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Analysis of 15 Different Genome Types of Adenovirus Type 7 Isolated on Five Continents

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A total of 15 different genome types of adenovirus type 7 (Ad7), i.e., Ad7p, Ad7p1, Ad7a, Ad7a1 to Ad7a5, Ad7b, Ad7c, Ad7d, Ad7d1, Ad7e, Ad7f, and Ad7g, were identified among 40 selected strains isolated in Europe, Asia, North America, South America, and Australia by using restriction endonucleases *Bam*HI, *Bcl*I, *Bgl*I, *Bgl*II, *Bst*EII, *Eco*RI, *Hin*dIII, *Hpa*I, *Sal*I, *Sma*I, *Xba*I, and *Xho*I. Eight of them, Ad7p1, Ad7a1 to Ad7a5, Ad7d1, and Ad7g, are newly discovered. All 15 genome types could be distinguished by the four restriction endonucleases *Bam*HI, *Bcl*I, *Bgl*I, and *Xba*I. At least four restriction sites differed beween Ad7d and Ad7g. Pairwise analyses of comigrating DNA restriction fragments of all 15 Ad7 genome types were performed and presented in a schematic fashion. According to the degree of comigration of DNA restriction fragments, the 15 genome types could be divided into three clusters. Ad7b was the dominant genome type in different parts of the world and may have evolved in China into Ad7d and further to Ad7d1.

To date, 41 adenovirus serotypes have been recognized (5). Of these, adenovirus type 7 (Ad7) is the serotype which is most frequently associated with severe disease. In children under 2 years of age, clinical features are epidemic outbreaks of severe and fatal pneumonia, pharyngoconjunctival fever, myocarditis, gastroenteritis, and other acute symptoms of systemic infection (4, 6, 8, 15, 20, 22, 26). Ad7 can also cause chronic lung damage (19). Ad7 infection of older children has a better prognosis (27). The impact of acute respiratory diseases among conscripts has warranted the development of a live enteric-coated Ad7 vaccine (6, 12, 16, 21).

Berge et al. (2) isolated the prototype strain Gomen of Ad7 (Ad7p), whereas Rowe et al. (17) recognized subtype Ad7a (strain 1058) in cross-neutralization experiments.

We previously analyzed 314 Ad7 strains by using restriction endonucleases *Bam*HI and *Sma*I and identified five additional genome types of Ad7, i.e., Ad7b, Ad7c, Ad7d, Ad7e, and Ad7f (24–27).

We have now selected 40 Ad7 strains which represent five continents, Asia, Europe, North America, Australia, and South America (Table 1). Analysis with restriction endonucleases *Bam*HI, *Bcl*I, *Bgl*I, *Bgl*II, *Bst*EII, *Eco*RI, *Hin*dIII, *Hpa*I, *Sal*I, *Sma*I, *Xba*I, and *Xho*I (13) revealed 15 different genome types among the strains isolated from the five continents. They have tentatively been designated Ad7p, Ad7p1, Ad7a, Ad7a1 to Ad7a5, Ad7b, Ad7c, Ad7d, Ad7d1, Ad7e, Ad7f, and Ad7g. Eight of them (Ad7p1, Ad7a1 to Ad7a5, Ad7d1, and Ad7g) are newly discovered.

The approximate sizes, expressed as base pairs (bp) of each DNA fragment, of the 15 genome types obtained after digestion with the 12 restriction endonucleases are presented in Table 2.

Nine different Ad7a strains, recovered from China, Holland, and the United States, that may differ in virulence were subjected to detailed analysis. The DNA restriction patterns of Ad7a and Ad7a1 to Ad7a5 obtained with *Bam*HI and *Sma*I were very similar. However, after digestion with three other restriction endonucleases, *Bcl*I, *Bgl*I, and *Xba*I, they could be divided into six distinctly different genome types.

Only four restriction endonucleases, *Bam*HI, *Bcl*I, *Bgl*I, and *Xba*I, could be used to distinguish all 15 Ad7 genome types.

