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Whole genome-based surveillance for human adenovirus-related diseases in Kobe City, Japan, 2018–2022

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Abstract

Background Human adenoviruses (HAdVs) cause various diseases, and they frequently undergo recombination. As adenoviruses are susceptible to recombination, whole-genome sequencing and analysis are essential approaches to understanding viral properties. In the present study, we performed whole-genome sequencing to characterize adenoviruses and assess the local epidemic genotypes of adenovirus-related diseases in Kobe, Japan.

Methods Eighty HAdV cell culture-positive strains isolated from clinical specimens in Kobe City, Japan, between 2018 and 2022 were used. Whole-genome sequencing, phylogenetic analysis, and recombination analysis were performed.

Results Disease-specific HAdV species were detected, with species B and C being the primary species detected for pharyngoconjunctival fever and species F for infectious gastroenteritis. All species B strains belonged to the HAdV-3 genotype. Multiple genotypes were detected in species C, including five strains in which the new genotype, P85H5F5, was identified in pharyngoconjunctival fever specimens.

Conclusions Whole-genome analysis of HAdV is an important approach not only for understanding local epidemics, but also for monitoring the emergence of recombinant genotypes.

Keywords Human adenovirus, Japan, Whole genome sequence, Genotyping, Surveillance

Background

Human adenoviruses (HAdVs) are non-enveloped double-stranded DNA viruses with a genome size of 35–36 Kb. Infection with HAdVs can cause various symptoms, including pharyngitis, keratoconjunctivitis, diarrhea, pneumonia, and hepatitis, mainly in children [1]. HAdV is transmitted via inhalation, aerosols, person-to-person contact, contaminated feces, or the fecal-oral route [2]. HAdV infections rarely cause serious illness or death. However, infants, immunocompromised individuals, and those with respiratory or cardiac diseases are at a higher risk of experiencing serious illness from HAdV infection [3].

HAdVs are classified into seven species (A–G) based on their biochemical characteristics, DNA homology, and genome sequences. Species B, C, and E mainly infect the respiratory tract; species D targets the conjunctiva; and species A, F, and G target the digestive tract [4]. Regions of the HAdV genome commonly associated with homologous recombination include the penton base, hexon, and fiber coding regions, which are also the most frequently mutated regions [5]. At least 114 HAdV genotypes have been reported based on the sequences of three genes: penton, hexon, and fiber (http://hadvwg.gmu.edu/). Recombination shuffles genomic fragments within species, but not between species [6].

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Monitoring the emergence of novel genotypes (including recombinant types) is important because they may be associated with immune escape through natural exposure to other genotypes [7]. As adenoviruses are susceptible to recombination, whole-genome sequencing and analysis are essential approaches in this regard. In the present study, we performed whole-genome analysis on strains that tested positive for adenovirus in Kobe from 2018 to 2022, with the aim of detecting local epidemic strains and genotypes.

Materials and methods

Specimens

Specimens were collected as part of sentinel surveillance for pharyngoconjunctival fever (PCF); infectious gastroenteritis; pneumonia; epidemic keratoconjunctivitis (EKC); herpangina; and hand, foot, and mouth disease (HFMD) in Kobe City between 2018 and 2022. The sentinel surveillance system in Kobe City is based on the National Epidemiological Surveillance of Infectious Diseases (NESID) in Japan [8]. All specimens were inoculated into Vero-E6, RD-18 S, FL, HEp-2, and A549 cells to attempt virus isolation. For PCF and EKC, PCR was performed for HAdV. For herpangina and HFMD, PCR for HAdV was performed on specimens with negative results for enterovirus PCR. Infectious gastroenteritis specimens were subjected to PCR for norovirus, rotavirus, sapovirus, adenovirus, and enterovirus. For pneumonia specimens, after the cytopathic effects of inoculated cells were confirmed, the cultured virus was identified using PCR. Specimen types are indicated in Table S1. The specimens were suspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 0.3 mg/mL kanamycin and then filtered through a 0.45 μm filter. DNA extraction was performed using 200 μL of the flowthrough using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. HAdVs were detected via real-time PCR using TB Green Premix Ex Taq II (Tli RNaseH Plus) (Takara Bio, Shiga, Japan) and conventional PCR using PrimeSTAR GXL DNA Polymerase (Takara Bio). The primer sequences are listed in Table S2.

