

Isolation of a novel intertypic recombinant human mastadenovirus B2 from two unrelated bone marrow transplant recipients

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Abstract

Human adenoviruses (HAdV) are well-known opportunistic pathogens of immunocompromised adult and pediatric patients but specific associations between HAdV species or individual HAdV types and disease are poorly understood.

In this study we report the isolation of a novel HAdV-B2 genotype from two unrelated immunocompromised patients, both recipients of a hematopoietic cell transplant. In both patients, the course of HAdV infection is consistent with a scenario of reactivation of a latent virus rather than a primary opportunistic infection.

Archived HAdV PCR-positive plasma, urine, and stool specimens were processed for virus isolation and detailed molecular characterization. Virus isolates were recovered from patient 1 from PCR-positive urine specimens obtained at days 103 and 116 after transplant in association with gross hematuria, and from a stool specimen obtained 138 days after transplant in association with diarrhea. An isolate was recovered from patient 2 from a PCR-positive urine specimen. Hexon and fiber gene amplification and sequencing were carried out for initial molecular typing, identifying the isolates as an intertypic recombinant with a HAdV-11-like hexon gene and a HAdV-77-like fiber gene. Comprehensive restriction fragment length polymorphism (RFLP) analysis was performed on viral DNA purified from urine and stool isolates, and next generation whole genome sequencing was carried out on purified viral genomic DNA. The genomes of the two isolated strains are 99.5% identical and represent the same RFLP genomic variant. The identified virus is a novel HAdV-B2 genotype designated HAdV-78 exhibiting a HAdV-11-like penton base, a HAdV-11-like hexon and a HAdV-77-like fiber (P11H11F77).

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Background

Human adenoviruses (HAdV) currently constitute a group of 51 antigenic (sero) types and over 90 genotypes defined on the basis of their genomic characteristics, classified into seven

species designated human mastadenovirus A to G (HAdV-A to HAdV-G) [1–3]. HAdVs are frequently detected as opportunistic pathogens in paediatric and adult patients undergoing iatrogenic immunosuppression for bone marrow or solid organ transplantation, as well as in patients receiving chemotherapy, or with other immunocompromising conditions such as acquired immunodeficiency syndrome [4–10]. Paediatric allogeneic haematopoietic stem cell transplant recipients with an active HAdV infection resulting from either virus reactivation from a latent infection or a primary exposure are at particularly high risk of developing severe disease involving one or more organ systems [11–16].

The natural history, epidemiology and pathobiology of HAdV infections detected in immunocompromised individuals are still poorly understood. Opportunities to study specific virus–host interactions and aspects of viral pathogenesis in this complex context where multiple opportunistic infections are often detected are very limited.

Objectives

To describe the clinical and virological findings associated with the isolation of a novel intertypic recombinant human adenovirus of species HAdV-B from a paediatric and an unrelated adult haematopoietic cell transplant (HCT) recipient. This work is part of an ongoing collaborative effort to establish more comprehensive data on the impact of HAdV infections in paediatric patients undergoing HCT and to contribute to the characterization of the still poorly defined molecular epidemiology of HAdV infections in HCT recipients and other immunocompromised patient populations.

Study design

Case identification and clinical data abstraction

Patient 1 was originally investigated as part of a cohort of HAdV-positive transplant recipients assembled for an observational retrospective study performed at the Children's Hospital of Philadelphia (CHOP) in partnership with the Lovelace Respiratory Research Institute (LRRRI). Patient 2 was originally investigated based on the clinical interest it elicited and the initial virological findings. The medical charts of these two individuals were retrospectively reviewed at CHOP (patient 1) and Memorial Sloan Kettering Cancer Center (MSKCC) (patient 2), respectively.

Adenovirus detection in clinical specimens

At CHOP, the DNA was extracted from 200 µL of each clinical specimen for real-time TaqMan® quantitative PCR-based detection of adenovirus using an automated MagNAPure LC instrument and a total nucleic acid isolation kit from Roche Diagnostics (Indianapolis, IN, USA). A laboratory-developed quantitative real-time TaqMan® PCR was performed in 50-µL volumes based on the primers and probe targeting the hexon gene developed by Heim et al. [17].

For adenovirus testing, MSKCC submitted clinical specimens to a reference laboratory (Viracore, Lee Summit, MO, USA) where they were processed for a proprietary laboratory-developed quantitative PCR assay that targets two conserved regions of the HAdV hexon gene (Test code 7500, <https://www.viracor-eurofins.com/test-menu/7500-adenovirus-real-time-qpcr>).