The Ad7b genome type was identified in 190 of the 336 Ad7 strains so far analyzed in our laboratory by use of restriction endonucleases *Bam*HI and *Sma*I. It is widely spread and has been the dominating genome type in the United States at least since 1967, in Europe since 1969, and in Australia since 1974 (24, 25). Three Ad7 strains isolated in Beijing, China, in 1958, 1965, and 1981 are all Ad7b. Since that time we have identified only Ad7d, which is unique to China and may have first appeared there.

The three Ad7b strains from China isolated during 23 years were compared with the KCH4 Ad7 strain representative of the large outbreak of Ad7 infections in England in 1973 (27) and found to be identical by use of the 12 restriction

TABLE 1. Origin of the 40 Ad7 strains identified as 15 different genome types

Genome type	Representative strain	Place (yr) of isolation	No. of strains
p	Gomen	United States (1954)	1
p1	BC 3423	China (1981)	1
a	S1058	United States (1958)	1
a1	BC 62	China (1958)	1
a1	A116873	Australia (1973)	1
a2	VW 12822	United States (1967)	1
a3	0-0410	United States (1970)	2
a3	68-11002	Holland (1968)	1
a4	BC 73	China (1958)	1
a5	67-8607	Holland (1967)	1
b	BC 3541	China (1958–1981)	3
b	KCH4	England (1973)	1
c	37300	Sweden (1964)	1
d	4492	China (1981–1984)	18
d1	4653	China (1984)	2
e	B762	Brazil	1
e	A10841	Australia (1975)	1
f	A18787	Australia (1980)	1
g	BC25	China (1958)	1

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TABLE 2. Molecular size of fragments of DNA from 15 different genome types of Ad7 obtained after digestion with 12 restriction endonucleases[a]

Restriction enzyme	*Bam*HI										*Bcl*I					
Pattern	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6
Genome type	p	p1	a	a1–5	b	c	d,d1	e	f	g	p,a1, a3–4, b,c,d	p1,e	a,f	a2	a5,d1	g
Size of fragments (bp)	8,400	8,400	9,600	9,600	9,600	9,600	13,500[b]	9,600	9,600	9,600	12,700	12,700	12,700	12,700	12,700	12,700
	7,200	7,200	8,400	8,400	8,400	8,400	8,400	8,100[b]	4,600	7,000[b]	6,800	6,800	6,800	6,800	6,800	6,800
	4,600	4,600	4,600	4,600	4,400	6,300[b]	4,400	4,600	4,400[b]	4.400	5,800	5,800	5,800	5,800	5,800	5,800
	3,700	3,750[b]	3,700[b]	3,620[b]	3,850[b]	4,600	3,580	3,750	3,850	3,850	3,280	3,280	3,280	3,280	3,280	5,700[b]
	3,580	3,580	3,580	3,580	3,580	3,580	2,650	3,580	3,750[b]	3;580	2,950	2,950	2,950	2,950	2,950	2,950
	2,650	2,650	2,650	2,650	2,650	1,170	1,170	2,650	3,580	2,650	2,800	2,900[b]	2,870[b]	2,800	2,800	1,180
	2,250	2,250[b]	1,170	1,170	1,170	960	960	1,170	2,650	1,170	1,180	1,180	1,180	1,180	2,350[b]	1,070
	1,650	1,650	960	960	960	830	830	960	1,170	1,150[b]	1,070	1,070	1,070	1,020[b]		
	1,170	1,170	830	830	830	790	790	830	960	960						
	830	830	790	790	790			790	830	830						
									790	790						

