Pantó, Laura; Podgorski, Iva I.; Jánoska, Máté; Márkó, Orsolya; Harrach, Balázs (2015)
Taxonomy proposal for Old World monkey adenoviruses: characterisation of several non-human, non-ape primate adenovirus lineages.
Archives of Virology, 160 (12). pp. 3165-3177. ISSN 0304-8608

Taxonomy proposal for Old World monkey adenoviruses − existence of several non-human, non-ape primate adenovirus lineages

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†Máté Jánoska, gifted scientist and beloved colleague passed away during the research period of this paper.

Abstract

A preliminary species classification is proposed for the (serotyped) Old World monkey adenovirus prototypes. Based on partial sequences of the IVa2, DNA-dependent DNA polymerase (pol), penton base, and hexon genes acquired by consensus PCR from earlier not sequenced simian adenoviruses (SAdVs), we found most of the SAdVs to belong to one or the other of the two earlier accepted species that contain monkey AdV serotypes. *Human mastadenovirus G* has been established for HAdV-52, but SAdV-1, -2, -7, -11, -12, and -15 also belong to it. The species *Simian mastadenovirus A* includes SAdV-3, -4, -6, -9, -10, -14, and -48. Several SAdVs (SAdV-5, -8, -49, -50) seemed to be members of the earlier proposed species *Simian mastadenovirus B*, together with baboon AdV-1, and rhesus monkey AdV strains A1139, A1163, A1173, A1258, A1285, A1296, A1312, A1327 and A1335. *Simian mastadenovirus C* should contain SAdV-19, together with baboon AdV-2/4 and -3. Our study revealed the existence of four further virus lineages. These candidate species are *Simian mastadenovirus D* (SAdV-13), *Simian mastadenovirus E* (SAdV-16), *Simian mastadenovirus F* (SAdV-17, -18), and *Simian mastadenovirus G* (SAdV-20). Several biological and genomic properties such as host origin, hemagglutination panel, number of fiber genes, and GC content of the genome support this proposed classification. Three SAdV strains originating from the American Type Culture Collection turned out to be mixtures of at least two virus types, either of the same species (SAdV-12 and -15 types from *Human mastadenovirus G*) or even of two different species (SAdV-5 types from *Human mastadenovirus G* and *Simian mastadenovirus B*).

Introduction

Simian adenoviruses (SAdVs) are members of the genus *Mastadenovirus* in family *Adenoviridae* [16]. The first mentioning of SAdVs in the literature was about a chimpanzee AdV [39], today known as SAdV-21 within the species *Human mastadenovirus B* (HAdV-B). The first monkey AdVs were discovered (with other, sort of mainly enteric simian viruses) while testing poliomyelitis vaccines on kidney tissue cultures of two macaque species [18]. Further monkey AdV serotypes, characterized by the lack of cross-neutralization [1], were found by screening other macaques and monkeys from two additional species, grivet and baboon [12, 19, 20, 24, 31]. Later on, when investigating chimpanzees suffering from kuru, experiments resulted in the discovery of four further ape AdVs [38].

The first classification of monkey AdVs was based on hemagglutination-inhibition test as a tool of taxon demarcation [37]. With the use of this biological assay, the 16 studied monkey AdV strains were separated to four hemagglutination groups (HG I–IV; Table 1). By analysing the DNA sequence of the genomes` left end, researchers inferred that SAdV-16 (originally named as SA7; Table 1) shares similar organization with HAdV-12, a member of HAdV-A species [25]. The similarity of chimpanzee AdVs to some HAdVs, namely HAdV-4 strains from the HAdV-E species, was recognized quite early [29].

Comparative molecular analysis of the 25 recognized SAdV types, SAdV-1 to 20, isolated from Old World monkeys (OWM), and SAdV-21 to 25 from chimpanzees has been performed by PCR amplification and sequencing of the virus-associated RNA (VA RNA) gene [23]. Two tandem VA RNA genes were detected in the genome of all chimpanzee AdVs, while in monkey AdVs only one (or none, most probably because of the PCR imperfections). These data were applied in making the first phylogenetic tree of SAdVs [23]. The short VA RNA sequences and the first partial hexon sequences, gained from chimpanzee AdVs in our lab, prompted us to place SAdV-21 into the species HAdV-B, and SAdV-22 to 25 into species HAdV-E [1]. These assumptions regarding the chimpanzee AdVs were confirmed later by the phylogenetic analysis of other, longer and finally full genome sequences [2, 10, 36, 40]. The first completed chimpanzee AdV genome was that of SAdV-25 [10]. It was followed by the genomes of the other four chimpanzee AdV types: SAdV-21 to 24 [40], and that of two further chimpanzee AdV genomes (under the strain names of ChAd3 and ChAd6) that had been deposited in GenBank but formally published later only [8, 35].

Ape AdVs have been isolated not only from chimpanzee, but also from bonobo and gorilla [41]. These viruses were suggested to be members of species HAdV-B, -C or -E, respectively, as they are definitely part of those lineages [41]. Partial genome analysis of further gorilla AdVs confirmed the theory on the mixed host-range of HAdV-B members [47]. HAdV-D species had only representatives of human source, however, a recent study suspected that some chimpanzee (and in case of HAdV-F, a gorilla) AdVs might belong to the above recited species [48] and a chimpanzee AdV belonging to HAdV-A has been even fully sequenced [50]. Production of vector vaccines from chimpanzee AdVs was started more than 10 years ago [49], and it has been the subject of growing interest [5]. Consequently, there is a growing number of chimpanzee AdVs available and tested. Colloca and co-workers [8] screened more than thousand chimpanzee and bonobo fecal samples and isolated AdVs from around 50% of them. The full sequence of some of these viruses indicated that they are closest to members of the species HAdV-B, -C or -E [8].

