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Genome Analysis of Bat Adenovirus 2: Indications of Interspecies Transmission

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Abstract

The genome of bat adenovirus 2 was sequenced and analyzed. It is similar in size (31,616 bp) to the genomes of bat adenovirus 3 and canine adenoviruses 1 and 2. These four viruses are monophyletic and share an identical genome organization, with one E3 gene and four E4 genes unique to this group among the mastadenoviruses. These findings suggest that canine adenoviruses may have originated by interspecies transfer of a vespertilionid bat adenovirus.

Adenoviruses (AdVs) have been described in every vertebrate class and in general exhibit a strong species specificity. Nevertheless, a study published recently (2011) by Chen et al. described the zoonotic transmission potential of AdVs from New World monkeys to humans (2). Also, bats have been recognized particularly as potential reservoir hosts of numerous zoonotic viruses (9).

The first bat AdV was isolated from a fruit bat in the suborder Megachiroptera (12). In 2008, we isolated the first AdV (bat adenovirus 2 *Pipistrellus pipistrellus* virus 1 [BtAdV-2 PPV1]) from a microchiropteran bat in Germany (13). A second microchiropteran bat AdV (BtAdV-3 strain TJM) was isolated from an Asian bat (*Myotis ricketti*), and an almost complete genome sequence has been reported (11). Twenty-nine additional bat AdVs have since been detected in China, Hungary, and Germany (5, 7, 15), and, by metagenomic study of bat guano specimens, the United States (10). In an initial study of BtAdV-2, a close phylogenetic relationship to canine adenoviruses (CAdVs) was observed (13). Although most AdVs are strictly host specific, CAdVs have been detected in an unusually broad range of carnivores (e.g., bears, wolves, raccoons, and sea lions) (1). Moreover, in their respective hosts, CAdVs are more severely pathogenic than the majority of adenoviruses. These observations and the close phylogenetic relationship led us to hypothesize an interspecies transmission event of a bat AdV to a carnivore. To explore this hypothesis, the whole genome of BtAdV-2 PPV1 was determined and analyzed.

In 2008, we detected a novel adenovirus in 3 of 55 free-ranging bats from Germany (13). We have now increased the number of animals examined to 330 European bats, with 116 belonging to two species of pipistrelles (*Pipistrellus pipistrellus* and *P. nathusii*). A full necropsy was performed on each bat, followed by histopathological examination.

For detection of BtAdV-2 DNA, a real-time PCR targeting the DNA polymerase gene was utilized (13). In addition to the initial three bats carrying BtAdV-2 (13), we detected the virus (sequence confirmed) by real-time PCR in an additional nine bats from Germany. Molecular biological examinations indicated distinct organ tropisms for intestine, kidney, and liver (Table 1), whereas histopathological changes were mostly limited to lung and spleen.

Shotgun pyrosequencing was carried out using a 454 Genome Sequencer FLX (454 Life Sciences, Branford, CT) in accordance with the manufacturer's protocol. A total of 15,565 reads (3,651,529 bp) were used for the Newbler and MIRA assembly, with an average coverage of 115.8 (3). Protein-coding open reading frames (ORFs) and splice sites were identified by comparative genomics with other mastadenoviruses.

The genome sequence (GenBank accession no. JN252129) consists of 31,616 bp, with an average G+C content of 53.5%, has an inverted terminal repeat (ITR) of 146 bp, and contains 31 predicted genes (Table 2).

Several features of the BtAdV-2 genome (Fig. 1) are worthy of comment. The BtAdV-2 genome is actually the first full genome of a bat AdV as the ITRs of BtAdV-3 were not determined. The first 40 bp (in the ITR) of BtAdV-2 are identical to those of CAdV-1and CAdV-2.

Each of the early regions E1, E3, and E4 contains at least one conserved genus-specific gene or ORF.

All three E1 genes (E1A, E1B 19K, and E1B 55K) are present in BtAdV-2, and their ORFs have typical lengths (Table 2).

In the middle part of the genome, 18 genes are conserved among all mastadenoviruses, including BtAdV-2 (4). The spliced nature of some of these genes is also evident. Each of the IVa2, pTP, DNA polymerase, and 33K genes consists of two exons, as in other mastadenoviruses.

