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Genome Analysis of Adenovirus Type 7 and Adenovirus Type 11

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Purpose: To study the epidemiology of adenovirus type 7 (Ad 7) conjunctivitis and adenovirus type 11 (Ad 11) conjunctivitis in the Japanese population by determining the genome type and sub-genome type.

Methods: For Ad 7, we used 12 strains from patients with acute viral conjunctivitis and one strain from a patient with pneumonia. For Ad 11, we used 17 strains from patients with acute viral conjunctivitis and 3 strains from patients with cystitis. For Ad 7 genome typing, we used 11 DNA restriction endonucleases (REs) recognizing 6-or 7-base pair sequences, and for Ad 11 genome typing, we used 7 REs. For Ad 7 and Ad 11 sub-genome typing, we used *Taq* I and *Hin*f I that recognize 4- or 5-base pair sequences.

Results: The 13 Ad 7 strains all belonged to the same genome type and sub-genome type (Ad 7dH1T1). Adenovirus type 11 strains showed six genome types, with Ad 11p being the most frequent strain. Fifteen Ad 11p strains showed three sub-genome types, but none was the same as the prototype.

Conclusions: Adenovirus type 7 seemed quite stable and an epidemic may occur again. On the other hand, Ad 11 showed several different types. This finding suggests that Ad 11 did not cause an epidemic in Japan during the first half of the 1990s. **Jpn J Ophthalmol 2001;45:22–30** © 2001 Japanese Ophthalmological Society

Key Words: Adenovirus type 7, adenovirus type 11, genome type, sub-genome type, viral conjunctivitis.

Introduction

Of the human adenoviruses (Ad) belonging to the B subgenera, it is known that Ad types 3, 7, and 11 cause conjunctivitis. Adenovirus type 3 and Ad 7, which belong to the B1 group, cause inflammation of the upper respiratory tract and pneumonia in addition to conjunctivitis, while Ad 11, which belongs to Group B2, causes diseases such as cystitis and nephritis. Over the last 10 years in Japan, Ad 3 has been the most frequently isolated.[1] On the other hand, Ad 7 and Ad 11 received attention in the latter half of the 1950s as the causative agents in epidemic keratoconjunctivitis (EKC) and pharyngoconjunctival fever (PCF).[2,3] Only a few adenoviruses have been isolated in Japan in the past 10 or more years. However, Ad 7 attained high frequencies of isolation beginning in early 1995 and is now receiving attention as an Ad that causes recurrent infection.[4] There was also a report[5] that Ad 11 was the cause of a large-scale epidemic of conjunctivitis in Southeast Asia in the 1980s.

Epidemiological analyses based on genome type differences have been conducted in recent years. Various genome types circulated in certain countries and continents in different years and caused outbreaks with varying degrees of severity. Wadell et al[6] classified Ad 7 into Ad 7p and 7a to 7f genome types based on the differences in the cleavage patterns obtained with restriction endonucleases (REs) that recognize 6-base pair sequence. Adenovirus 7p is a genome type of prototype, of which a strain named *Gomen* was detected in throat swab abrasions of a patient with pharyngitis. They have described repeated outbreaks of Ad 7b in Europe, the United States, and Australia, and of Ad 7d in China, and reported that Ad 7b and Ad 7d were closely related because of the high percentage of iden-

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tical restriction fragments. It was reported that the three strains isolated in Japan from 1969 to 1979 were Ad 7p.[6,7] The Ad 7 epidemic strains isolated in Japan since 1995 were classified as Ad 7d according to Wadell's classification. However, it has been reported that the cleavage pattern obtained with *Bst*E II is different from the genome types reported thus far.[4,8] This genome type may be the same as the genome type of the strain isolated in Israel.[9] Furthermore, Ad 7h, which has been reported to be an epidemic strain in South America,[10] was isolated in Aichi prefecture, Japan, in 1996.[11] The majority of strains described in these reports were isolated from pharyngeal exudates. In the more than 100 strains isolated, only a few were isolated from conjunctival abrasions.[4,12] There are very few reports on Ad 7 from conjunctival abrasions in international literature even though it is recognized as being the causative agent in PCF.

Adenovirus type 11 has been classified into five genome types (Ad 11p, 11a to 11d) by Guo et al[13] based on the differences in the cleavage patterns generated by REs which recognize 6-base pair sequence. Adenovirus 11p is a genome type of prototype, in which the strain named *Slobitski* was detected in the stool of a patient with paralytic polio. There have been several reports in Japan on the isolation of strains from conjunctivitis abrasions and genome types. In these reports, the majority of genome types are Ad 11p. Multitype genome types also have been reported.[14–16]

In the present study, we conducted an epidemiological examination of the genome types of strains isolated from the abrasions of Ad 7 conjunctivitis patients infected from 1995 onward, with prevalent recurrent infection, as well as the genome types of strains isolated from the abrasions of Ad 11 conjunctivitis, which until now has not been so prevalent in Japan. We also compared the clinical symptoms associated with these two types of conjunctivitis. Itakura et al[17] and Shiao et al[18] reported that they were able to classify not only the genome type, but also the sub-genome type of the same genome types by determining the differences in cleavage patterns using REs that recognize 4- or 5-base pair sequences, and that they could predict the stability of the genome type. We also studied the sub-genome types of strains that expressed the same genome type to monitor the alterations occurring in prevalent strains.

Materials and Methods

Viral Strains

Twelve strains of Ad 7 detected in conjunctival abrasions of 12 patients with acute conjunctivitis were used, after obtaining informed consent from each patient for use in this study. They were isolated in Sapporo and Kanagawa from 1995 to 1996 with the serotype being determined by neutralization test. A strain of Ad 7 (Ad 7 Hiroshima strain) isolated from pharyngeal exudates and which is also presently common in Japan was obtained from the Hiroshima Public Health Institute. An Ad 7 prototype purchased from the American Tissue Culture Center (ATCC) was used as a control. Detailed clinical symptoms of 10 of the 12 patients are presented in Table 1. The conjunctivitis findings were classified into 4 stages (0 = none, 1+ = mild, 2+ = moderate, and 3+ = severe) according to their degree of severity. For minor hemorrhages, only the absence or presence of hemorrhaging was determined. The degree of severity of conjunctivitis was scored from 1+ to 3+ based on the following criteria: 1+: acute follicular conjunctivitis findings in the inferior conjunctiva were positive, 2+: positive findings in the fornix region, 3+: positive findings in the superior conjunctiva.

