

SHORT REPORT

Characterisation of hexon and fibre genes of a novel strain of adenovirus involved in epidemic keratoconjunctivitis

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Aims: To characterise a novel strain (M86) of adenovirus (Ad) involved in epidemic keratoconjunctivitis (EKC).

Methods/Results: The virus strain was neutralised by antisera to both Ad35 and Ad11. Restriction endonuclease analysis of genomic DNA showed 98% and 88% homology with Ad11 and Ad35, respectively. The deduced amino acid sequence of the hypervariable regions of (HVRs) of the hexon gene showed a higher homology with Ad35 (94.4%) than with Ad11 (83.7%). However, it was 100% homologous to Ad35 in HVRs 1, 2, 3, and 6 and to Ad11 in HVRs 4 and 6. In the fibre knob, the isolate was more homologous to Ad11 (99.4%) than to Ad35 (29.1%).

Conclusion: This novel strain of adenovirus showed similarities with both Ad11 and Ad35. The isolation of a novel strain like Ad35+11 is important because of its association with EKC.

patterns of Ad11, Ad35, and M86, using the percentage of pair wise co-migrating restriction fragments of a pair divided by the total number of bands in the pair. The isolate (M86) showed 98% and 88% homology with Ad11 and Ad35, respectively (fig 1). Higher homology of the new strain in restriction endonuclease analysis with Ad35 and Ad11 provide supportive evidence that the new strain might have evolved from the recombination of these two parent viruses.⁷

The fibre knob enables the virus to attach to the cellular receptor and, together with the hexon protein, defines the serological specificity of the adenoviruses. Therefore, the hexon gene and the fibre gene were analysed to compare the immunological data with the molecular biological results, in addition to looking for any possible variation that might be related to ocular pathogenicity. The hypervariable regions

Adenovirus type 11 (Ad11) and adenovirus type 35 (Ad35) belong to subgenus B2, and cause opportunistic infections mainly among the immunocompromised patients.¹ Ad35 was isolated for the first time from a renal transplant recipient with interstitial pneumonia,² whereas Ad11 was isolated from a faecal specimen of a child with poliomyelitis.³ Here, we report the isolation of a novel strain of adenovirus from a 25 year old otherwise healthy male patient with severe clinical manifestations of epidemic keratoconjunctivitis (EKC) in southern Japan.

METHODS AND RESULTS

Immunochromatography confirmed the causative agent as adenovirus (strain M86).⁴ Conjunctival scrapings were isolated in A549 cells and the viral titre was also determined in a microtitre plate containing a confluent monolayer of A549 cells. Aliquots (25 µl) of 100 tissue culture infectious doses of virus (100TCID₅₀) were incubated with 25 µl of serially diluted type specific antisera at 37°C for 60 minutes and then inoculated into A549 cells. Viral growth was inhibited by two different type specific antisera, anti-Ad11 and anti-Ad35, at a 256-fold dilution, and the strain was identified as Ad35+11. Although Ad11 infrequently causes keratoconjunctivitis, Ad35 or a novel strain like M86 (Ad35+11) has never been reported as an ocular pathogen.⁵ Therefore, this strain was subjected to a detailed study at the molecular level.

Viral DNA extraction and restriction endonuclease analysis of M86 with BamHI, BglII, BstEII, EcoRI, HindIII, PstI, Sall, SmaI, XbaI, and XhoI (Boehringer Mannheim, Mannheim, Germany) were carried out to investigate homology with the serologically related prototypes (Ad35 and Ad11), as described previously.⁶ Genomic homology between M86, Ad35, and Ad11 was calculated from published restriction