Virus isolation and initial rounds of molecular typing

All HAdV PCR-positive clinical specimens available for patient 1 (plasma, urine and stool) and the only specimen (urine) available for patient 2 were inoculated into conventional virus-culture tubes of A549 cells (ATCC® CCL-185) for virus isolation. Infected monolayers were monitored for the development of cytopathic effect for 1 week and harvested when extensive cytopathic effect was observed. Isolates were recovered only from the stool and urine specimens from patient 1 and from the urine specimen from patient 2. Intracellular viral DNA was isolated from infected A549 cell monolayers in 75-cm² flasks for restriction enzyme analysis as previously described [18]. Briefly, ~1 µg of viral genomic DNA was digested with 10 units of each endonuclease following the manufacturer's recommendations (New England Biolabs, Ipswich, MA, USA) and digests were analysed by horizontal 1% agarose gel electrophoresis. Initial typing at the species level was accomplished by analysis of BamHI digestion profiles. A more detailed characterization was subsequently performed by digestion with BclI, BglII, BstEII, HindIII, HpaI, PstI, SmaI and XbaI, the panel of endonucleases described by Li et al. [19] for the analysis of subspecies HAdV-B2 types. Molecular typing was initially carried out by PCR amplification and sequencing of hypervariable regions 1–7 of the hexon gene and the complete fibre gene followed by BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) analysis [20]. Molecular type identities were assigned based on the identity of the closest match. For initial molecular typing of patient 2's specimen, total DNA was extracted from 200 µL of the sample using a NUCLESENS easyMAG (bioMérieux, Durham, NC, USA) and processed for PCR amplification of hexon gene hypervariable regions 1–6 using the primers and cycling conditions developed by Okada et al. [21]. Sanger sequencing of the amplicon was performed on a 3130 analyser (ThermoFisher Scientific Inc., Waltham, MA, USA) using the amplification primers. The resulting sequences were assigned a hexon type identity by BLAST analysis.

Complete genomic sequencing of adenovirus genomes

Viral DNA purified from a urine isolate from patient 1 (2146-13-VT10810), and from the urine isolate obtained from patient 2 were further processed for whole-genome sequencing. Next-generation whole-genome sequencing was performed on an Illumina MiSeq using the standard protocol recommended by the manufacturer (Illumina Inc., San Diego, CA, USA). In brief, barcoded libraries were prepared with the Nextera XT DNA Library Prep kit (Illumina Inc.) and quantified using the Qubit dsDNA HS assay (ThermoFisher Scientific Inc.). The library size was determined using the Agilent Bio-analyser (Agilent Technologies, Santa Clara, CA, USA). Paired-end sequencing with the 2 × 250 bp kit was performed using the MiSeq 500 cycle v2

TABLE 1. Nucleotide and predicted amino acid differences between HAdV-78 strains 2146-13-VT10870 (KT970441) and NYS12/12146 (KT970442)

Nucleotide position	Gene	CDS	Nucleic acid change		Amino acid change	
			KT970442	KT970441	KT970442	KT970441
120	ITR	NCR	T	C	NCR	NRC
3036	E1B	E1B large T antigen	T	C	SYN	SYN
4903	IVa2	IVa2	A	G	Ile	Met
6254	E2B	DNA polymerase	T	C	Ser	Pro
16335–16637	pTP/E2B/L1	NCR	—	TTT	NCR	NCR
17205	L2	x core	G	A	SYN	SYN
17320–17323	NCR	NCR	—	AAAA	NCR	NCR
23204	E2A	ssDNA binding protein	A	C	Glu	Asp
29867	E3	E3 RID-β	T	C	Leu	Ser
30170	E3	E3 RID-beta	C	A	SYN	SYN
32486	E4	E4 control protein (34 KDa)	A	T	SYN	SYN
33852	E4	E4 control protein ORF2	C	T	SYN	SYN
34260	E4	E4 control protein ORF1	G	A	Arg	Lys
34680	ITR	NCR	A	G	NTR	NTR

ITR, inverted terminal repeat; NCR, non-coding region; SYN, synonymous.
 Non-synonymous mutations and their corresponding locations and resulting amino acid changes are presented in bold font.

kit. Contiguous sequences (contigs) were *de novo* assembled using the SPAdes 3.5 application in BASESPACE (Illumina Inc.), and the resulting contigs from each of the samples were used in all further genetic analyses.

Genomic sequence data analysis

The complete genomic sequences of the prototype strains of HAdV-7 (Gomen/GenBank AY594255), HAdV-11 (Slobitski/GenBank AF32578), HAdV-34 (Compton/GenBank AY737797), HAdV-35 (Holden/GenBank AY271307), and that of a recently described intertypic recombinant P35H34F7 (HAdV-77, strain DEU/HEIM_00092/1985/GenBank KF268328) were included in the analysis. These genomic sequences and those obtained in this study for strains CHOP2146/10810 and NY12/12146 were aligned using MAFFT in GENEIOUS R11 (Biomatters Inc., Auckland, New Zealand, www.geneious.com). Multiple alignments were further analysed with SIMPLOT [22] using the genome of urine isolate 2146-13-VT10810 as a query sequence. SIMPLOT settings were set at a window of 200 bp with a step of 20 bp. The neighbour-joining phylogenetic tree was constructed based on the Kimura two-parameter model [23,24] using MEGA X software (<https://www.megasoftware.net>) with 500 bootstrap replicates.

In silico restriction enzyme analysis of viral genomes was carried out using GENEIOUS R11.

Results

Case reports

Patient 1 was a 17-year-old male who received a matched unrelated B- and T-cell-depleted peripheral haematopoietic cell transplant for high-risk acute lymphoblastic leukaemia