SAdVs have been found to be associated with several diseases of primates, including diarrhea, pneumoenteritis, conjunctivitis, hepatitis [4, 24, 45, 51], and some of them were reported to induce tumors when

injected into neonate rodents [21]. Short sequences from various genes of monkey AdVs are often reported from captive macaque colonies, either suffering from diarrhea [46] or not showing any clinical signs ascribed to AdVs [30, 48]. The study of monkey AdVs, compared to the ape AdVs, is left behind by now. The first full monkey AdV genome published was that of SAdV-3 (isolated from rhesus macaque) [27]. SAdV-3 was proposed to be the first member of a new species, SAdV-A. This species was accepted by the International Committee on Taxonomy of Viruses, and is still the sole species containing OWM AdVs exclusively [17]. The next sequenced OWM AdV genome was that of SAdV-1 (isolated from crab-eating macaque). This virus was classified into the species HAdV-G [28] together with a HAdV type, HAdV-52 [22]. Further full genomes were published from SAdV-48, -49 and -50 [41], as well as some partial sequences [4, 33]. Meantime, as the interest in OWM AdVs as potential gene delivery tools increased, additional SAdV genomes were fully sequenced. These included SAdV-7 [42] followed by SAdV-6, -18 and -20 [43]. Phylogenetic analysis of the novel AdVs isolated from rhesus macaques indicated the need to establish a new species, *Simian mastadenovirus B* (SAdV-B), together with previously sequenced SAdV-49 and -50 [43]. SAdV-48 was shown to belong to the SAdV-A species. Novel AdV strains, found in olive baboons and named baboon AdV-1 (BaAdV-1), BaAdV-2, -3 and -4, have been recently sequenced and proposed to form a third species *Simian mastadenovirus C* (SAdV-C) (BaAdV-2/4, -3), or to belong to the previously proposed SAdV-B (BaAdV-1) [7]. Recent publications reported AdVs also in New World monkeys (NWMs) [6, 14, 15, 48].

Nowadays, the recognized diversity of SAdVs is approaching that of the HAdVs. However, while ape AdVs are well characterized and fully classified, most monkey AdVs still await classification, and only very short or no sequence is published from their genome. Some short sequences (e.g., those acquired from the VA RNA by PCR) are inappropriate for comparative analysis. Therefore we targeted multiple genes of monkey AdVs, hoping that these new sequences will help the better understanding of the phylogenetic relationships of SAdV types suspected to have several distinct lineages.

Materials and Methods

Viruses

OWM SAdV strains (SAdV-1 to 20) deposited in the American Type Culture Collection (ATCC) were studied initially by PCR and compared with other SAdV sequences from other laboratories (Table 1). Purified DNA of SAdV-1 to 20, or cell culture supernatants were used. Because of its low quantity, the DNA of SAdV-5 was subjected to isothermal random amplification (Repli-g Mini Kit, Qiagen), in accordance with the instructions of the manufacturer, to provide more starting material.

PCR and DNA sequencing

Shorter or longer fragments were obtained by PCR amplification from four genes including the IVa2, DNA-dependent DNA polymerase (pol), penton base and hexon of 14 non-sequenced SAdVs (Table 1). The primers and PCR products are presented in Table 2. The IVa2 and penton base gene fragments were amplified with degenerate primers designed in our laboratory based on highly conserved amino acid (aa) motifs of numerous mastadenoviruses (IVa2) or only SAdVs (penton base) (Table 2). To connect the 5’ end of the gained IVa2 gene fragment and the 3’

end of the pol gene fragment, degenerate PCR primers targeting monkey AdV genes (designated as "sasu") were designed based on conserved nucleotide sequences. Sequencing primers were designed if needed for primer walking (Table 3). PCRs were performed using GoTaq DNA polymerase (Promega Corp.) with the following parameters (final concentration in 50 μl): 3 mM $MgCl_2$, 0.2 mM dNTP, 1 μM of both primers, GoTaq Buffer and 1.5 unit GoTaq enzyme. SAdV-24 was used as positive control, except in cases when sasu or specific primers were applied. The PCR program consisted of an initial denaturation step at 94˚C for 5 min followed by 45 cycles (94˚C, 30 s; 46˚C, 60 s; 72˚C, 60 s) and final elongation step at 72˚C for 3 min. PCRs with sasu primers were modified to fewer cycles (35), annealing at 52˚C for 30 s and elongation at 72˚C for 90 s, with final elongation of 7 min. Product size, quality and amount were checked by loading 10 μl of the reaction mixture on agarose gels. Amplified fragments were purified using the Nucleospin Extract II Kit (Macherey-Nagel), and sequenced by the use of the Big Dye Terminator v3.1 Cycle Sequencing Kit (Life Technologies Inc.) and 3500 Series Genetic Analyzer (Life Technologies). When heterogeneous sequences were gained, amplicons were molecularly cloned with use of the pJET1.2/blunt Cloning Vector (Thermo Fischer Scientific Inc.). Chemically competent DH5α *E. coli* strain was transformed with the ligated vector by heat-shock (90 s, 42˚C) and positive plasmids were purified with the alkaline lysis method.

Phylogenetic analysis

The identity of the sequences was checked with BLASTX program at NCBI. Primate AdV sequence alignments were edited with MEGA6 program [44]. The tree shrew AdV-1 (TSAdV-1) was included as outgroup. Although several of the studied SAdVs were recently fully sequenced (Table 1), phylogenetic calculations were based only on the gene fragments available for every studied virus. Phylogenetic relationships were based on partial aa sequence alignments for the IVa2 and pol, whereas on partial nucleotide sequence alignments for the penton and hexon genes. Protein sequence analyses were performed by using protdist and PhyML on the Mobyle portal provided by the Institute Pasteur, and the ProtTest program [9]. The protdist analyses were run with JTT substitution model followed by Fitch-Margoliash analysis applying the global rearrangements option. PhyML calculations [13] were based on aprotdist based user tree and a model gained by the ProtTest (JTT with the invariable sites and gamma distribution options). Nucleotide sequence analyses were performed by using PhyML in TOPALi v2 platform [34] with TrNef model for the penton base and TIMef model for the hexon gene, both with invariable sites and gamma distribution options (proposed by the Model Selection module of TOPALi). Bootstrap analysis with 100 sampling was applied for all the trees, which were then visualized by MEGA6 program [44] using TSAdV-1 as outgroup.

