AKADÉMIAI KIADÓ

Complete genome analysis confirms that the pygmy marmoset adenovirus is a variant of the skunk adenovirus 1 – Short communication

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RESEARCH ARTICLE

ABSTRACT

The complete genomic sequence along with phylogenetic analyses of an adenovirus (AdV), isolated from a dead captive pygmy marmoset (*Callithrix pygmaea*) from a Hungarian zoo is reported. Earlier, based on the phylogenetic analysis of the sequence of a PCR-amplified fragment from the DNA polymerase gene, the pygmy marmoset AdV (PMAdV) has been reported to cluster closest to certain chiropteran AdVs. In the following years similar AdVs were discovered in additional mammalian hosts, including a skunk (*Mephitis mephitis*), African pygmy hedgehogs (*Atelerix albiventris*), North American porcupines (*Erethizon dorsatum*) and grey fox (*Urocyon cinereoargenteus*). After the full genome analysis of the skunk adenovirus (SkAdV-1), a novel species *Skunk mastadenovirus A* (SkAdV-A) has been established. The AdVs, originating from the African pygmy hedgehogs, have been found to belong to virus species SkAdV-A. Partial gene sequences from the porcupine AdVs have also implied their very close genetic relatedness to SkAdV-A. The complete genomic sequence of PMAdV, examined in this study, was found to share 99.83% nucleotide identity with SkAdV-1, thus unequivocally represents a genomic variant of SkAdV-1. The observation that viruses classifiable as SkAdV-A are able to infect and cause diseases in several, distantly related mammals seems to deserve further studies to elucidate the infection biology of this intriguing AdV.

KEYWORDS

Adenoviridae, *Skunk mastadenovirus A*, complete genome, cross-species transmission

Adenoviruses (AdVs) are non-enveloped, dsDNA viruses infecting all classes of vertebrates, from fish to humans (Harrach et al., 2011). They are generally considered to be host-specific viruses with usually one host species (Davison et al., 2003). However, exceptions are also known. For example, canine adenovirus 1 (CAdV-1) has been reported from several carnivores (bears, foxes, sea lions and wolves) other than dog (Burek et al., 2005; Buonavoglia and Martella, 2007; Balboni et al., 2019a, 2019b). According to the phylogenetic calculations, CAdV-1 shares close common ancestry with certain AdVs found in small bats from the family Vespertilionidae (Li et al., 2010; Jánoska et al., 2011). Another example is the newly discovered skunk adenovirus 1 (SkAdV-1). A novel AdV has been isolated from a skunk (*Mephitis mephitis*), found dead in the wild in Canada. At necropsy, acute hepatitis and interstitial pneumonia were seen. The phylogeny inferences showed that the SkAdV-1 belongs to the cluster of CAdVs and chiropteran AdVs (Kozak et al., 2015). Subsequently, the presence of SkAdV-1 has repeatedly been reported in African pygmy hedgehogs (*Atelerix albiventris*), kept as pets in Japan (Madarame et al., 2016, 2019; Ochiai et al., 2020). These animals had respiratory diseases. Later, the virus was also detected in pet pygmy hedgehogs with bronchopneumonia in the USA (Needle et al., 2019). Most recently, SkAdV-1 has also been reported from North American porcupines (*Erethizon dorsatum*) with respiratory diseases (Balik et al., 2020) and from grey fox (*Urocyon cinereoargenteus*) (Needle et al., 2020).

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A few years before the isolation of SkAdV-1 in Canada, a yet unknown AdV had been isolated from the internal organs of a dead pygmy marmoset (*Callithrix pygmaea*) originating from a Hungarian zoo; prior to its death the marmoset showed severe respiratory signs (Gál et al., 2013). A PCR-amplified fragment from the DNA polymerase gene has been sequenced. Surprisingly, in the phylogeny analysis, the virus did not cluster with simian or human AdVs (Pantó et al., 2015; Podgorski et al., 2016), but appeared close to certain chiropteran AdVs (Gál et al., 2013). In the present study, we completed the whole genome sequencing and analysis of the pygmy marmoset AdV (PMAdV).