with induction failure. He was conditioned with total body irradiation, cyclophosphamide and thiotepa and received cyclosporine for prophylaxis against graft-versus-host disease (GVHD). His initial post-transplant course was complicated by mucositis, *Capnocytophaga* sp. bacteraemia, and stage III skin GVHD, that was controlled with prednisone and tacrolimus. The patient was discharged home 29 days after transplant (day +29) but had to be readmitted on day +33 for gross haematuria, urinary frequency, urgency and dysuria. At that time, his urine and blood were both PCR-positive for BK virus but negative for HAdV. He was given cidofovir at a dose of 0.25 mg/kg for presumed BK virus haemorrhagic cystitis and his haematuria resolved on day +40. Bone marrow studies showed absence of minimal residual disease and 100% donor engraftment by day +35, and he was eventually discharged to home on day +47. One week later he presented at the emergency department complaining of fever, nausea, vomiting, diarrhoea, weight loss and poor oral intake. His initial work-up including blood cultures, stool cultures and *Clostridium difficile* was negative. Viral PCR tests of his blood for cytomegalovirus (CMV), Epstein–Barr virus (EBV) and HAdV were negative. A viral gastrointestinal PCR panel that includes HAdV-40 and HAdV-41 was also negative. A lumbar puncture was negative for leukaemia and negative for CMV, EBV, human herpesvirus-6, HAdV, enterovirus and parechovirus by PCR. Upper gastrointestinal endoscopy revealed oesophagitis caused by *Candida albicans* and *Candida tropicalis* and the patient was started on fluconazole. After 21 days of hospitalization, the patient was discharged to home but was readmitted on day +99 for poor nutrition and skin and liver GVHD. His GVHD was treated with tacrolimus and methylprednisolone followed by prednisone. On day +103 he had recurrence of gross haematuria and this time, both his urine and blood

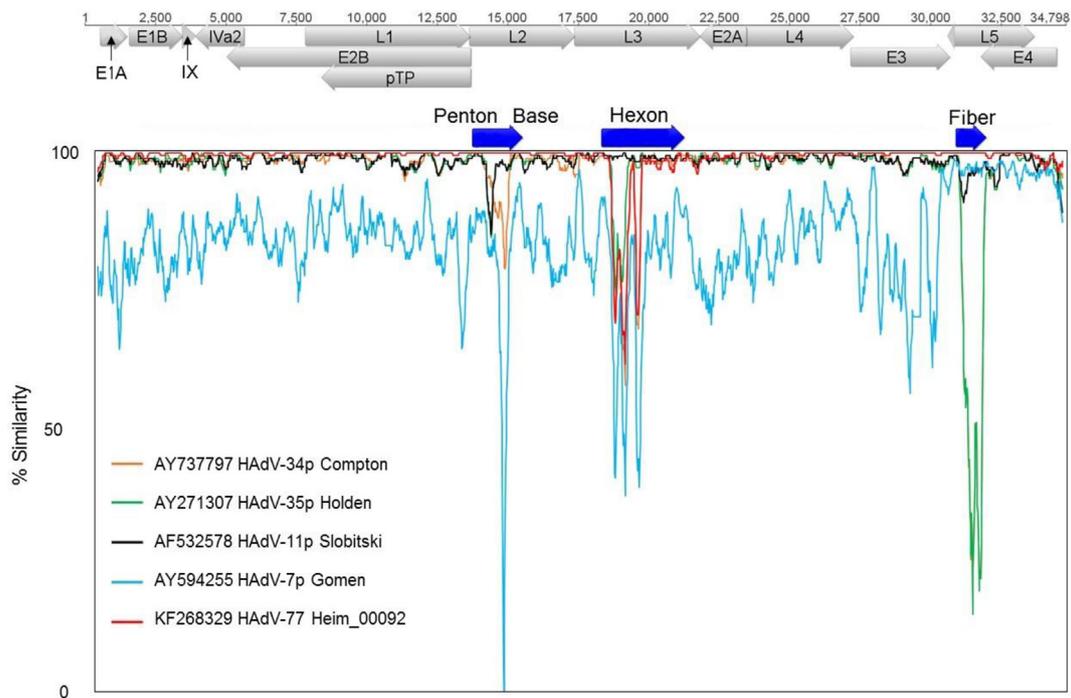


FIG. 1. Analysis of sequence similarities between isolate 2146-13-VT10810 and closely related human adenovirus B (HAdV-B) types HAdV-7, HAdV-11, HAdV-34, HAdV-35 and HAdV-77. Plots of similarity were generated by SIMPLOT [22]. Each curve is a comparison between the genome being analysed and the genome of urine isolate 2146-13-VT10810 as a query sequence. Each point plotted is the percentage identity within a 200 bp wide sliding window centred on the position plotted, with a step size between points of 20 bp (GapSrip:On, J-C Correction: On). The horizontal bars above the curves are a cartoon of the coding regions of the HAdV-B genome. The colours indicate the type to which that part of the genome is most similar, based on the plot below.

tested positive for HAdV (Ct 22.4 and Ct 39.66, respectively) and BK virus. Cidofovir was restarted at a dose of 0.25 mg/kg and then increased up to 2 mg/kg administered weekly for a total of six doses. Despite this therapy his presentation progressed to kidney failure requiring dialysis. At this time

(day + 138) a plasma specimen, a nasopharyngeal aspirate and a stool specimen tested positive for HAdV by PCR with Ct values of 28.88, 41.99 and 25.39, respectively. These PCR test results were obtained in the setting of radiographic evidence of pneumonitis and diarrhoea, raising concern for

TABLE 2. Analysis of sequence similarities between clinical isolates 2146-13-VT18017 and NY12/12146, and closely related viruses of species HAdV-B