Adenovirus type 11 was obtained from 17 strains in which Ad 11 was detected in the conjunctival abrasions of patients with acute conjunctivitis, after having obtained informed consent from each patient. They were isolated mainly in Sapporo and Kanagawa between 1990 and 1994, and in three strains detected in the urine of 3 patients with acute hemorrhagic cystitis. Their serotype was determined by the neutralization test. An Ad 11 prototype was purchased from ATCC. Detailed clinical symptoms of 8 of the 17 patients with conjunctivitis are presented in Table 2. The criteria used in the assessment were identical to those for Ad 7.

Extraction of Virus DNA

Hep-2 cells inoculated with the virus were cultured for 2 to 4 days with the infected cells being collected when the cytopathic effect (CPE) was almost complete. The DNA was then extracted using a modification of the Shinagawa[19] method.

Restriction Enzyme Treatment and Electrophoresis

The Ad 7 genome types were studied using the 6- and 7-base pair REs, *Bam*H I, *Bcl* I, *Bg1* I, *Bg1* II, *Bst*E II, *Eco*R I, *Hind* III, *Hpa* I, *Sma* I, *Xba* I, and *Xho* I, while the Ad 11 genome types were investigated using *Bam*H I, *Bg1* II, *Bst*E II, *Hind* III, *Pst* I, *Sac* I, and *Sma* I. The sub-genome types of the dominant strains were also studied using *Hin*f I, a RE which recognizes 5-base pairs, and *Taq* I, an RE

Table 1. Clinical Symptoms of Ad7 Conjunctivitis

Patient No.	Isolate Number	Age	Sex*	Date of Isolate	Diagnosis†	Site of Conjunctivitis	Objective Findings: Degree of severity		Objective Findings: Palpebral Conjunctiva						Objective Findings: Epithelial Kerapathy	Extraocular Symptoms: Periauricular Lymphoadenopathy	Extraocular Symptoms: Systemic Symptoms	In-family Infections	Region of Isolate
							Right	Left	Injection	Papillae	Folliculae	Turbidity	Edema	Spot Hemorrhage					
1	V12729	19	M	95.6	EKC	Bilateral	1+	3+							−	−	−	−	Kanagawa
2	Ad7	13	M	95.8	EKC	Bilateral	3+	1+							+	+	−	−	Kanagawa
3	95-5385	21	M	95.11	EKC	Bilateral	2+	3+	2+	1+	2+	1+	1+	−	+	+	Pharyngalgia Fever, Headache	−	Hokkaido
4	95-5504	32	F	95.11	Acute conjunctivitis	Bilateral	1+	2+	2+	1+	2+	1+	1+	1+	−	−	Cough	+	Hokkaido
5	95-5505	37	F	95.12	EKC	Unilateral	0	3+	3+	2+	1+	2+	2+	1+	−	+	Fever	−	Hokkaido
6	96-5642	66	M	96.2	Acute conjunctivitis	Bilateral	2+	1+	3+	1+	2+	3+	3+	1+	−	−	−	−	Hokkaido
7	96-5795	44	F	96.3	Acute conjunctivitis	Unilateral	1+	0	2+	2+	1+	2+	2+	2+	−	+	Rhinorrhea	−	Hokkaido
8	96-5166	29	M	96.6	Acute conjunctivitis	Unilateral	1+	0	2+	1+	1+	2+	2+	1+	−	−	Pharyngalgia	−	Hokkaido
9	96-5167	20	M	96.6	Acute conjunctivitis	Bilateral	2+	3+	2+	1+	2+	2+	2+	1+	+	−	−	−	Hokkaido
10	96-5288	31	M	96.7	EKC	Unilateral	0	3+	3+	2+	2+	2+	1+	1+	−	+	Fever	−	Hokkaido

*M: Male, F: Female.
†EKC: Epidemic keratoconjunctivitis.

Table 2. Clinical Symptoms of Ad 11 Conjunctivitis

Patient No.	Isolate Number	Age	Sex*	Date of Isolate	Diagnosis	Site of Conjunctivitis	Objective Findings: Degree of Severity	Objective Findings: Blepharoconjunctiva						Objective Findings: Epithelial Kerapathy	Extraocular Symptoms: Periauricular Lymphoadenopathy	Extraocular Symptoms: Systemic Symptoms	In-family Infections	Region Isolate
								Papillae	Folliculae	Turbidity	Edema	Spot Hemorrhage	Pseudo Membrane					
1	90-78	38	M	90.4	Acute conjunctivitis	Unilateral	3+	2+	2+	2+	2+	3+	−	−	−	Tympanitis	−	Hokkaido
2	90-340	17	M	90.10	Acute conjunctivitis	Bilateral	3+	3+	3+	2+	2+	1+	−	−	−	Rhinorrhea	−	Hokkaido
3	91-191	35	M	91.4	Acute conjunctivitis	Bilateral	3+	1+	2+	1+	2+	1+	−	−	−	−	+	Hokkaido
4	91-465	1	F	91.8	Acute conjunctivitis	Unilateral	2+	0	2+	1+	2+	0	−	−	−	Tonsillitis, Fever	−	Hokkaido
5	92-174	42	M	92.2	Acute conjunctivitis	Bilateral	3+	1+	2+	2+	1+	1+	−	+	+	Pharyngalgia	−	Hokkaido
6	94-682	21	M	94.11	Acute conjunctivitis	Bilateral	3+	1+	2+	2+	2+	1+	−	−	+	Pharyngalgia		Hokkaido
7	Tc19872	23	F	92.6	Acute conjunctivitis	Unilateral	1+							−	−	Pharyngalgia		Kanagawa
8	Tc21220	22	F	92.1	Acute conjunctivitis	Unilateral	3+							+	+	−	−	Kanagawa

*M: Male, F: Female.