The DNA of the PMAdV (HUN/2009 strain) was extracted using the innuPREP Virus DNA/RNA kit (Analytic Jena AG). DNA library was prepared and sequenced using the Ion Torrent PGM platform (Life Technologies) (Homonnay et al., 2014). Sequences were assembled with the use of the CLC GW v7.0 software (www.qiagen.com). To determine the inverted terminal repeat (ITR) regions at both ends of the genome, 5′/3′ RACE Kit, 2nd Generation (Roche) was applied. Open reading frame (ORF) prediction was carried out by FgenesV (www.softberry.com) and DNA Javascript Translator 1.1 software (Perry, 2002). Phylogenetic relations within the genus *Mastadenovirus* were inferred from the analysis of the alignment of 1,080 deduced amino-acid-long sequences of the DNA polymerase gene. For the multiple alignment the online Mafft version 7 (Kuraku et al., 2013) was used with default parameters. After removal of the gaps, maximum likelihood method (Phyml) was used for phylogenetic calculations with the RTRev amino acid substitution model. Phyml was applied within the TOPALi v2.5 program package (Milne et al., 2004) (1,000 samplings).

The genome of the PMAdV proved to be 31,809 bp long (GenBank accession number: MN482116) and it showed 99.83% nucleotide (nt) identity to SkAdV-1 (31,848 bp). In total, 31 ORFs were predicted as putative genes. The nt differences resulted in minor (1–2) amino acid substitutions in some of the encoded proteins. The major structure proteins (hexon, penton and fibre) showed 100% amino acid identity to that of the SkAdV-1. The very simple E3 region contained only two genes. One is the homologue of the 12.5 K. The other putative gene is ORF A, where the largest indels were found. Amino acid alignments of the protein encoded by this ORF in the skunk, pygmy hedgehog and pygmy marmoset AdVs are presented in Fig. 1. The most divergent part was at the end (carboxy-terminal) of the protein. Here a frameshift mutation was observed in the PMAdV genome caused by the insertion of two thymine nucleotides at the position of 26,582–26,583. The sequence of the ORF A in the PMAdV was confirmed by PCR and Sanger sequencing as well. The phylogenetic tree reconstruction based on the adenoviral DNA polymerase sequence is shown in Fig. 2. In accordance with the high nt identity, PMAdV appeared in the genus *Mastadenovirus* in a common branch with SkAdV-1. In the same clade appeared the CAdVs, equine AdV-1 and certain AdVs from bats, classified into the family Vespertilionidae.

The genomic analysis and identification of PMAdV as an isolate of SkAdV-1 extended the host range of this peculiar virus. Although AdVs are generally considered as host species specific viruses, SkAdV-1 was found to be able to infect at least five, evolutionarily distantly related host animals. The pygmy marmoset is the smallest New World monkey, a member of the family Callitrichidae within the order Primata. The skunk and the grey fox belong to the order Carnivora, the pygmy hedgehog is classified into the order Eulipotyphia (that includes hedgehogs, shrews, and moles), whereas the North American porcupine belongs to the order Rodentia. Moreover, the two isolates of SkAdV-1 have been propagated successfully on simian, porcine, bovine, chiropteran, canine and even human cells, though with varying efficiency (Gál et al., 2013; Kozak et al., 2015).

The gene composition of the E3 region of AdVs is highly divergent. In the well-studied human AdVs, the genes of the E3 region proved to have immunomodulatory functions (Oliveira and Bouvier, 2019). Although similar studies have not been conducted on the ORF A gene, one can speculate