Virus culture

Each HAdV-PCR-positive specimen was inoculated into Vero-E6, RD-18 S, FL, HEp-2, and A549 cells in DMEM supplemented with 2% FBS and 0.3 mg/mL kanamycin and incubated at 37 °C in a CO_2 incubator. When cytopathic effects were observed, cells and cell supernatants were collected. DNA was extracted from 200 μL of sample using the QIAamp DNA Mini Kit (Qiagen). The DNA concentration was measured using a Qubit dsDNA HS Kit (Thermo Fisher Scientific, Waltham, MA, USA). A total of 80 HAdV culture-positive strains were obtained and used in this study.

Whole-genome sequencing

Fragmentation, adapter ligation, and PCR amplification were performed using viral DNA as a template with a QIAseq FX DNA Library Kit (Qiagen). Short-read sequences were obtained using the MiSeq Reagent Kit v2 (300 cycles, Illumina, San Diego, CA, USA) on the MiSeq System (Illumina). Complete genomes were determined through bioinformatics analysis performed using the following packages: adapter and quality trimming: fastp v0.23.4 [9]; assembly: SPAdes v3.15.5 [10]; mapping analysis and SNP/InDel detection: bwa v0.7.17 [11] and VarScan v2.4.4 [12]; and annotation: prokka v.1.14.6 [13].

Genome analysis

Multiple alignments of DNA sequences were performed using MAFFT [14]. A phylogenetic tree was constructed with IQ-TREE v2.2.6 [15] or MEGA11 [16] using the maximum likelihood (ML) method [15] and visualized with iTOL [17]. Recombination analysis was performed in the RDP4 using RDP, GENECONV, BOOTSCAN, MaxChi, CHIMAERA, SiSCAN, and 3SEQ methods [18], and SimPlot++V1.3 [19] with the following parameters: window length, 500 bp; step size, 100 bp; and Kimura 2-parameters.

Accession

Nucleotide sequence data generated in this study are available in the DDBJ/EMBL/GenBank databases under accession numbers LC791110–LC791189.

Results

The HAdV positivity rate was 74.4% (67/90) for PCF, 8.2% (7/85) for infectious gastroenteritis, 16.7% (1/6) for pneumonia, 40.0% (2/5) for EKC, 5.3% (2/38) for herpangina, and 0.5% (1/195) for HFMD (Table 1). Species B and

Table 1 Number of cases and adenovirus positivity rate for each disease

Diagnosis	Year					Total
	2018	2019	2020	2021	2022	
Pharyngoconjunctival fever (PCF)	26/39 (66.7%)	37/47 (78.7%)	1/1 (100%)	1/1 (100%)	2/2 (100%)	67/90 (74.4%)
Infectious gastroenteritis	5/55 (9.1%)	2/24 (8.3%)	0/0 (0%)	0/3 (0%)	0/3 (0%)	7/85 (8.2%)
pneumonia	0/4 (0%)	1/1 (100%)	0/0 (0%)	0/0 (0%)	0/1 (0%)	1/6 (16.7%)
epidemic keratoconjunctivitis (EKC)	1/1 (100%)	0/0 (0%)	0/0 (0%)	1/4 (25.0%)	0/0 (0%)	2/5 (40.0%)
Herpangina	2/17 (11.8%)	0/17 (0%)	0/1 (0%)	0/0 (0%)	0/3 (0%)	2/38 (5.3%)
Hand, Foot, and Mouth Disease (HFMD)	0/80 (0%)	1/94 (1.0%)	0/4 (0%)	0/11 (0%)	0/6 (0%)	1/195 (0.5%)
Total	34/196 (17.3%)	41/183 (22.4%)	1/6 (16.7%)	2/19 (10.5%)	2/15 (13.3%)	80/419 (19.1%)

C accounted for more than 80% of the total HAdV samples over the five years (Table 2; Fig. 1). All species F were detected in cases of infectious gastroenteritis. One species, A, was detected in case of infectious gastroenteritis, while species D was found in EKC. Species E and G were not detected.

