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Recently Added Frameshift Mutation in Human Monkeypox Virus (hMPXV) *OPG191* Gene

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Human monkeypox virus (hMPXV) has caused sporadic outbreaks intermittently across countries in recent years, with the largest outbreak in 2022. However, the underlying mechanisms remain unclear. This study searched for recently developed structural variants of the viral genome. A total of 22 hMPXV whole genome sequences were randomly selected from the National Center for Biotechnology Information GenBank sequence database for initial screening. As a result, a recent frameshift mutation based on a 2-base insertion in a coding region was identified at the 3' terminal of the *OPG191* gene, which encodes MPXVgp168 (B7R) protein. With this insertion, the protein was prematurely truncated, and the last 11 amino acids were missing, with 3 alternative amino acids added. Among the hMPXV genome sequences registered in the GenBank database as of January 2023, 61 sequences lacked the 2-base insertion and 3,362 sequences were inserted. All 61 sequences without mutations were collected before 2020, whereas 3,358 (99.9%) of the 3,362 sequences with the insertion were collected during or after 2022. These findings imply that a 2-base insertion has recently emerged and has been fixed among the virus population that prevailed in 2022. In summary, a recently emerged frameshift mutation with a 2-base insertion was identified in hMPXV *OPG191* gene. Although the structural and functional consequences of this mutation on virulence and infectivity are unknown, research on the possible associations between this mutation and recent hMPXV outbreaks is warranted.

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Introduction

The monkeypox virus (MPXV) was first discovered in 1958 during an outbreak in an animal facility in Denmark (Parker and Buller 2013). The first human case with MPXV infection was reported in 1970 in the Democratic Republic of the Congo (Meo and Jawaid 2022). Since then, the virus has caused intermittent sporadic outbreaks worldwide (Bunge et al. 2022). The outbreak of human MPXV (hMPXV) in 2022 was the largest, infecting more than 80,000 individuals across the countries worldwide via human-to-human transmission (Centers for Disease Control and Prevention 2023). Previously, the virus primarily caused human-to-human transmission in African countries (Durski et al. 2018; Mauldin et al. 2022); however, it caused outbreaks in many European countries and North America in 2022 (World Health Organization 2022). Genome sequence analyses have suggested that the 2022 outbreak originated during the 2017 Nigeria outbreak (Velavan and Meyer 2022; Wassenaar et al. 2022). hMPXV is one of the largest double-stranded DNA viruses, with approximately 200,000 bases (Kugelman et al. 2014), making it difficult to fully elucidate mutation profiles across the whole viral genome. Previous studies have mainly focused on point mutations in the viral genome. Currently, the presence and role of structural variants in viral spread and evolution remain largely unknown. Therefore, this study aimed to determine the presence, site, and estimated timing of recently added structural variants in the hMPXV population.

Methods

Multiple sequence alignments and sequence search

The primary objective of this study was to identify recently fixed structural variants in the coding regions of the hMPXV genome that may have been fixed in the last

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decade. As an initial screening for fixed structural variants, 22 whole-genome sequences collected between 1979 and 2022 from various countries worldwide were randomly selected from the National Center for Biotechnology Information (NCBI) GenBank sequence database. A list of these 22 sequences, together with the GenBank Accession ID, collection date and locations, is shown in Table 1. The whole-genome sequences of these 22 strains were used for multiple sequence alignments, which was performed using the Molecular Evolutionary Genetics Analysis Version 11 (MEGA11) software (Tamura et al. 2021). For the identified candidate sites with fixed major mutations, sequence similarity searches were performed using the Basic Local Alignment Search Tool (BLAST) offered by NCBI, to investigate the number of registered sequences with or without the identified major mutations.

Ethics and data availability

This study was approved by the Institutional Review Board of Tohoku University Graduate School of Medicine (approval number: 2022-1-720). The genome sequences from the 22 hMPXV genome sequences evaluated are available in the NCBI GenBank database with the accession ID numbers listed in Table 1.

