

Nucleotide sequence and polymerase chain reaction/restriction fragment length polymorphism analyses of Aleutian disease virus in ferrets in Japan

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Abstract. Two ferrets with spontaneous Aleutian disease (AD) were found in Japan. The diagnosis was verified by polymerase chain reaction (PCR) amplification of part of the capsid gene specific to AD virus (ADV). The nucleotide sequences (365 bp in length) of the amplified fragments from the 2 ferrets differed by a single nucleotide, producing an amino acid alteration. Compared with other types of ADV, these isolates had 96% sequence similarity to a published ferret ADV (FADV) in contrast to <91% homology to various types of mink ADV (MADV). The phylogenetic tree of ADVs indicates that these 2 isolates and the published FADV belong to the same genetic group and definitely are divergent from MADVs. The predicted amino acid sequence of the hypervariable segment in the capsid gene was conserved among the 3 types of FADV. These results indicated that the 2 isolates found in Japan were new DNA types of FADV and could have been derived from FADV(s). A restriction fragment length polymorphism (RFLP) method to distinguish the ferret types of ADV from the mink types of ADV was developed on the basis of differences in their nucleotide sequences. Digestion of the PCR products with *AfaI* or *ScaI* provided different cleavage patterns for FADV and MADV. This PCR/RFLP analysis of the ADV capsid gene will be a valuable asset for diagnosis of this virus infection in ferrets.

Aleutian disease virus (ADV) is the causative agent of Aleutian disease (AD) in ferrets (*Mustela putorius furo*) and mink (*Mustela vison*). ADV can be transmitted from ferret to ferret or from mink to ferret.^{2,5,8–11} Mink ADV (MADV) and ferret ADV (FADV) strains appear to be biologically distinct and species specific in their ability to cause disease, although these viruses are closely related antigenically.^{10,11,13}

A ferret with pathologically apparent Aleutian disease (AD) was observed for the first time in Japan in 1999.¹⁶ The diagnosis was confirmed by a polymerase chain reaction (PCR) assay using consensus primers¹⁴ directed against a part of the capsid gene of several strains of ADV. The PCR assay allowed rapid identification of ADV in another ferret, which was independent of the first infected ferret, representing the second case of ferret AD recognized in Japan and suggesting that the virus may be prevalent in Japan. Although this PCR method is suitable for the detection of diverse types of ADV, it cannot be used to determine a particular type of ADV. To characterize the ADV in the 2 ferrets, the nucleotide sequences of the amplified segments of the capsid gene from both specimens were analyzed.

PCR amplification was performed with samples of the formalin-fixed kidney from the first ferret and with heat-treated⁴ serum from the second ferret. A single fragment corresponding in length to the expected ADV capsid gene segment (401 bp) were obtained after 40 cycles of PCR, using a pair of primers as previously described.¹⁴ The PCR products were subsequently treated with exonuclease I^a and shrimp alkaline phosphatase^a and then were directly sequenced in both directions with the amplification primers using a DNA sequencer.^b The segments from both specimens were 365 bp in length aside from the primer annealing regions.

The nucleotide sequences of the ADV of the first ferret (FADV1) differed from those of the second ferret (FADV2) by only 1 nucleotide at position 292, which caused an amino acid change from lysine in the first virus to arginine in the second virus (Fig. 1). These ADVs were thought to be 2 different types of ADV at the molecular level. However, it is unknown whether this amino acid change has any effect on biological function. The fact that 2 different types of FADV were obtained from only 2 ferrets is not surprising; MADV is characterized by an usually high genetic diversity and the mutation of this virus is even biased toward amino acid changes.^{6,7,12} The sequence analysis indicated possible existence in Japan of various FADV types. The sequences were subsequently compared with those of other known ADV strains including an FADV strain, 7 representative MADV strains, which were available from the GenBank/EMBL/DDBJ DNA databases, and the newly determined sequences from 2 types of MADV (MADV1 and MADV2; Fig. 1), which appeared in Japan a few years ago. Each accession number is indicated in parentheses in Fig. 1. Only the nucleotides different from those of the reference sequence of FADV1 are shown. The location of the entire sequence (365 bp) corresponds to positions 3043–3407 on the complete sequence of the mink ADV-G. The sequences of FADV1 and FADV2 were very similar (96%) to that of a published FADV. In contrast, sequence similarity to various types of MADV was at most 90%. In a phylogenetic tree of ADV strains, FADV1, FADV2, and the published FADV formed a monophyletic group completely separate from the MADVs (100% bootstrap values) (Fig. 2). Thus, FADV1 and FADV2 probably were derived from a ferret ADV rather than a mink ADV. The hypervariable region of the capsid gene, spanning nucleotides 3093–3131 in the mink ADV-G strain (corresponding to positions 51–89; solid lines with arrows in Fig. 1), is considered a major determinant of host range and pathogenicity.¹ The predicted 13 amino acids of the region in FADVs 1 and 2 were identical to those from the published FADV. A translated isoleucine in the region,