Gene	Nucleotide or amino acid	Strain ID	HAdV-7p (Gomen) AY594255	HAdV-11p (Slobitski) AF32578	HAdV-34p (Compton) AY737797	HAdV-35p (Holden) AY271307	HAdV-77 (Heim-00092) KF268328
Penton base	Nt	2146-13-VT10810	80.4%	97.3%	94.2%	97.3%	100%
	Nt	NY12/12146	80.4%	97.3%	94.2%	97.3%	100%
	AA	2146-13-VT10810	84.4%	98%	95.4%	97.9%	100%
	AA	NY12/12146	84.8%	98%	95.4%	97.9%	100%
Hexon	Nt	2146-13-VT10810	78.9%	98.8%	92%	95.3%	91.7%
	Nt	NY12/12146	78.9%	98.8%	92%	95.5%	91.7%
	AA	2146-13-VT10810	85.7%	99.3%	92.2%	95.1%	92.3%
	AA	NY12/12146	86%	99.3%	92.2%	95.1%	92.3%
Fibre	Nt	2146-13-VT10810	96.2%	94.8%	67.5%	67.7%	100%
	Nt	NY12/12146	96.2%	94.8%	67.5%	67.7%	100%
	AA	2146-13-VT10810	95.1%	94.5%	61.8%	61.8%	100%
	AA	NY12/12146	95.1%	94.5%	61.8%	61.8%	100%
Whole-genome sequence		2146-13-VT10810	83.7%	98.4%	96.9%	97.4%	99%
		NY12/12146	83.6%	98.3%	96.9%	97.3%	99%

AA, amino acid, Nt, nucleotide.

disseminated adenoviral disease. His hospitalization was further complicated by *Staphylococcus epidermidis* bacteraemia, and by CMV and EBV reactivation that were managed with valganciclovir and rituximab, respectively. Unfortunately, on day +150 the patient died. His death was considered possibly attributable to disseminated HAdV disease.

Patient 2 was a 50-year-old male with chronic lymphocytic leukaemia and small lymphocytic lymphoma treated with pentostatin, rituximab, fludarabine and bendamustine who was admitted to MSKCC for a double umbilical cord HCT. His medical history was significant for an episode, 3 years previously, of HAdV-associated haemorrhagic cystitis treated with intravenous cidofovir, and for a viscus perforation requiring an ileocollectomy. His post-transplant course was significant for fever close to the time of engraftment, rhinorrhoea, watery eyes and cough. At day +12 a nasopharyngeal specimen tested negative with the FilmArray RVP version 1.6 respiratory virus panel (Idaho Technology, Salt Lake City, UT, USA). At day +19 a plasma specimen tested positive for HAdV (1200 copies/mL) but negative for CMV and EBV. At day +32, in the context of dysuria and haematuria, a urine specimen tested positive for HAdV (8.1×10^6 copies/mL) and HAdV viraemia was still positive and elevated (43 200 copies/mL). He also tested positive for human herpesvirus-6. Over the following 3 weeks, to address his persistent HAdV viruria and viraemia, the patient was treated with intravenous cidofovir at a dose of 3–5 mg/kg. Urine samples were tested multiple times for BK virus during the course of his hospitalization and were always negative. His condition worsened in the context of significant HAdV viraemia and viruria, further complicated by CMV reactivation, fatigue and weakness on walking and recurrent fevers, even under broad-spectrum antibiotic treatment. At day +45 in the context of diarrhoea, a stool specimen tested positive for HAdV. Renal failure limited cidofovir use. At day +124 the HAdV load in urine was 11×10^6 copies/mL and at day +132 the patient died.

Molecular typing of virus isolates

The initial molecular typing for patient 1 isolates was carried out by PCR amplification and sequencing of hexon and fibre genes, and by restriction fragment length polymorphism (RFLP) analysis of viral DNA purified from a stool isolate from a specimen collected 138 days after the transplant (+138), and from two urine isolates from specimens collected at days +103 and +116. The three isolates exhibited identical HAdV-11-like partial hexon gene sequences and identical HAdV-7-like fibre gene sequences (data not shown). The urine isolate from patient 2 was initially typed as HAdV-11-like based on PCR amplification and sequencing of hypervariable regions 1–6 of the hexon gene.

RFLP analysis with a panel of nine endonucleases showed the three clinical isolates from patient 1 and the urine isolate from patient 2 to correspond to subspecies HAdV-B2, to be identical to one another (data not shown), and also to be highly similar to paediatric respiratory isolate Arg10817/00, an intertypic recombinant with an HAdV-11-like hexon and an HAdV-7-like fibre gene originally typed as RFLP variant 11c4 [20].

Complete genomic sequences were generated for urine isolate 2146-13-VT10810 from patient 1, and for urine isolate NYS12/12146 from patient 2. Sequences were deposited in GenBank under accession numbers KT970441 and KT970442. The sequences corresponded to a novel subspecies HAdV-B2 genotype and were 99.5% identical, with only 19 nucleotide differences scattered along the entire genome as described in Table 1. SIMPLOT analysis (Fig. 1) identified the virus as an intertypic recombinant with a type 11-like penton base gene, a type 11-like hexon gene and a type 77-like fibre gene (P11H11F77). A detailed comparison of nucleotide and amino acid sequence similarities with other species HAdV-B types included in the analysis is presented in Tables 2 and 3.

The Human Adenovirus Working Group (<http://hadvwg.gmu.edu>) assigned number 78 to designate the novel genotype reported in this paper. Phylogenetic analysis (Fig. 2) revealed HAdV-78 to be most closely related to genotype HAdV-77, which was recently described by bioinformatics analysis of the whole-genome sequence of a German isolate from 1985 (KF268328).

