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Isolation of an Intertypic Recombinant Human Adenovirus (Candidate Type 56) from the Pharyngeal Swab of a Patient with Pharyngoconjunctival Fever

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Human adenoviruses (HAdVs) are non-enveloped, double-stranded DNA viruses of the family *Adenoviridae*, genus *Mastadenovirus*. HAdVs have been categorized into seven species, A to G, on the basis of various biological and morphological criteria, nucleic acid characteristics, and homologies. The main tropism sites of HAdVs are determined by the species (Table 1).

HAdV-15/29/H9 reported by Kaneko et al. (1) is a novel intertypic recombinant type of adenovirus. HAdV candidate type 56 (HAdV-56) (2) has the same sequence as HAdV-15/29/H9.

HAdV-15/29/H9 (HAdV-56) caused epidemic keratoconjunctivitis (EKC) throughout Japan (1) and other countries (2). In this short report, we present the first case of pharyngoconjunctival fever (PCF) caused by

Table 1. Species of human adenoviruses and their main tropism

Species	Adenovirus type	Main tropism site
A	12, 18, **31**	Gut
B1	**3**, **7**, 16, 21, 50	Respiratory tract and/or eye
B2	**11**, 14, 34, 35, 55*	Urogenital system, respiratory tract (severe infection among immunocompromised patient)
C	**1**, **2**, **5**, **6**	Respiratory tract (ARD among children) and gut
D	**8**–10, 13, 15, 17, **19**, 20, 22–30, 32, 33, 36, **37**–39, 42–49, 51, **53**, **54**, **56***	Eye (epidemic keratoconjunctivitis)
E	**4**	Respiratory tract
F	**40**, **41**	Gut
G	52	Gut

* Human adenovirus types 1–56 are shown in this table.
Those most commonly associated with particular syndromes are in bold type.
The asterisk indicates the candidate type as of May 2012.

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Table 2. Complete nucleotide sequences of hexon-, fiber-, and penton base-coding regions of the HAdV in this study

Strain	Definition	Accession no.	bp	Reference	Hyogo6180 strain		
					Accession no. AB719408 2,856 bp	AB719409 1,089 bp	AB719410 1,560 bp
					Hexon-coding region	Fiber-coding region	Penton base-coding region
HAdV-15/29/H9 (isolate, 2307-S)	full genome	AB562588	35,067	1	100% identical (position, 17,727–20,582)	100% identical (position, 30,896–31,984)	100% identical (position, 13,486–15,045)
HAdV-56 (candidate)	full genome	HM770721	35,066	2	100% identical (position, 17,726–20,581)	100% identical (position, 30,895–31,983)	100% identical (position, 13,485–15,044)

HAdV-56.

On May 12, 2011, an immunocompetent 11-year-old boy from Himeji city, Hyogo Prefecture, developed a fever of 38.0°C. The following day, when his fever increased to 39.0°C, he visited Okafuji Pediatric Clinic in Himeji. He presented with symptoms of upper respiratory tract infection, such as red pharynx, as well as excessive redness of the right eye. Based on his clinical presentation, the patient was diagnosed with PCF but not with EKC. A bedside immunochromatographic test (Check Ad; Alfresa Pharma Corp., Osaka, Japan) conducted using a pharyngeal swab confirmed the presence of HAdV. Another pharyngeal swab was collected for type identification, as a part of the protocol for infectious agent surveillance in Hyogo Prefecture. Informed written consent for the laboratory test was obtained from the patient's guardian. The patient did not have any significant underlying disease.

The virus was isolated from the pharyngeal swab sample, as described previously (3). Briefly, the sample was inoculated into A549 and RD-18S cell lines. On the 7th day of the culture, cultured cells were passaged to a fresh culture of the same cell type. The typical cytopathic effect (CPE) of adenovirus appeared 6 days after passage into A549 cells. Nevertheless, no viral growth was observed in RD-18S cells. After culture isolation (strain: Hyogo6180), complete sequences of the hexon- (accession no. AB719408), fiber- (accession no. AB719409), and penton- (accession no. AB719410) coding regions were determined by the direct sequencing method, as described previously (1). All these sequences of the isolate were identical to those of HAdV-15/29/H9 (1) and HAdV-56 (2) (Table 2).

HAdV-56 had originally isolated in France in 2008 from the pulmonary biopsy of a 10-day-old neonate who had died because of a fatal respiratory infection, and from the conjunctival swabs of three healthcare workers who cared for the neonate and subsequently developed keratoconjunctivitis (2). An identical strain has been identified from cases of EKC throughout Japan since 2008. Genome sequence analysis revealed that the new strain in Japan is actually a recombinant virus carrying genetic material from different species D HAdVs, i.e., type 15 (and 29) in the hexon-conding region (hyper variable region), type 9 (and 26) in the penton-coding region, and type 9 in the fiber-coding region. Therefore, the new strain was designated as a novel intertypic recombinant, AdV-15/29/H9 (1). Robinson et al. (2) later performed a computational analysis of the genome sequence of the strain isolated in France from both the neonate and a healthcare worker, and found extensive recombination between HAdV-9, -15, -26, -29, and/or another adenovirus. They assigned the strain as a novel type, HAdV-56.

The present study is the first to indicate HAdV-56 as a causative agent of PCF. PCF is a highly contagious disease that affects young children more frequently than adults, resulting in outbreaks among institutionalized children. Worldwide data show that HAdV-3, HAdV-7 (species B), HAdV-1, HAdV-2, HAdV-5, HAdV-6 (species C), and HAdV-4 (species E) are the most frequent causative agents of PCF (4,5). In PCF, the virus infects epithelial cells of the respiratory tract and may or may not cause conjunctiva. The isolation of HAdV-56 from a patient with PCF signifies that specimens from respiratory tract infection cases should be examined for the presence of HAdV, including type 56. To identify HAdV-56, adenoviral fiber- and penton base-coding regions should be sequenced in addition to the hexon-coding region.

Since 2011, HAdV-56 has been included in reports to the National Epidemiological Surveillance of Infectious Disease (NESID) system in Japan. A total of 30 isolates were reported until April 2012, and 27 (90%) of these isolates came from the eye swabs of EKC patients. Following our isolation of HAdV-56 from PCF, another PCF case (detection from pharyngeal swab) and a ''fever of unknown origin'' (detection from feces) case associated with HAdV56 were reported (detailed data were not available). The tissue tropism of HAdV-56 has not yet been sufficiently resolved. This case underscores the necessity for continuous surveillance in order to understand the epidemiological nature of HAdV-56.

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Conflict of interest None to declare.

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