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Characterisation of the *Equine adenovirus 2* genome

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ABSTRACT

Equine adenovirus 2 (EAdV-2) is one of two serotypes of adenoviruses known to infect equines. Initial studies did not associate EAdV-2 infections with any specific clinical syndromes, although more recent evidence suggests that EAdV-2 may be associated with clinical and subclinical gastrointestinal infections of foals and adults respectively. In contrast, *Equine adenovirus 1* is well recognised as a pathogen associated with upper respiratory tract infections of horses. In this study the complete genome sequence of EAdV-2 is reported. As expected, genes common to the adenoviruses were identified. Phylogenetic reconstructions using selected EAdV-2 genes confirmed the classification of this virus within the *Mastadenovirus* genus, and supported the hypothesis that EAdV-2 and EAdV-1 have evolved from separate lineages within the adenoviruses. One spliced open reading frame was identified that encoded for a polypeptide with high similarity to the pIX and E1b_55K adenovirus homologues and was designated pIX_E1b_55K. In addition to this fused version of E1b_55K, a separate E1b_55K encoding gene was also identified. These polypeptides do not appear to have evolved from a gene duplication event as the fused and unfused E1b_55K were most similar to E1b_55K homologues from the *Atadenovirus* and *Mastadenovirus* genera respectively. The results of this study suggest that EAdV-2 has an unusual evolutionary history that warrants further investigation.

1. Introduction

Equine adenovirus (EAdV) was first isolated in the United States of America (Todd, 1969), and subsequently reported in Australia (Wilks and Studdert, 1972) with initial characterisations published in 1973 (Ardans et al., 1973). Later studies suggested associations between EAdV infections and variable clinical presentations in foals and horses (Bell et al., 2006; Dunowska et al., 2002; Studdert et al., 1974; Wilks and Studdert, 1972). The adenoviruses were initially divided into two serotypes and subsequent molecular phylogenetic studies confirmed these serotypes as separate viral species now referred to as *Equine adenovirus 1* (EAdV-1) and *Equine adenovirus 2* (EAdV-2) (Cavanagh et al., 2012; Reubel and Studdert, 1997). Of the two species, EAdV-1 is predominantly associated with respiratory tract infections while EAdV-2 has mainly been isolated from horses with gastrointestinal tract infections (Studdert and Blackney, 1982). Giles et al. (2010) described a widespread prevalence of antibodies to EAdV-1 and EAdV-2 in the horse populations of New South Wales, Australia. Cavanagh et al. (2012) recently described the complete sequencing and characterisation of EAdV-1 genome which suggests this virus has a close molecular relationship to bat and canine adenoviruses within the genus *Mastadenovirus*. In comparison there is little information available on the genomic organisation and genetic structure of the EAdV-2 genome. The aim of this this study was to sequence and analyse the EAdV-2 genome. Molecular reconstruction phylogenetic analyses using selected genes from the viral genome confirmed EAdV-2 as a member of the genus *Mastadenovirus*. Genome analyses identified one open reading frame (ORF) which encodes a polypeptide which is a fusion of the pIX and E1b_55K homologues. Interestingly the pIX and E1b_55K regions of this polypeptide were most similar to homologues from different genera of the Adenoviruses. Other unusual features such as several putative genes for which no AdV homologues were identified are also discussed.

2. Materials and methods

2.1. Sequencing the EAdV-2 genome

EAdV-2 viral (Strain ID: EAdV2.385/75.9) (Studdert and Blackney, 1982) stocks were obtained from the University of Melbourne.

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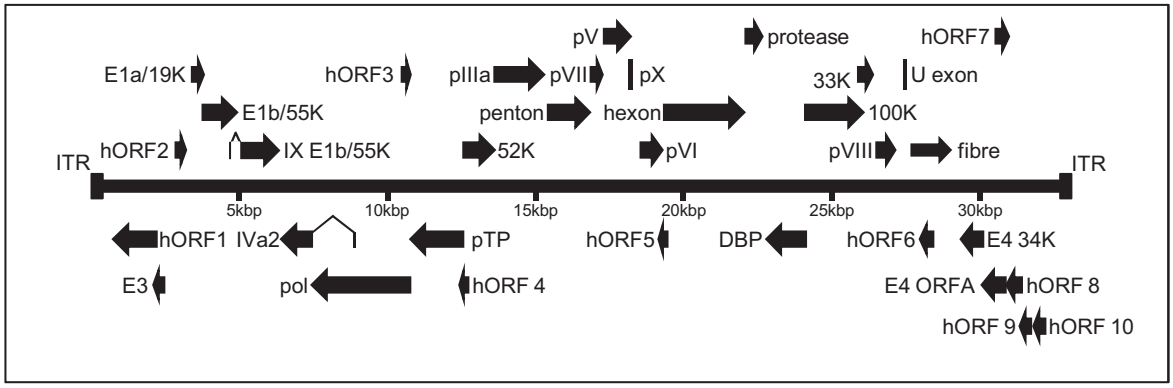

Fig. 1. The genomic organisation of the *Equine adenovirus 2* genome. Viral genes are represented as solid arrows with known protein products illustrated and named using adenovirus conventional nomenclature. Hypothetical open reading frames with unidentified polypeptides were labelled as hORF and numbered sequentially from the 5' end of the genome. The arrows represent the direction of gene transcription. Predicted spliced transcripts are shown as black lines linking the coding regions. The inverted terminal repeat (ITR) sequences are also illustrated at respective ends of the genome.

