associated with severe lingual papillomatosis in an immunocompromised individual

Jie-Mei Yu [a,1], Ze-Yin Liang [b,1], Yuan-Hui Fu [a], Xiang-Lei Peng [a], Yan-Peng Zheng [a], Yu-Jun Dong [b,*], Jin-Sheng He [a,*]

[a] College of Life Sciences and Bioengineering, Beijing Jiaotong University, Beijing, 100044, China
[b] Department of Hematology, Peking University First Hospital, Beijing, 100034, China

Dear Editor,

Patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT) often experience a significant reduction in immune function due to factors such as severe lymphopenia, immunosuppressive treatment, and the development of graft-versus-host disease, rendering them more susceptible to severe infections (Sahin et al., 2016). Traditional diagnostic approaches are limited in sensitivity, speed, and assay targets, thus failing short of meeting the diagnostic demands (Qu et al., 2022). However, next-generation sequencing (NGS) has greatly improved the accuracy and efficiency of diagnosing various diseases (Schuler et al., 2022), and has been widely utilized for pathogen detection in samples from HSCT patients (Armstrong et al., 2019; Peng et al., 2021). In this study, we employed viral metagenomics analysis using high-throughput sequencing on fecal and tissue samples from a patient with HSCT experiencing severe lingual papillomatosis. Our findings revealed the co-detection of adeno-associated virus 2 (AAV2) and its potential helper viruses, human adenovirus serotype 18 (AdV18) and herpesvirus type 1 (HSV-1) in the fecal and tissue samples, respectively.

In June 2018, a fecal sample (coded MI-F), along with a tissue sample (coded MI-T) of excised lingual papilloma from a 13-year-old allo-HSCT recipient at Peking University First Hospital were collected. This patient was diagnosed with acute lymphoblastic leukemia in 2017 and underwent allo-HSCT. During the transplant period, the patient experienced prolonged severe oral mucositis. Four months post-transplant, the patient presented with multiple proliferative lesions on the tongue (Supplementary Fig. S1A). Blood tests indicated the presence of cytomegalovirus (CMV) and Epstein-Barr virus (EBV). Following treatment with ganciclovir, both CMV and EBV became undetectable. Subsequently, the proliferative lesion on the tongue was surgically removed, and tissue samples from the excised lingual papilloma were collected. Total nucleic acids were extracted from the two samples, MI-F and MI-T, which then respectively underwent random RT-PCR amplifications using primers with unique barcodes before undergoing NGS. Results showed that in the MI-F sample, the *Parvoviridae* family was the predominant viral family, accounting for up to 85.6% of the viral reads. Additionally, *Caudovirales* and *Adenoviridae* constituted 10.2% and 2.1% of the viral reads, respectively. Other virus families, including *Anelloviridae* and phages, contributed to the remaining 2.1% of viral reads (Fig. 1A). Similarly, in the MI-T sample, *Anelloviridae*, *Parvoviridae*, *Herpesvirales*, and *Caudovirales* collectively represented over 93% of the viral reads, with proportions of 36.6%, 34.6%, 14.5%, and 6.7% respectively. The remaining 7.7% of viral reads encompassed nine virus families (Fig. 1B). Notably, there was less than 0.01% of *Herpesvirales* in the fecal samples and 0.3% of *Adenoviridae* in the tissue samples, which may be attributed to potential low-level cross-contamination between samples during NGS. Alternatively, it is plausible that viral genomes with low abundance are genuinely present in the samples. The viral metagenomic data were provided in Supplementary Table S1.

Remarkably, our analysis revealed that 85.6% of the reads in the fecal sample and 34.6% of the reads in the tissue sample were identified as belonging to the *Parvoviridae* family. Subsequent investigation revealed that these *Parvoridae*-related reads corresponded to AAV2. As the AAV2 reads were not detected in other samples from the same run of the NGS (data not shown), it was speculated that the presence of the AAV2 reads in the fecal and tissue samples of the patient was not due to contamination. The nearly full-length genome of the AAV2 detected in this study, designated as MI-AAV2, was assembled from the NGS data (4518 bp) and has been deposited in public databases (GenBank under accession number: PP621512, Science Data Bank: https://doi.org/10.57760/sciencedb.j00001.00828). Sequence analysis showed that MI-AAV2 exhibited the highest nucleotide homology of 97.3% with an AAV2 sequence obtained from a liver biopsy conducted in the UK in 2022,