All species B strains belonged to the HAdV-B3 genotype, while all species F belonged to the HAdV-F41 genotype. The species A strain was identified as HAdV-A31, and the species D strain was HAdV-D37. No recombinant strains were detected for species A, B, D, or F (Fig. 1 and Table S1). In contrast, multiple genotypes

Table 2 HAdV genotypes detected by diagnosis in this study

Species		Diagnosis						
	Genotype	PCF	Infectious gastroenteritis	pneumonia	EKC	Herpangina	HFMD	Total
A	HAdV-31		1					1
B	HAdV-3	31			1			32
C	HAdV-1	10				2		12
	HAdV-2	16	1	1				18
	HAdV-5	2					1	3
	HAdV-89	3						3
	P89H5F5	5						5
D	HAdV-37				1			1
F	HAdV-41		5					5
Total		67	7	1	2	2	1	80

PCF: Pharyngoconjunctival fever, EKC: Epidemic keratoconjunctivitis, HFMD: Hand, Foot, and Mouth Disease

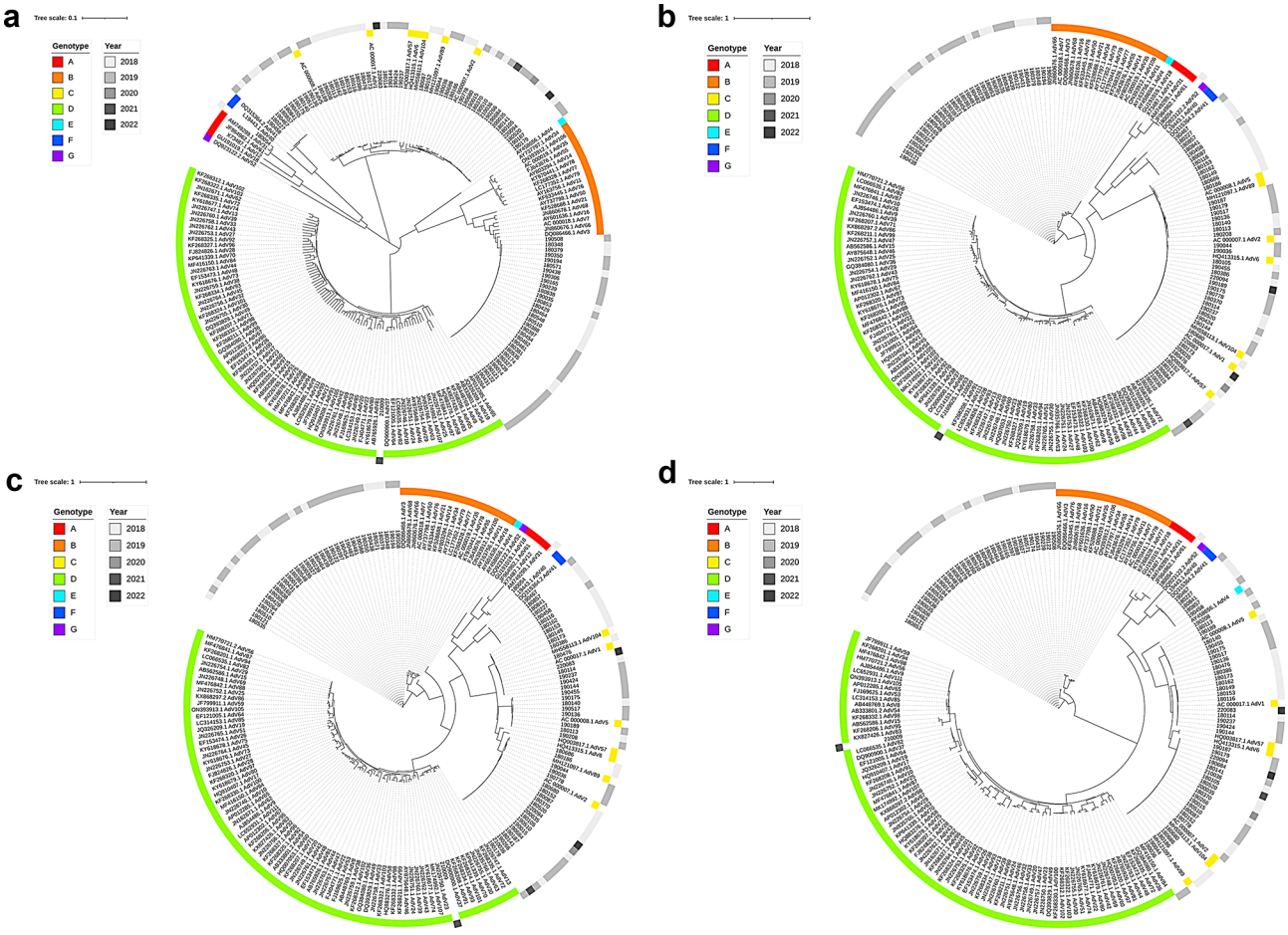

Fig. 1 Phylogenetic analysis of HAdV detected in this studyPhylogenetic tree based on the complete sequences (**a**), penton base (**b**), hexon (**c**), and fiber (**d**) genes using the maximum likelihood (ML) method. Color strips indicate reference strains, and gray strips indicate strains sequenced in this study

were detected for species C. Based on the hexon and fiber sequences, the genotypes were determined to be type 1, 2, and 5, whereas eight strains were classified as type 89 based on the penton sequence. The arginine-glycine-aspartate (RGD) loop region of the penton base protein of these eight strains had characteristic A363E and P364del mutations (Fig. 2a). Of these eight strains, three were HAdV-C89 with P89H2F2, and five were P89H5F5 (Fig. 2b-d). Recombination analysis revealed that the five strains exhibited breakpoint positions around the penton gene (Table 3; Fig. 3).