Table 3. Genome Type and Subgenome Type of Ad 7

Isolate Number	Diagnosis	Region Isolated	Date of Isolation	Genome Type	Sub-genome Type
V12729	Conjunctivitis	Kanagawa	1995 6.23	Ad 7d	Ad 7d H1T1
Ad7	Conjunctivitis		8.9	Ad 7d	Ad 7d H1T1
95-5385	Conjunctivitis	Hokkaido	1995	Ad 7d	Ad 7d H1T1
95-5504	Conjunctivitis		12.18	Ad 7d	Ad 7d H1T1
95-5505	Conjunctivitis		Unknown	Ad 7d	Ad 7d H1T1
96-5642	Conjunctivitis		1996 2.9	Ad 7d	Ad 7d H1T1
96-5795	Conjunctivitis		3.27	Ad 7d	Ad 7d H1T1
96-5166	Conjunctivitis		6.1	Ad 7d	Ad 7d H1T1
96-5167	Conjunctivitis		6.4	Ad 7d	Ad 7d H1T1
96-5288	Conjunctivitis		7.22	Ad 7d	Ad 7d H1T1
96-5648	Conjunctivitis		11.26	Ad 7d	Ad 7d H1T1
96-5686	Conjunctivitis		12.4	Ad 7d	Ad 7d H1T1

which recognizes 4-base pairs. The reaction buffer solutions (Takara Shuzo, Kyoto) used for the respective enzymes were the optimum buffers described in the package inserts. One unit of enzyme was added to 1 μg of virus DNA and the DNA was digested by reacting for 4 hours or more at the optimum temperature of the respective enzyme.

DNA digested with an RE that recognizes 6- or 7-base pairs was electrophoresed at room temperature for 3 hours at 120 V using 1.2% agarose gel and then stained for 15 minutes with ethidium bromide (1 μg/mL). DNA digested with an RE that recognizes 4- or 5-base pairs was electrophoresed at room temperature for 4 hours at 100 V using a 5% polyacrylamide gel and then stained for 5 minutes with ethidium bromide (1 μg/mL).

The cleavage pattern of the DNA obtained by electrophoresis was observed under ultraviolet irradiation and photographed.

Results

The genome types and sub-genome types of Ad 7 are shown in Table 3 and Figure 1. All 12 strains had identical cleavage patterns. The patterns were the same as those reported for Ad 7d, with the exception of *Bst*E II. The *Bst*E II cleavage pattern was different from previous maps, and did not have 4300 bp and 4000 bp fragments although it did have a new fragment of about 9000 bp. These demonstrated the same cleavage patterns as Ad 7, which is currently prevalent in Japan (Figures 2–4) The cleavage patterns (H1T1) of the sub-genome types of the 12 strains were the same as Ad 7 (Hiroshima strain) and all were very different from the prototype cleavage pattern (HpTp) (Figures 5 and 6).

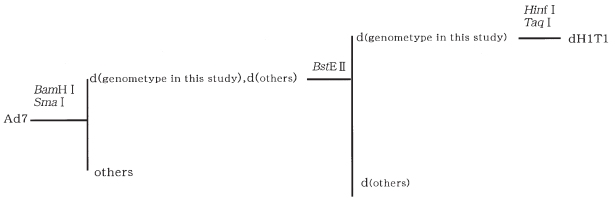

Figure 1. Genome type and sub-genome type of adenovirus type 7 (Ad 7) in this study.

The Ad 11 genome types are shown in Table 4 and Figure 7. Of the 17 conjunctivitis-derived strains, 12 were Ad 11p with the same cleavage pattern as the prototype, and 3 were Ad 11d. One is Ad 11e in which the cleavage pattern of *Bam*H I and *Hind* III were identical to Ad 11d while *Sma* I had a new cleavage pattern. Another one is Ad 11f in which the

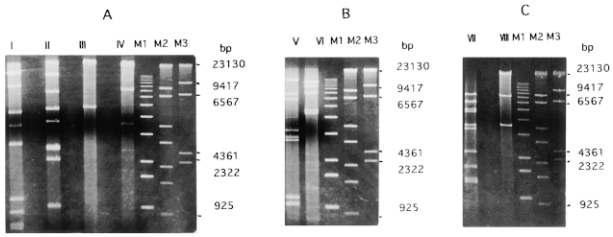

Figure 2. Electrophoresis photograph of adenovirus type 7 (Ad 7) digested with restriction endonucleases *Bam*H I, *Sma* I, *Eco*R I, *Xho* I, *Bcl* I, *Xba* I, *Hind* III, or *Hpa* I. A-I: Ad 7 clinical isolates (isolate number V12729) digested with *Bam*H I. A-II: Ad 7 clinical isolates (isolate number V12729) digested with *Sma* I. A-III: Ad 7 clinical isolates (isolate number V12729) digested with *Eco*R I. A-IV: Ad 7 clinical isolates (isolate number V12729) digested with *Xho* I. B-V: Ad 7 clinical isolates (isolate number V12729) digested with *Bcl* I. B-VI: Ad 7 clinical isolates (isolate number V12729) digested with *Xba* I. C-VII: Ad 7 clinical isolates (isolate number V12729) digested with *Hind* III. C-VIII: Ad 7 clinical isolates (isolate number V12729) digested with *Hpa* I. M1: 1 kb DNA ladder (molecular weight marker). M2: λ-*Eco*T14I (molecular weight marker). M3: λ-*Hind* III (molecular weight marker).