Results

Identification of recent frameshift mutation site

Screening based on multiple sequence alignments with the initially recruited 22 whole genome sequences identified a recently emerged and fixed structural variant with a frameshift mutation in the *OPG191* gene, which encodes the MPXVgp168 (B7R; similar to Vaccinia virus strain Copenhagen B6R) protein. This frameshift mutation was based on a 2-base insertion, substituting the last 11 amino acids at the C-terminus of MPXVgp168 with 3 alternative amino acids. Multiple sequence alignments of this frameshift mutation site are shown in Fig. 1. There were several other sites with relatively short insertions or deletions in the coding regions, but most of these sites were with in-frame mutations involving multiple of three nucleotides. The aforementioned frameshift mutation site in the *OPG191* gene was the only frameshift site, based on the initially selected 22 sequences, that could have emerged within the last decade and prevailed among the virus populations during the outbreak in 2022. Moreover, there was no confirmed pair of neighboring point mutations involving two or more adjacent amino acids across the whole hMPXV genome sequences.

BLAST sequence search

To investigate the prevalence and spread of the aforementioned frameshift mutations in the viral population, an NCBI BLAST search was performed for each sequence with or without the 2-base insertion. As of January 26, 2023, 3,565 whole-genome sequences of MPXV have been registered in the NCBI GenBank database. Among these,

Table 1. List of the 22 MPXV genome sequences used for the initial screening with multiple sequence alignments.

GenBank Accession ID	Collection date	Country	2-base deletion in *OPG191*
DQ011155.1	1979	Zaire	No
KC257460.1	1985	Republic of Congo	No
DQ011157.1	2003	USA	No
DQ011154.1	2003	Republic of Congo	No
JX878415.1	Dec 2006	Republic of Congo	No
JX878421.1	Mar 2007	Republic of Congo	No
MK783027.1	Nov 2017	Nigeria	Yes
MN648051.1	Oct 2018	Israel	Yes
MT250197.1	2019	Singapore	No
OP612678.1	Mar 2019	Nigeria	Yes
OL504741.1	Dec 2019	UK	Yes
ON676708.1	Nov 2021	USA	Yes
OP415196.1	Mar 2022	UK	Yes
ON563414.3	May 2022	USA	Yes
ON959131.1	May 2022	USA	Yes
ON674051.1	May 2022	USA	Yes
ON838178.1	Jun 2022	Slovenia	Yes
OP320555.1	Jul 2022	Peru	Yes
OP936000.1	Sep 2022	Vietnam	Yes
OP890547.1	Oct 2022	USA	Yes
OP920698.1	Nov 2022	USA	Yes
OP935997.1	Nov 2022	USA	Yes