The restriction profiles obtained *in silico* for the genomes of the two isolated strains of HAdV-78 together with those of other closely related genotypes of species HAdV-B are shown in Fig. 3. Consistent with the results of our initial gel-based RFLP analysis, strains 2146-13-VT10810 and NYS12/12146 yielded identical profiles with nine different endonucleases. The analysis of *in silico*-generated profiles also confirmed the close resemblance between the genomes of the HAdV-78 isolates and HAdV-77 demonstrated by our phylogenetic analysis.

TABLE 3. Analysis of sequence similarities between clinical isolate 2146-13-18017 and closely related viruses of the same molecular type identity (P11H11F7)

Strain ID	CDS	Nucleotide or amino acid	NYS12/12146	Arg18017/2000
2146-13-VT10810	Penton	Nt	100%	NA
		AA	100%	NA
	Hexon	Nt	100%	99.9% (X034750) ^a
		AA	100%	100%
	Fibre	Nt	100%	99.8% (X034751) ^a
		AA	100%	100%
WGS	Nt		99.5%	99.4%

AA, amino acid; CDS, coding sequence; NA, sequence not available; Nt, nucleotide; WGS, whole-genome sequence.
^aDescribed in Kajon et al. 2013 [20].

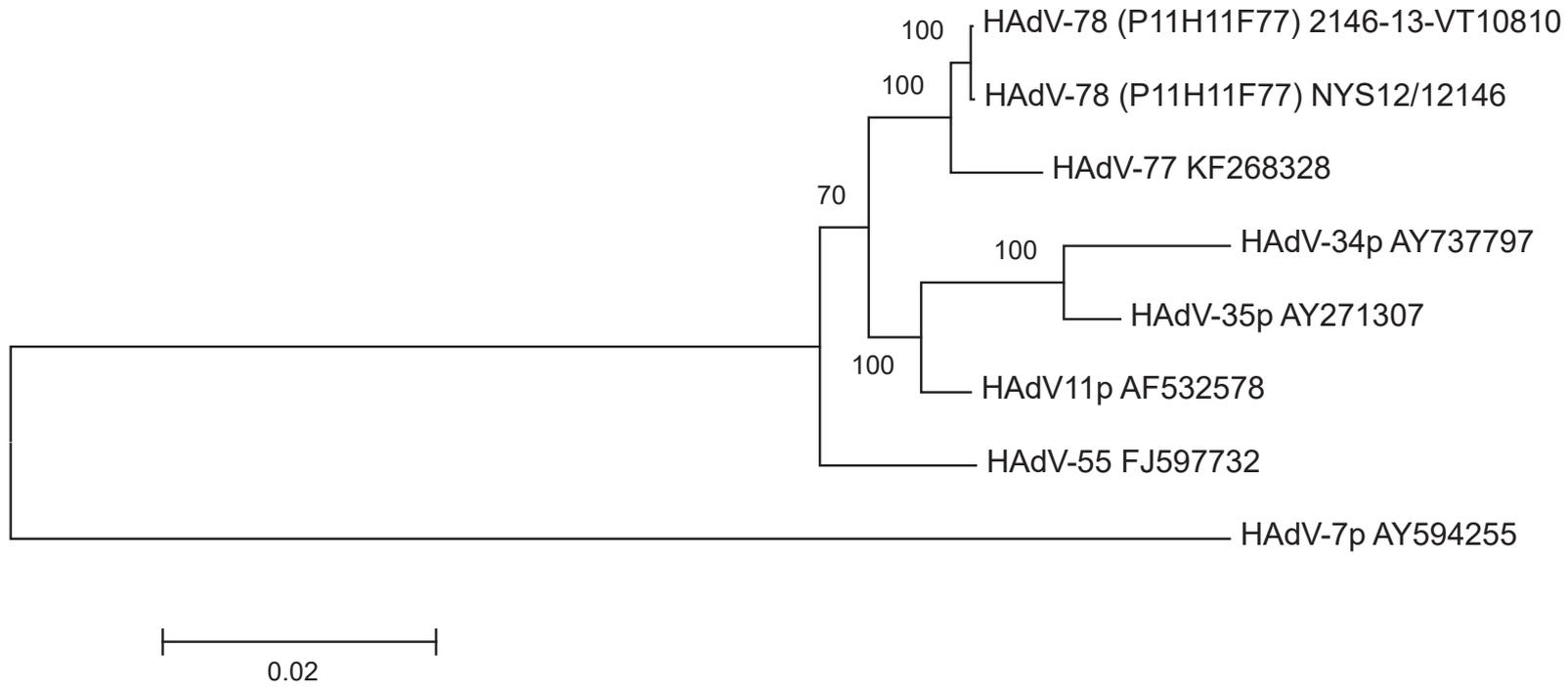


FIG. 2. Phylogenetic analysis of whole-genome sequences of subgroup B human adenoviruses (HAdV). Eight whole-genome sequences including those of HAdV-7p (AY594255), HAdV-11p (AF532578), HAdV-34p (AY737797), HAdV-35p (AY271307), HAdV-55 (FJ597732), HAdV-77 (KF268328), and those obtained in this study for the newly identified HAdV-78 strains 2146-13-VT10810 and NYS12/12146 were aligned using MAAFT in GENEIOUS R1.1 (Biomatters, Auckland, New Zealand; www.geneious.com). The phylogenetic tree was reconstructed in MEGAX using the neighbour-joining method based on the Kimura two-parameter model with 500 bootstrap replicates. Support values <70 not shown.