The E3 region of BtAdV-2, BtAdV-3, and the CAdVs contains two genes. The first is the 12.5K gene, which is present in the majority of mastadenoviruses. Although this gene was not mentioned in the original description of the BtAdV-3 genome, it is clearly present (11). The second gene is E3 ORF1 and is present in BtAdV-3 and the CAdVs but not in other AdVs studied to date.

Also, the U exon, which is present in almost all AdVs (8, 14), is present in BtAdV-2 between E3 ORF1 and the fiber gene. Sequence conservation in this gene can be detected within but not among AdV genera, and downstream exons have been detected thus far only in members of the mastadenovirus species *Human adenovirus C*.

The protein playing the most crucial role for virus attachment is the antenna-like projection called “fiber.” All nonprimate mastadenoviruses have a single fiber gene, and BtAdV-2 and BtAdV-3 are no exceptions.

The E4 region is the second most variable region in mastadenovirus genomes (after the E3 region) both in its length and in its genetic contents (14). Adjacent to the fiber gene is the spliced ORF6/7 gene, which occurs in many mastadenoviruses. The adjacent 34K gene is the most conserved gene in the E4 region and consists of the first exon of ORF6/7 extended into the intron. In some mastadenoviruses (e.g., bovine AdV-3 and porcine AdV-5) and seemingly in all atadenoviruses, the 34K gene is duplicated (6, 14), whereas BtAdV-2, BtAdV-3, and CAdVs have only a single instance. To the right of the 34K gene, four novel putative genes (ORFA to -D) have been described in CAdVs and BtAdV-3, although the functions of their predicted protein products are not known (11). Homologous ORFs are present in BtAdV-2.

Similarities between bat and canine AdVs are apparent not only in their genome organization (including unique arrangements of the E3 and E4 genes), but also in their phylogenetic relationship.

Multiple alignments of predicted amino acid sequences were prepared with the MultAlin 5.4.1 program, and phylogenetic tree reconstructions were performed on six large viral proteins (DNA-dependent RNA polymerase, terminal protein precursor, pIIIa, penton base, hexon, and 100K) by three different methods (distance matrix, maximum likelihood, and Bayesian). In each Bayesian (Fig. 2), maximum likelihood, and distance matrix analysis tree, BtAdV-2, BtAdV-3, and the CAdVs grouped monophyletically, thus strongly supporting the origin of these viruses from a common ancestor.

The overall genetic distance between BtAdV-2 and BtAdV-3 (and also their distance to the cluster of CAdVs) exceeds 5%, which is a prerequisite for assigning AdVs into separate species. As the first bat AdV for which extensive sequence data were derived, BtAdV-3 has recently been proposed as the founding member of a new species called *Bat adenovirus A*. We now propose BtAdV-2 as the founding member of a new species called *Bat adenovirus B*. Official approval of these two species is currently awaiting voting by the International Committee on Taxonomy of Viruses (ICTV).

In summary, the results of histopathological and molecular biological investigations indicate that BtAdV-2 may be associated in bats with either an enteric course of infection or a mild to inapparent infection of other organs and strongly suggest that BtAdV-2, BtAdV-3, CAdV-1, and CAdV-2 have descended from a common ancestor. As there are several other vespertilionid AdVs similar to BtAdV-2 and BtAdV-3, the most obvious hypothesis is that the CAdVs originated from a bat AdV by host switching at some point in the past. Incomplete adaptation to the new host is consistent with the high pathogenicity of CAdV-1 and the ease with which it can cross the host species barrier between different carnivore hosts.

Nucleotide sequence accession number.

The GenBank accession number of the sequence reported in this paper is JN252129.

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Tables and Figures

Table 1 Molecular biological and histopathological results for pipistrelle bats found positive for BtAdV-2