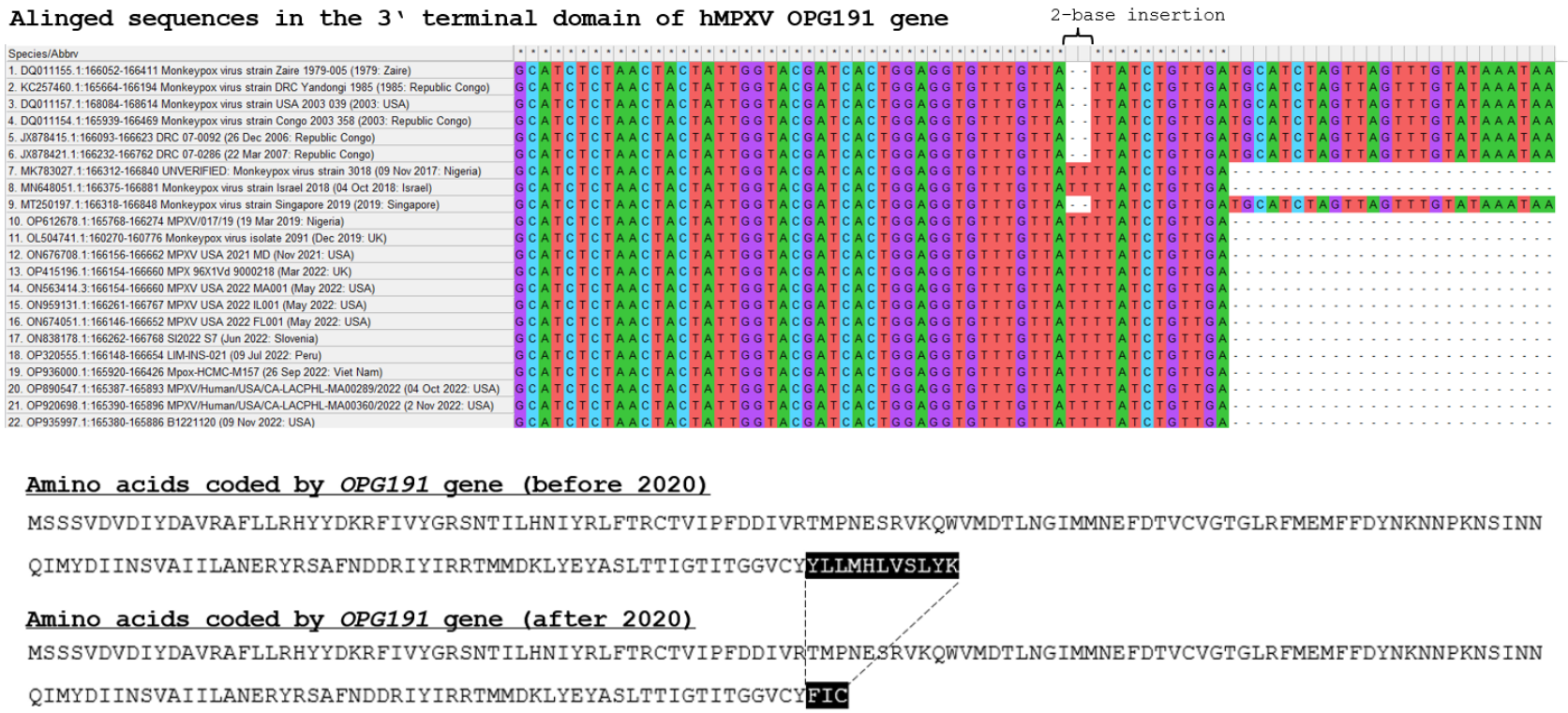

Fig. 1. Nucleotide and amino acid sequences at the identified fixed frameshift mutation site of the human monkeypox virus (hMPXV) genome.
A 2-base insertion causing a frameshift mutation in the 3′ terminal of *OPG191* gene (coded protein: MPXVgp168) was identified as a recently fixed structural variant in the hMPXV population. The upper panel shows the results of multiple sequence alignments with 22 initially selected hMPXV genome sequences. The lower panel shows the coded amino acid sequences at the identified fixed frameshift mutation sites. The encoded amino acid sequence in the C-terminal domain of MPXVgp168 was replaced and shortened by this frameshift mutation.

61 sequences were confirmed to be absent from the 2-base insertion, all of which were collected before 2020. The other 3,362 sequences were confirmed to have a 2-base insertion. Among them, 3,358 sequences (99.88%) were collected in 2022, and the remaining 4 sequences that were collected before 2022 were as follows: MPXV_Nig_2017_297957 (GenBank: MG693723.1; collection date: 2017 in Nigeria); MPXV_Nig_2017_298464 (GenBank: MG693724.1; collection date: 2017 in Nigeria); MPX_Nig_2017_298481 (GenBank: MG693725.1; collection date: 2017 in Nigeria); Israel_2018 (GenBank: MN648051.1; collection date: Oct 2018 in Israel) (Faye et al. 2018). These findings indicate the possibility that the hMPXV virus strains that caused the outbreak in Nigeria in 2017 and the international outbreak in 2022 share a common ancestor (Ogoina et al. 2019).

Discussion

The present study identified a frameshift mutation in the C-terminal domain of the MPXVgp168 (B7R) protein encoded by *OPG191*, which could have been fixed in the viral population that caused the recent hMPXV outbreaks since 2017. This frameshift mutation was based on a 2-base insertion that truncated the C-terminal domain of the encoded protein. The results obtained from the BLAST sequence search indicated that almost all sequences collected during the 2022 international outbreak were associated with this mutation. Collectively, these data indicated that this frameshift mutation could have played an unknown role in the sporadic outbreaks of the virus in Nigeria since 2017 and the largest international outbreak in 2022. The virus is also known to sometimes incorporate a large-scale deletion involving dozens of bases in addition to traditional single nucleotide substitutions or short insertions/deletions (Sereewit et al. 2022). Such reductive evolution with gene loss in orthopoxviruses is intimately associated with adaptation of the host range (Senkevich et al. 2021). Moreover, accessory gene loss events are usually associated with host antiviral innate immunity responses and changes in virus-host interactions (Senkevich et al. 2021; Forni et al. 2022). A previous accessory gene loss event in West Africa was implied to have increased the human-to-human transmissibility of the virus and caused a local outbreak (Kugelman et al. 2014). However, the exact impact of major mutations in the MPXV genome, such as an accessory gene loss events or frameshift mutations, on human-to-human transmissibility and virulence remains uncertain (Xiang and White 2022). Further studies are needed to determine whether genetic mutations underlay the recent hMPXV outbreaks and which of the mutations could have increased viral infectivity and survival.