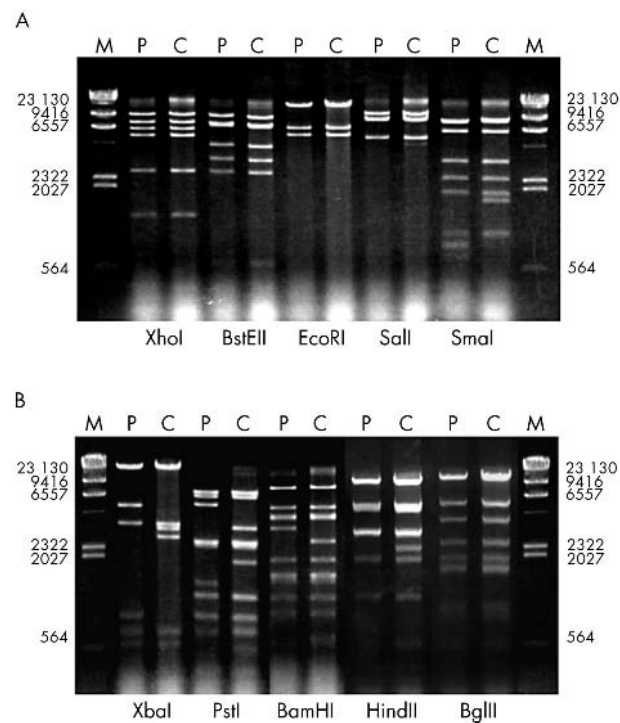


Figure 1 Restriction patterns obtained after cleavage of Ad35p (P) and M86 (C). (A) Restriction endonucleases XhoI, BstEII, EcoRI, Sall, and SmaI; (B) XbaI, PstI, BamHI, HindIII, and BglII. The samples were electrophoresed on a 1.2% agarose gel. A HindIII digest of λ DNA (lane M) was run as a molecular weight standard.

Abbreviations: AA, amino acid; Ad, adenovirus; EKC, epidemic keratoconjunctivitis; HVR, hypervariable region

Table 1 Hexon gene amino acid homologies (%) of M86 with other members of subgenus B and subgenus D

Ad	HVR1	HVR2	HVR3	HVR4	HVR5	HVR6	HVR7	Overall
Ad3	11.7	36.8	33.3	28.5	12.5	36.3	20.0	56.5
Ad7	11.7	41.1	33.3	38.0	31.2	50.0	16.2	61.2
Ad11	45.4	50.0	86.6	100.0	87.5	100.0	85.0	86.7
Ad34	48.9	50.0	100.0	28.5	62.5	50.0	15.3	70.7
Ad35	100.0	100.0	100.0	42.8	87.5	100.0	95.0	94.4
Ad8	12.7	9.5	12.1	42.2	12.5	41.6	10.0	51.3
Ad19	10.8	22.2	18.1	28.5	25.0	66.6	10.0	53.2
Ad37	10.8	41.1	18.1	25.0	10.6	66.6	10.0	52.7

Per cent of homology between the indicated hypervariable regions (HVRs) of M86 and the HVRs of adenovirus (Ad) subgenus B and subgenus D.

(HVRs) of the hexon gene and all regions of the fibre gene were sequenced by overlapping primers from genomic DNA by direct cycle sequencing. Multiple sets of primers for the hexon and fibre genes were selected based on alignment of the hexon gene (x76549 (Ad3), x76551 (Ad7), AB018424 (Ad11), AB018425 (Ad14), x74662 (Ad16), AY008279 (Ad21), AB018246 (Ad34), and AB018427 (Ad35)) and fibre gene sequences (m12411 (Ad3), m23696 (Ad7), L08231 (Ad11), AB065116 (Ad14), u06106 (Ad16), u06107 (Ad21), u10271 (Ad34), and u10272 (Ad35)) available from GeneBank. The sequences were determined by a Genetic Analyser 310 (Applied Biosystem, Foster City, California, USA). DNASIS software (Hitachi Software Ltd, Tokyo, Japan) was used for sequence alignment and analysis. The amino acid (AA) sequences of these residues were deduced. The AA sequences of M86 were compared with the available sequences of Ad3, Ad7, Ad11, Ad34, Ad35, Ad8, Ad19a, and Ad37 involved in keratoconjunctivitis. The nucleotide sequence data reported in our paper will appear in the DDBJ/GeneBank nucleotide sequence database with the accession numbers AB098564 (hexon gene) and AB098563 (fibre gene).

Table 2 Fibre gene nucleotide and amino acid homologies (%) of M86 with other members of adenovirus (Ad) subgenus B and subgenus D

	Ad11	Ad34	Ad35	Ad3	Ad7	Ad8	Ad19/37
Tail							
DNA	100.0	99.2	99.2	80.6	97.6	48.8	48.0
Protein	100.0	97.6	97.6	86.0	97.6	37.2	30.0
Shaft							
DNA	98.1	71.7	71.7	55.2	90.1	32.9	31.8
Protein	98.9	63.7	63.7	52.2	91.2	16.4	15.3
Knob							
DNA	99.8	45.0	45.4	50.6	93.7	27.9	32.2
Protein	99.4	28.9	29.1	28.8	91.6	8.3	10.6
Overall							
DNA	99.3	59.7	59.9	56.3	93.2	30.4	34.1
Protein	99.3	47.8	47.8	41.8	92.3	12.6	12.6

Per cent of homology between different fibre regions of M86 and those of adenovirus (Ad) subgenus B and subgenus D.