Results

The IVa2 nested PCR resulted in considerable amount of product after the first round from a few samples only. Therefore, the product (253 bp without the primers) of the second round was used for analyses. Degenerate primers targeting the pol gene of the primate AdVs generated sufficient amount of amplicon in the first round of the nested PCR [47], so we did not perform the second round. For the sequencing of these longer fragments, we designed an internal consensus primer (4466; Table 3). Assembling of the sequences resulted in a sequence of 952 bp (from nucleotide position 5269 to 6220 in SAdV-1 genome of GenBank). PCR with sasu primers (connecting the partial IVa2 and pol genes) resulted in a product of 2154 to 2226 bp (coding 502 aa from the pol and 287 aa from the IVa2

gene that could be used for phylogenetic analyses). Product length of the penton base PCR varied from 319 to 331 bp (316 bp were used for phylogenetic analyses), whilst from the hexon gene 253 bp were determined.

Phylogenetic calculation showed usually well separated, distinct lineages supported by high bootstrap values (Fig. 1). When preparing the hexon-based tree (Fig. 1d), the corresponding sequence from several additional novel AdVs of rhesus monkeys [4, 11, 33, 48] could also be included. Considering the tree topologies as well as other features of the examined viruses, the existence of minimum four distinct lineages, most probably meriting the species-level demarcation, was revealed besides the already established HAdV and SAdV species (Table 4). Species HAdV-G and SAdV-A appeared in two clear lineages on all four trees, with a great number of types. Two OWM ATCC strains (SAdV-5 and -8) together with the earlier described lineage involving SAdV-49, -50 [41], plus nine AdVs isolated from rhesus macaques [43], and one AdV isolated from olive baboon (BaAdV-1) [7] appeared on the trees as a monophyletic clade corresponding to the recently proposed SAdV-B species. SAdV-13 alone seemed to represent an independent lineage, candidate species SAdV-D. A sister clade was formed by the closest virus, a novel AdV strain (23336) reported from rhesus macaque most recently [32]. It is negotiable if the distance between these two viruses warrants separate species classification, or both of them should fall in the proposed species SAdV-D. Similarly, SAdV-20 also formed alone an independent branch most closely diverging from the clade species SAdV-A. In this case, the establishment of a novel species SAdV-G seems to be justified. SAdV-16 always appeared closest to the clade of species SAdV-B, but as a long distinct branch proposed SAdV-E on every tree. SAdV-17 and -18 were sister clades closest to the HAdV-F species members, but always well separated from them. SAdV-19 was on the branch together with other baboon AdVs of the SAdV-C species proposed recently [7].

Direct sequencing of the PCR products obtained from the penton base gene showed that three viruses, namely SAdV-5, -12 and -15, were mixtures. Molecular cloning resulted in the separation of two viruses in each mixture: both “SAdV-12” and “SAdV-15” had two different and considerably distinct HAdV-G members, respectively, whereas “SAdV-5” containing also two AdVs, had an HAdV-G member and another belonging to the candidate species SAdV-B (Fig. 1c).

Discussion

We amplified partial gene fragments from four different locations of the genome (between the genes of IVa2 and hexon), and the studied genes are different also by being expressed in different stages of the viral life cycle (representing both early and late genes). The pol is important because the species demarcation is based on this protein [16]. The amplified penton base gene fragment proved to be well applicable for detecting different types/variants in SAdV strains earlier supposed to be clean isolates, because the amplified region is a highly variable part. Molecular cloning of amplified penton base gene fragments proved that three of the ATCC strains (SAdV-5, -12 and -15) are mixtures of multiple AdVs. Consequently, even the validity of their original serological comparison with other strain can be questioned. IVa2 nested PCR was found to detect mastadenoviruses very effectively, and for the primate AdVs it proved to be even more sensitive than the pol PCR. The amplified partial hexon gene coding the highly conserved N-terminal part is a very popular genome fragment for general AdV PCR [4, 33, 48] thus further SAdVs could be compared to the studied ones on the hexon tree (Fig. 1d). Many of them could be safely assigned to several previously established or proposed species (SAdV-A, HAdV-G, SAdV-B), while a few of them (red colobus

3 [48], rh15 and rh50 [4]) seem to belong to still further species to be established in the future. The fully sequenced titi monkey AdV (a New World species) [6] and isolate 23336 from rhesus monkey [32] proved to be members of further species, too.

As only partial gene sequences were amplified, the validity of the phylogenetic trees is an important question. The bootstrap values mirror the length and conservation status of the amplified fragment: the highest values appeared on the IVa2 (Fig. 1a) and pol (Fig. 1b) aa based trees being 84-100, and 89-100 for different primate AdV species, respectively. The most trustable pol tree seems to show correctly also the "time" of the presumed acquisition of a second fiber gene (shown by a black arrow on Fig. 1). From that time-point, all the OWM AdVs and even HAdV members of HAdV-F and HAdV-G (both of them supposed to have originated from OWM AdVs), have two fiber genes. The only exception is SAdV-18, which probably lost it during a presumed host-switch and adaptation to grivet.

