



Molecular and serological characterization of species B2 adenovirus strains isolated from children hospitalized with acute respiratory disease in Buenos Aires, Argentina



Adriana E. Kajon ^{a,*}, Jan C. de Jong ^b, Laura M. Dickson ^a, Georgina Arron ^b, Patricia Murtagh ^c, Diana Viale ^c, Guadalupe Carballal ^d, Marcela Echavarria ^d

^a Infectious Disease Program, Lovelace Respiratory Research Institute, 2425 Ridgecrest Drive SE, Albuquerque, NM 87108, USA

^b Erasmus Medical Centre, Viroscience Lab, 3015 GE Rotterdam, The Netherlands

^c Hospital de Pediatría Juan P. Garrahan, Buenos Aires, Argentina

^d Clinical Virology Laboratory, Centro de Educacion Medica e Investigaciones Clinicas (CEMIC) University Hospital, Buenos Aires, Argentina

ARTICLE INFO

Article history:

Received 7 January 2013

Received in revised form 11 June 2013

Accepted 24 June 2013

Keywords:

Adenovirus

Restriction enzyme analysis

Genome typing

Neutralization

HAdV-11

Molecular typing

ABSTRACT

Background: Between September 2000 and November 2005, approximately 10% of the retrospectively examined human adenovirus (HAdV)-positive pediatric cases of acute respiratory disease (ARD) requiring hospitalization at the Hospital Nacional de Pediatría Juan P. Garrahan in Buenos Aires, Argentina, were found to have a HAdV-B2 infection.

Objective: To characterize genetically and antigenically the HAdV-B2 virus isolates.

Study design: Restriction enzyme analysis (REA), hexon and fiber gene sequencing and virus neutralization assays (VN) were carried out on 8 HAdV-B2 respiratory virus isolates.

Results: REA showed that the 8 examined HAdV-B2 virus isolates were HAdV11, belonging to two genomic variants: HAdV11a and a *Bcl1* variant of HAdV11c which we designated 11c4. Molecular analysis of the hexon genes showed that both REA variants had a HAdV11-like hexon gene. Confirming previous reports, the 7 HAdV11a virus isolates were found to have HAdV14-like fiber genes and therefore are HAdV H11/F14. The fiber gene of the HAdV11c4 virus isolates most closely resembled that of various strains of HAdV7. In VN assays, the 4 tested HAdV11a strains were serotyped as HAdV11-14. The HAdV11c4 strain was serotyped as HAdV11 but also showed a weak but significant reactivity with antiserum to HAdV7. Compared with the other HAdV-positive cases in our study, infection with HAdV11 caused a similarly severe disease.

Conclusions: Our results provide evidence to the long term world-wide circulation of HAdV H11/F14 as a causative agent of ARD. Combined, our molecular and serology data support the rationale to base the molecular typing and designation of recombinant viruses on the sequences of the hexon and fiber genes.

© 2013 Elsevier B.V. All rights reserved.

1. Background

According to genomic criteria, human adenoviruses (HAdV) are primarily grouped in 7 species, called A–G [1]. Intertypic recombination is frequent within the same species but rare between types of different species. The species classification is closely associated with differences in the affected human organs (tropism). Within each species, serotypes are distinguished based on their reactivity in virus neutralization (VN) assays. This reactivity is mainly due to antibodies to the major capsid protein, the hexon, but also to

antibodies to the penton base and fiber proteins [2–8]. Presently, 51 HAdV serotypes have been recognized [9]. Within many serotypes, variants have been defined by restriction enzyme analysis (REA) of genomic DNA [10].

Currently, the serological type definition is being replaced by genomic criteria. On this basis, 3 more HAdV types have been listed by the International Committee on Taxonomy of Viruses (ICTV) and registered as HAdV52, HAdV53, and HAdV54 [1].

HAdV of subspecies B2 (HAdV-B2, serotypes 11, 14, 34 and 35) have been reported to infect the airways and, more rarely, the conjunctiva, kidney and bladder. The circulation of HAdV11 and more specifically REA variant HAdV11a in association with acute respiratory disease (ARD) and conjunctivitis has been amply documented since the 1980s, especially in Asia [11–21] but also in the Middle East, Europe and the Americas [22–28]. The other three HAdV-B2 serotypes have been detected less frequently. A non-recombinant

Abbreviations: ARD, acute respiratory disease; HAdV, human adenovirus; HVR, hypervariable region; VN, virus neutralization; REA, restriction enzyme analysis.

* Corresponding author. Tel.: +1 505 348 9159; fax: +1 505 348 8567.

E-mail address: akajon@lrri.org (A.E. Kajon).

Table 1

Clinical characteristics of pediatric cases of HAdV-B2-associated ARD.