Although HAdV-B3 was dominant in 2018 and 2019, HAdV-C1 and C2 were still detected in 2020 and beyond (Table 4). HAdV-C89 was detected only in 2018, HAdV-C5 was detected only in 2019, and P89H5F5 was detected in both 2018 and 2019.

Discussion

HAdV causes various diseases in humans and is known to undergo recombination. The detection of partial sequences using the commonly used Sanger sequencing method alone is insufficient to evaluate recombination. The present study shows the whole-genome sequences of HAdVs isolated from various diseases in Kobe, Japan.

During the study period of 2018–2022, adenovirus detection decreased after 2020, likely attributed to the impact of COVID-19. The COVID-19 pandemic changed the dynamics of the infectious disease and reduced the number of patients infected with adenovirus. In various countries and regions, a reduction in respiratory infections and PCF was reported during the COVID-19 pandemic [20–23]. In this study, HAdV-C2 was consistently detected throughout the 2020–2022 period, although in small numbers, suggesting that it was the predominant strain during the COVID-19 pandemic. According to the Infectious Diseases Weekly Report of Japan, the proportion of adenovirus-associated infections, especially PCF, has increased since 2023 [24]. Trends in adenovirus infections following major outbreaks are of interest.

PCF includes conjunctivitis, pharyngitis, and upper respiratory tract inflammation and should be monitored individually. Most PCF specimens collected in this study were throat swabs, with pharyngitis and upper respiratory tract inflammation as the main foci. In previous reports, the detection rates of adenoviruses in throat swabs by specimen type were in the following order: HAdV-C2, HAdV-C1, and HAdV-B3 [25]. Other studies conducting surveillance for respiratory infections also