Comparing the GC percentages of AdV genomes, members of the different species proved to have well-distinguishable values and it is a further species demarcation criterion [16]. The limited sequence length of AdVs could lead to controversial inferences. However, data of completed genomes irrefutably make the difference between the proposed species. SAdV-B and -F are GC rich species (60.1-62.9%), SAdV-A, -E, and HAdV-G are moderately GC rich (54.4-57.9%), SAdV-C, -D, -G and HAdV-F have almost balanced GC content (47.8–52.6%), and strain 23336 has the lowest GC content (46.7%; Table 4).

All phylogenetic calculations indicate that 12 earlier described types and a recent isolate (SAdV-48) belong either to the species SAdV-A or HAdV-G, established earlier [22, 27]. Recently published AdV genotypes indicate that the prevalence of these two groups is high in macaques [4, 30]. Besides these two monkey AdV lineages, other genetic clusters appeared on the calculated trees as well. The third most numerous cluster is that of SAdV-5, -8, -49, -50, nine other rhesus macaque isolates [41, 43], and BaAdV-1 [7]. This cluster has been proposed to be named species SAdV-B [43], and the presently analyzed SAdV-5 and -8 are just further proved members of it, albeit the first ones that had been even serotyped by virus neutralization [37]. Other earlier publications based on partial hexon sequences supported this clade as well [4, 11, 33, 48]. SAdV-19 proved to be a further member of the previously proposed SAdV-C species [7]. This was supported by all of the calculated trees, the GC content, and also by the uniqueness and uniformity of the host (baboon).

SAdV-13 turned out to be the only member of a newly suggested species, SAdV-D. The exact host species of this AdV type (*Macaca sp.*) is not known, unfortunately. All the constructed phylogenetic trees inferred that SAdV-13 diverged from the other monkey AdVs at an early time. GC content of this virus is also different from all the other viruses (Table 4). The phylogenetically closest AdV is the rhesus monkey isolate 23336, but both the phylogenetic distance and the GC content (46.7 contra 49.9%) differentiate them.

SAdV-16 is closely related to the proposed species SAdV-B. However, its phylogenetic distance seems to be large enough to propose it to be the first member of a separate species. The different host species, grivet (*Chlorocebus aethiops*), supports the establishment of a new taxon for SAdV-16. However, as host switch is supposed to be relatively common among AdVs [48], it cannot be excluded that this virus has a macacue origin because the two other grivet AdVs (SAdV-17 and -18) are phylogenetically very different from SAdV-16, while very similar to each other. The GC content of the full genome of SAdV-16 is 57.9%, which is remarkably different from

those of the members of SAdV-B (~62%). Based on all data available for this virus, we propose this type to be classified as representative of novel species named SAdV-E.

SAdV-17 and -18 compose the sister taxon of HAdV-F. The phylogenetic distance and host species difference (*Chlorocebus aethiops*) seems to be sufficient to propose a new species, SAdV-F. Also the genome organization of SAdV-18 shows very important differences compared to HAdV-40 and -41 (the two known members of HAdV-F). SAdV-18 has one fiber gene, while HAdV-40 and -41 have two of it. The HAdV-F members are unique among the primate AdVs, as they do not have RGD motif in the penton-base gene, and they also lack the 12.5K gene in their E3 region, while SAdV-18 has this gene. The GC content of HAdV-40 and -41 is 51%, while SAdV-18 has a GC rich genome (61.4%). We think that the proposal to establish a separate species (SAdV-F) for SAdV-17 and -18 is adequate.

Phylogenetic trees show SAdV-20 as the sister group of SAdV-A, but adequately distant to be a valid separate species (SAdV-G). The only exception is the hexon, where SAdV-20 is not separated clearly from SAdV-A members. This may be caused by some recombination, which occurs often in this gene. Difference in host species and radically different GC content of the SAdV-20 genome (47.8%) from that of SAdV-A members (54.4-55.8% on the full genome length) confirm our proposal for the new species, SAdV-G.

The penton base PCR was the best method in this work to detect different variants in three ATCC deposits. Our conclusion is that SAdV-12 and -15 are mixtures of two types of the HAdV-G species (Fig. 1c), respectively, whilst the SAdV-5 seems to be a mixture of a HAdV-G and a putative SAdV-B member. The signs of non-homogeneity were observed also in previously conducted cross neutralization experiments [20]. SAdV-5 (a mixture of HAdV-G and SAdV-B members) showed one way cross neutralization, and SAdV-12 (seemingly a mixture of two HAdV-G members) had two way cross-neutralization with different putative HAdV-G types. This early experiment did not study SAdV-15, so our statement on the mixed type is based on the described penton base sequences, but also on a shot-gun sequencing [27] attempt that revealed heterogeneity of SAdV-15 (unpublished).

The first classification of monkey AdVs, based on hemagglutination-inhibition test as an early tool of taxon separation [37] also helped us in the species demarcation. Every HAG group II member belongs to SAdV-A (Table 4). HAG III group members SAdV-5 and -8 belong to the SAdV-B, however this group includes several members of HAdV-G, too. This indicates that the HAG probe is not sensitive enough to make difference at the species level. Still, it may help to distinguish species from each other, thus this biological property is one of the applicable species demarcation criteria [16]. For example, HAG classification confirmed the uniqueness of SAdV-13 as it is the sole member of the HAG group I. Similarly, SAdV-16 is the sole member of HAG group IV, which confirms its classification as a new taxon.

The tissue tropism of the ATCC strains does not seem to be determined by phylogenetic clustering, i.e., AdVs isolated from internal organs (such as liver and kidney) share common species with apparent enteric SAdVs. It would be interesting to find out if different SAdV species have different tissue tropism in macaques, just as it is with HAdVs. E.g., members of HAdV-F, typical enteric AdVs, are found generally in human stool (and are common in waste water), while some HAdV-D members are notorious to infect the cornea and cause epidemic keratoconjunctivitis [3]. An early study reported that SAdVs can cause epidemic conjunctivitis in macaques [45]. By

comparing the results with our phylogenetic clustering, it is notable that seemingly only SAdV-A members cause this disease.