Discussion

A number of novel HAdV genotypes representing examples of intertypic recombination have been recently described through the molecular characterization of clinical isolates or by direct next-generation whole-genome sequencing of viral DNA recovered from patient specimens [25–28]. HAdV-78 is a novel subspecies HAdV-B2 genotype, closely related phylogenetically to other members of the subspecies and in particular to HAdV-77, an intertypic recombinant (P35H34F7) isolated in Germany in 1985 from an unreported source and described in the GenBank accession as corresponding to serotype 34 (KF268328).

The high similarity of the restriction profiles and the identical predicted amino acid sequences of hexon and fibre proteins

suggest that the virus reported in association with a paediatric case of acute respiratory infection sampled in 2000 in Argentina [20] may represent a genomic variant of HAdV-78. The identical hexon and fibre genes also support the hypothesis that HAdV-78 may neutralize as serotype 11 based on the serology data reported for Argentina strain 18017/2000 [20]. Although no sequence or RFLP analysis data are available for the HAdV-B detected in a blood specimen of a neonatal case of severe disseminated HAdV following water delivery, the reported description of a recombinant virus possessing an HAdV-11-like hexon gene and an HAdV-7-like fibre gene with comparable percentage similarities to reference strains [29] is strongly suggestive of a closely related genome.

Taken together, the clinical and virology diagnostic test data available for the two unrelated individuals presented in

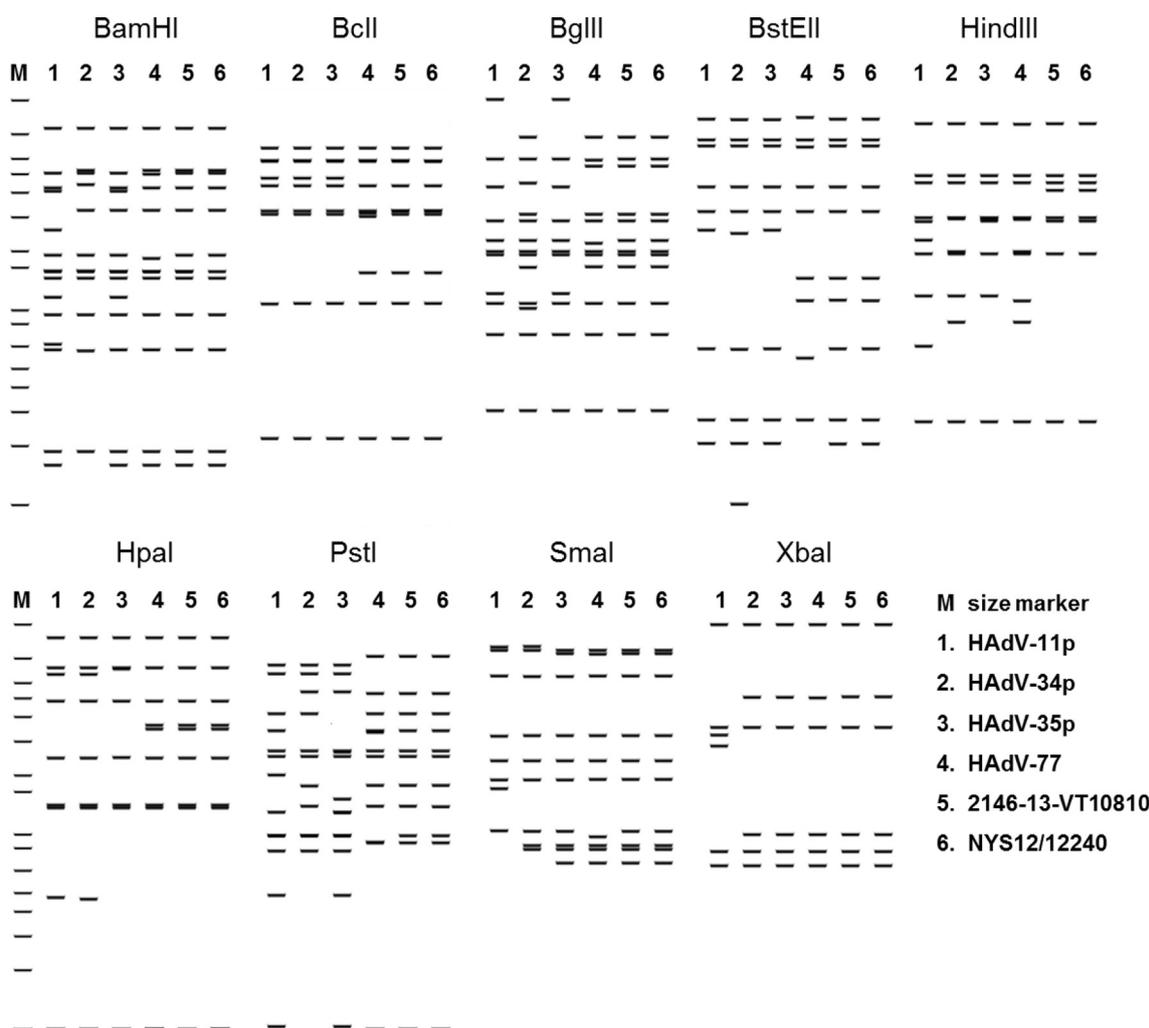


FIG. 3. *In silico* restriction enzyme analysis of HAdV-78 and closely related genotypes of species human adenovirus B (HAdV-B). Virtual restriction enzyme analysis of viral genomic DNAs of urine isolates in comparison with those of genomic DNA of HAdV-11 (JN226748), HAdV-34 (JN226749) and HAdV-35 (JN226761), and HAdV-77. M: virtual 1 kb Plus™ ladder (100–12 000 bp, Life Technologies, Carlsbad, CA, USA).

this study, and the information available for the above mentioned unrelated cases described in the cited publications, support the hypothesis that infection by HAdV-78 is initially acquired through the respiratory tract and that the virus has the ability to persist in the gut and possibly also in the renal epithelium or vasculature, from where it can reactivate and be shed under certain immunocompromising or stressful conditions.