* Corresponding authors.
E-mail addresses: jshhe@bjtu.edu.cn (J.-S. He), dongy@bjmu.edu.cn (Y.-J. Dong).
[1] Jie-Mei Yu, Ze-Yin Liang contributed equally to this article.

https://doi.org/10.1016/j.virs.2024.06.006
Received 16 October 2023; Accepted 19 June 2024
Available online 22 June 2024

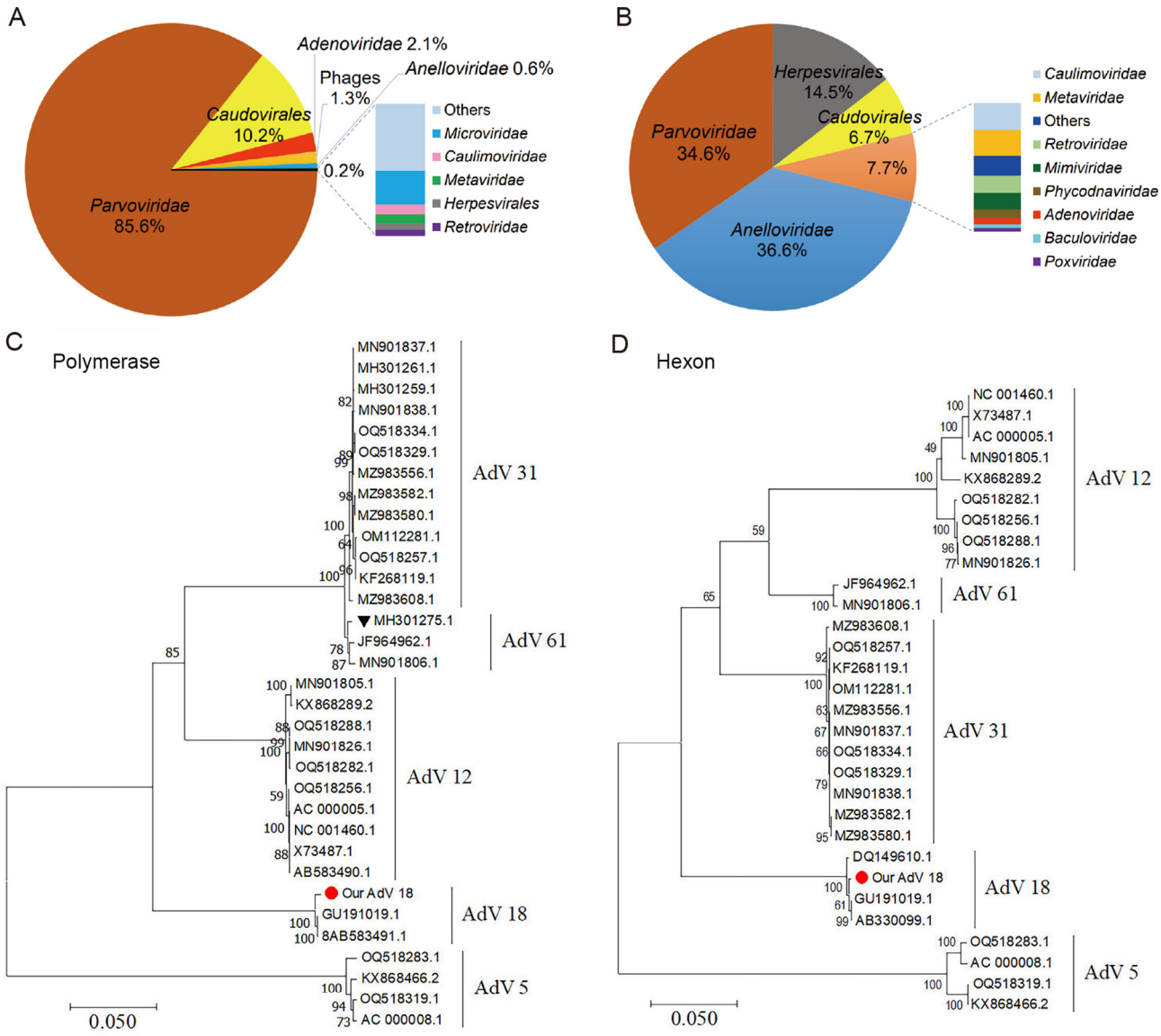

Fig. 1. Viral metagenomic analysis of the patient with lingual papillomatosis. **A** Viral metagenomics of fecal sample MI-F. **B** Viral metagenomics of tissue sample MI-T. High reads of *Parvoviridae* and *Adenoviridae* were detected in feces, while *Parvoviridae* and *Herpesvirales* were detected in tissue. **C, D** Phylogenetic trees for polymerase and hexon genes by neighbor-joining method with 1000 bootstrap replicates using MEGA 7.0.26. Our AdV18 differed from the previous ones in one amino acid in the hexon (G349C) and three in the polymerase (E303D, S359T and Q1079H). A close relationship was observed between both regions of AdV18 in our study and previously reported AdV18 sequences. Sequences acquired in this study were indicated by "•" in red.