TABLE 2—Continued

Restriction enzyme	*Bgl*I				*Bgl*II								*Bst*EII					
Pattern	1	2	3	4	1	2	3	4	5	6	7	8	1	2	3	4	5	6
Genome type	p,p1	a,a1–3, a5,b,c, d,d1,e,f	a4	g	p	p1	a	a1,a3–5, b,c,d,d1	a2	e	f	g	p,p1	a,d,e	a1–5,c	b,f	d1	g
Size of fragments (bp)	13,000	13,000	13,000	13,000	8,100	8,100	8,100	8,100	8,100	8,100	8,100	8,100	12,700	10,700	10,700	10,700	10,700	10,700
	9,000	9,000	9,000	9,000	5,400	6,200[b]	5,400	5,400	5,400	5,400	6,200[b]	3,700	10,000	10,000	10,000	10,000	10,000	10,000
	5,100[b]	4,170	4,170	4,170	5,100	5,400	3,700	3,700	3,700	3,700	5,400	3,450[b]	4,350	4,350	4,350	4,350	4,780[b]	4,280[b]
	4,170	3,300	3,300	3,300	3,700	5,100	3,220	3,220	3,220	3,220	3,220	3,220	4,030	4,030	4,030	4,030	4,350	4,030
	2,040	2,400[b]	2,750[b]	2,040	3,220	3,220	2,720	2,720	2,840[b]	2,720	2,720	2,720	2,750	2,750	2,750	2,750	2,750	2,750
	1,830	1,830	1,830	1,830	2,380	2,340	2,470	2,470	2,720	2,470	2,470	2,470	1,950	2,200[b]	2,170[b]	2,170	2,200	1,950
	940	1,700	1,700	1,700[b]	2,300	1,880	2,380	2,380	2,470	2,380	2,300	2,380	930	1,950	1,950	1,950	1,950	930
	780	940	940	940	1,880	1,550	2,300[b]	2,240[b]	2,380	2,340[b]	1,850[b]	2,180		930	930	830[b]		
				780[b]	1,550	1,270	1,880	1,850	1,850	1,880	1,550	2,000[b]						
					1,270	1,020	1,550	1,550	1,550	1,550	1,270	1,880						
					1,020	630	1,270	1,270	1,270	1,270	1,020	1,550						
					630		1,020	1,020	1,020	1,020	630	1,270						
							630	630		630		1,020						
												630						

TABLE 2—Continued

Restriction enzyme	*Eco*RI			*Hin*dIII			*Hpa*I				*Sal*I	*Sma*I			*Xba*I			*Xho*I
Pattern	1	2	3	1	2	3	1	2	3	4	1	1	2	3	1	2	3	1
Genome type	p	p1,a,e,f	a1–5,b, c,d, d1,g	p,p1	a	a1–5,b, c,d,d1, e,f,g	p,p1	a,a1–5, b,c,d, e,f	d1	g	All	p,p1	a,a1–5, c,e	b,d,d1, f,g	p,p1,g	a,a1,a4–5,b,c, d,d1,e,f	a2–3	All
Size of fragments (bp)	31,000	31,000	32,000[b]	7,300	7,300	7,300	12,000	25,000	25,000	14,000[b]	18,000	9,800	7,700[b]	12,700[b]	15,300	10,400[b]	13,500[b]	19,000
	4,600	4,600	4,600	5,800	7,000[b]	5,800	7,900	7,400	7,400	12,000	12,000	6,900	6,900	6,900	9,100	9,100	10,400	8,800
	880	800[b]		4,800	5,800	4,800	7,400	3,500	2,780[b]	7,200	6,500	5,300	5,100	4,700	7,300	7,300	7,300	3,900
				4,500	4,500	4,500	5,300[b]		1,020	3,400		4,700	4,700	3,700	4,400	4,650	4,650	2,650
				3,500	3,500	3,500	3,500					3,700	3,700	2,600		4,400[b]		1,250
				3,300	3,300	3,300						2,500	2,600	2,500				700
				1,730	1,730	2,230[b]						2,150	2,500	2,150				
				1,420	1,420	1,730						1,070	2,150	1,070				
				1,370	1,370	1,420							1,070					
				1,200		1,370												
				1,050														

[a] All strains were propagated in A-549 cells. Viral DNA was extracted by a modification of the method of Shinagawa (18).
[b] Characteristic fragments or members of a characteristic pair of fragments.