Case	Isolate designation	Genome type	Date of collection of clinical sample	Sex	Age	Diagnosis	Other symptoms/findings	Preexisting conditions	Outcome of disease
1	Arg/18017/00	11c4	Sept/2000	M	18m	Multifocal pneumonia	Bacterial sepsis by <i>Staphylococcus aureus</i>	None	Subglottis stenosis from prolonged intubation
2	Arg/18060/00	11a	Nov/2000	F	3.2m	Bronchiolitis	Bacterial sepsis by <i>Pseudomonas aeruginosa</i> Atelectasis	None	Bronchiectasis in left lower lobe
3	Arg/3992/04	11a	Feb/2004	M	7.5 y		Diarrhea Herpes zoster	Hodgkin's lymphoma Treated with chemotherapy and radiotherapy	Recovered
4	Arg/2314/05	11a	Feb/2005	F	8 y	Pharyngitis/otitis	Vomiting	Urea metabolism disorder (deficit of ornithine transcarbamylase)	Recovered
5	Arg/7138/05	11a	Apr/2005	M	3.8m	Bronchiolitis	Conjunctivitis	Prematurity	Recovered
6	Arg/7204/05	11a	May/2005	F	8m	Bronchiolitis	Bilateral pseudomembranous conjunctivitis with periorbital cellulitis	Down Syndrome, interventricular communication, biliary lithiasis, hydronephrosis, transitory pancytopenia	Recovered
7	Arg4922/05	11a+AAV	Oct/2005	M	5.7m	Upper respiratory infection	Conjunctivitis	None	Recovered
8	Arg/4245/05	11a	Nov/2005	F	9m	Pharyngitis/otitis	Intussusception Conjunctivitis diarrhea	None	Recovered

genomic variant of prototype HAdV14, HAdV14p1, was identified as an emerging respiratory pathogen in the United States, Ireland, and Canada over the last 5 years [29–34]. Although rare, outbreaks and individual cases of severe HAdV35-associated ARD have also been described [35,36].

The isolation or detection of HAdV-B2 from respiratory specimens of pediatric patients hospitalized for ARD in Buenos Aires, Argentina, was previously reported but no detailed molecular characterization of the viruses was carried out [26,28].

2. Objectives

To characterize genetically and antigenically 8 HAdV-B2 viruses isolated from pediatric cases of ARD requiring hospitalization in Buenos Aires, Argentina between 2000 and 2005.

3. Study design

3.1. Patients

Patient data are summarized in Table 1. Informed consent was obtained from the parents or guardians of all children participating in the study.

3.2. Clinical specimens and specimen processing for virus isolation and rapid viral diagnosis

Nasopharyngeal aspirates (NPAs) collected on admission to the hospital were initially tested for HAdV by indirect immunofluorescence. PCR-based confirmation of the diagnosis and species identification, was carried out on total DNA extracted from HAdV-positive NPAs with the QIAamp DNA blood minikit (QIAGEN Inc., Valencia, CA) using a commercial PCR-hybridization-immunoenzymatic assay (PCR Adenovirus consensus, Argene, Varilhes, France) as previously described [37,38]. Virus isolation was carried out on A549 cells following standard protocols.

HAdV-isolates were shipped on dry ice to the USA and The Netherlands, for detailed molecular and antigenic characterization, respectively.

3.3. Viral DNA extraction and restriction enzyme analysis (REA)

At Lovelace Respiratory Research Institute, virus isolates were further grown in A549 cells for viral DNA extraction as previously described [39]. REA was performed and interpreted using the panel of endonucleases and approach described by Li et al. [40].

3.4. Molecular typing and phylogenetic analysis

HAdV hexon and fiber genes were amplified by PCR using iProof high fidelity DNA Polymerase (BIO-Rad, Temecula, CA, USA) following the manufacturer's recommendations. Amplicons were generated and sequenced using primers listed in Table 2. Sequences were assembled and analyzed using Lasergene software (DNASTAR, Inc., Madison, WI, USA) and deposited in Genbank under accession numbers JX034750–JX034755 and KC999101–KC999108. For

Table 2

Primers used for amplification and sequencing of hexon and fiber genes of HAdV-B2 isolates.

Primer	Sequence 5' → 3'
<i>Hexon</i>	
Forward amp/seq hex1	cgtcgacgctgagttac
Reverse amp/seq hex 6	acatcgggatcataactgtcaac
HVR-7 forward	gtcttatgtactataacagtactgg
HVR-7 reverse	gtggttgaatgggtgcac
End of hex forward	agggtggctctggatctatgg
End of hex reverse	gccatgggtcagctgc
Internal end of hex forward	gtacccttacctgaaccac
Internal end of hex reverse	aagtgtggttcaggtagaaagg
<i>Fiber</i>	
Forward amp/seq primer	agccggcatacttctccatac
Reverse amp/seq primer	gggaggccaaataactctcg

Table 3

HAdV-B strains included in the phylogenetic analysis of Argentine HAdV-B2 isolates.

Strain	Genome type (REA variant)	GenBank accession number	
		Hexon gene	Fiber gene
HAdV3 USA/GB/1953 prototype	3p	AY599834	AY599834
HAdV7 USA/Gomen/1954 prototype	7p	AY594255	AY594255
HAdV7 USA/FS2154/2009	7d2	JN860677	JN860677
HAdV11 USA/Slobitski/1957 prototype	11p	AY163756	AY163756
HAdV11 S. Dakota/97026382/1997	11a	FJ841907	FJ841899
HAdV11 Singapore/SGN1222/2005	11a	FJ597732	FJ597732
HAdV11*(aka HAdV55) China/QS-DLL/2006	11a	FJ643676	FJ643676
HAdV11*(aka HAdV55) CHN/BJ01/2011	11a	JX491639	JX491639
HAdV11 Spain/273/1969	11a1	FJ841900	FJ841908
HAdV14 The Netherlands/De Wit/1957 prototype	14p	AY803924	AY803924
HAdV14 USA/303600/2007	14p1	FJ822614	FJ822614
HAdV16 Saudi Arabia/Ch 79/1955 prototype	16p	AY601636	AY601636
HAdV21 Saudi Arabia/AV-1645/1956 prototype	21p	AY601633	AY601633
HAdV34 USA/Compton/1972 prototype	34p	AY737797	AY737797
HAdV35 USA/Holden/1973 prototype	35p	AY128640	AY128640

assignment of molecular identity and identification of the closest match, sequence alignments were performed using the Basic Local Alignment Search Tool (BLAST) against NCBI GenBank data base (<http://www.ncbi.nlm.nih.gov>). Phylogenetic analysis was conducted in MEGA 5.1 [41] using the Maximum Likelihood method. The analysis involved the reference virus strains described in Table 3.