The host range of several newly proposed species, and the previously acknowledged SAdV-A and HAdV-G is mixed (Table 4). AdVs infecting different simian genera usually belong to separate AdV species. However, there are AdV species that contain AdVs of several different monkey species. In contraty, in some cases, AdVs from the same monkey species may belong to different viral species. This is a general feature of SAdV and HAdV species to have mixed host origins, e.g., human, chimpanzee, gorilla and bonobo AdVs were suggested as members of HAdV-B and HAdV-C [41]. But, crossing the host barrier occurs usually very rarely and mainly only among evolutionary close primate species, most characteristically between apes and humans or among OWMs.

Based on comparisons of the phylogenetic analyses and biological properties of OWM AdVs, we confirmed the need to establish two earlier proposed species SAdV-B and SAdV-C. Furthermore, we propose the establishment of four new SAdV species, SAdV-D, -E, -F and -G. All of these newly proposed species would contain only OWM AdVs for now. There is a need to study AdVs also from other monkey species in future, especially NWM and prosimian AdVs, to get better insight in the evolution and host characteristics of the complete primate AdV lineage. We assume that our proposal of SAdV species will be confirmed in the near future by finding further AdVs and analyzing the phylogeny and full genome characteristics of a large number of SAdVs.

Acknowledgements

We would like to thank Andor Doszpoly for kindly providing the PCR primers he designed for the penton base gene of SAdVs. Thanks are also due to Alistair Kidd and Alexander Zakhartchouk for generously sharing some samples with us. The research project was supported by the Hungarian Scientific Research Fund grant OTKA NN107632 and EU grant ADVance FP7-290002.

References

1. Benkő M, Harrach B, Russell WC (2000) Family *Adenoviridae*. In: Van Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, Maniloff J, Mayo MA, McGeoch DJ, Pringle CR, Wickner RB (eds) Virus Taxonomy, Classification and Nomenclature of Viruses: Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press, San Diego, pp 227-238
2. Benkő M, Harrach B (2003) Molecular evolution of adenoviruses. Curr Top Microbiol Immunol 272:3-35
3. Benkő M (2008) Adenoviruses: Pathogenesis. In: Mahy BWJ, Van Regenmortel HV (eds) Encyclopedia of Virology (Third edition). Elsevier, Oxford, pp 24-29
4. Bányai K, Esona MD, Liu A, Wang Y, Tu X, Jiang B (2010) Molecular detection of novel adenoviruses in fecal specimens of captive monkeys with diarrhea in China. Vet Microbiol 142:416-419
5. Capone S, D'Alise AM, Ammendola V, Colloca S, Cortese R, Nicosia A, Folgori A (2013) Development of chimpanzee adenoviruses as vaccine vectors: challenges and successes emerging from clinical trials. Expert Rev Vaccines 12:379-393
6. Chen EC, Yagi S, Kelly KR, Mendoza SP, Tarara RP, Canfield DR, Maninger N, Rosenthal A, Spinner A, Bales KL, Schnurr DP, Lerche NW, Chiu CY (2011) Cross-

species transmission of a novel adenovirus associated with a fulminant pneumonia outbreak in a new world monkey colony. PLoS Pathog 7:e1002155

7. Chiu CY, Yagi S, Lu X, Yu G, Chen EC, Liu M, Dick EJ, Carey KD, Erdman DD, Leland MM, Patterson JL (2013) A novel adenovirus species associated with an acute respiratory outbreak in a baboon colony and evidence of coincident human infection. MBio 4:e00084

8. Colloca S, Barnes E, Folgori A, Ammendola V, Capone S, Cirillo A, Siani L, Naddeo M, Grazioli F, Esposito ML, Ambrosio M, Sparacino A, Bartiromo M, Meola A, Smith K, Kurioka A, O'Hara GA, Ewer KJ, Anagnostou N, Bliss C, Hill AV, Traboni C, Klenerman P, Cortese R, Nicosia A (2012) Vaccine vectors derived from a large collection of simian adenoviruses induce potent cellular immunity across multiple species. Sci Transl Med 4:115ra112

9. Darriba D, Taboada GL, Doallo R, Posada D (2011) ProtTest 3: fast selection of best-fit models of protein evolution. Bioinformatics 27:1164-1165

10. Farina SF, Gao GP, Xiang ZQ, Rux JJ, Burnett RM, Alvira MR, Marsh J, Ertl HC, Wilson JM (2001) Replication-defective vector based on a chimpanzee adenovirus. J Virol 75:11603-11613

11. Foytich KR, Deshazer G, Esona MD, Liu A, Wang Y, Tu X, Jiang B (2014) Identification of new provisional simian adenovirus species from captive monkeys, China. Emerg Infect Dis 20:1758-1759

12. Fuentes-Marins R, Rodriguez AR, Kalter SS, Hellman A, Crandell RA (1963) Isolation of enteroviruses from the normal baboon (*Papio doguera*). J Bacteriol 85:1045-1050

13. Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52:696-704

14. Gál J, Hornyák Á, Mándoki M, Bakonyi T, Balka G, Szeredi L, Marosán M, Ludányi T, Forgách P, Sós E, Demeter Z, Farkas SL (2013) Novel mastadenovirus infection and clinical disease in a pygmy marmoset (*Callithrix* [*Cebuella*] *pygmaea*). Vet Microbiol 167:695-699

15. Hall NH, Archer LL, Childress AL, Wellehan JF (2012) Identification of a novel adenovirus in a cotton-top tamarin (Saguinus oedipus). J Vet Diagn Invest 24:359-363