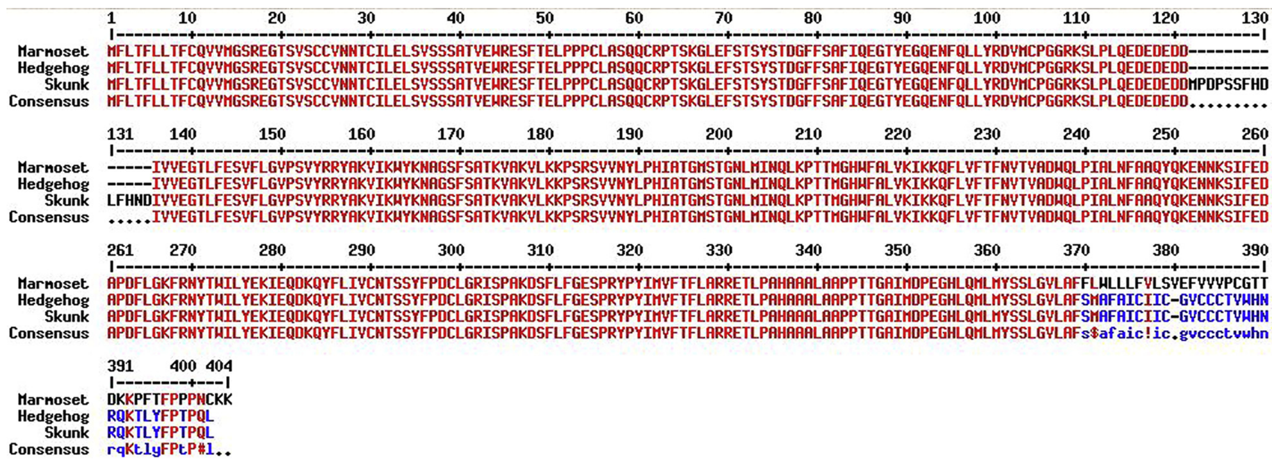

Fig. 1. Alignment of the deduced amino acid sequences of the putative genes, named ORF A in the SkAdV-1 variants from pygmy marmoset, pygmy hedgehog and skunk