3.5. Virus neutralization assays

Virus neutralization (VN) assays were carried out at the Viroscience lab, Erasmus Medical Center in A549 cells in microplates following the procedure described by Kasel [42]. Antisera were prepared in rabbits as previously described [9]. The viruses were used in a concentration which produced cytopathic effect (CPE) in all cells at 7 days post infection. VN titers were read on the first day on which all cells in the virus control wells showed CPE, and expressed as the reverse of the highest dilution of antiserum preventing any CPE in the cells (100% neutralization) on that day.

4. Results

4.1. Prevalence of HAdV-B2 infections in the study cohort and associated disease

Using a commercial PCR-hybridization-immunoenzymatic assay [37], 8 of 81 of the retrospectively examined HAdV-positive pediatric cases of ARD were found to have HAdV-B2 infection. Thirty-nine of the HAdV-positive cases were diagnosed as HAdV-B1 infections, 23 as HAdV-C, 10 as HAdV-E, and 1 as a HAdV-A infection. Co-infections by HAdVs of different species were not detected. Compared with the other 73 HAdV-positive cases in the cohort, infection with HAdV-B2 was not associated with different severity of the course of the disease (Table 1). Virus isolates were recovered from respiratory specimens from all 8 cases of HAdV-B2 infection and characterized by molecular and serological methods.

4.2. REA of HAdV-B2 virus isolates

REA of viral DNA with *Bam*HI, *Bcl*II, *Bgl*III, *Bst*Ell, *Hind*III, *Hpa*I, *Pst*I, *Sma*I, and *Xba*I identified 7 of the 8 HAdV-B2 virus isolates as belonging to REA variant HAdV11a originally described by Li and colleagues [40] and one virus isolate as a novel *Bcl*II variant of HAdV11c [40] tentatively designated HAdV11c4. REA profiles of DNA from 3 virus isolates representing each of the 2 REA variants and a mixture of HAdV11a with adeno-associated virus (AAV) are shown in Fig. 1.

4.3. Phylogenetic analysis of hexon and fiber gene sequences

The hexon and fiber gene sequences of the 7 HAdV11a-like virus isolates from Argentina (18060/2000, 3992/2004; 2314/2005; 7138/2005; 7204/2005; 4922/2005, and 4245/2005) and those of the HAdV11c4-like isolate (18017/2000) were used to construct phylogenetic trees (Figs. 2A and 3, respectively). Previously described virus strains of HAdV11a and other HAdV-B1 and HAdV-B2 types available from Genbank and used in our study are listed in Table 3.

All 7 Argentine strains of HAdV11a exhibited identical hexon and fiber genes (data not shown). Molecular typing assignments were based on the identity of the closest matching sequence(s) after BLAST analysis. The complete hexon sequences of the HAdV11a and HAdV11c4 viruses displayed 98–100% nucleotide (nt) identity among each other and to the hexon of HAdV11p strain Slobitski, and lower sequence identity to the hexon of HAdV14p and HAdV14p1 strains (92–93%).

The sequences of the fiber genes of the 7 HAdV11a strains were 99.5% identical to those of HAdV14p and HAdV14p1 and to those of other previously described strains of HAdV11a [19,20,31]. These typing results indicate that the 7 HAdV11a strains are intertypic recombinants of HAdV11 and HAdV14 that can be designated as molecular type HAdV H11/F14.

Interestingly, the hypervariable region 1 (HVR-1) [43] of the hexon of the HAdV11c4 strain 18017/2000 showed distinct sequence features compared to that of HAdV-11p, including a 9 nucleotide insertion and two non-synonymous point mutations (Fig. 2B). At variance with the 7 HAdV11a strains, the fiber gene of the HAdV11c4 strain showed the highest sequence identity (97%) to the fiber gene of strains of HAdV7 although it was also related to the fiber genes of HAdV11p (95% identity) and HAdV14p (94%) (Fig. 3). Based on the identity of the closest matches and the results of phylogenetic analysis, this virus is possibly an intertypic recombinant of HAdV11 and HAdV7 that can be designated as molecular type HAdV H11/F7.

4.4. Virus neutralization (VN) assays

Because in clinical virology antigenic properties of HAdV are more relevant than molecular characteristics, 5 HAdV11-like strains, Spain/273/1969, S. Dakota/97026382/1997, Singapore/1222/2005, Argentina/2314/2005, and Argentina/18017/2000, were examined in VN tests to serotype them and to assess the implications of the revealed genetic make-up on antigenic properties. For a complete serotype designation of HAdV, the reactivity of the virus in hemagglutination inhibition (HI) tests is required