In HVRs of the hexon, M86 showed an overall 94.4% AA homology with Ad35 and 86.7% with Ad11 (table 1). However, it was 100% homologous to Ad35 in HVRs 1, 2, 3, and 6 and to Ad11 in HVRs 4 and 6 (fig 2). The fibre knob showed high AA homology (99.4%) with Ad11, but only 29.1% homology with Ad35 (table 2).

DISCUSSION

Members of subgenus D adenoviruses (Ad8, Ad19, and Ad37) are the common agent of EKC. Occasionally, the members of subgenus B (Ad3 and Ad7) and subgenus E (Ad4) are also related to EKC. The tropism of adenoviruses for conjunctival or corneal cells depends on the presence of certain amino acids in the knob, which attaches the virus to the specific cellular receptor.⁸ The fact that the fibre knob of M86 has 99.4% homology with Ad11, with only a single AA difference, but only 29.1% homology with Ad35, means that it is able to attach to conjunctival and corneal cells (fig 3).

		L1<		HVR 1		
Ad35	1	SGTAYNSLAP	KGAPNASQWI	AKGVPTAAAA	GNGEEEHETE	EKTATYTFAN
M86	1	SGTAYNSLAP	KGAPNASQWI	AKGVPTAAAA	GNGEEEHETE	EKTATYTFAN
Ad11	1	SGTAYNSLAP	KGAPNTSQWI	AEGVKNTTGE	EHVTEE---E	TNTTYTTFGN
		>	<	HVR 2	>	<
Ad35	51	APVKAEAQIT	KEGLPIGLEI	SAENESKPIY	ADKLYQPEPQ	VGDETWTDDL
M86	51	APVKAEAQIT	KEGLPIGLEI	SAENESKPIY	ADKLYQPEPQ	VGDETWTDDL
Ad11	51	APVKAEAEIT	KEGLPVGLEV	S-DEESKPIY	ADKTYQPEPQ	LGDETWTDDL
		>	<	HVR 4	>	<
Ad35	101	GKTEEYGGRA	LKPTTNMKPC	YGSYAKPTNL	KGGQAKPKNS	EPSSEKIEYD
M86	101	GKTEEYGGRA	LKPDTKMKPC	YGSFAKPTNV	KGGQAKQKTT	EQPNQKVEYD
Ad11	101	GKTEKYGGRA	LKPDTSMKPC	YGSFAKPTNV	KGGQAKQKTT	EQPNQKVEYD
		<	>	HVR 5	>	<
Ad35	151	IDMEFFDNSS	QRTNFSPIKIV	MYAENVGLET	PDTHVVKPG	TEDTSSEANL
M86	151	IDMEFFDAAS	QRTNFSPIKIV	MYAENVGLET	PDTHVVKPG	TEDTSSEANL
Ad11	151	IDMEFFDAAS	QKTNLSPKIV	MYAENVNLET	PDTHVVKPG	TEDTSSEANL
		>	<	HVR 6	>	<
Ad35	201	GQQSMNRPNP	YIGFRDNFIG	LMYYNSTGNM	GVLGQASQL	NAVVDLQDRN
M86	201	GQQSMNRPNP	YIGFRDNFIG	LMYYNSTGNM	GVLGQASQL	NAVVDLQDRN
Ad11	201	GQQSMNRPNP	YIGFRDNFIG	LMYYNSTGNM	GVLGQASQL	NAVVDLQDRN
		>	<	HVR 7	>	<
Ad35	251	TELSYQLLLD	SLGDRTRYFS	MWNQAVDSYD	PDVRVIENHG	VEDELPNYCF
M86	251	TELSYQLLLD	SLGDRTRYFS	MWNQAVDSYD	PDVRVIENHG	VEDELPNYCF
Ad11	251	TELSYQLLLD	SLGDRTRYFS	MWNQAVDSYD	PDVRVIENHG	VEDELPNYCF
		>	<	HVR 7	>	<
Ad35	301	PLDGIGVPTT	SYKSIVPNGE	DNNNWKPEEV	NGTSEIGQGN	LFAMEINLQA
M86	301	PLNGIGVPTT	SYKSIVPNGE	DNNNWKPEEV	NGTSEIGQGN	LSAMEINLQA
Ad11	301	PLDGIGVPTT	SYKSIVPNGD	NAPNWKPEEV	NGTSEIGQGN	LFAMEINLQA
Ad35	351	NLWRSFLY..
M86	351	NLWRSFLY..
Ad11	351	NLWRSFLY..