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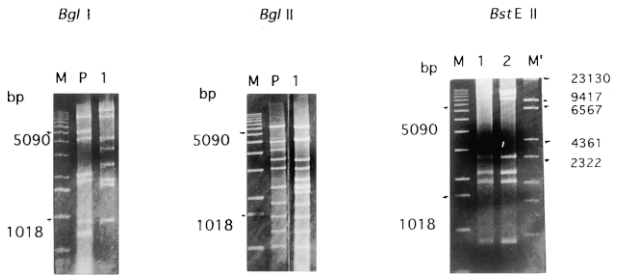

Figure 3. Electrophoresis photograph of adenovirus type 7 (Ad 7) digested with restriction endonucleases *Bg1* I, *Bg1* II, or *Bst*E II. *Bg1* I-M: 1 kb DNA ladder (molecular weight marker). *Bg1* I-P: Ad 7 prototype (from American Tissue Culture Center [ATCC]) digested with *Bg1* I. *Bg1* I-1: Ad 7 clinical isolates (isolate number V12729) digested with *Bg1* I. *Bg1* II-M: 1 kb DNA ladder (molecular weight marker). *Bg1* II-P: Ad 7 prototype (from ATCC) digested with *Bg1* II. *Bg1* II-2: Ad 7 clinical isolates (isolate number V12729) digested with *Bg1* II. *Bst* E II-M: 1 kb DNA ladder (molecular weight marker). *Bst* E II-3: Ad 7 clinical isolates (isolate number V12729) digested with *Bst* E II. *Bst* E II-4: Ad 7 clinical isolates (isolate number Ad 7) digested with *Bst* E II. *Bst* E II-M′: λ-*Hind* III (molecular weight marker).

cleavage pattern of *Hind* III and *Sma* I were identical to Ad 11p while *Bam*H I had a new cleavage pattern. In contrast, the three hemorrhagic cystitis strains had the same cleavage pattern as Ad 11p. In the cleavage patterns of the DNA digested with *Bg1* II, *Bst*E II, *Pst* I, and *Sac* I, all 20 strains digested with *Bst*E II and *Sac* I had cleavage patterns identical to that of the prototype. Two types of patterns were seen with *Bg1* II and all three strains of Ad 11d had the same pattern. The other 17 strains all had another pattern. Four types of cleavage patterns were obtained with *Pst* I, of which the two types of Ad 11d were divided into Ad 11d1 and Ad 11d2 for convenience. One strain was Ad 11d1 and two were Ad 11d2. The one case which corresponds with Ad 11e also demonstrated a different cleavage pattern, while the remaining 16 strains had the same cleavage pattern (Figures 8–11). The dates and regions of the isolates

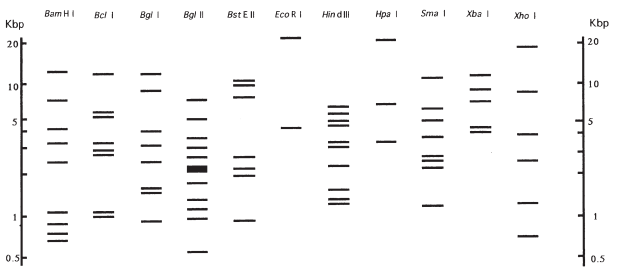

Figure 4. Diagram of cleavage pattern using restriction endonucleases, which recognizes 6- and 7-base pairs in Ad 7.

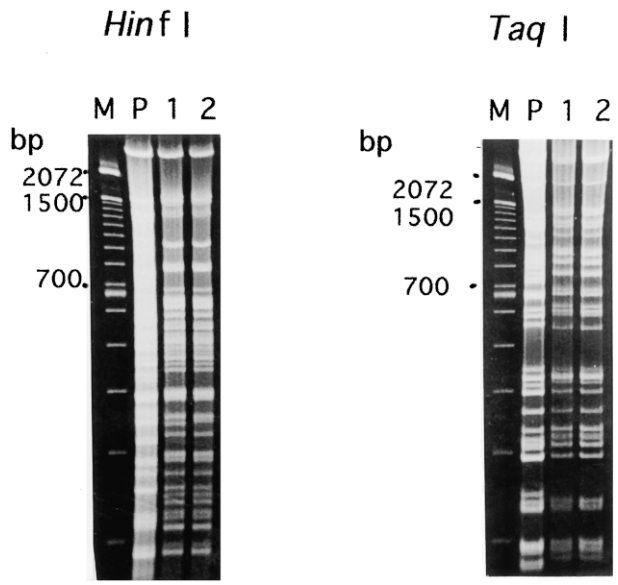

Figure 5. Electrophoresis photograph of cleavage of adenovirus type 7 (Ad 7) by *Hin*f I and *Taq* I. *Hin*f I-M: Molecular weight marker (100-base ladder). *Hin*f I-1: Ad 7 dH1 (strains from pharyngeal exudates of respiratory disease patients) digested with *Hin*f I. *Hin*f I-2: Ad 7dH1 (isolate number V12729) digested with *Hin*f I. *Taq* 1-M: molecular weight marker (100-base ladder). *Taq* 1-1: Ad 11 dT1 (strains isolated from pharyngeal exudates of respiratory disease patients) digested with *Taq* 1. *Taq* 1-2: Ad 7dT1 (isolate number V12729) digested with *Taq* 1.

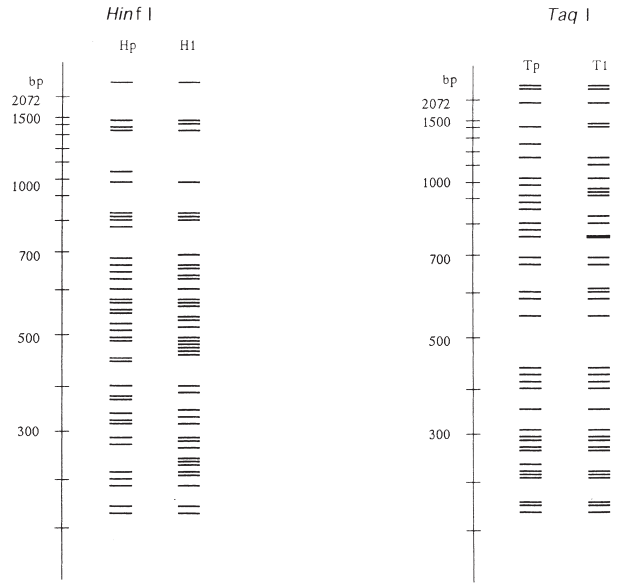

Figure 6. Diagram of cleavage pattern using *Hin*f I and *Taq* I in Ad 7. *Hin*fI-p: Ad 7pHp (prototype). *Hin*f I-1: Ad 7dH1 (isolate number V12729). *Taq* I-P: Ad 7pTp (prototype). *Taq* I-2: Ad 7dT1 (isolate number V12729).