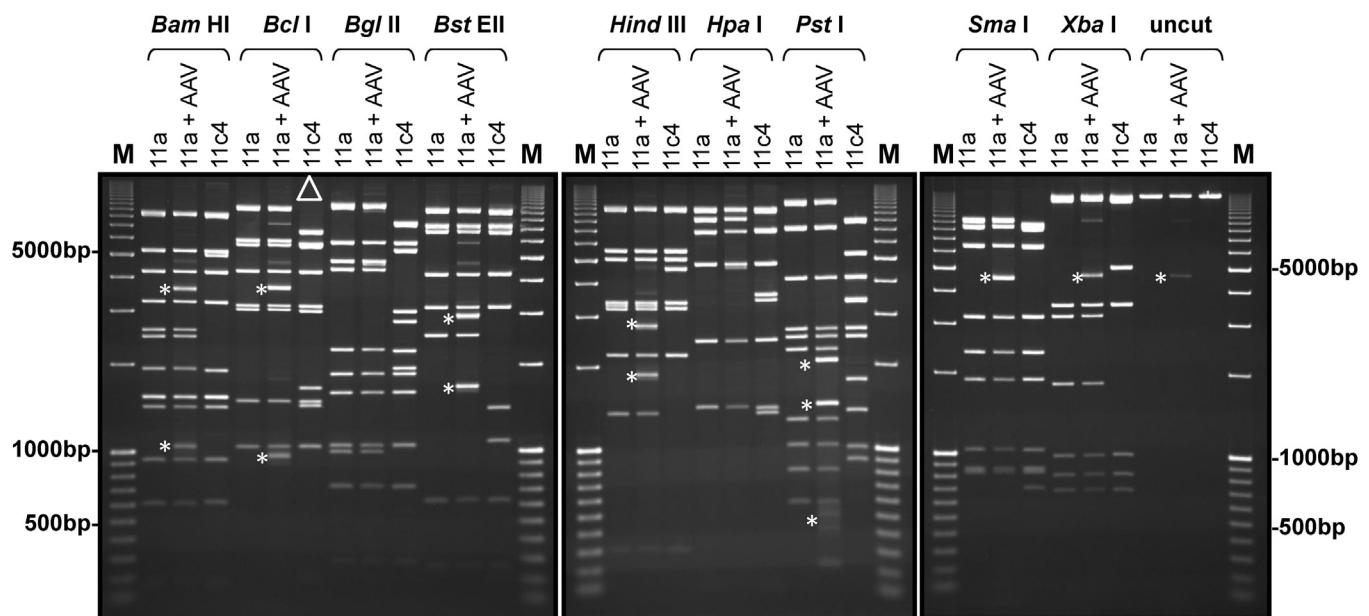


Fig. 1. REA of genomic DNA from Argentine HAdV-B2 virus isolates representative of genome types HAdV11a and HAdV11c4. Uncut DNA was run in gels to detect the presence of adeno-associated viral DNA in the HAdV DNA preparations. Asterisks indicate the presence of DNA fragments resulting from endonuclease digestion of adeno-associated virus DNA. Δ indicates novel *Bcl* I profile not described by Li et al. [40].

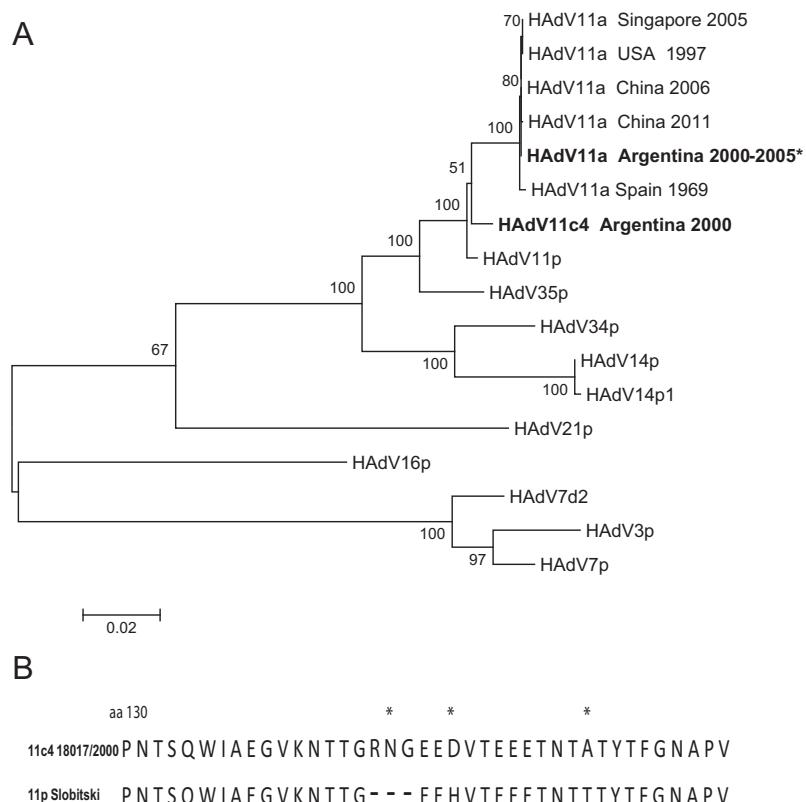


Fig. 2. (A) Phylogenetic analysis of hexon genes of HAdV-B2 strains isolated from pediatric patients in Argentina between 2000 and 2005. Phylogenetic analysis was performed by maximum likelihood method in MEGA 5.1 [41]. All positions containing gaps were eliminated. The analysis involved the nucleotide sequences for HAdV-Bs listed on Table 3. Taxon names include country and year of isolation and corresponding genome type. Scale bars indicate nucleotide substitutions per site. Numbers on branches and at nodes indicate bootstrap proportions from 1000 replications. *Only one hexon gene sequence (2841 bp) representing the 7 identical Argentine HAdV11a strains was included in the analysis. (B) Alignment of amino acid sequences corresponding to hypervariable region 1 of the predicted hexon proteins of HAdV11c4 strain Argentina/18017/2000 and HAdV11p strain de Wit showing distinct mutations including a 3 amino acid indel.