Figure 2 Comparison of predicted amino acid sequences of seven hypervariable regions (HVRs) of M86 with that of adenovirus type 11 (Ad11) and Ad35. The sequences of loop 1 (L1) and loop 2 (L2) were aligned to obtain maximal homology. Deduced amino acid sequences of Ad11 and Ad35 were obtained from GeneBank (accession numbers AB018424 (Ad11) and AB018427 (Ad35)).

M86	1	WTGVNPTKAN	CQIMNSSES	DKLILTLVK	TGALVTAFFVY	VIGVSNFNM	50
Ad11	1	WTGVNPTKAN	CQIMNSSES	DKLILTLVK	TGALVTAFFVY	VIGVSNFNM	50
Ad35	1	WTGINPP-PN	CQIVENTNTN	DGKLTLLVVK	NGGLVNGYVS	LVGVSDTVNQ	50
M86	51	LTHRNINFT	AELFFDSTGN	LLTRLSSLKT	PLNHKSGQNM	ATGAIITNAKG	100
Ad11	51	LTHRNINFT	AELFFDSTGN	LLTRLSSLKT	PLNHKSGQNM	ATGAIITNAKG	100
Ad35	51	MFTQKTANIQ	LRLYFDSSGN	LLTEESDLKI	PLKNKSSTA-	TSETVASSKA	100
M86	101	FMPSTTAYPF	NDNSREKENY	IYGTCYYAAS	-DRTAFPIDI	SVMLNRRAIN	150
Ad11	101	FMPSTTAYPF	NDNSREKENY	IYGTCYYTAS	-DRTAFPIDI	SVMLNRRAIN	150
Ad35	101	FMPSTTAYPF	NTTTRDSENY	IHGICYMYS	YDRSLFPLNI	SIMLNSRMIS	150
M86	151	DETSYCIRIT	WSWNTGDAPE	VQTSATTLVT	SPPTFYIYIRE	DD.....	200
Ad11	151	DETSYCIRIT	WSWNTGDAPE	VQTSATTLVT	SPPTFYIYIRE	DD.....	200
Ad35	151	SNVAYAIQFE	WNLNASESPE	--SNIATLTT	SPFFFSYITE	DD.....	200

Figure 3 Comparison of predicted fibre knob sequences of M86, adenovirus type 11p (Ad11p), and Ad35p. The sequences were aligned to obtain maximal homology. The deletions are represented by dashes. Deduced amino acid sequences of Ad11p and Ad35p were obtained from GeneBank (accession numbers L08231 (Ad11p) and U10272 (Ad35p)).

Neutralisation of the infectivity of adenoviruses is primarily carried out by antibodies against the hexon protein. Antigenic determinants (epitopes) located in two or more of the seven HVRs in loop 1 and loop 2 of the hexon react with neutralising antibodies.⁹ These HVRs are highly conserved within the serotype.¹⁰ However, the position of the epitope in the HVRs and number of amino acids forming them are yet to be determined. The construction of a chimaera in the HVRs of the hexon could change the antigenic specificity of the virus, enabling it to escape type specific neutralisation.¹¹ M86 was 100% homologous to Ad35 in HVRs 1, 2, 3, and 6 and to Ad11 in HVRs 4 and 6. This sequence variation reflects the preceding mutation or recombination events involving the HVRs of the hexon, which is expressed by a mixed antigenic character in the neutralisation test. This novel arrangement in the HVRs might enable the virus to circumvent existing immunity.

“The construction of a chimaera in the hypervariable regions of the hexon could change the antigenic specificity of the virus, enabling it to escape type specific neutralisation”

The isolation of a strain like M86 as a new aetiological agent of EKC is medically and epidemiologically important because it shows that recombination or mutation involving the HVRs of the hexon gene can enable the non-ocular adenoviruses to become ocular pathogens. It is also possible that such strains can circumvent existing immunity and

might be responsible for outbreaks of EKC in the future; this may be especially important in developing countries, where the detection of adenoviruses in the clinical setting is not available. It is also important to accumulate data on the HVRs of the hexon gene and fibre gene sequences of the EKC strains to predict their possible role in keratoconjunctivitis.

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Take home messages

- We have characterised a novel strain (M86) of adenovirus (Ad) involved in epidemic keratoconjunctivitis
- The virus strain showed homology with both Ad35 and Ad11 using both immunological and molecular biological techniques
- It high homology with the fibre knob sequences of Ad11 may provide it with the ability to invade ocular cells
- The mixed antigenic characteristics of virus strains such as this may enable them to circumvent existing immunity



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