TABLE 3. Percentage of PCRF of 15 genome types of Ad7 obtained from cleaving with 12 restriction endonucleases

Genome type	% PCRF with:													
	p1	a	a1	a2	a3	a4	a5	b	c	d	d1	e	f	g
p	93	78	74	71	72	74	72	72	73	74	68	75	72	73
p1		75	71	68	70	71	70	69	71	72	66	78	75	71
a			91	87	88	89	90	86	90	88	83	94	88	75
a1				94	97	99	98	95	98	94	89	91	88	78
a2					97	93	93	87	91	88	84	86	83	74
a3						96	95	92	95	91	86	88	85	76
a4							97	93	97	93	87	90	87	78
a5								93	96	92	90	89	86	76
b									94	96	92	87	92	80
c										93	88	91	88	77
d											95	89	88	79
d1												83	84	74
e													89	77
f														76

endonucleases. This may be taken as a sign of a remarkable genetic stability of the most widely spread Ad7 genome type.

Three restriction sites differed in the genomes of Ad7b and Ad7d (Table 2). The approximately 13,500-bp *Bam*HI fragment of Ad7d corresponds to the Ad7b *Bam*HI fragments of 9,600 and 3,850 bp. The Ad7b *Bst*EII 2,170- and 830-bp fragments may, after duplication, correspond to the 2,200- and 930-bp fragments of Ad7d. Ad7b may have evolved into Ad7d, since Ad7d first appeared later than Ad7b. Ad7b was discovered from 1958 to 1981, and Ad7d has been discovered since 1981 in China only.

In the comparison of Ad7d and Ad7d1, only three restriction sites differed. The approximately 2,350-bp *Bcl*I fragment of Ad7d1 corresponds to the 1,180- and 1,070-bp *Bcl*I fragments of Ad7d. The *Bst*EII fragment of Ad7d1 of approximately 4,780 bp can be expected to be formed by the 4,030- and 930-bp fragments of Ad7d. The approximately 3,500-bp *Hpa*I fragment of Ad7d corresponds to the 2,780- and 1,020-bp *Hpa*I fragments of Ad7d1. It is possible that Ad7d has evolved into Ad7d1. The Ad7d genome type predominated from 1981 to 1984 in Beijing, China, whereas Ad7d1 first appeared in December 1984.

So far, genome types Ad7d and Ad7g have only been found in China. Restriction site maps of the Ad7d and Ad7g genomes obtained with *Eco*RI, *Hin*dIII, *Hpa*I, *Sal*I, *Sma*I, *Xba*I, and *Xho*I have been elaborated (Fig. 1). At least four restriction sites, at map units 29.0, 38.3, 69.6, and 71.0, differed between Ad7d and Ad7g.

To estimate the relationships among these fifteen genome types, pairwise analyses of comigrating DNA restriction fragments of 15 Ad7 genome types were performed after digestion with the 12 restriction endonucleases. From 165 to 171 restriction fragments were compared in each pair of genome types (Table 3). The concentration of pairwise comigrating restriction fragments (PCRF) varied from 66 to 99% among the 15 Ad7 genome types.

Ad7p and Ad7p1 were closely related and displayed 93% comigrating fragments. Ad7e genome types, which were found in South America and Australia only, were closely related to Ad7a; the percentage of PCRF reached 94%. The percentage of PCRF between Ad7b and Ad7f reached 92%.

The degree of genetic relatedness, expressed as links from a given genome type to the two most closely related genome types, is presented in a diagrammatic fashion (Fig. 2). A degree of relation defined as 80% PCRF was used to divide all 15 genome types into three clusters. Cluster 1 contained