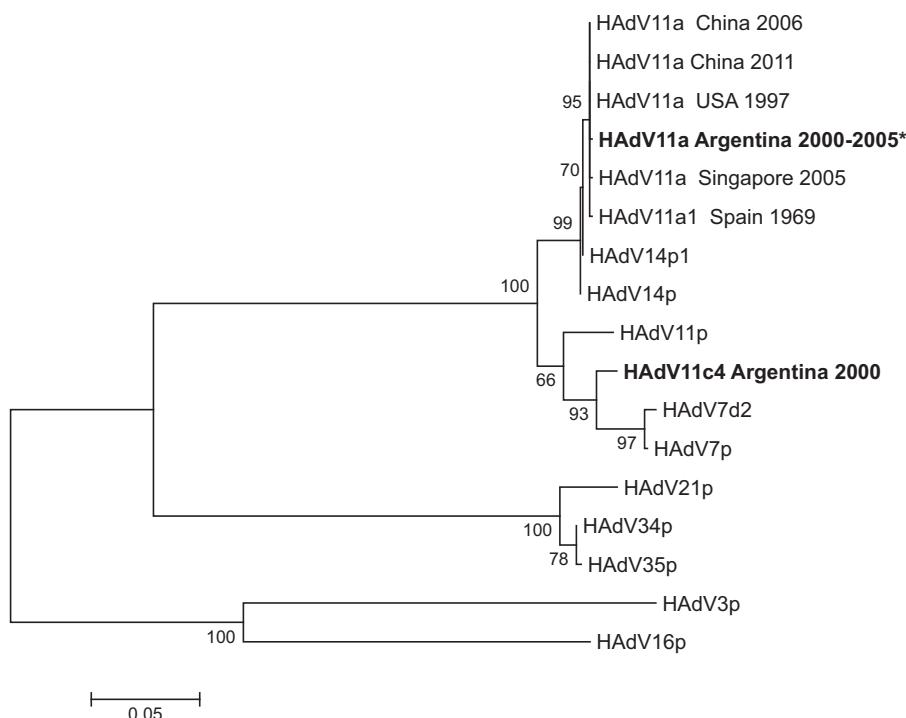


Fig. 3. Phylogenetic analysis of fiber genes of HAdV-B2 strains isolated from pediatric patients in Argentina between 2000 and 2005. Phylogenetic analysis was performed by maximum likelihood method in MEGA 5.1 [41]. All positions containing gaps were eliminated. The analysis involved the nucleotide sequences for HAdV-Bs listed on Table 3. Taxon names include country and year of isolation and corresponding genome type. Scale bars indicate nucleotide substitutions per site. Numbers on branches and at nodes indicate bootstrap proportions from 1000 replications. *Only one fiber gene sequence (978 bp) representing the 7 identical Argentine HAdV11a strains was included in the analysis.

[44]. Unfortunately, in the present study such assays could not be carried out due to lack of suitable erythrocytes. Two patterns of reactivity in VN assays could be distinguished (Table 4).

Pattern 1: Virus strains Spain/273/1969, S. Dakota/97026382/1997, Singapore/1222/2005 and Argentina/2314/2005 were neutralized by antiserum to HAdV11p to homologous or even higher titers, and by antiserum to HAdV14p to titers 4 to 8-fold lower than the homologous titer (Table 4).

Pattern 2: Compared with the 4 HAdV11a strains, the HAdV11c4 virus Argentina/18017/2000 showed weaker reactivity with the antisera to HAdV11p and HAdV14p (Table 4). The titer of antiserum to HAdV11p against Argentina/18017/2000 was 8-fold lower than the homologous titer. The threshold titer difference for assigning HAdVs into a given serotype is 20, therefore Argentina/18017/2000 classified as serotype HAdV11. Because the VN titer of antiserum to HAdV14p against this virus was 256-fold lower than the

homologous titer, the virus could not be designated as HAdV11-14. At variance with the Ad11-14 viruses, the fiber gene of Argentina/18017/2000 resembled mostly that of HAdV7. Because antibodies to the fiber can exhibit neutralizing activity [4], we tested the virus against antiserum to HAdV7p and observed that, in contrast to HAdV11-14 viruses, antiserum to HAdV7p reacted significantly with Argentina/18017/2000, although the titer was borderline too low to designate the virus as serotype HAdV7-11 (Table 4).

5. Discussion

In the present study, 7 of 8 HAdV-B2 Argentine virus isolates were classified by REA as HAdV11a and by sequence analysis as HAdV H11/F14. One of these 7 virus strains were serotyped in VN assays as HAdV11-14. The 8th HAdV-B2 virus was classified by

Table 4
Virus neutralization (VN) titers of antisera to HAdV7, HAdV11, HAdV14 and HAdV35 against HAdV-B2 strains.