16. Harrach B, Benkő M, Both GW, Brown M, Davison AJ, Echavarria M, Hess M, Jones MS, Kajon A, Lehmkuhl HD, Mautner V, Mittal SK, Wadell G (2011) Family Adenoviridae. In: King AMQ, Adams MJ, Lefkowitz E, Carstens EB (eds) Virus Taxonomy, Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier, Oxford, pp 125-141

17. Harrach B (2014) Adenoviruses: General features. Reference Module in Biomedical Sciences. Elsevier

18. Hull RN, Minner JR, Smith JW (1956) New viral agents recovered from tissue cultures of monkey kidney cells. I. Origin and properties of cytopathogenic agents S.V.1, S.V.2, S.V.4, S.V.5, S.V.6, S.V.11, S.V.12 and S.V.15. Am J Hyg 63:204-215

19. Hull RN, Minner JR (1957) New viral agents recovered from tissue cultures of monkey kidney cells. II. Problems of isolation and identification. Ann N Y Acad Sci 67:413-423

20. Hull RN, Minner JR, Mascoli CC (1958) New viral agents recovered from tissue cultures of monkey kidney cells. III. Recovery of additional agents both from cultures of monkey tissues and directly from tissues and excreta. Am J Hyg 68:31-44

21. Hull RN, Johnson IS, Culbertson CG, Reimer CB, Wright HF (1965) Oncogenicity of the simian adenoviruses. Science 150:1044-1046

22. Jones MS, Harrach B, Ganac RD, Gozum MM, Dela Cruz WP, Riedel B, Pan C, Delwart EL, Schnurr DP (2007) New adenovirus species found in a patient presenting with gastroenteritis. J Virol 81:5978-5984
23. Kidd AH, Garwicz D, Oberg M (1995) Human and simian adenoviruses: phylogenetic inferences from analysis of VA RNA genes. Virology 207:32-45
24. Kim CS, Sueltenfuss ES, Kalter SS (1967) Isolation and characterization of simian adenoviruses isolated in association with an outbreak of pneumoenteritis in vervet monkeys (Cercopithecus aethiops). J Infect Dis 117:292-300
25. Kimelman D, Miller JS, Porter D, Roberts BE (1985) E1a regions of the human adenoviruses and of the highly oncogenic simian adenovirus 7 are closely related. J Virol 53:399-409
26. Kiss I, Matiz K, Allard A, Wadell G, Benkö M (1996) Detection of homologous DNA sequences in animal adenoviruses by polymerase chain reaction. Acta Vet Hung 44:243-251
27. Kovács GM, Davison AJ, Zakhartchouk AN, Harrach B (2004) Analysis of the first complete genome sequence of an Old World monkey adenovirus reveals a lineage distinct from the six human adenovirus species. J Gen Virol 85:2799-2807
28. Kovács GM, Harrach B, Zakhartchouk AN, Davison AJ (2005) Complete genome sequence of simian adenovirus 1: an Old World monkey adenovirus with two fiber genes. J Gen Virol 86:1681-1686
29. Li QG, Wadell G (1988) The degree of genetic variability among adenovirus type 4 strains isolated from man and chimpanzee. Arch Virol 101:65-77
30. Lu J, Wang Q, Wang H, Li G, Gao G (2011) Molecular characterization of adenoviruses in fecal samples of captively bred rhesus macaques in China. Vet Microbiol 149:461-466
31. Malherbe H, Harwin R (1963) The cytopathic effects of vervet monkey viruses. S Afr Med J 37:407-411
32. Malouli D, Howell GL, Legasse AW, Kahl C, Axthelm MK, Hansen SG, Früh K (2014) Full genome sequence analysis of a novel adenovirus of rhesus macaque origin indicates a new simian adenovirus type and species. Virol Rep 3-4:18-29
33. Maluquer de Motes C, Hundesa A, Almeida FC, Bofill-Mas S, Girones R (2011) Isolation of a novel monkey adenovirus reveals a new phylogenetic clade in the evolutionary history of simian adenoviruses. Virol J 8:125
34. Milne I, Lindner D, Bayer M, Husmeier D, McGuire G, Marshall DF, Wright F (2009) TOPALi v2: a rich graphical interface for evolutionary analyses of multiple alignments on HPC clusters and multi-core desktops. Bioinformatics 25:126-127
35. Peruzzi D, Dharmapuri S, Cirillo A, Bruni BE, Nicosia A, Cortese R, Colloca S, Ciliberto G, La Monica N, Aurisicchio L (2009) A novel chimpanzee serotype-based adenoviral vector as delivery tool for cancer vaccines. Vaccine 27:1293-1300
36. Purkayastha A, Ditty SE, Su J, McGraw J, Hadfield TL, Tibbetts C, Seto D (2005) Genomic and bioinformatics analysis of HAdV-4, a human adenovirus causing acute respiratory disease: implications for gene therapy and vaccine vector development. J Virol 79:2559-2572
37. Rapoza NP (1967) A classification of simian adenoviruses based on hemagglutination. Am J Epidemiol 86:736-745
38. Rogers NG, Basnight M, Gibbs CJ, Gajdusek DC (1967) Latent viruses in chimpanzees with experimental kuru. Nature 216:446-449