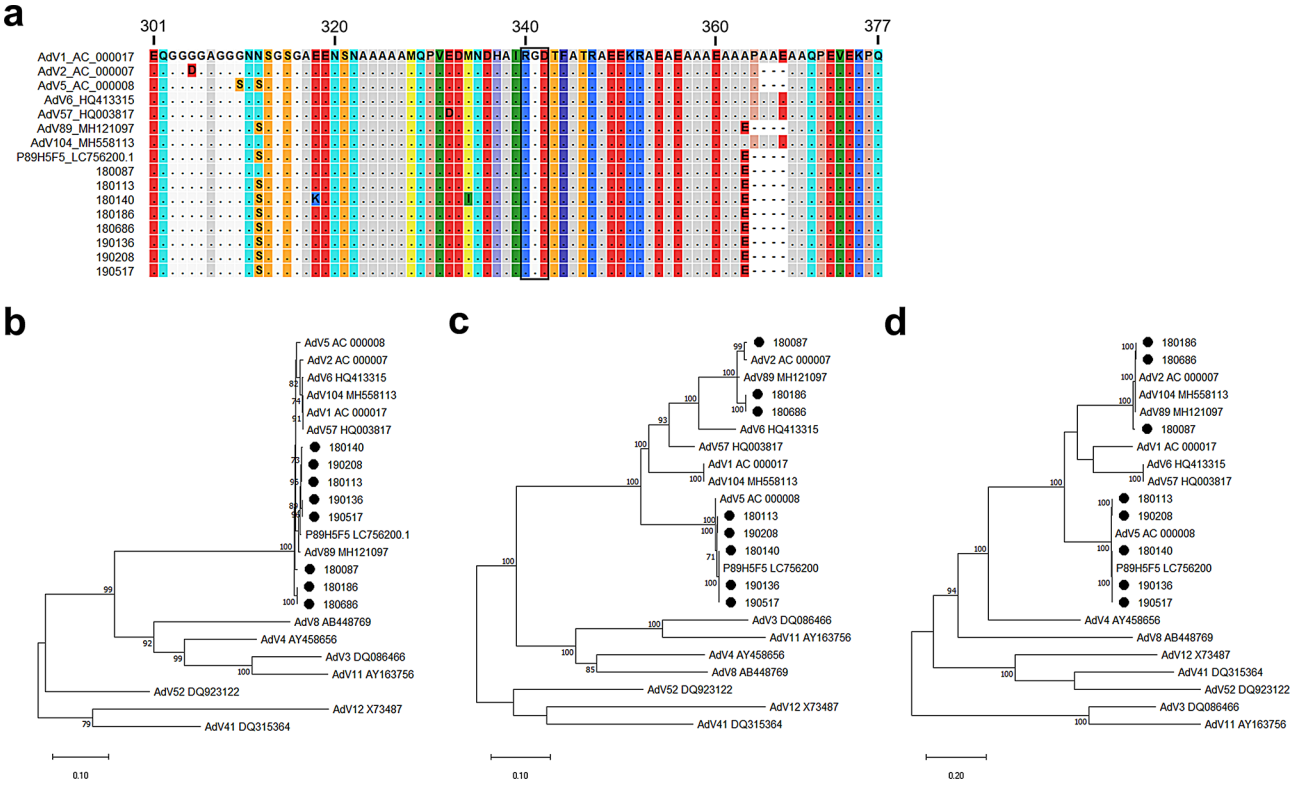

Fig. 2 Phylogenetic analysis of the new HAdV genotype detected in this study(**a**) Multiple amino acid alignments of the RGD variable loop sequence (amino acid position: 301–377) of the penton base. RGD is boxed in the black square. Residues identical to AdV1 are shown as dots. Hyphens indicate positions where residues are missing. (**b**-**d**) Phylogenetic tree based on the penton base (**b**), hexon (**c**), and fiber (**d**) genes using the maximum likelihood (ML) method. Bootstrap values (> 70) are indicated for the corresponding nodes. Circles indicate the samples sequenced in this study