Virus strain and REA variant	VN titers of rabbit antiserum to*				Serotype
	HAdV7p	HAdV11p	HAdV14p	HAdV35p	
HAdV7p (prototype, Gomen)	8000	<32	<256	<32	
HAdV11p (prototype, Slobitski)	<400	512	16	8	
HAdV14 p (prototype, de Wit)	<400	16	4096	2	
HAdV35 p (prototype, Holden)	<400	4	<4	512	
HAdV11a1 (Spain/273/1969)	NA**	1024	512	8	HAdV11-14
HAdV11a (S. Dakota/97026382/1997)	NA	2048	1024	32	HAdV11-14
HAdV11a (Singapore/SGN1222/2005)	NA	1024	1024	16	HAdV11-14
HAdV11a (Argentina/2314/2005)	NA	512	512	32	HAdV11-14
HAdV11c4 (Argentina/18017/2000)	200	64	16	8	HAdV11

Homologous titers are highlighted in bold.

* VN titers are expressed as reciprocal values of the highest dilution that completely neutralizes the concerned strain after 7 days, when the virus control shows complete cytopathic effect.

** NA: not assayed.

REA as HAdV11c, by sequence analysis as HAdV H11/F7 and by VN assays as HAdV11, displaying also weak but significant reactivity with antiserum to HAdV7p.

The relations between the detected recombination events and the antigenic differences between the studied virus strains and prototype HAdV11 can be explained by the prevailing opinions about the antigen specificity of HAdV neutralizing antibodies. Neutralizing antibodies against HAdV can be directed to all 3 major capsid proteins, hexon, fiber, and penton [4]. The relative proportions of the various specificities vary with the virus strain and the host producing the antibodies. In most cases, the largest fraction of neutralizing antibodies targets the hexon protein [4,8] but antibodies to fibers can also have high neutralizing activity [44], and penton-specific antibodies have been reported to exert synergistic neutralizing activities [3]. It is therefore likely that the enhanced reactivity of HAdV11-14, compared to HAdV11p, with antiserum to HAdV14p is the consequence of the replacement of the HAdV11-like fiber gene by a HAdV14-like fiber gene. The replacement of the HAdV11-like penton gene by a HAdV14-like penton gene described for several HAdV H11/F14 strains including Singapore/1222/2005 [20] may have also contributed to the increased reactivity of antiserum to HAdV14p against HAdV11-14.

The designation serotype HAdV11-14 confirms the label Ad11-14 given by Wigand et al. to similar HAdV11-like strains, including Spain/273/1969 [45,46]. Wigand et al. could not perform HI assays with these virus strains either and therefore also restricted their designation to Ad11-14. Of note, Spain/273/1969 was originally characterized by Hierholzer et al. as HAdV11/H14 [45], meaning related to HAdV11p in VN assays and to HAdV14p in HI tests; threshold titer differences with the homologous virus are in both assays 20-fold. In contrast with the results of Wigand et al. [45] and our study, virus strain Spain/273/1969 did not react with antiserum to HAdV14p in VN assays in the studies of Hierholzer et al. [22,46]. The discrepancy may be attributable to the different source of the concerned antisera to the prototypes. Hierholzer et al. used equine antisera [46] while Wigand et al. [45] and our study used rabbit antisera. Combining the results of the 3 studies, the serotype designation of virus strain Spain/273/1969 would become HAdV11-14/H14.

Our findings show interesting associations between genomic data and the clinically more relevant antigenic characteristics of the tested viruses. They underscore the importance of determining the molecular identity of the hexon and fiber genes in order to establish the species, discover intertypic recombinants, and get valuable clues regarding the antigenic reactivity and tropism.

Importantly, our observations support the proposal to base the molecular typing and designation of recombinant viruses on the sequences of the hexon and fiber genes [47] as they confirm that these genes accommodate the major epitopes involved in VN. Several PCR-based approaches have been developed and used successfully for this purpose [48–51].

We discourage the proposed use of “HAdV55” to designate the Chinese intertypic recombinant HAdV H11/F14, virus strain QS-DLL [52], as well as other recently characterized Chinese HAdV strains (Genbank accession #JX491639 and JX123027–JX123029) because this generates confusion regarding the antigenic identity of the virus. The sequences of the hexon and fiber genes of these Chinese viruses show them to be almost indistinguishable from those of the Argentine, North American, Singapore and Spanish strains of HAdV11a examined in our VN assays (Figs. 2 and 3) and strongly suggest that they are also serotype HAdV11-14.

Virus strain Argentina/18017/2000 exhibited a rare genetic make-up. Intertypic recombinants with a HAdV11-like hexon gene and a HAdV7-like fiber gene have so far been reported in the literature only once [53]. From a case of neonatal severe pneumonia and disseminated HAdV-infection, a HAdV was isolated that exhibited

molecular characteristics similar to Argentina/18017/2000. The sequence of the hypervariable regions 1–6 (HVR1–6 [43]) of the hexon gene of the neonatal virus most closely matched those of HAdV11p and the sequences of the fiber gene most closely matched those of HAdV7p, suggesting that the two virus isolates are closely related.

Extending previously reported data from molecular typing [28] and genetic characterization [23] of HAdV strains circulating in Argentina and elsewhere [52], the present study provides additional evidence of the long term worldwide circulation of HAdV H11/F14.

Funding

The work of AEK was supported in part by the US Department of Defense (DoD) Global Emerging Infections Surveillance and Response System (GEIS).

Competing interests

None declared.

Ethical approval

The study was approved by the Institutional Ethics and Review Committees of the Hospital de Pediatría Juan P. Garrahan and CEMIC University Hospital. Because the personal and demographic data for the clinical specimens were deidentified prior to the study, the protocol was exempt of IRB review at the Lovelace Respiratory Research Institute.