followed by 96.9% homology with an AAV2 sequence derived from a liver tumor in France in 2015. Furthermore, comparison of the amino acid sequences of each protein encoded by MI-AAV2 demonstrated homologies exceeding 99% with previously reported AAV2 sequences.

Typically, AAV is inherently deficient in replication and relies on co-infection with a helper virus such as adenovirus and herpesvirus for productive propagation (Meier et al., 2020). Initially considered non-pathogenic and safe for human use, AAV has been extensively utilized as a gene therapy vector (Wang et al., 2019). However, a recent study has highlighted that recurrent clonal AAV2 insertions were associated with the development of hepatocellular carcinoma (La Bella et al., 2020). Furthermore, AAV2 infection has been implicated in acute pediatric hepatitis outbreaks in both the United States and the United Kingdom (Gates et al., 2023; Ho et al., 2023). In this study, we identified the sequences from *Adenoviridae* in the fecal sample specifically as AdV18, while the sequences from *Herpesvirales* in the tissue sample were identified as HSV-1. This observation suggested that these two viruses could potentially serve as helper viruses facilitating AAV2 replication within host cells. The freshly excised tissue was promptly frozen in a cryostat, sectioned, and then placed in a fixative solution. Rapid hematoxylin and eosin (HE) staining was conducted, revealing the inflammatory hyperplasia of the lingual papilloma (Supplementary Figs. S1B and C). Unfortunately, we were unable to conduct the in situ hybridization experiment to ascertain the presence of the virus within the tissue due to insufficient specimen quantity. Thus, we cannot exclude the possibility that the lingual papilloma may be linked to AAV2 with AdV18 and/or HSV-1 acting as helper viruses. Further validation from clinical cases is imperative to elucidate this potential association.

Significantly, AdV18 is a rarely detected serotype of adenovirus that has garnered considerable interest due to its oncogenic potential in neonatal rodents (Huebner et al., 1962). Initially isolated from an anal swab of a child with Niemann-Pick syndrome in the 1950s, AdV18 has been sporadically detected in children's fecal samples, as evidenced by a study from Tanzania in 2014 (Moyo et al., 2014). Currently, only one complete genome sequence of AdV18 (GU191019.1) is available in public databases, originating from a sample collected in 1954 in the

United States. Additionally, there are 22 AdV18-related fragments of varying lengths (ranging from 235 bp to 3164 bp) in public databases, located at different positions within the viral genome (Supplementary Fig. S1D). Among the three relatively long sequences, one represents a near full-length sequence of the polymerase, the other two are full-length sequences of the hexon (Liu et al., 2000; Hoeben and Uil, 2013). Our sequence differed from the previous sequences by one amino acid in the hexon (G349C) and three in the polymerase: E303D, S359T and Q1079H. The first two alterations in the polymerase are located in the exonuclease domain, while the latter resides in the thumb domain. Further investigation is warranted to determine whether these changes impact polymerase activity. Evolutionary trees were constructed using nucleotide sequences from the polymerase and hexon regions of related AdVs. Our results demonstrated a close association between both regions of AdV18 in our study and previously reported AdV18 sequences, forming a coherent evolutionary cluster (Fig. 1C and D).

The role of AdV18 in human health remains poorly understood, including its potential association with the lingual papillomatosis observed in the patient of this study. Although AdV18 has been recognized for its oncogenic potential since its initial discovery in 1954, it has been rarely reported in the literature. Currently, only one complete genome sequence of AdV18 is available in public databases. In our investigation, we successfully obtained a complete genome sequence of AdV18 through high-throughput sequencing (GenBank under accession number: OR609364, Science Data Bank: https://doi.org/10.57760/sciencedb.j00001.00828), which exhibited 99.3% nucleotide identity with the previously reported sequence. This represents the second complete genome sequence accessible in the public domain. The newly acquired data serve as a valuable resource for future studies focusing on the molecular evolution, origins, and pathogenicity of adenoviruses.

