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Epidemic Keratoconjunctivitis Cases Resulting from Adenovirus Types 8 and 54 Detected at Fukuoka University Hospital between 2014 and 2015

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Epidemic keratoconjunctivitis (EKC) is mainly caused by various types of *Human mastadenovirus* type *D* (HAdV), including HAdV-8, HAdV-64 (previously known as 19a), HAdV-37, HAdV-53, HAdV-54, and HAdV-56, and with less severe manifestations by *H. mastadenovirus* types *B* and *E*. Infectious outbreaks often result in potentially severe clinical manifestations of EKC. After the first reports of HAdV-53 in 2007, EKC epidemics caused by HAdV-53, HAdV-54 and HAdV-56 have frequently been observed in Japan. Here, we retrospectively detected and typed EKC cases resulting from HAdV-8 and HAdV-54 at the Fukuoka University Hospital and compared their clinical manifestations.

This study focused on adenoviral EKC at the Fukuoka University Hospital; therefore, the enrolment criteria for patients were clinical diagnosis of EKC and a positive result obtained by an immunochromatography (IC) kit (Adenocheck, Santen Co. Ltd., Osaka, Japan). Conjunctival swab specimens from patients were collected from 2014 through 2015, and were stored in a refrigerator until they were used for polymerase chain reaction (PCR) amplifications. This study was approved by the Ethics Committee of Fukuoka University (16-2-24). Deoxyribonucleic acid was extracted from these specimens using a High Pure Viral Nucleic Acid Kit (Roche, Mannheim, Germany), and real-time PCR and nested PCR were performed as reported previously (1,2). In addition, the hexon loop 1 region was amplified using nested PCR (3), sequenced using a direct sequencing method, and typed by BLAST comparisons to other publicly available sequences. To characterize the circulating strain of HAdV-8, 2 samples were fully sequenced by Macrogen Corp., Kyoto, Japan, using the HiSeq 2500 (Illumina, San Diego, CA, USA), assembled using the CLC Genomics Workbench (CLC bio, Aarhus, Denmark), and deposited in the International Nucleotide Sequence Database Collaboration database (http://www.insdc.org/), with the accession numbers LC312461 and LC312462. The clinical symptoms of individual patients were summarized from past medical records.

Details of 13 specimens collected from 12 patients, including one patient with bilateral ocular infection, are described in Table 1. The average age of the patients was 42.54 ± 29.6 years. Five patients from 2014 who suffered from HAdV-8 infection were believed to have been infected during an implicated nosocomial outbreak.

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Table 1.	Thirteen	specimens	collected	from	twelve	patients	of	EKC
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Patient	Age (y)	Date of sample collection	From onset to sample collection (d)	Ad-IC	Туре	Symptom	Route of infection	Multiple subepithelial corneal infiltrates	Two kinds of PCR
1	52	3/Feb./2014	4	+	8	Bilateral	nosocomial	+	+
2 ¹⁾	82	4/Feb./2014	1	+	8	Unilateral	nosocomial	-	+
3	13	7/Feb./2014	No data	+	8	Unilateral	nosocomial	+	+
4	60	17/Feb./2014	1	+	8	Bilateral	nosocomial \rightarrow family	-	+
5 ¹⁾	89	18/Feb./2014	No data	+	8	Unilateral	nosocomial	-	+
6	37	13/Apr./2015	0	+	N.D.	Unilateral	community	-	-
7	No data	17/Jul./2015	No data	+	54	Unknown	No data	No data	+
8	27	10/Aug./2015	0	+	N.D.	Bilateral	No data	No data	_
9 Left	36	17/Aug./2015	1	+	54	Bilateral	family	+	+
9 Right	36	17/Aug./2015	7	-	54	Bilateral	family	-	+
10	71	29/Sep./2015	4	+	54	Bilateral	community	-	+
11	1	26/Oct./2015	0	+	54	Unknown	No data	No data	+
12	0	10/Nov./2015	0	+	N.D.	Unilateral	community	-	-

Ad-IC, Adenovirus immunochromatography; +, positive result; -, negative result.