Table 3 Recombinant analysis using RDP4

Breakpoint Positions					Detection Methods								
Begin	**End**	**Recombinant Sequence(s)**	**Minor Parental Sequence(s)**	**Major Parental Sequence(s)**	**RDP**	**GENECONV**	**Bootscan**	**Maxchi**	**Chimaera**	**SiSscan**	**PhylPro**	**LARD**	**3Seq**
9489	10,841	180,113	AdV89_MH121097	AdV5_AC_000008	2.75E-12	3.74E-07	2.68E-12	1.77E-03	1.91E-02	NS	NS	NS	1.23E-06
14,522	16,331	180,113	AdV89_MH121097	AdV5_AC_000008	2.05E-16	NS	5.35E-05	NS	1.57E-02	NS	NS	NS	2.31E-09
Breakpoint Positions					**Detection Methods**								
Begin	**End**	**Recombinant Sequence(s)**	**Minor Parental Sequence(s)**	**Major Parental Sequence(s)**	**RDP**	**GENECONV**	**Bootscan**	**Maxchi**	**Chimaera**	**SiSscan**	**PhylPro**	**LARD**	**3Seq**
1214	3520	180,140	AdV89_MH121097	AdV5_AC_000008	1.12.E-16	1.25.E-11	2.67.E-14	1.58.E-02	6.27.E-04	NS	NS	NS	1.12.E-02
13,724	15,977	180,140	AdV89_MH121097	AdV5_AC_000008	6.56.E-18	NS	1.20.E-04	4.14.E-02	6.99.E-03	NS	NS	NS	4.34.E-03
Breakpoint Positions					**Detection Methods**								
Begin	**End**	**Recombinant Sequence(s)**	**Minor Parental Sequence(s)**	**Major Parental Sequence(s)**	**RDP**	**GENECONV**	**Bootscan**	**Maxchi**	**Chimaera**	**SiSscan**	**PhylPro**	**LARD**	**3Seq**
1676	2720	190,136	AdV89_MH121097	AdV5_AC_000008	1.01.E-17	5.18.E-12	6.10.E-15	1.44.E-02	1.81.E-03	NS	NS	NS	NS
7313	11,516	190,136	AdV89_MH121097	AdV5_AC_000008	5.13.E-40	NS	1.15.E-17	6.97.E-05	1.22.E-05	NS	NS	NS	1.39.E-02
Breakpoint Positions					**Detection Methods**								
Begin	**End**	**Recombinant Sequence(s)**	**Minor Parental Sequence(s)**	**Major Parental Sequence(s)**	**RDP**	**GENECONV**	**Bootscan**	**Maxchi**	**Chimaera**	**SiSscan**	**PhylPro**	**LARD**	**3Seq**
9453	10,636	190,208	AdV89_MH121097	AdV5_AC_000008	8.51.E-09	3.75.E-07	2.70.E-12	2.82.E-02	NS	NS	NS	NS	1.23.E-06
14,522	16,331	190,208	AdV89_MH121097	AdV5_AC_000008	2.05.E-16	NS	5.37.E-05	NS	NS	NS	NS	NS	2.31.E-09
Breakpoint Positions					**Detection Methods**								
Begin	**End**	**Recombinant Sequence(s)**	**Minor Parental Sequence(s)**	**Major Parental Sequence(s)**	**RDP**	**GENECONV**	**Bootscan**	**Maxchi**	**Chimaera**	**SiSscan**	**PhylPro**	**LARD**	**3Seq**
690	3740	190,517	AdV89_MH121097	AdV5_AC_000008	1.02.E-21	2.03.E-16	6.02.E-18	2.34.E-04	7.73.E-03	NS	NS	NS	6.30.E-03
7480	11,562	190,517	AdV89_MH121097	AdV5_AC_000008	3.85.E-30	NS	2.05.E-17	4.43.E-07	3.06.E-03	NS	NS	NS	9.75.E-04
13,724	15,983	190,517	AdV89_MH121097	AdV5_AC_000008	9.10.E-19	NS	6.20.E-07	4.68.E-03	6.28.E-03	NS	NS	NS	3.43.E-12
16,739	16,913	190,517	AdV89_MH121097	AdV5_AC_000008	1.04.E-03	6.24.E-06	9.59.E-09	NS	NS	NS	NS	NS	NS