Table 4. Genome Type and Subgenome Type of Ad 11

Isolate Number	Diagnosis	Region Isolated	Date of Isolation	Genome Type	Sub-genome Type
90-78	Conjunctivitis	Hokkaido	1990 4/28	Ad 11p	Ad 11p HpT1
90-340	Conjunctivitis		10/2	Ad 11p	Ad 11p HpT1
91-191	Conjunctivitis		1991 4/18	Ad 11p	Ad 11p HpT1
91-465	Conjunctivitis		8/29	Ad 11p	Ad 11p HpT1
92-174	Conjunctivitis		1992 2/27	Ad 11p	Ad 11p HpT1
92-578	Conjunctivitis		8/27	Ad 11d1	
93-847	Conjunctivitis		1993 11/12	Ad 11e	
94-163	Conjunctivitis		1994 3/10	Ad 11d2	
94-682	Conjunctivitis		11/10	Ad 11p	Ad 11p HpT1
94-685	Conjunctivitis		11/10	Ad 11p	Ad 11p HpT1
Tc19872	Conjunctivitis	Kanagawa	1992 6/7	Ad 11d2	
Tc21220	Conjunctivitis		12/8	Ad 11p	Ad 11p HpT1
Tc20764	Conjunctivitis	Kumamoto	1992 9/28	Ad 11p	Ad 11p HpT2
Tc20847	Conjunctivitis		10/12	Ad 11p	Ad 11p HpT1
P-008	Conjunctivitis	Unknown	Unknown	Ad 11f	
P-060	Conjunctivitis			Ad 11p	Ad 11p HpT1
P-061	Conjunctivitis			Ad 11p	Ad 11p HpT1
Tc-24184	Hemorrhagic cystitis	Unknown	1994	Ad 11p	Ad 11p HpT3
Tc-24235	Hemorrhagic cystitis		1994	Ad 11p	Ad 11p H1T2
Tc-24753	Hemorrhagic cystitis		1994	Ad 11p	Ad 11p H1T2

in Table 4 show that Ad 11p was the most prevalent strain. The sub-genome types of the 12 conjunctivitis and the 3 hemorrhagic cystitis Ad 11p strains are shown in Table 4 and Figures 12 and 13. Two types of patterns were obtained with *Hin*f I. The one identical to the prototype was designated Hp and the other H1. Three types were obtained with *Taq* I, designated T1, T2, and T3; however, none had the same cleavage pattern as the prototype (Tp). Eleven of the 12 conjunctivitis strains were Ad 11pHpT1 and one was Ad 11pHpT2. Two of the 3 hemorrhagic cystitis strains were Ad 11pH1T2 and one was Ad 11HpT3.

Discussion

Currently more than 10 different Ad 7 genome types, based on the classification of Wadell et al[6] who used two types of REs (*Bam*H I and *Sma* I), have been reported from around the world.[6,7,9,10] In the present study we investigated genome types using 11 DNA REs, including the two REs used by Wadell et al, and found that the 12 strains all had the same

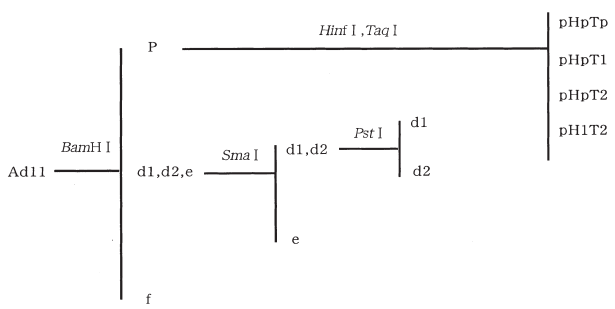

Figure 7. Genome type and sub-genome type of adenovirus type 11 (Ad 11) in this study.

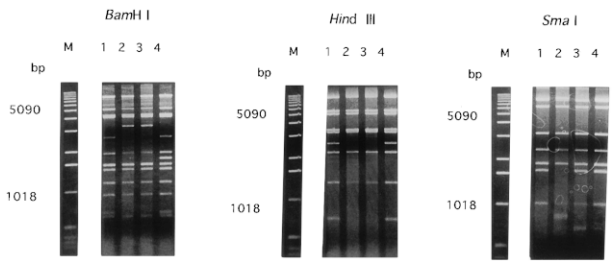

Figure 8. Electrophoresis photograph of cleavage of adenovirus type 11 (Ad 11) using *Bam*H I, *Hin*d III, and *Sma* I. *Bam*H I-M: molecular weight marker (1 kb ladder). *Bam*H I-1: Ad 11p (isolate number Tc21220) digested with *Bam*H I. *Bam*H I-2: Ad 11d (isolate number 92-578) digested with *Bam*H I. *Bam*H I-3: Ad 11e (isolate number 93-847) digested with *Bam*H I. *Bam*H I-4: Ad 11f (isolate number P-008) digested with *Bam*H I. *Hin*d III-M: molecular weight marker (1 kb ladder). *Hin*d III-1: Ad 11p (isolate number Tc21220) digested with *Hin*d III. *Hin*d III-2: Ad 11d (isolate number 92-578) digested with *Hin*d III. *Hin*d III-3: Ad 11e (isolate number 93-847) digested with *Hin*d III. *Hin*d III-4: Ad 11f (isolate number P-008) digested with *Hin*d III. *Sma* I-M: molecular weight marker (1 kb ladder). *Sma* I-1: Ad 11p (isolate number Tc21220) digested with *Sma* I. *Sma* I-2: Ad 11d (isolate number 92-578) digested with *Sma* I. *Sma* I-3: Ad 11e (isolate number 93-847) digested with *Sma* I. *Sma* I-4: Ad 11f (isolate number P-008) digested with *Sma* I.