The virus was grown on primary equine kidney cells with a minimal essential media (Life Sciences), 10% foetal bovine serum, 2% L-glutamine (Life Sciences) and 2% antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin) (Life Sciences) at 37°C, 5% CO_2. Virus was recovered by polyethylene glycol precipitation and genomic DNA extracted using phenol-chloroform (Mahy and

Table 1
Summary of the predicted genes encoded by the *Equine adenovirus 2* (EAdV-2) genome. The nucleotide (nt) location of each gene in the viral genome is shown, along with the predicted amino acid (aa) length of the encoded polypeptides. The identified adenovirus homologues for each of the EAdV-2 polypeptide are illustrated. The details of the AdV homologues with the highest polypeptide similarity are also shown as the percentage similarity (%) to the EAdV-2 polypeptide, the adenovirus species, amino acid residues in the homologue and the relevant accession number. The genomic locations of hypothetical open reading frames (hORF) greater than 300 nucleotides for which no polypeptide homologues were identified are also shown. ^Encodes a putative fusion polypeptide of pIX (residues 1-68) and E1B_55K (residues 69-457). Polypeptide homologue identification and similarity scores were conducted separately. *Genes or ORFs predicted to be transcribed from the complementary strand of the viral genome are denoted by "c". #Abbreviations of adenovirus species used; BtAdV, bat adenovirus; BAdV, bovine adenovirus; CAdV, canine adenovirus; EAdV, equine adenovirus; HAdV, human adenovirus; OAdV, ovine adenovirus; PAdV, porcine adenovirus; SAdV, *Simian adenovirus*; TSAdV tree shrew adenovirus.

Gene	Location (nt)*	Length (aa)	AdV homologue	Percentage similarity (%); virus#; length (aa); accession number
hORF1	533–1984c	484	None identified	
E3	1965–2324c	120	12.5 kDa-like protein	46; CAdV-2; 119; AP_000629.1
hORF2	2678–3079	134	None identified	
E1a_19K	3254–3667	138	Small T-antigen	48; SAdV-1; 183; YP_213962.1
E1b_55K	3613–4773	387	Large T-antigen	48; SAdV-7; 471; ABH01040.1
IX_E1b_55K^	4578–4599 4833–6181	457	Hexon associated protein IX (1-68)	46; HAdV-52; 135; ABK35033
			E1b large T protein (69-457)	65; OAdV-7; 382; NP_659513
IVa2	6223–7562c 8852–8953c	479	IVa2 protein	70; SAdV-18; 447; YP_008520225
pol	7311–10655c	1115	DNA polymerase	75; OAdV-7; 1017; NP_064286.1
hORF3	10372–10671	100	None identified	
pTP	10583–12388c	602	Terminal protein precursor	80; BtAdV TJM; 609; YP_005271183
hORF4	12327–12629c	101	None identified	
52K	12458–13519	354	Late L1 protein, encapsidation	78; SAdV-6; 396; AFG19589.1
pIIIa	13528–15204	559	Precursor protein	75; TSAdV-1; 540; YP_068063
penton	15336–16757	474	Penton base protein	87; TSAdV-1; 476; YP_068064
pVII	16776–17192	139	Core protein	70; TSAdV-1; 131; YP_068065
pV	17222–18121	300	Minor core protein	55; HAdV-23; 333; AGW47841
pX	18137–18388	84	Late L2 mu core protein	76; BAdV-2; 70; Q96626
pVI	18469–19203	245	Capsid protein precursor	75; TSAdV-1; 247; YP_068068
hORF5	18999–19319c	107	None identified	
hexon	19259–21967	903	Hexon capsid protein	97; EAdV-2; 903; AAB88060 86; Caprine adenovirus 2; 908; ABG22146
protease	21995–22600	202	Adenovirus protease	96; EAdV-2; 201; PRO_ADE E2 79; BAdV-3; 204; NP_046324
dbp	22646–23953c	436	DNA binding protein	66; BAdV-3; 432; AEW91341
100K	24037–26028	664	Hexon assembly protein	73; BAdV-3; 850; AEW91343
33K	25838–26323	162	33 kDa protein	48, BAdV-3;274; AEW91344
pVIII	26465–27106	559	pVIll protein	78, PAdV-5; 222; NP_108672
U exon	27423–27599c	59	U exon protein	58, BAdV-3; 55; AP_000040
fibre	27601–29019	473	Fibre protein	57; BAdV-2; 548; AAN75194
hORF6	27921–28391c	157	None identified	
E4 34K	29264–30025c	254	Early E4 protein	54, BAdV-1; 253; YP_094053
E4 ORFA	30039–30827c	263	deoxyuridine 5′-triphosphate nucleotidohydrolase	60; BAdV-1; 142; YP_094055.1
hORF7	30525–30932	136	None identified	
hORF8	30814–31245c	144	None identified	
hORF9	31301–31702c	134	None identified	
hORF10	31790–32179c	130	None identified	