The detection of other HAdV-B2 types—including serologically ‘intermediate’ strains—has been reported in the allograft tissue and in the urine of renal transplant recipients, and also in the urine of HCT recipients and other immunocompromised patients presenting with symptoms of haemorrhagic cystitis, fever, renal dysfunction, or systemic disseminated disease [30–34]. These findings support the hypothesis that this subgroup of HAdV-B features the distinct ability to establish a latent infection in the kidney and become active again as a consequence of immunological impairment. Interestingly, the isolation from urine specimens of intertypic recombinants with restriction enzyme digestion profiles similar to those of HAdV-77 and HAdV-78 was actually reported in the USA in the 1980s [35,36]. Unfortunately, the lack of available sequence data for these strains prevented their inclusion in our phylogenetic analysis.

Although molecular typing data were not retrievable for the HAdV genome detected by PCR in the nasopharyngeal aspirate obtained from patient 1 on day +138 concomitantly with radiographic evidence for pneumonitis, or from the stool specimen obtained from patient 2 at day +45, the clinical evidence interpreted in conjunction with available virology data is strongly indicative of a disseminated HAdV infection in both individuals.

Ethical approval

The study was approved by the Institutional Ethics and Review Committees of the Children’s Hospital of Philadelphia and Memorial Sloan Kettering Cancer Center. Because the examined clinical specimens were de-identified before the study, the protocol was exempt from IRB review at the Lovelace Respiratory Research Institute and Wadsworth Center.

Author contributions

AEK contributed to funding acquisition, supervision, conceptualization, investigation, data curation, writing and original draft preparation. DL contributed to methodology, investigation, analysis and data curation. ES contributed to original case

report draft preparation; NEB contributed to original draft preparation, diagnostic test data curation, reviewing and editing. AMC contributed to supervision and diagnostic test data curation. BF contributed to funding acquisition, supervision, reviewing and editing; and KSG contributed to funding acquisition, reviewing and editing.

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Conflicts of interest

The authors have no competing interests or conflicts to declare.

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References

- [1] Wold WSM, Ison MG. Adenovirus. In: Knipe DM, Howley PM, editors. *Fields virology*. 6th ed. Philadelphia: Lippincott, Williams & Wilkins; 2013. p. 1732–67.
- [2] Houldcroft CJ, Beale MA, Sayeed MA, Qadri F, Dougan G, Mutreja A. Identification of novel adenovirus genotype 90 in children from Bangladesh. *Microb Genom* 2018;4(10). <https://doi.org/10.1099/mgen.0.000221>.
- [3] Dhingra A, Hage E, Ganzenmueller T, Böttcher S, Hofmann J, Hamprecht K, et al. Molecular evolution of human adenovirus (HAdV) species C. *Sci Rep* 2019;9:1039.
- [4] Hale GA, Heslop HE, Krance RA, Brenner MA, Jayawardene D, Srivastava DK. Adenovirus infection after pediatric bone marrow transplantation. *Bone Marrow Transplant* 1999;23:277–82.
- [5] de Mezerville MH, Tellier R, Richardson S, Hébert D, Doyle J, Allen U. Adenoviral infections in pediatric transplant recipients: a hospital-based study. *Pediatr Infect Dis J* 2006;25:815–8.
- [6] Echavarría M. Adenoviruses in immunocompromised hosts. *Clin Microbiol Rev* 2008;21:704–15.
- [7] Tebruegge M, Curtis N. Adenovirus infection in the immunocompromised host. *Adv Exp Med Biol* 2010;659:153–74.
- [8] Lion T. Adenovirus infections in immunocompetent and immunocompromised patients. *Clin Microbiol Rev* 2014;27:441–62.
- [9] Lynch JP, Kajon AE. Adenovirus: epidemiology, global spread of novel serotypes, and advances in treatment and prevention. *Semin Respir Crit Care Med* 2016;37:586–602.