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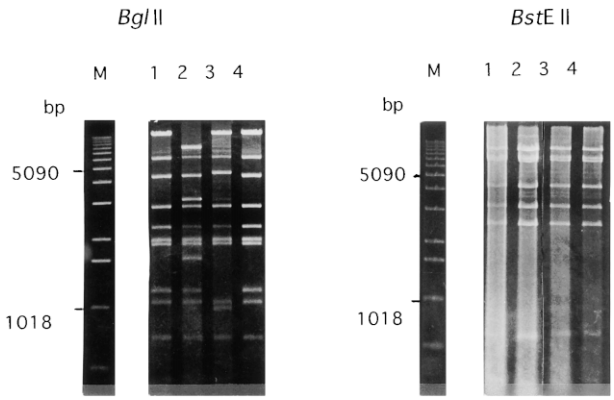

Figure 9. Electrophoresis photograph of adenovirus type 11 (Ad 11) cleaved with *Bg1* II and *Bst*E II. *Bg1* II-M: molecular weight marker (1 kb ladder). *Bg1* II-1: Ad 11p (isolate number Tc21220) digested with *Bg1* II. *Bg1* II-2: Ad 11d (isolate number 92-578) digested with *Bg1* II. *Bg1* II-3: Ad 11e (isolate number 93-847) digested with *Bg1* II. *Bg1* II-4: Ad 11f (isolate number P-008) digested with *Bg1* II. *Bst*E II-M: molecular weight marker (1 kb ladder). *Bst*E II-1: Ad 11p (isolate number Tc21220) digested with *Bst*E II. *Bst*E II-2: Ad 11d (isolate number 92-578) digested with *Bst*E II. *Bst*E II-3: Ad 11e (isolate number 93-847) digested with *Bst*E II. *Bst*E II-4: Ad 11f (isolate number P-008) digested with *Bst*E II.

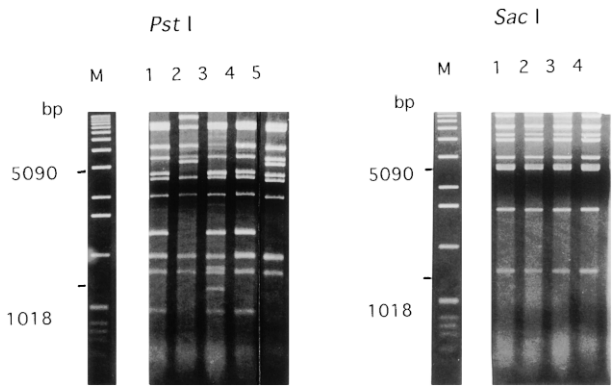

Figure 10. Electrophoresis photograph of adenovirus type 11 (Ad 11) cleaved with *Pst* I and *Sac* I. *Pst* I-M: molecular weight marker (1 kb ladder). *Pst* I-1: Ad 11p (isolate number Tc21220) digested with *Pst* I. *Pst* I-2: Ad 11D1 (isolate number 92-578) digested with *Pst* I. *Pst* I-3: Ad 11e (isolate number 93-847) digested with *Pst* I. *Pst* I-4: Ad 11f (isolate number P-008) digested with *Pst* I. *Pst* I-5: Ad 11d2 (isolate number 94-163) digested with *Pst* I. *Sac* I-M: molecular weight marker (1 kb ladder). *Sac* I-1: Ad 11p (isolate number Tc21220) digested with *Sac* I. *Sac* I-2: Ad 11d (isolate number 92-578) digested with *Sac* I. *Sac* I-3: Ad 11e (isolate number 93-847) digested with *Sac* I. *Sac* I-4: Ad 11f (isolate number P-008) digested with *Sac* I.

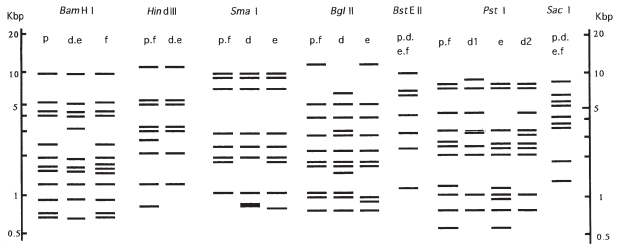

Figure 11. Diagram of a cleavage pattern of adenovirus type 11 (Ad 11) cleaved by enzymes that recognize 6-base pairs. p, d, e, f, d1, and d2 represent the cleavage patterns of Ad 11p, Ad 11d, Ad 11e, Ad 11f, Ad 11d1, and Ad 11d2, respectively.

cleavage pattern, which was classified as Ad 7d using *Bam*H I and *Sma* I. However, the pattern of digestion with *Bst*E II is different from that for Ad 7d. This pattern indicated that it was the same genome type as Ad 7, which has been prevalent in Japan since 1995. The genome types of all 12 strains isolated from the conjunctivitis patients were identical so we decided to investigate the sub-genome types, using Ad 7 (Hiroshima strain) as a control, in order to determine if there were any differences between the genome types. All the sub-genome types were identical. That points to one possibility, that the Ad 7 strains that