Bat host	Species	Age	Sex	Origin	Organ tropism (PCR results)		Histopathological results[b]	
					Organ(s) PCR positive	No. of viral DNA copies[a]	Histopathological change(s)	Autolysis
198/07	*P. pipistrellus*	Adult	Male	Bavaria, Germany	Intestine Liver Kidney	$>1.0 \times 10^6$ 1.4×10^3 3.1×10^5	Lung, nonsuppurative interstitial pneumonia (+)	+++
199/07	*P. pipistrellus*	Adult	Female	Bavaria, Germany	Intestine Liver Kidney	$>1.0 \times 10^6$ 2.0×10^3 2.0×10^3	Lung, nonsuppurative interstitial pneumonia (++) and leucocytostasis of blood vessels (+++)	++
200/07	*P. pipistrellus*	Adult	Male	Bavaria, Germany	Intestine Spleen Kidney	$>1.0 \times 10^6$ 1.4×10^3 2.6×10^2	Lung, nonsuppurative interstitial pneumonia (++); spleen, follicular hyperplasia (+++)	++
279/08	*P. pipistrellus*	Adult	Male	Lower Saxony, Germany	Intestine	7.9×10^3	Lung, nonsuppurative interstitial pneumonia (++) and leucocytostasis of blood vessels (+++)	+
332/08	*P. nathusii*	Adult	Male	Bavaria, Germany	Intestine Kidney	2.0×10^3 3.2×10^1	Lung, leucocytostasis of blood vessels (+); spleen, follicular hyperplasia (+)	–
348/08	*P. pipistrellus*	Subadult	Female	Bavaria, Germany	Intestine Salivary glands	3.2×10^1 6.9×10^1	Lung, neutrophilic infiltration of alveolar septa (+); spleen, follicular hyperplasia (+++) and colliquative necrosis (+++)	+
097/09	*P. pipistrellus*	Subadult	Male	Bavaria, Germany	Intestine	4.4×10^5	Skin, focal purulent ulcerative dermatitis (+++)	+++
142/09	*P. pipistrellus*	Subadult	Male	Bavaria, Germany	Intestine	7.9×10^3	Lung, nonsuppurative interstitial pneumonia (++)	–
173/09	*P. pipistrellus*	Adult	Male	Berlin, Germany	Intestine	3.2×10^1	Spleen, follicular hyperplasia (+)	++
198/09	*P. pipistrellus*	Juvenile	Male	Berlin, Germany	Intestine	6.7×10^2	Lung, nonsuppurative interstitial pneumonia (+++)	–
199/09	*P. pipistrellus*	Adult	Male	Berlin, Germany	Intestine	2.9×10^2	Spleen, follicular hyperplasia (++)	–
228/09	*P. pipistrellus*	Adult	Male	Berlin, Germany	Intestine	1.6×10^1	Spleen, follicular hyperplasia (+++)	+

a Number of copies of viral DNA per ml of homogenized organ tissue (average sample size, 8 mm3 per ml).

b Degree of severity: - , none; +, mild; ++, moderate; +++, severe.

Table 2 Characteristics of the genes of the two bat and two canine adenovirus types, with human adenovirus 2 included for comparison

Gene name, type, or product	Coding sequence positions[a]	Size of gene (bp or aa) from virus of species[b]: BtAdV-B[c] (virus BtAdV-2 [31,616 bp])	BtAdV-A[c] (virus BtAdV-3 [>31,680 bp])[d]	CAdV: Virus CAdV-1 (30,288 bp)	CAdV: Virus CAdV-2 (31,323 bp)	HAdV-C (virus HAdV-2 [35,937 bp])
ITR	1–146, 31471–31616	146	ND[e]	199	198	102
E1A	490–1000, 1085–1305	243	217	230	232	289
E1B 19K	1474–2016	180	182	169	169	175
E1B 55K	1836–3170	444	459	444	444	495
IX	3237–3548	103	106	103	103	140
IVa2	3545–4881, 5160–5172 c	449	442	446	446	449
pol	4654–8061, 12628–12636 c	1,138	1,142	1,149	1,150	1,198
pTP	7917–7740, 12628–12636 c	610	609	608	610	671
52K	9777–10970	397	438	389	388	415
pIIIa	10843–12591	582	574	563	567	585
III	12660–14093	477	525	477	477	571
pVII	14125–14508	127	134	170	172	198
V	14582–15895	437	433	421	428	369
pX	15852–16133	93	69	68	69	80
pVI	16186–16932	248	273	238	249	250
Hexon	17000–19723	907	908	905	905	968
Protease	19735–20355	206	206	206	206	204
DBP	20399–21783 c	461	472	454	454	529
100K	21796–23895	699	682	689	689	805
33K	23732–23860, 24099–24401	143	164	149	149	228
22K	23732–24262	176	155	128	128	195
pVIII	24405–25094	229	222	224	224	227
E3 12.5K	25081–25431	116	118[f]	117	119	107
E3 ORF1	25454–26626	390	382	216	364	
U exon	26658–26821 c	55	67	55	55	54
Fiber	26820–28493	557	555	543	542	582
E4 ORF6/7	28508–28725, 29512–29533 c	79	96	86	86	150
E4 34K	28748–29533 c	261	260	265	259	294
E4 ORFD	29534–29986 c	150	130	124	124	
E4 ORFC	29862–30275	137	124	132	123	
E4 ORFB	30302–30661	119	119	128	134	
E4 ORFA	30746–31141	131	187	153	131	

a The letter “c” indicates the product is coded on the complementary strand.
b The sizes of the ITR are given in base pairs (bp). All other sizes are given in numbers of amino acids (aa).
c Proposal is waiting for final approval by ICTV.
d The genome ends have not been determined.
e ND, not determined.
f Not determined in the original submission.