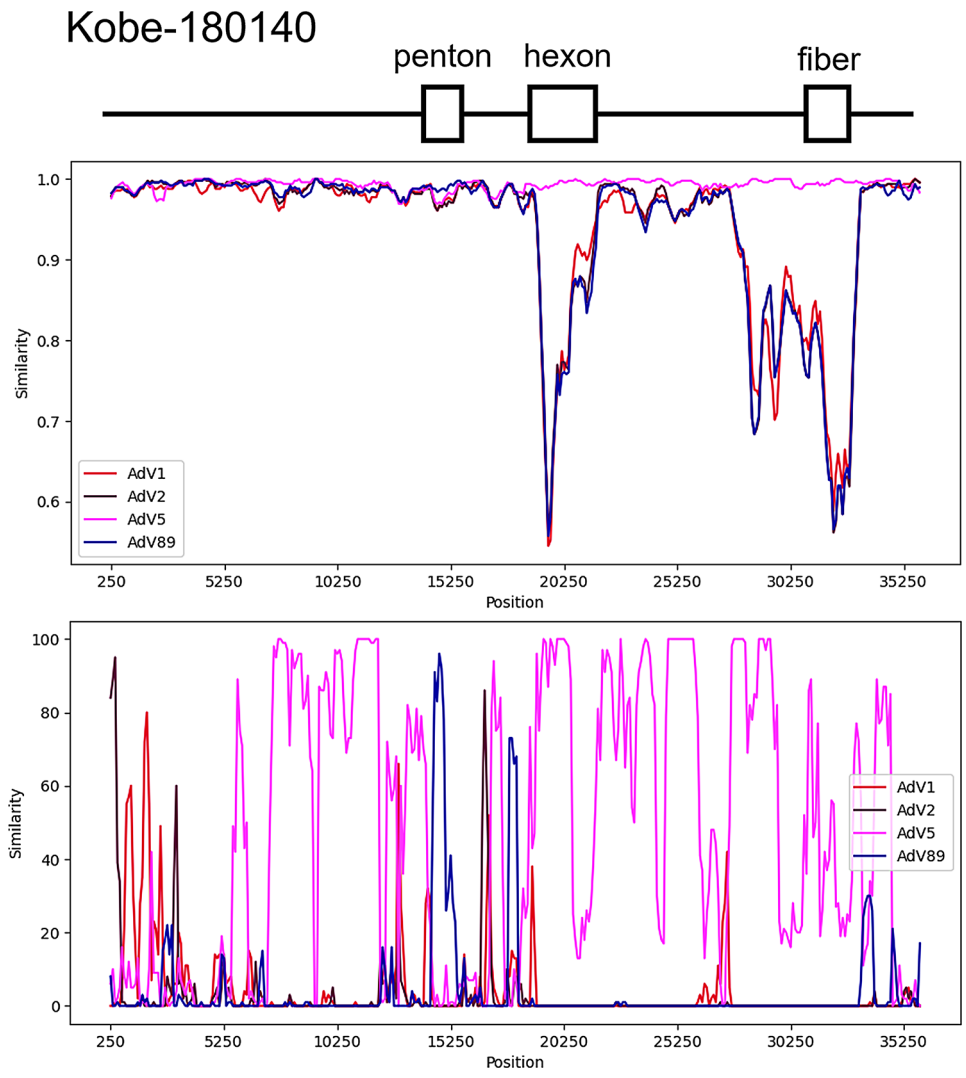

Fig. 3 Recombinant analysis using Simplot Representative Simplot (upper panel) and BootScan (lower panel) analysis of the new genotype P89H5F5, strain Kobe-180,140