- [10] Ison MG, Hirsch HH. Community-acquired respiratory viruses in transplant patients: diversity, impact, unmet clinical needs. *Clin Microbiol Rev* 2019;32. pii: e00042-19.
- [11] Morfin F, Boucher A, Najjoulah F, Bertrand Y, Bleyzac N, Poitevin-Later F, et al. Cytomegalovirus and adenovirus infections and disease among 75 pediatric unrelated allogeneic bone marrow transplant recipients. *J Med Virol* 2004;72:257–62.
- [12] Mynarek M, Ganzenmueller T, Mueller-Heine A, Mielke C, Gonnermann A, Beier R, et al. Patient, virus, and treatment-related risk factors in pediatric adenovirus infection after stem cell transplantation: results of a routine monitoring program. *Biol Blood Marrow Transplant* 2014;20:250–6.
- [13] Kosulin K, Geiger E, Vécsei A, Huber WD, Rauch M, Brenner E, et al. Persistence and reactivation of human adenoviruses in the gastrointestinal tract. *Clin Microbiol Infect* 2016;22:381. e1–8.
- [14] Boge CLK, Fisher BT, Petersen H, Seif AE, Purdy DR, Galetaki DM, et al. Outcomes of human adenovirus infection and disease in a retrospective cohort of pediatric solid organ transplant recipients. *Pediatr Transplant* 2019;23:e13510.
- [15] Dailey Garnes NJM, Ragoonanan D, Aboulhosn A. Adenovirus infection and disease in recipients of hematopoietic cell transplantation. *Curr Opin Infect Dis* 2019;32:591–600.
- [16] Lion T. Adenovirus persistence, reactivation, and clinical management. *FEBS Lett* 2019 Aug 14. <https://doi.org/10.1002/1873-3468.13576>.
- [17] Heim A, Ebnet C, Harste G, Pring-Akerblom P. Rapid and quantitative detection of human adenovirus DNA by real-time PCR. *J Med Virol* 2003;70:228–39.
- [18] Kajon AE, Erdman DD. Assessment of genetic variability among subspecies B1 human adenoviruses for molecular epidemiology studies. *Methods Mol Med* 2007;131:335–55.
- [19] Li QG, Hambraeus J, Wadell G. Genetic relationship between thirteen genome types of adenovirus 11, 34, and 35 with different tropisms. *Intervirology* 1991;32:338–50.
- [20] Kajon AE, de Jong JC, Dickson LM, Arron G, Murtagh P, Viale D, et al. Molecular and serological characterization of species B2 adenovirus strains isolated from children hospitalized with acute respiratory disease in Buenos Aires, Argentina. *J Clin Virol* 2013;58:4–10.
- [21] Okada M, Ogawa T, Kubonoya H, Yoshizumi H, Shinozaki K. Detection and sequence-based typing of human adenoviruses using sensitive universal primer sets for the hexon gene. *Arch Virol* 2007;152:1–9.
- [22] Lole KS, Bollinger RC, Paranjape RS, Gadkari D, Kulkarni SS, Novak NG, et al. Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. *J Virol* 1999;73:152–60.
- [23] Kimura M. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–20.
- [24] Kumar S, Stecher G, Li M, Knyaz C, Tamura K, Mega X. Molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 2018;35:1547–9.
- [25] Kajon AE, Dickson LM, Murtagh P, Viale D, Carballal G, Echavarría M. Molecular characterization of an adenovirus 3-16 intertypic recombinant isolated in Argentina from an infant hospitalized with acute respiratory infection. *J Clin Microbiol* 2010;48:1494–6.
- [26] Kajon AE, Lamson D, Shudt M, Oikonomopoulou Z, Fisher B, Klieger S, et al. Identification of a novel intertypic recombinant species D human adenovirus in a pediatric stem cell transplant recipient. *J Clin Virol* 2014;61:496–502.
- [27] Hage E, Gerd Liebert U, Bergs S, Ganzenmueller T, Heim A. Human mastadenovirus type 70: a novel, multiple recombinant species D mastadenovirus isolated from diarrhoeal faeces of a haematopoietic stem cell transplantation recipient. *J Gen Virol* 2015;96:2734–42.
- [28] Hashimoto S, Gonzalez G, Harada S, Oosako H, Hanaoka N, Hinokuma R, et al. Recombinant type Human mastadenovirus D85 associated with epidemic keratoconjunctivitis since 2015 in Japan. *J Med Virol* 2018;90:881–9.
- [29] Soileau SL, Schneider E, Erdman DD, Lu X, Ryan WD, McAdams RM. Case report: severe disseminated adenovirus infection in a neonate following water birth delivery. *J Med Virol* 2013;85:667–9.
- [30] Harnett GB, Bucens MR, Clay SJ, Saker BM. Acute haemorrhagic cystitis caused by adenovirus type 11 in a recipient of a transplanted kidney. *Med J Aust* 1982;1:565–7.
- [31] Hierholzer JC, Adrian T, Anderson LJ, Wigand R, Gold JW. Analysis of antigenically intermediate strains of subgenus B and D adenoviruses from AIDS patients. *Arch Virol* 1988;103:99–115.
- [32] Miyamura K, Takeyama K, Kojima S, Minami S, Matsuyama K, Morishima Y, et al. Hemorrhagic cystitis associated with urinary excretion of adenovirus type 11 following allogeneic bone marrow transplantation. *Bone Marrow Transplant* 1989;4:533–5.
- [33] Abe T, Furukawa T, Masuko M, Sugimoto A, Okazuka K, Honma K, et al. Sequential adenovirus infection of type 14 hemorrhagic cystitis and type 35 generalized infection after cord blood transplantation. *Int J Hematol* 2009;90:421–5.
- [34] Pettengill MA, Babu TM, Prasad P, Chuang S, Drage MG, Menegus M, et al. Probable donor-derived human adenovirus type 34 infection in 2 kidney transplant recipients from the same donor. *Open Forum Infect Dis* 2018;6:ofy354.
- [35] de Jong PJ, Valderrama G, Spigland I, Horwitz MS. Adenovirus isolates from urine of patients with acquired immunodeficiency syndrome. *Lancet* 1983;1(8337):1293–6.
- [36] Flomenberg PR, Chen M, Munk G, Horwitz MS. Molecular epidemiology of adenovirus type 35 infections in immunocompromised hosts. *J Infect Dis* 1987;155:1127–34.