Frameshift mutations are rarely fixed in viral populations, as coded proteins with frameshifts are often dysfunctional, and viral fitness is reduced (Huang et al. 2020). Meanwhile, several previous studies have suggested that frameshift mutations in some viruses could benefit the viruses, possibly facilitating immune evasion (García-Barreno et al. 1990; Biacchesi et al. 2007). In addition to frameshift mutations in viral genome sequences, translational frameshifting based on frameshift signals, known as programmed ribosomal frameshifting, is considered a widely distributed post-transcriptional mechanism that regulates gene expression (Dinman 2006; Penn et al. 2020). According to a recent study, the genome sequences of various microbes have been optimized for frameshift tolerance, with high similarity between frameshift and wild-type protein sequences (Wang et al. 2022). Further studies are warranted to elucidate the potential role of frameshifting in hMPXV evolution.

As a limitation of this study, the exact effects of the identified frameshift mutation on the viral infectivity to humans remain unknown. Although the protein encoded by *OPG191*, MPXVgp168 (B7R), is known to be an ankyrin-like protein, whether the coded protein also acts as a protein mediating the attachment of membrane proteins to the submembranous cytoskeleton in the virus is uncertain (Cunha and Mohler 2009). Further studies regarding the structural and functional changes caused by the observed frameshift mutation to the MPXVgp168 protein are needed to clarify the significance of this mutation.

In summary, the present study identified a recently added frameshift mutation with 2-base insertion at the 3′ terminal of hMPXV *OPG191* gene. With this insertion, the protein was prematurely truncated, and the last 11 amino acids were missing, with addition of 3 alternative amino acids. A sequence database search suggested that this frameshift mutation emerged between 2017 and 2022, and was fixed in the viral population that prevailed in 2022. Further studies are needed to elucidate the potential effects of this frameshift mutation on viral infectivity and virulence.

Author Contributions

T.A. contributed to the conception, performed formal analysis, and prepared the figures and the manuscript.

Conflict of Interest

The author declares no conflict of interest.

References

Biacchesi, S., Murphy, B.R., Collins, P.L. & Buchholz, U.J. (2007) Frequent frameshift and point mutations in the SH gene of human metapneumovirus passaged in vitro. *J. Virol.*, **81**, 6057-6067.

Bunge, E.M., Hoet, B., Chen, L., Lienert, F., Weidenthaler, H., Baer, L.R. & Steffen, R. (2022) The changing epidemiology of human monkeypox-a potential threat? A systematic review. *PLoS Negl. Trop. Dis.*, **16**, e0010141.

Centers for Disease Control and Prevention (2023) 2022 Mpox Outbreak Global Map. https://www.cdc.gov/poxvirus/monkeypox/response/2022/world-map.html [*Accessed*: May 26, 2023]

Cunha, S.R. & Mohler, P.J. (2009) Ankyrin protein networks in membrane formation and stabilization. *J. Cell. Mol. Med.*, **13**, 4364-4376.

Dinman, J.D. (2006) Programmed Ribosomal Frameshifting Goes Beyond Viruses: organisms from all three kingdoms use frameshifting to regulate gene expression, perhaps signaling a paradigm shift. *Microbe Wash. DC*, **1**, 521-527.

Durski, K.N., McCollum, A.M., Nakazawa, Y., Petersen, B.W., Reynolds, M.G., Briand, S., Djingarey, M.H., Olson, V., Damon, I.K. & Khalakdina, A. (2018) Emergence of monkeypox - West and Central Africa, 1970-2017. *MMWR Morb. Mortal. Wkly. Rep.*, **67**, 306-310.

Faye, O., Pratt, C.B., Faye, M., Fall, G., Chitty, J.A., Diagne, M.M., Wiley, M.R., Yinka-Ogunleye, A.F., Aruna, S., Etebu, E.N., Aworabhi, N., Ogoina, D., Numbere, W., Mba, N., Palacios, G., et al. (2018) Genomic characterisation of human monkeypox virus in Nigeria. *Lancet Infect. Dis.*, **18**, 246.

Forni, D., Cagliani, R., Molteni, C., Clerici, M. & Sironi, M. (2022) Monkeypox virus: the changing facets of a zoonotic pathogen. *Infect. Genet. Evol.*, **105**, 105372.