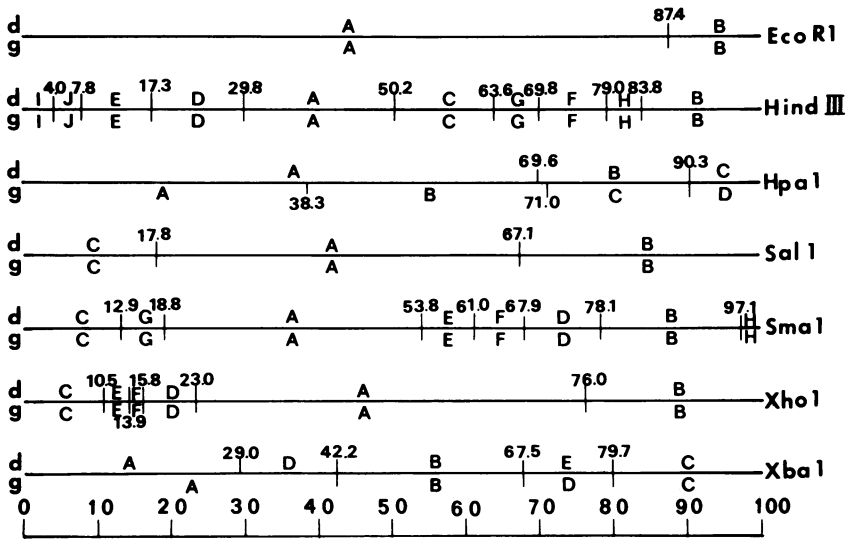

FIG. 1. Restriction site maps of the genomes of Ad7 and Ad7g obtained with the indicated restriction endonuclease.

Ad7p and Ad7p1, whereas Ad7g was the only member of cluster 2. All the other 12 genome types were grouped into cluster 3.

The degree of comigration between Ad7 genome types of the three different clusters (Fig. 2) is on the same level as that between different serotypes of the same subgenus: subgenus D Ad9 and Ad10, 79%; subgenus C Ad2 and Ad6, 77%; Ad1 and Ad2, 76%; subgenus B Ad3 and Ad7, 80%; Ad34 and Ad35, 78% (Adrian et al., Arch. Virol., in press). The expressed genetic variability within the Ad7 serotype is no less than that among different related adenovirus serotypes.

A denominating system for genome types is required as more and more new genome types are discovered after the analysis of adenovirus strains with restriction endonucleases. Up to now, the following genome types of human adenoviruses have been identified and designated: Ad2a (1), Ad3a (23), Ad3b to Ad3e (14), Ad4a (23), Ad5a (3), Ad7a to Ad7f (24), Ad8a and Ad8b (7, 9), Ad40a (11), and Ad41a (10). After digesting 40 Ad7 strains with the 12 restriction endonucleases, we found that *Bam*HI can distinguish 10 genome types. They have been designated p, a, b, c, etc., by the order in which they were identified.

The prototype is abbreviated p. We considered the following modification of the previously applied terminology. Restriction-fragment-length polymorphism was frequently limited to less than 3% of the fragment. Under these conditions, restriction-fragment-length-polymorphism variants were designated by the same letter.

The arabic numeral after p, a, b, c, etc., describes the different genome types that were distinguished by use of additional restriction endonucleases. The genome types were numbered in the order in which the strain was identified.

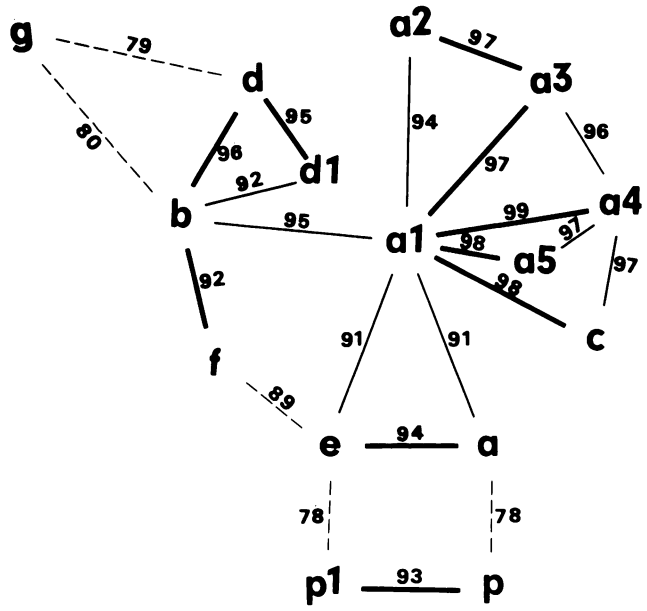

FIG. 2. Schematic map of the relationships among 15 genome types. The numbers are percentages of comigrating fragments. Degrees of homology in PCRF: ▬, highest; ——, second highest; – – –, the highest or second highest is below 90%.

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