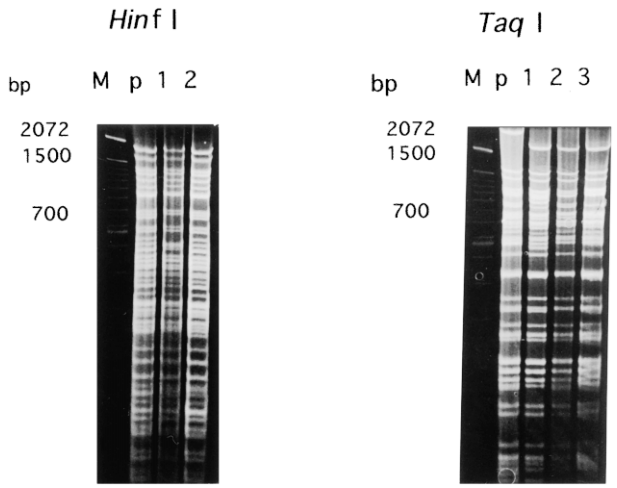

Figure 12. Electrophoresis photograph of adenvirus type 11 (Ad 11) cleaved using *Hin*f I and *Taq* I. *Hin*f I-M: molecular weight marker (100-base ladder). *Hin*f I-p: prototype digested with *Hin*f I. *Hin*f I-1: Ad 11pHp (isolate number Tc24235) digested with *Hin*f I. *Hin*f I-2: Ad 11pH1 (isolate number 92-174) digested with *Hin*f I. *Taq* I-M: molecular weight marker (100-base ladder). *Taq* I-p: prototype digested with *Taq* I. *Taq* I-1: Ad 11pT3 (isolate number Tc24184) digested with *Taq* I. *Taq* I-2: Ad 11pT2 (isolate number 92-174) digested with *Taq* I. *Taq* I-3: Ad 11pT1 (isolate number Tc24235) digested with *Taq* I.

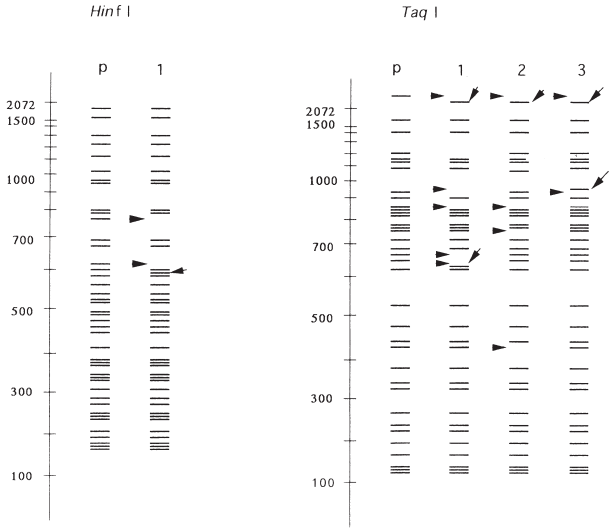

Figure 13. Cleavage diagram of Ad 11 cleaved with *Hin*f and *Taq* I. Hinf I-p: Ad 11pHp (prototype and that with same cleavage pattern), 1: Ad 11pH1. Taq I-p: Ad 11p Tp (prototype), 1: Ad 11pT1, 2: Ad 11pT2, 3: Ad 11pT3. arrowhead: Loss of fragment different from prototype. arrow: Acquisition of fragment different from prototype.

caused the respiratory disease and those that caused conjunctivitis were most likely identical. Accordingly, the different symptoms were due not to the virus but rather to differences in the host or the route of infection. This agrees with the findings of a report that determined, using genetic analysis of the hexon hypervariable region of current epidemic strains, that the homology of the nucleic acids and amino acids in conjunctivitis and pharyngeal-derived strains was 99.6 to 100%.[20] However, a study that examined the base sequences of all the amino acids of the previous Ad 7d and the currently epidemic Ad 7 reported differences between the two residues in four locations.[8]

Concerning the sub-genome types of Ad 3, it has been reported that there was one sub-genome type of Ad 3g during the epidemic, while there were two sub-genome types of Ad 3, that there were many changes from year to year, and that Ad 3f was a comparatively unstable form.[17]

From this study of Ad 7, it is thought that the Ad 7 epidemic strains, which were all identical in the present study, are comparatively stable forms, and in the future the epidemic may continue, or even if it disappears temporarily, it may once again reappear. This is not due to a viral factor only, but can also be due to the low retention state of the Ad 7 antibody titer.[21] Furthermore, the fact that the genome type of the strain that causes conjunctivitis was identical to the genome type of the respiratory disease strain indicates that it is conceivable that a droplet infection from an ophthalmological location may be responsible for the serious respiratory disease. In considering the risk of an in-hospital infection, we must take precautions to prevent future epidemics of Ad 7 conjunctivitis.

The Ad11 genome type was based upon the classification by Guo et al,[13] who used three types of REs (*Bam*H I, *Hind* III, and *Sma* I). The Ad 11p was the dominant form in 15 of the 20 strains in the present study. Aoki et al[14] studied the genome types of 6 strains of Ad 11 isolated from conjunctivitis patients in Sapporo, Japan, between 1983 and 1986, 3 strains from Manila, the Philippines, and one strain from Melbourne, Australia. There were also several reports in the Japanese patients.[15,16] All three of these Japanese studies identified the strain as Ad 11p. Although there were slight differences in the REs used in the studies conducted in Japan, in the report on the strain isolated in Hiroshima,[15] 6 of the 9 cases of conjunctivitis were Ad 11p, as were the strains in both the hemorrhagic cystitis patients and in 6 of the 7 strains reported in Tottori Prefecture.[16]

Therefore, we studied the sub-genome types of 12 conjunctivitis derived strains and 3 hemorrhagic cystitis derived strains that were Ad 11p. No strain that had a cleavage pattern identical to the Ad11HpTp of the prototype was observed. This suggests that, betweeen 1990 and 1994, there were strains that were not the prototype but very similar to it.

From this study of Ad 11, the genome type of the most frequently detected strain was similar to the prototype and a slight DNA alteration compared with Ad 7. Even though close to 500 strains have been isolated from Ad 11 infections over the past 10 years, there have been no reports of the virus causing a large-scale epidemic. The results of the present study support that. As in previous reports,[13–16] we also detected multiple types of genome types in the same period. Shiao et al,[18] studying the sub-genome type, have reported that multiple forms appear during non-epidemic periods.[19] This report also supports the contention that there had not been a large-scale outbreak of Ad 11 from 1990 to 1994. Another major reason why there has not been a major epidemic is believed to be that the Ad 11 indigenous to Japan is Ad 11p. Virtually no prototypes have been detected from epidemic strains in other Ad conjunctivitis epidemics. Since the antibody retention rate for Ad 11 is not as low as with Ad 7, and because there have been no reports in the 1990s of repeated epidemics overseas, it is thought that at the present time, the chance of a large-scale conjunctivitis infection in Japan is comparatively low. However, it is uncertain whether a different genome

type may enter Japan from overseas. Multiple types of Ad 11 genome are appearing and continue to mutate. The Ad 11 genome type during an epidemic in Southeast Asia has not been identified, so we still need to be vigilant about future epidemics.