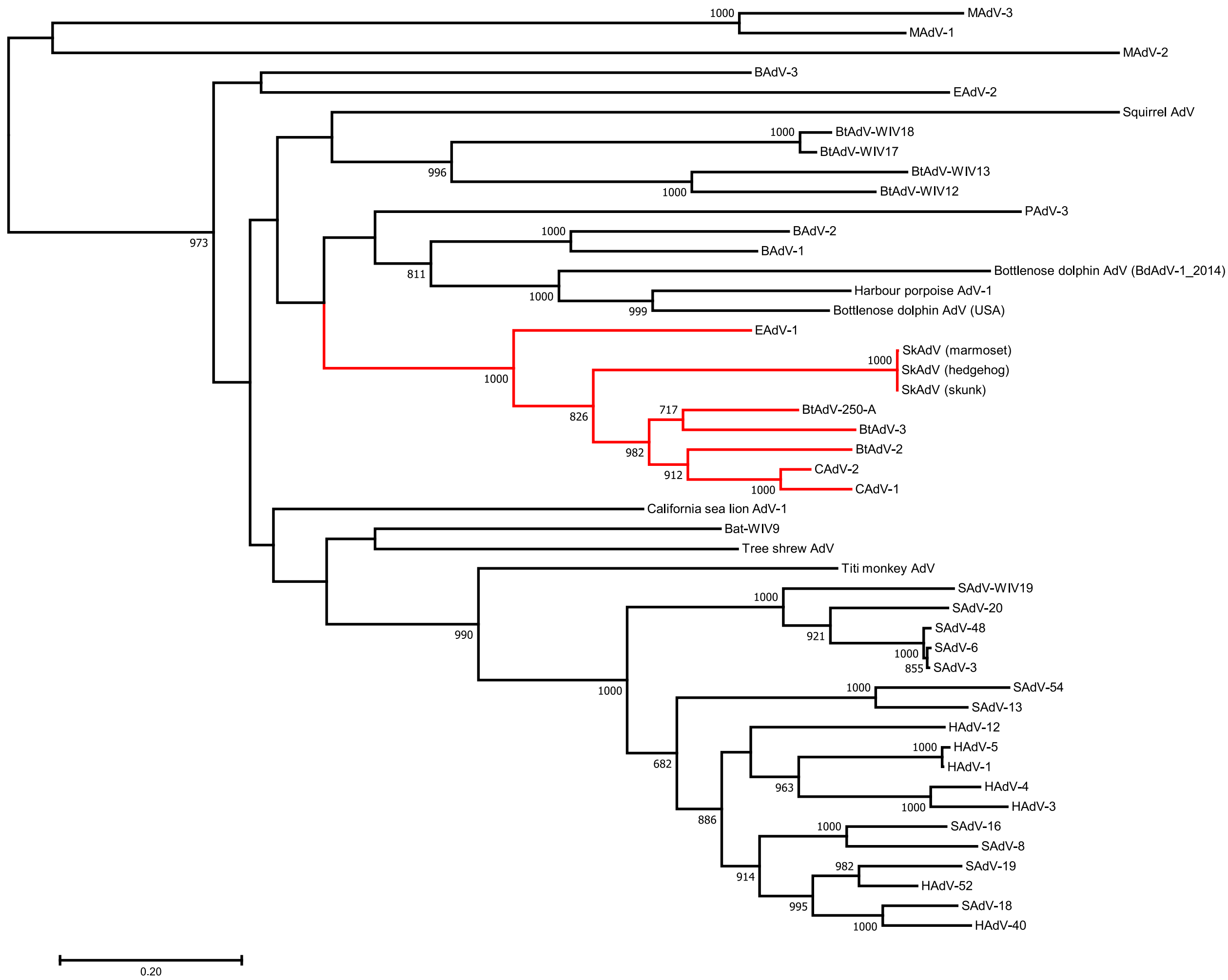

Fig. 2. Unrooted maximum-likelihood phylogenetic tree, based on the deduced amino acid sequence (1080 aa) of the DNA polymerase genes. The tree was rooted on midpoint. Bootstrap values greater than 600 are shown at the branch nodes. The branches of the cluster that, besides the SkAdV-1, also contains adenoviruses of certain vespertilionid bats, the two canine and one equine AdV, are highlighted in red. Abbreviations: BAdV = bovine adenovirus; BtAdV = bat adenovirus; EAdV = equine adenovirus; HAdV = human adenovirus; CAdV = canine adenovirus; MAdV = murine adenovirus; PAdV = porcine adenovirus; SAdV = simian adenovirus; SkAdV =skunk adenovirus (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

that the variability of this gene within the different SkAdV-1 isolates might contribute to the successful multiplication of the virus in different hosts. The homologues of ORF A can be found only in chiropteran, canine and equine-1 AdVs. The SkAdV also seems to be widely distributed, since it was reported from three continents (Asia, Europe and North America) from captive (zoo and pets) and wild animals (Gál et al., 2013; Kozak et al., 2015; Madarame et al., 2016; Balik et al., 2020; Needle et al., 2020). SkAdV-1 was detected from dead or diseased animals, usually displaying respiratory disease signs (Gál et al., 2013; Madarame et al., 2016; Needle et al., 2019; Ochiai et al., 2020). This is not surprising since cross-species transmission of AdVs and other dsDNA viruses often result in a more severe outcome of an infection (Doszpoly et al., 2011; Jánoska et al., 2011; Kohl et al., 2012; Vidovszky et al., 2015). The true host species of SkAdV-1 is currently unknown. The GC content of the SkAdV-1 isolates was balanced (48.71%), which seems to exclude the possibility that SkAdV-1 strains in different hosts undergo adaptive evolutionary processes that may be accompanied by a decrease of GC content. If this reasoning is correct, we should consider that some, if not all, of the identified hosts serve as dead-end hosts, in which the virus was able to efficiently replicate and cause morbidity, a feature that is rarely seen in AdVs. However, none of the animals positive for this virus exhibit large, intercontinental distribution. Therefore it seems reasonable to speculate that the true host of SkAdV-1 might be an animal widely dispersed geographically, which may be contacting both wild animals and captive animals at various locations of the world. Some bats and rodents, or even domestic mammals, fulfil these criteria, therefore they seem to be the primary candidates for being the true host of SkAdV-1. Irrespectively, the unusual phenotype of SkAdV, that is the ability to readily switch hosts among distantly related mammalian species, is compelling, and this finding deserves further investigations to be initiated. In this respect the zoonotic potential of SkAdV-1 needs exploration in order to estimate the risk of infection, if any, among pet owners and zookeepers.

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