Table 4 HAdV genotypes detected by years

Species	Genotype	Year				
		2018	2019	2020	2021	2022
A	HAdV-31	1				
B	HAdV-3	11	21			
C	HAdV-1	8	3			1
	HAdV-2	6	9	1	1	1
	HAdV-5		3			
	HAdV-89	3				
	P89H5F5	2	3			
D	HAdV-37				1	
F	HAdV-41	3	2			
Total		34	41	1	2	2

detected high percentages of HAdV-B3 and HAdV-C1 [26, 27]. HAdV detection in our patients with PCF was consistent with the results of these reports. In contrast, surveillance for conjunctivitis has been reported more frequently in patients with EKC, with HAdV-D8 being the genotype detected primarily [28], followed by other genotypes of HAdV-B3, HAdV-E4, HAdV-D37, HAdV-D53, HAdV-D64, and HAdV-D85 [29, 30]. In Japan, HAdV-D54 (P54H54F8) was the predominant genotype detected in EKC surveillance [31]. As the number of EKC specimens was small (one each of HAdV-B3 and HAdV-D37), it is essential to accumulate additional data in the future to accurately determine the epidemic type.

In other HAdV gastroenteritis surveillance studies, HAdV-F41 was the most common genotype, with HAdV-C1, HAdV-C2, and HAdV-B3 being subdominant genotypes [32–34]. In the present study, five HAdV-F41, one HAdV-C2, and one HAdV-A31 were detected in patients with infectious gastroenteritis, which is consistent with the results of previous studies.

In this study, adenoviruses were detected in two cases of herpangina and one case of HFMD. HAdV-B7 has been detected in patients with HFMD in whom enterovirus 71 was detected [35]. HAdV has been detected in suspected measles or rubella cases with rash as the primary symptom [36, 37]. While these are important indicators suggesting that the rash may be caused by adenovirus, details regarding the genotype are not clear, requiring further investigation.

Currently, there are eight known species C of HAdV—four original types, namely HAdV-C1, C2, C5, and C6, and four recombinant types, namely HAdV-C57 (P1H57F6) [38], C89 (P89H2P2) [39], C104 (P1H1F2) [40], and C108 (P1H2F2). Of the 41 strains of species C, eight had A363E and P364del mutations in the penton base sequence, which are characteristic of type 89 [39]. Of these eight strains, five had type 5 hexons and fibers, and were considered as a potential new genotype, P89H5F5. P89H5F5 was also detected in Aichi Prefecture, Japan, in 2017 (accession number: LC756200). This sequence has been submitted to the HAdV Working Group and is currently under review to determine whether it is a new genotype. Additionally, this genotype was also detected in the Kobe strain in 2018 and 2019, suggesting a small outbreak during this period in Japan, which then disappeared and was overshadowed by the COVID-19 pandemic.

The lack of respiratory virus surveillance specimens is a limitation of this study, which likely affected the number and percentage of genotypes detected. To resolve this limitation, multiplex PCR for respiratory viruses has been performed on all specimens in Kobe City since 2024. In addition, the national acute respiratory infections surveillance will begin in April 2025, which is expected to enable a more accurate identification of causative viruses of respiratory tract infections.

Conclusion

The present study highlights the prevalent genotypes of HAdV in Kobe City, Japan, using whole-genome sequencing. Whole-genome analysis of HAdV is an important approach not only for understanding local epidemics but also for identifying new recombinant events.

Limitations

This study was conducted in Kobe, Japan, and does not represent the epidemiology of a broad spectrum of adenoviruses. Another limitation is the small number of specimens for respiratory diseases. The decrease in adenovirus infections due to COVID-19 outbreak must be carefully discussed, and the fact that the surveillance function was temporarily impaired must be taken into account.

Abbreviations

HAdV	Human adenovirus
PCF	Pharyngoconjunctival fever
EKC	Epidemic keratoconjunctivitis
HFMD	Hand, foot, and mouth disease
ML	Maximum likelihood

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13104-025-07225-z.

Supplementary Material 1

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Author contributions

YT designed the research. YT, MO, EI, KA, YO, and AM conducted the study. YT, MO, and RN analyzed the data. YT and MO drafted the manuscript. All the authors have read and approved the final version of the manuscript.

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Data availability

Nucleotide sequence data generated in this study is available in the DDBJ/EMBL/GenBank databases under accession numbers LC791110–LC791189.

Declarations

Ethical approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Kobe Institute of Health (permit number: SenR3-6). The requirement for written informed consent was waived by the Ethics Committee, as this study was a part of routine surveillance activities.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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