Acknowledgements

The authors gratefully acknowledge Carmen Ricarte and Beatriz Ebekian for technical assistance with specimen processing for viral isolation. AEK is a member of the Center for Infectious Disease and Immunity, University of New Mexico.

References

- [1] Harrach B, Benkő M, Both GW, Brown M, Davison AJ, Echavarria M, et al. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, editors. Family adenoviridae in virus taxonomy: classification and nomenclature of viruses: ninth report of the international committee on taxonomy of viruses. San Diego: Elsevier; 2011. p. 125–41.
- [2] Toogood CI, Crompton J, Hay RT. Antipeptide antisera define neutralizing epitopes on the adenovirus hexon. *J Gen Virol* 1992;73:1429–35.
- [3] Gahéry-Ségard H, Farace F, Godfrin D, Gaston J, Lengagne R, Tursz T, et al. Immune response to recombinant capsid proteins of adenovirus in humans: anti-fiber and anti-penton base antibodies have a synergistic effect on neutralizing activity. *J Virol* 1998;72:2388–97.
- [4] Sumida SM, Truitt DM, Lemckert AA, Vogels R, Custers JH, Addo MM, et al. Neutralizing antibodies to adenovirus serotype 5 vaccine vectors are directed primarily against the adenovirus hexon protein. *J Immunol* 2005;174:7179–85.
- [5] Roy S, Clawson DS, Calcedo R, Lebherz C, Sanmiguel J, Wu D, et al. Use of chimeric adenoviral vectors to assess capsid neutralization determinants. *Virology* 2005;333:207–14.
- [6] Pichla-Gollon SL, Drinker M, Zhou X, Xue F, Rux JJ, Gao GP, et al. Structure-based identification of a major neutralizing site in an adenovirus hexon. *J Virol* 2007;81:1680–9.
- [7] Cheng C, Gall JG, Nason M, King CR, Koup RA, Roederer M, et al. Differential specificity and immunogenicity of adenovirus type 5 neutralizing antibodies elicited by natural infection or immunization. *J Virol* 2010;84:630–8.
- [8] Qiu H, Li X, Tian X, Zhou Z, Xing K, Li H, et al. Serotype-specific neutralizing antibody epitopes of human adenovirus type 3 (HAdV-3) and HAdV-7 reside in multiple hexon hypervariable regions. *J Virol* 2012;86:7964–75.
- [9] De Jong JC, Wermebol AG, Verweij-Uijterwaal MW, Slaterus KW, Wertheim-van Dillen P, van Doornum GJJ, et al. Adenoviruses from human immunodeficiency virus-infected individuals, including two strains that represent new candidate serotypes Ad50 and Ad51 of species B1 and D, respectively. *J Clin Microbiol* 1999;37:3940–5.
- [10] Wadell G, Varsányi. Demonstration of three different subtypes of adenovirus type 7 by DNA restriction site mapping. *Infect Immun* 1978;21:238–46.