In conclusion, our analysis identified a notable abundance of reads for AAV2, AdV18 in the feces and HSV-1 in the tissue of an individual exhibiting severe lingual papillomatosis following HSTC. Despite failing to establish a direct causal relationship between these viral infections and the development of lingual papillomatosis, this study provides the first documentation of the concurrent presence of non-replicative AAV2 alongside potential helper viruses AdV18 or HSV-1 in fecal or tissue samples of a patient.

Footnotes

This work was supported by the Natural Science Foundation of Beijing Municipality (L222074) and Key R&D Program of Shandong Province, China (2019JZZY011011). We acknowledge the support and resources from the Center for High Performance Computing at Beijing Jiaotong University (https://hpc.bjtu.edu.cn). None of the authors declare a conflict of interest. The study protocol was approved by the Ethics Committee of the Peking University First Hospital. Due to the retrospective nature of the study, informed written consent was waived.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2024.06.006.

References

Armstrong, A.E., Rossoff, J., Hollemon, D., Hong, D.K., Muller, W.J., Chaudhury, S., 2019. Cell-free DNA next-generation sequencing successfully detects infectious pathogens in pediatric oncology and hematopoietic stem cell transplant patients at risk for invasive fungal disease. Pediatr. Blood Cancer 66, e27734.

Gates, S., Andreani, J., Dewar, R., Smith, D.B., Templeton, K., Child, H.T., Breuer, J., Golubchik, T., Bassano, I., Wade, M.J., et al., 2023. Postpandemic rebound of adeno-associated virus type 2 (AVV2) infections temporally associated with an outbreak of unexplained severe acute hepatitis in children in the United Kingdom. J. Med. Virol. 95, e28921.

Ho, A., Orton, R., Tayler, R., Asamaphan, P., Herder, V., Davis, C., Tong, L., Smollett, K., Manali, M., Allan, J., et al., 2023. Adeno-associated virus 2 infection in children with non-A-E hepatitis. Nature 617, 555–563.

Hoeben, R.C., Uil, T.G., 2013. Adenovirus DNA replication. Cold Spring Harbor Perspect. Biol. 5, a013003.

Huebner, R.J., Rowe, W.P., Lane, W.T., 1962. Oncogenic effects in hamsters of human adenovirus types 12 and 18. Proc. Natl. Acad. Sci. U.S.A. 48, 2051–2058.

La Bella, T., Imbeaud, S., Peneau, C., Mami, I., Datta, S., Bayard, Q., Caruso, S., Hirsch, T.Z., Calderaro, J., Morcrette, G., et al., 2020. Adeno-associated virus in the liver: natural history and consequences in tumour development. Gut 69, 737–747.

Liu, H.T., Naismith, J.H., Hay, R.T., 2000. Identification of conserved residues contributing to the activities of adenovirus DNA polymerase. J. Virol. 74, 11681–11689.

Meier, A.F., Fraefel, C., Seyffert, M., 2020. The interplay between adeno-associated virus and its helper viruses. Viruses 12, 662.

Moyo, S.J., Hanevik, K., Blomberg, B., Kommedal, O., Nordbo, S.A., Maselle, S., Langeland, N., 2014. Prevalence and molecular characterisation of human adenovirus in diarrhoeic children in Tanzania; a case control study. BMC Infect. Dis. 14, 666.

Peng, J.M., Du, B., Qin, H.Y., Wang, Q., Shi, Y., 2021. Metagenomic next-generation sequencing for the diagnosis of suspected pneumonia in immunocompromised patients. J. Infect. 82, 22–27.

Qu, Y.H., Ding, W.J., Liu, S., Wang, X.J., Wang, P.F., Liu, H.Y., Xia, H., Chen, Y., Jiang, H., 2022. Metagenomic next-generation sequencing vs. Traditional pathogen detection in the diagnosis of infection after allogeneic hematopoietic stem cell transplantation in children. Front. Microbiol. 13, 868160.

Sahin, U., Toprak, S.K., Atilla, P.A., Atilla, E., Demirer, T., 2016. An overview of infectious complications after allogeneic hematopoietic stem cell transplantation. J. Infect. Chemother. 22, 505–514.

Schuler, B.A., Nelson, E.T., Koziura, M., Cogan, J.D., Hamid, R., Phillips, J.A., 2022. Lessons learned: next-generation sequencing applied to undiagnosed genetic diseases. J. Clin. Invest. 132, e154942.

Wang, D., Tai, P.W.L., Gao, G.P., 2019. Adeno-associated virus vector as a platform for gene therapy delivery. Nat. Rev. Drug Discov. 18, 358–378.