¹⁾: the genome was sequenced.



Fig. 1. Phylogenetic tree of complete genomes inferred with MrBayes using a transversion substitution model allowing for invariable sites (TVM + I). Posterior probabilities of the branches are shown. The two sequences obtained in this study are highlighted in bold font. Accession numbers of the sequences are shown in parentheses. The scale bar shows the number of base substitutions per site.

Although no potential route of nosocomial infection with HAdV-8 could be clearly identified in this study, disinfection measures were taken at the hospital to mitigate potential risk. EKC symptoms caused by HAdV-8 and HAdV-54 were quite severe, and one patient with HAdV-54 infection reported eye pain. Although eye swabs from both eyes in one patient were collected with high HAdV copy numbers, the sample from the right eye yielded negative results on the IC kit test (Table 1). This result suggested that infections caused by HAdV-54 cannot be ruled out solely based on a negative single IC kit test result performed during the 7 days following the onset of symptoms. Three samples yielded positive results on the IC kit test but negative results on PCR. This could be explained as a false-positive reaction of the IC kit, because these 3 samples showed negative findings in both real-time and nested PCR. As previously described (4), local anesthesia applied while collecting

samples might affect the reaction on an IC kit test. The partial nucleotide sequences of all HAdV-8 samples displayed 100% identity to epidemic strains from Germany sampled in 2005 (accession no. KT862548.1), 2006 (KT862546.1), and 2014 (KT862547.1, KT862549.1) (5) and to epidemic strains from the USA isolated in 2012 (KT340070.1 and KT340055.1). The nucleotide sequence from the partial hexon region of HAdV-54 strains displayed 100% identity to the strain identified in Kobe in 2000 (AB333801.2).

The clinical manifestations of EKC caused by HAdV-8 are severe, and several cases of major nosocomial outbreaks have been reported in Japan and elsewhere (5,6). Interestingly, more cases were reported annually during 1982–1996 than during 1997–2007 in Japan (7). According to the Infectious Agents Surveillance Report (IASR), a total of 46 HAdV-8 cases were reported between 2012 and 2014 in Japan (https://www. niid.go.jp/niid/en/iasr-vol38-e/7371-idx449-e.html). Outside of Japan, HAdV-8 has been a major cause of EKC epidemics (8,9).

The analysis of 2 whole-genome sequences from the samples revealed identical sequences in 2 different EKC patients, thereby suggesting that the same viral strain was behind the reported cases. Additionally, these whole genome sequences differed from other publicly available German HAdV-8 sequences. The German strains from 2009 (KT862547) and 2010 (KT862549) displayed 25 and 27 differences, respectively, from our sequenced samples (Fig. 1).

In recent years, large-scale epidemics of EKC have been caused by HAdV-54 in Japan. HAdV-54 cases have been reported to involve a higher frequency of multiple subepithelial corneal infiltrates, and some cases of persistent corneal infiltrates and secondary bacterial infections (10,11). HAdV-54 was the most frequently detected viral type in EKC cases reported in Japan from 2015 to 2016 according to the IASR available at (https://nesid4g. mhlw.go.jp/Byogentai/Pdf/data41j.pdf). (Japanese). In the present study, HAdV-8 and HAdV-54 infections have similar clinical manifestations, with severe keratoconjunctival symptoms noted in infections by both types. At our hospital, severe precautionary measures are frequently taken to reduce the risk of nosocomial HAdV infections. Early diagnosis using PCR remains important for type identification and preventing false diagnosis of EKC by IC kit; however, its application is limited to laboratory conditions, as reflected by the 3 samples that vielded positive results by the IC kit at the bedside but showed negative findings on laboratory PCR.

In this study, we reported EKC cases resulting from HAdV-8 and HAdV-54. Recent reports describe other severe cases resulting from HAdV-54 (10,12). This highlights the burden of this adenoviral type on the Japanese

healthcare system. In Japan, the annual number of EKC cases resulting from HAdV-8 appears to be decreasing; however, our results clearly demonstrated that HAdV-8 remains a threat in ophthalmological hospitals in Japan.

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Conflict of interest None to declare.

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