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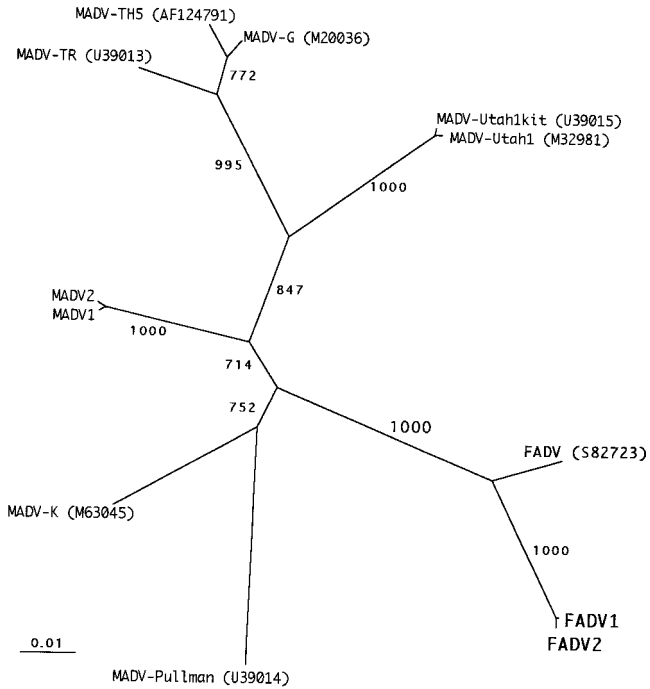


Figure 2. Phylogenetic tree of ADVs obtained by the neighbor-joining method.¹⁵ Numbers at the nodes indicate the bootstrap values from 1,000 replications. Scale bar is for branch lengths.

samples from ferrets with AD and from mink with AD, respectively.

Sources and manufacturers

- a. Amersham Pharmacia Biotech, Buckinghamshire, UK.
- b. 373A DNA sequencer, Applied Biosystems, Foster, CA.

FADV1 aa	1 QKAAQSTLEWTAI 13
FADV2 aa	1 13
FADV (S82723) aa	1 13
MADV-G (M20036) aa	1 ..V.TE..T.D.V 13
MADV-Utah1 (M32981) aa	1 ..LG.EQ....GT 13
MADV-Utah1kit (U39015) aa	1 ..MG.EQ....GT 13
MADV-K (M63045) aa	1 ..S...Q....GT 13
MADV-Pullman (U39014) aa	1P....GT 13
MADV-TH5 (AF124791) aa	1 ..V.GE..T.D.V 13
MADV-TR (U39013) aa	1 ..V.TE..T.D.V 13
MADV1 aa	1E.Q....GT 13
MADV2 aa	1ETQ....GT 13

Figure 3. The predicted amino acid (aa) sequences of the hypervariable region from nucleotide positions 51–89 in Fig. 1. Dots indicate amino acids identical to those of FADV1. The last amino acid, I (isoleucine), was conserved for all 3 types of FADV but was replaced by T (threonine) or V (valine) in MADV.

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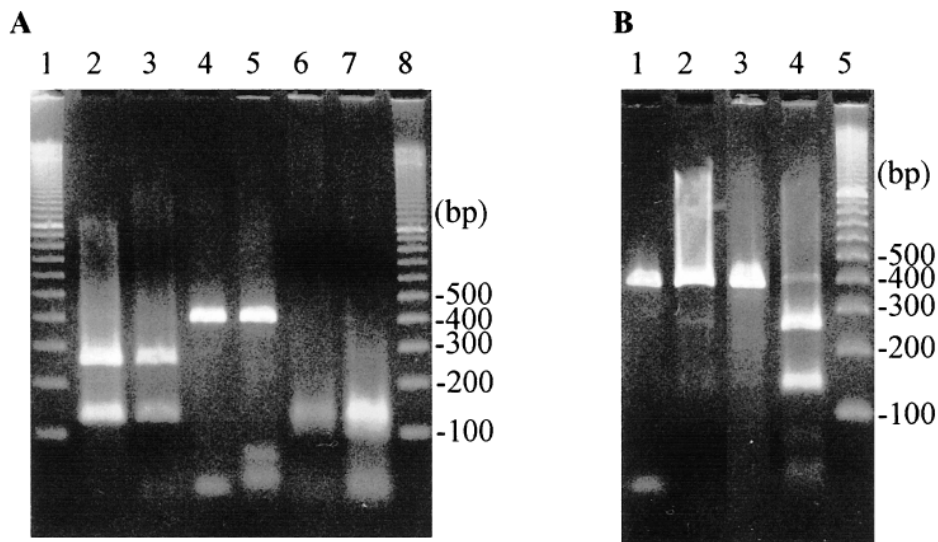


Figure 4. RFLP analysis of capsid gene PCR products from FADV and MADV. **A.** *AfaI*. Lanes 1 and 8: 100-bp markers; lane 2: digested FADV1; lane 3: digested FADV2; lane 4: nondigested FADV1; lane 5: nondigested MADV1; lane 6: digested MADV1; lane 7: digested MADV2. **B.** *ScaI*. Lane 1: nondigested FADV2; lane 2: digested FADV2; lane 3: nondigested MADV2; lane 4: digested MADV2; lane 5: 100-bp marker.

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