39. Rowe WP, Hartley JW, Huebner RJ (1956) Additional serotypes of the APC virus group. Proc Soc Exp Biol Med 91:260-262
40. Roy S, Gao G, Clawson DS, Vandenberghe LH, Farina SF, Wilson JM (2004) Complete nucleotide sequences and genome organization of four chimpanzee adenoviruses. Virology 324:361-372
41. Roy S, Vandenberghe LH, Kryazhimskiy S, Grant R, Calcedo R, Yuan X, Keough M, Sandhu A, Wang Q, Medina-Jaszek CA, Plotkin JB, Wilson JM (2009) Isolation and characterization of adenoviruses persistently shed from the gastrointestinal tract of non-human primates. PLoS Pathog 5:e1000503
42. Roy S, Clawson DS, Adam VS, Medina A, Wilson JM (2011) Construction of gene transfer vectors based on simian adenovirus 7. J Gen Virol 92:1749-1753
43. Roy S, Sandhu A, Medina A, Clawson DS, Wilson JM (2012) Adenoviruses in fecal samples from asymptomatic rhesus macaques, United States. Emerg Infect Dis 18:1081-1088
44. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 30:2725-2729
45. Vasileva VA, Ivanov MT, Rumel NB, D'Yachenko AG, Kakubava VV, Danelyan GA (1978) Isolation and biological characterization of an adenovirus of rhesus macaques. Acta Biol Med Ger 37:1281-1287
46. Wang Y, Tu X, Humphrey C, McClure H, Jiang X, Qin C, Glass RI, Jiang B (2007) Detection of viral agents in fecal specimens of monkeys with diarrhea. J Med Primatol 36:101-107
47. Wevers D, Leendertz FH, Scuda N, Boesch C, Robbins MM, Head J, Ludwig C, Kühn J, Ehlers B (2010) A novel adenovirus of Western lowland gorillas (*Gorilla gorilla gorilla*). Virol J 7:303
48. Wevers D, Metzger S, Babweteera F, Bieberbach M, Boesch C, Cameron K, Couacy-Hymann E, Cranfield M, Gray M, Harris LA, Head J, Jeffery K, Knauf S, Lankester F, Leendertz SA, Lonsdorf E, Mugisha L, Nitsche A, Reed P, Robbins M, Travis DA, Zommers Z, Leendertz FH, Ehlers B (2011) Novel adenoviruses in wild primates: a high level of genetic diversity and evidence of zoonotic transmissions. J Virol 85:10774-10784
49. Xiang Z, Gao G, Reyes-Sandoval A, Cohen CJ, Li Y, Bergelson JM, Wilson JM, Ertl HC (2002) Novel, chimpanzee serotype 68-based adenoviral vaccine carrier for induction of antibodies to a transgene product. J Virol 76:2667-2675
50. Zhou C, Tian H, Wang X, Liu W, Yang S, Shen Q, Wang Y, Ni B, Chen S, Fu X, Fei R, Zhang W (2014) The genome sequence of a novel simian adenovirus in a chimpanzee reveals a close relationship to human adenoviruses. Arch Virol 159:1765-1770
51. Zöller M, Mätz-Rensing K, Kaup FJ (2008) Adenoviral hepatitis in a SIV-infected rhesus monkey (*Macaca mulatta*). J Med Primatol 37:184-187

Figure legends

Fig. 1 Phylogenetic analysis based on (a) partial IVa2 and (b) DNA-dependent DNA polymerase (pol) aa sequences and (c) partial penton base and (d) hexon nucleotide sequences. Simian AdVs are shown by their type number followed by abbreviation of host: rh, rhesus macaque; cr, crab-eating macaque; yb, yellow baboon; gr, grivet. AdVs found in rhesus macaques [4] for which only the short hexon sequence is available in GenBank are designated as "rhxx" (xx=strain number). Some types on the hexon tree are hidden as follows: rh+CHN(e): rh20, 25, 30, 34, 39, 43 and 48, CHN-39, -43, and -48; rh+CHN(f): rh2, 8, 9, 24 and 40, CHN-8; rh+CHN(g): rh51, and 59-60, CHN-30 and -51; rh+CHN(h): rh23, CHN-23 and -24. Black arrow indicates the time point from which all the fully sequenced AdVs (except SAdV-18) have 2 fiber genes. On the pol tree, G+C content is shown in brackets next to the species names. Other abbreviations: HAdV, human AdV; BaAdV, baboon AdV; TSAdV, tree shrew AdV; TMAdV, titi monkey AdV; Cynom1, cynomolgus monkey AdV-1

Table 1. Names of the studied SAdVs and related information

Name	Old name	Host species	Source	ATCC strain	Acc. number	Ref.
SAdV-1	SV1	Crab-eating macaque, *Macaca fascicularis*	rectal swab	VR-195	**AY771780**	[28]
SAdV-2	SV11	Rhesus macaque, *Macaca mulatta*	tissue culture	VR-196	KP853120, KP853125, KP853112	present work
SAdV-3	SV15			VR-1449	**AY598782**	[27]
SAdV-4	SV17			VR-198	KP853121, KP853126, KP853113	present work
SAdV-5	SV20		rectal swab	VR-199	KP853111, KP853127, KP853128, KP853114	present work
SAdV-6	SV39	Macaque, *Macaca sp.*	tissue culture	VR-200	**CQ982401**	[43]
SAdV-7	SV25	Rhesus macaque, *Macaca mulatta*	rectal swab	VR-201	**DQ792570**	[41]
SAdV-8	SV30	Crab-eating macaque, *Macaca fascicularis*		VR-1539	**KP329561**	present work
SAdV-9	SV31	Macaque, *Macaca sp.*		VR-204	KP853122, KP853129, KP853115	present work
SAdV-10	SV32			VR-205	KP853110, KP853130, KP853116	present work
SAdV-11	SV33	Rhesus macaque, *Macaca mulatta*		VR-206	**KP329562**	present work
SAdV-12	SV34		tissue culture (CNS)	VR-207	KP853123, KP853131, KP853132, KP853117	present work
SAdV-13	SV36	Macaque, *Macaca sp.*	tissue culture	VR-208	**KP329563**	present work
SAdV-14	SV37	Rhesus macaque, *Macaca mulatta*		VR-209	KP853124, KP853133, KP853118	present work
SAdV-15	SV38		cervical cord	VR-355	KP853109, KP853134, KP853135, KP853119	present work
SAdV-16	SA7	Grivet, *Chlorocebus aethiops*	rectal swab	VR-941	**KP329564**	present work
SAdV-17	SA17		unknown	VR-942	-	manuscript in preparation
SAdV-18	SA18			VR-943	**CQ982407**	[43]
SAdV-19	AA153	Yellow baboon, *Papio cynocephalus*	stool	VR-275	**KP329565**	present work
SAdV-20	V340	Grivet, *Chlorocebus aethiops*	fatal pneumoenteritis	VR-541	**HQ605912**	[43]
SAdV-48		Crab-eating macaque, *Macaca fascicularis*	stool	-	**HQ241818**	[41]
SAdV-49					**HQ241819**	
SAdV-50					**HQ241820**	
BaAdV-1		Olive baboon, *Papio hamadryas anubis*	nasal swab		**KC693021**	[7]
BaAdV-2/4					**KC693022**	
BaAdV-3					**KC693023**	
A1139[a]		Rhesus macaque, *Macaca mulatta*	stool		**JN880448**	[43]
A1163[a]					**JN880449**	
A1173[a]					**JN880450**	
A1258[a]					**JN880451**	
A1285[a]					**JN880452**	
A1296[a]					**JN880453**	
A1312[a]					**JN880454**	
A1327[a]					**JN880455**	
A1335[a]					**JN880456**	
23336[a]					**NC_025678**	[32]