Figure 1 Genomic organization of BtAdV-2. The genome is represented by a black horizontal line marked at 2,000-bp intervals. ORFs assumed to encode proteins are shown as arrows. Coding regions in the first exons of spliced genes are represented by rectangles.

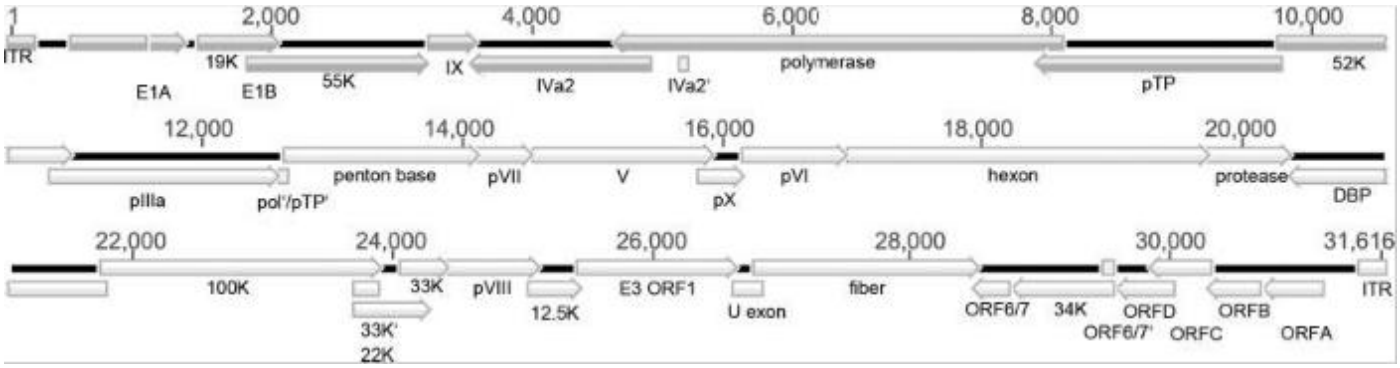

Figure 2 Phylogenetic (Bayesian) analysis of BtAdV-2 using amino acid sequences of a nonstructural protein and a structural protein of the four AdV genera containing multiple species. (a) DNA-dependent DNA polymerase; (b) hexon. Posterior probability values are depicted. BtAdV-2 is shown in boldface. The scale bar shows the evolutionary distance of 0.2 amino acid substitution per position. The calculations were unrooted, but for visualization, members of the genus *Siadenovirus* were used as the outgroup. Accession numbers: BtAdV-2, JN252129; BtAdV-3, GU226970; bovine AdV-1 (BAdV-1), NC_006324; BAdV-2, AC_000001; BAdV-3, AC_000002; BAdV-4, AF036092; CAdV-1, AC_000003; CAdV-2, AC_000020; duck AdV-1 (DAdV-1), AC_000004; fowl AdV-1 (FAdV-1), AC_000014; FAdV-4, AJ431719; FAdV-9, AC_000013; frog AdV-1 (FrAdV-1), AF224336; human AdV-3 (HAdV-3), DQ086466; HAdV-4, AY487947; HAdV-5, AC_000008; HAdV-12, X73487; HAdV-40, L19443; HAdV-54, AB333801; murine AdV-1 (MAdV-1), NC_000942; MAdV-2, HM049560; MAdV-3, EU835513; ovine AdV-7 (OAdV-7), U40839; porcine AdV-3 (PAdV-3), AF083132; PAdV-5, AF289262; raptor AdV-1 (RAdV-1), EU715130; simian AdV-1 (SAdV-1), AY771780; SAdV-3, AY598782; snake AdV-1 (SnAdV-1), DQ106414; tree shrew AdV-1 (TSAdV-1), NC_004453; turkey AdV-1 (TAdV-1), GU936707; and TAdV-3, AC000016.