García-Barreno, B., Portela, A., Delgado, T., Lopez, J.A. & Melero, J.A. (1990) Frame shift mutations as a novel mechanism for the generation of neutralization resistant mutants of human respiratory syncytial virus. *EMBO J.*, **9**, 4181-4187.

Huang, X., Chen, R., Sun, M., Peng, Y., Pu, Q., Yuan, Y., Chen, G., Dong, J., Du, F., Cui, X. & Tang, Z. (2020) Frame-shifted proteins of a given gene retain the same function. *Nucleic Acids Res.*, **48**, 4396-4404.

Kugelman, J.R., Johnston, S.C., Mulembakani, P.M., Kisalu, N., Lee, M.S., Koroleva, G., McCarthy, S.E., Gestole, M.C., Wolfe, N.D., Fair, J.N., Schneider, B.S., Wright, L.L., Huggins, J., Whitehouse, C.A., Wemakoy, E.O., et al. (2014) Genomic variability of monkeypox virus among humans, Democratic Republic of the Congo. *Emerg. Infect. Dis.*, **20**, 232-239.

Mauldin, M.R., McCollum, A.M., Nakazawa, Y.J., Mandra, A., Whitehouse, E.R., Davidson, W., Zhao, H., Gao, J., Li, Y., Doty, J., Yinka-Ogunleye, A., Akinpelu, A., Aruna, O., Naidoo, D., Lewandowski, K., et al. (2022) Exportation of monkeypox virus from the African Continent. *J. Infect. Dis.*, **225**, 1367-1376.

Meo, S.A. & Jawaid, S.A. (2022) Human monkeypox: fifty-two years based analysis and updates. *Pak. J. Med. Sci.*, **38**, 1416-1419.

Ogoina, D., Izibewule, J.H., Ogunleye, A., Ederiane, E., Anebonam, U., Neni, A., Oyeyemi, A., Etebu, E.N. & Ihekweazu, C. (2019) The 2017 human monkeypox outbreak in Nigeria-report of outbreak experience and response in the Niger Delta University Teaching Hospital, Bayelsa State, Nigeria. *PLoS One*, **14**, e0214229.

Parker, S. & Buller, R.M. (2013) A review of experimental and natural infections of animals with monkeypox virus between 1958 and 2012. *Future Virol.*, **8**, 129-157.

Penn, W.D., Harrington, H.R., Schlebach, J.P. & Mukhopadhyay, S. (2020) Regulators of viral frameshifting: more than RNA influences translation events. *Annu. Rev. Virol.*, **7**, 219-238.

Senkevich, T.G., Yutin, N., Wolf, Y.I., Koonin, E.V. & Moss, B. (2021) Ancient gene capture and recent gene loss shape the evolution of orthopoxvirus-host interaction genes. *mBio*, **12**, e0149521.

Sereewit, J., Lieberman, N.A.P., Xie, H., Bakhash, S., Nunley, B.E., Chung, B., Mills, M.G., Roychoudhury, P. & Greninger, A.L. (2022) ORF-interrupting mutations in monkeypox virus genomes from Washington and Ohio, 2022. *Viruses*, **14**, 2393.

Tamura, K., Stecher, G. & Kumar, S. (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.*, **38**, 3022-3027.

Velavan, T.P. & Meyer, C.G. (2022) Monkeypox 2022 outbreak: an update. *Trop. Med. Int. Health*, **27**, 604-605.

Wang, X., Dong, Q., Chen, G., Zhang, J., Liu, Y. & Cai, Y. (2022) Frameshift and wild-type proteins are often highly similar because the genetic code and genomes were optimized for frameshift tolerance. *BMC Genomics*, **23**, 416.

Wassenaar, T.M., Wanchai, V. & Ussery, D.W. (2022) Comparison of Monkeypox virus genomes from the 2017 Nigeria outbreak and the 2022 outbreak. *J. Appl. Microbiol.*, **133**, 3690-3698.

World Health Organization (2022) Monkeypox Key facts. https://www.who.int/news-room/fact-sheets/detail/monkeypox [*Accessed*: May 26, 2023]

Xiang, Y. & White, A. (2022) Monkeypox virus emerges from the shadow of its more infamous cousin: family biology matters. *Emerg. Microbes Infect.*, **11**, 1768-1777.