The accession numbers of full genome sequences are shown with bold letters.

[a] strain name

Table 2. PCR primers

Name	Target sequence	Sequence 5' → 3'	Target size[a]	Position[a] (SAdV-1 genome)	Ref.
HexAdB	hexon (mastadenoviruses)	GCCGCARTGGTCYTACATGCACATC	252	17558–17809	[26]
HexAdJ		CAGCRYRCCGCGGATGTCAAART			
4431s[b]	DNA-dependent DNA polymerase (primate AdVs)	GTNTWYGAYATHTGYGGHATGTAYGC	952	5269–6220	[47]
4428as		GAGGCTGTCCGTRTCNCCGTA			
IVa2 outfo	IVa2 (mastadenoviruses)	CCNNSNCCNGARACNGTNTTYTT	351	3998–4348	present work
IVa2 outre		GGRTTCATRTTRTGNARNACNAC			
IVa2 info		CCNCARRTNGAYATGATHCCNCC	253	4067-4319	
IVa2 inre		TTNSWNGGRAANGCRTGRAARAAYTT			
penton outfo	penton base (SAdVs)	ACNCARACNATHAAYTTYGAYGA	318	13461–13778	Andor Doszpoly
penton outre		GTRTANACNCCNGGCATNAC			
sasu4617F	IVa2-polymerase (SAdVs)	CARATYTGCATYTCCCASGC	1161	4307–5467	present work
sasu5821R		TACACHTACAAGCCAATCAC			

[a] Primer length and position are excluded from the target size and position

Table 3. Sequencing primers

Name	Sequenced PCR product	Sequence 5' → 3'	Position (SAdV-1 genome)	Ref.
4466	4431s-4428as	CGTGRSHTACACHTAYAARCCAA	5470	present work
sasu-5040F	Sasu	ATCTCGATCCARCARRYYTC	4729	
sasu-5040R		GARRYYTGYTGGATCGAGAT	4707	
sasu-5330R		TCCAARGGMAARCTKCGCGCC	4994	

Table 4. Proposal of taxonomy based on phylogenetic analyses and other criteria

Adenovirus	Existing/ Proposed species	Host species	HAG	Fiber genes	GC content[b]
SAdV-1	HAdV-G	Crab-eating macaque	III	2	56.4 (55.2)
SAdV-2[a]		Rhesus macaque			58.0
SAdV-7				2	56.8 (56.3)
SAdV-11[e]					57.9 (55.0)
SAdV-12[a]					58.1
SAdV-15[a]				2[c]	57.5
HAdV-52		Human		2	57 (55.1)
SAdV-3	SAdV-A	Rhesus macaque	II	1	58.1 (55.3)
SAdV-4[a]					59.6
SAdV-6		Macaque		1	58.3 (55.8)
SAdV-9[a]					59.4
SAdV-10[a]					59.8
SAdV-14[a]		Rhesus macaque			59.6
SAdV-48		Crab-eating macaque		1	57 (54.4)
SAdV-5[a]	SAdV-B	Rhesus macaque	III	2	65.3
SAdV-8[e]		Crab-eating macaque			63.1 (60.3)
SAdV-49					65.6 (62.8)
SAdV-50					65.4 (62.6)
BaAdV-1		Olive baboon			65.4 (62.7)
A1139[d]		Rhesus macaque			65.7 (62.6)
A1163[d]					65.1(62.0)
A1173[d]					63.0 (61.1)
A1258[d]					63.0 (60.1)
A1285[d]					62.7 (61.0)
A1296[d]					65.5 (62.6)
A1312[d]					65.5 (62.6)
A1327[d]					65.5 (62.9)
A1335[d]					65.6 (62.8)
SAdV-19[e]	SAdV-C	Yellow baboon		2	53.7 (52.2)
BaAdV-2		Olive baboon		2	52.3 (52.6)
BaAdV-3					52.3 (52.3)
SAdV-13[e]	SAdV-D	Macaque	I	1	50.0 (49.9)
SAdV-16[e]	SAdV-E	Grivet	IV	2	63.6 (57.9)
SAdV-17[a]	SAdV-F	Grivet			64.5
SAdV-18				1	63.4 (61.4)
SAdV-20	SAdV-G	Grivet		1	47.1 (47.8)
23336[d]	NEW?	Rhesus macaque		1	47.4 (46.7)

[a] full genome sequence not available

[b] GC content of the amplified fragments or corresponding region of the full genome; GC content of the full genome is shown in brackets ( ).

[c] unpublished data

[d] strain name

[e] full genome sequence available; manuscript in preparation