From this research we confirmed that genome typing is a powerful tool for investigating the epidemiology of adenovirus, but many types may be identified using many different REs. It is difficult to compare types that are used by different REs. The use of standardized REs to determine genome types, and the use of commonly recognized nomenclature of genome types, are desirable.

The detailed clinical symptoms of a number of patients infected with Ad 7 or Ad 11 are listed in Tables 1 and 2. If we compare the symptoms according to the genome type, it becomes evident that the degree of severity of the symptoms was not the same even though the Ad 7 strains were all the same genome type. Also, the symptoms associated with Ad 11 were not necessarily different, even though the genome type was different. The results of the present study indicate that differences in the clinical symptoms of conjunctivitis were not observed in the genome type. So there is no support for the theory that symptoms are related to different genome types. In the future, molecular epidemiological research on Ad conjunctivitis in the B group and studies on the relationship between the genome types and the clinical profiles are needed.

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References

1. Yamadera S, Yamashita K, Akatsuka M, et al. Adenovirus surveillance, 1982–1993, Japan. Jpn J Med Sci Biol 1995;48:199–210.
2. Seiji S, Kaichi W, Keiichi T. Ocular manifestations of pharyngoconjunctival fever which occurred in outbreaks among several elementary schools in Tokyo in Autumn 1958. Nippon Ganka Gakkai Zasshi (Acta Soc Ophthalmol Jpn) 1959;63:719–24.
3. Seiji S. Studies on the relationship between adenovirus and epidemic keratoconjunctivitis, and on the superficial punctate keratitis. Nippon Ganka Gakkai Zasshi (Acta Soc Ophthalmol Jpn) 1959;63:208–47.
4. Mamoru N, Miki K, Katuhiko A, Yoshifumi I, Kouji Y, Takeo O. Restriction enzyme analysis of adenovirus type 7 isolates in Japan. Infect Agents Surveill Rep 1996;17:102–4.
5. Yin-Murphy M, Lin KH, Chua PH. Adenovirus type 11 epidemic conjunctivitis in Singapore. Southeast Asian J Trop Med Public Health 1974;5:333–41.
6. Wadell G, Gooney MK, da Costa Linhares A, et al. Molecular epidemiology of adenoviruses: global distribution of adenovirus 7 genome types. J Clin Microbiol 1985;21:403–8.
7. Li QG, Wadell G. Analysis of 15 different genome types of adenovirus type 7 isolated on five continents. J Virol 1986; 60:331–5.
8. Inada T. Brief history, situation in the world, abroad, national, surveillance, diagnosis and molecular epidemiology of adenovirus type 7 infection. Clin Virol 1998;26:216–25.
9. Azar R, Varsano N, Mileguir F, Mendelson E. Molecular epidemiology of adenovirus type 7 in Israel: identification of two new genome types, Ad7K and Ad7dd2. J Med Virol 1998; 54:291–9.
10. Kajon AE, Mistchenko AS, Videla C, Hortal M, Wadell G, Avendano LF. Molecular epidemiology of adenovirus acute lower respiratory infections of children in the South Cone of South America (1991–1994). J Med Virol 1996;48:151–6.
11. Hashido M, Mukouyama J, Inada T, et al. Genotyping adenovirus type 7h isolated in 1996 in Japan. IASR 1998;19:8–9.
12. Ogino T. Epidemiological study on adenovirus type 7 infection. Clin Virol 1998;26:216–25.
13. Guo DF, Shinagawa M, Aoki K, Sawada H, Itakura S, Sato G. Genome typing of adenovirus strains isolated from conjunctivitis in Japan, Australia, and Philippines. Microbiol Immunol 1988;32:1107–18.
14. Aoki K, Sawada H, Shinagawa M, Kawana R. Molecular epidemiology of adenoviral conjunctivitis in Japan, the Philippines and Australia. Nippon Ganka Gakkai Zasshi (Acta Soc Ophthalmol Jpn) 1998;92:96–102.
15. Noda M, Miyamoto Y, Ikeda Y, Matsuishi T, Ogino T. Restriction enzyme analysis of adenovirus types 3 and 11 isolated in Hiroshima city. Clin Virol 1991;19:56–61.
16. Motida K, Itagaki A, Gomyouda T. Genome analysis of adenovirus type 11 which was detected in the patients with epidemic keratoconjunctivitis. Nihon Iji Shinpou 1995;3691:48–9 (in Japanese with English abstract).
17. Itakura S, Aoki K, Sawada H, Shinagawa M. Analysis with restriction endonucleases recognizing 4-or-5-base-pair sequences of human adenovirus type 3 isolated from ocular diseases in Sapporo, Japan. J Clin Microbiol 1990;28:2365–9.
18. Shiao S, Aoki K, Isobe K, et al. Genome analysis of adenovirus type 3 isolated in Japan. J Clin Microbiol 1996;34:413–6.
19. Shinagawa M, Matsuda A, Ishiyama T, Goto H, Sato G. A rapid and simple method for preparation of adenovirus DNA from infected cells. Microbiol Immunol 1983;27:817–22.
20. Takeuchi S, Oshima A, Ito N, et al. Analysis of adenovirus type 7 hexon hypervariable region. Nippon Ganka Gakkai Zasshi (J Jpn Ophthalmol Soc) 1998;102:570–5.
21. Nishio O, Matsui K, Akiyama M, et al. Change in antibody prevalence to adenovirus type 7 during the past twenty years in Japan. Clin Virol 1998;26:255–9.