- [11] Yin-Murphy M, Lim KH, Chua PH. Adenovirus type 11 epidemic conjunctivitis in Singapore. *Southeast Asian J Trop Med Public Health* 1974;5:333–41.
- [12] Tai FH, Chu S, Chi WH, Wei HY, Hierholzer JC. Epidemic haemorrhagic conjunctivitis associated with adenovirus type 11 in Taiwan. *Southeast Asian J Trop Med Public Health* 1974;5:342–9.
- [13] Zhang ZJ, Chen YZ, Gao LM, Wang ZL, Zhao JM. Symptomatology of adenovirus pneumonia type 11. *Acta Acad Med Wuhan* 1984;4:241–4.
- [14] Guo DF, Shinagawa M, Aoki K, Sawada H, Itakura S, Sato G. Genome typing of adenovirus strains isolated from conjunctivitis in Japan, Australia, and the Philippines. *Microbiol Immunol* 1988;32:1107–18.
- [15] Nakayama M, Miyazaki C, Ueda K, Kusuvara K, Yoshikawa H, Nishima S, et al. Pharyngoconjunctival fever caused by adenovirus type 11. *Pediatr Infect Dis J* 1992;11:6–9.
- [16] Iwaya M, Ueda K, Kadoya R, Kusuvara K, Miyazaki C, Hidaka Y, et al. Seroepidemiology of adenovirus type 11 in Fukuoka, Japan. *Acta Paediatr Jpn* 1995;37:413–5.
- [17] Kitamura N. Genome analysis of adenovirus type 7 and adenovirus type 11. *Jpn J Ophthalmol* 2001;45:22–30.
- [18] Chen HL, Chiu SS, Hsiao HP, Ke GM, Lin YC, Lin KH, et al. Respiratory adenoviral infections in children: a study of hospitalized cases in southern Taiwan in 2001–2002. *J Trop Pediatr* 2004;50:279–88.
- [19] Zhu Z, Zhang Y, Xu S, Yu P, Tian X, Wang L, et al. Outbreak of acute respiratory disease in China caused by B2 species of Adenovirus type 11. *J Clin Microbiol* 2009;47:697–703.
- [20] Kajon AE, Dickson LM, Metzgar D, Houngh HS, Lee V, Tan BH. Outbreak of febrile respiratory illness associated with adenovirus 11a infection in a Singapore military training camp. *J Clin Microbiol* 2010;48:1438–41.
- [21] Gu L, Liu Z, Li X, Qu J, Guan W, Liu Y, et al. Severe community-acquired pneumonia caused by adenovirus type 11 in immunocompetent adults in Beijing. *Clin Virol* 2012;54:295–301.
- [22] Hierholzer JC, Pumarola A, Rodriguez-Torres A, Beltran M. Occurrence of respiratory illness due to an atypical strain of adenovirus type 11 during a large outbreak in Spanish military recruits. *Am J Epidemiol* 1974;99:434–42.
- [23] Kajon AE, Mistchenko AS, Videla C, Hortal M, Wadell G, Avendaño LF. Molecular epidemiology of adenovirus acute lower respiratory infections of children in the south cone of South America (1991–1994). *J Med Virol* 1996;48:151–6.
- [24] Centers for Disease Control, Prevention (CDC). Civilian outbreak of adenovirus acute respiratory disease – South Dakota, 1997. *MMWR Morb Mortal Wkly Rep* 1998;47:567–70.
- [25] Chmielewicz B, Benzler J, Pauli G, Krause G, Bergmann F, Schweiger B. Respiratory disease caused by a species B2 adenovirus in a military camp in Turkey. *J Med Virol* 2005;77:232–7.
- [26] Metzgar D, Osuna M, Yingst S, Rakha M, Earhart K, Elyan D, et al. PCR analysis of Egyptian respiratory adenovirus isolates, including identification of species, serotypes, and coinfections. *J Clin Microbiol* 2005;43:5743–52.
- [27] Metzgar D, Osuna M, Kajon AE, Hawksworth AW, Irvine M, Russell KL. Abrupt emergence of diverse species B1 and B2 adenoviruses in US military recruit training centers. *J Infect Dis* 2007;196:1465–73.
- [28] Barrero PR, Valinotto LE, Tittarelli E, Mistchenko AS. Molecular typing of adenoviruses in pediatric respiratory infections in Buenos Aires, Argentina (1999–2010). *J Clin Virol* 2012;53:145–50.
- [29] Centers for Disease Control, Prevention (CDC). Acute respiratory disease associated with adenovirus serotype 14 – four states, 2006–2007. *MMWR Morb Mortal Wkly Rep* 2007;56:1181–4.
- [30] Louie JK, Kajon AE, Holodniy M, Guardia-LaBar L, Lee B, Petru AM, et al. Severe pneumonia due to adenovirus serotype 14: a new respiratory threat? *Clin Infect Dis* 2008;46:421–5.
- [31] Kajon AE, Lu X, Erdman DD, Louie J, Schnurr D, George KS, et al. Molecular epidemiology and brief history of emerging adenovirus 14-associated respiratory disease in the United States. *J Infect Dis* 2010;202:93–103.
- [32] Carr MJ, Kajon AE, Lu X, Dunford L, O'Reilly P, Holder P, et al. Deaths associated with human adenovirus-14p1 infections, Europe, 2009–2010. *Emerg Infect Dis* 2011;17:1402–8.
- [33] O'Flanagan D, O'Donnell J, Domegan L, Fitzpatrick F, Connell J, Coughlan S, et al. First reported cases of human adenovirus serotype 14p1 infection, Ireland, October 2009 to July 2010. *Euro Surveill* 2011;16:19801.
- [34] Girouard, et al. Adenovirus serotype 14 infection, New Brunswick, Canada, 2011. *Emerg Infect Dis* 2013;19:119–22.
- [35] Pinto A, Beck R, Jadavji T. Fatal neonatal pneumonia caused by adenovirus type 35. Report of one case and review of the literature. *Arch Pathol Lab Med* 1992;116:95–9.
- [36] Sanchez MP, Erdman DD, Torok TJ, Freeman CJ, Matyas BT. Outbreak of adenovirus 35 pneumonia among adult residents and staff of a chronic care psychiatric facility. *J Infect Dis* 1997;176:760–3.
- [37] Echavarria M, Maldonado D, Elbert G, Videla C, Rappaport R, Carballal G. Use of PCR to demonstrate presence of adenovirus species B, C, or F as well as coinfection with two adenovirus species in children with flu-like symptoms. *J Clin Microbiol* 2006;44:625–7.
- [38] Vabret A, Gouarin S, Joannes M, Barranger C, Petitjean J, Corbet S, et al. Development of a PCR-and hybridization-based assay (PCR Adenovirus Consensus) for the detection and the species identification of adenoviruses in respiratory specimens. *J Clin Virol* 2004;31:116–22.
- [39] Kajon AE, Erdman DD. Assessment of genetic variability among subspecies B1 human adenoviruses for molecular epidemiology studies. In: Wold WSM, Tolleson A, editors. *Methods mol. med. vol. 2. Adenovirus methods and protocols*, vol. 131, 2nd ed. Totowa, NJ: Humana Press Inc.; 2007. p. 335–55 [Chapter 23].
- [40] Li QG, Hambraeus J, Wadell G. Genetic relationship between thirteen genome types of adenovirus 11, 34, and 35 with different tropisms. *Intervirology* 1991;32:338–50.
- [41] Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011;28:2731–9.
- [42] Kasel JA. Adenoviruses. In: Lennette EH, Schmidt NJ, editors. *In diagnostic procedures for viral, rickettsial and chlamydial infections*. 5th ed. Washington, DC: American Public Health Association; 1979. p. 229–55 [Chapter 9].
- [43] Crawford-Miksza L, Schnurr DP. Analysis of 15 adenovirus hexon proteins reveals the location and structure of seven hypervariable regions containing serotype-specific residues. *J Virol* 1996;70:1836–44.
- [44] Yu B, Dong J, Wang C, Zhan Y, Zhang H, Wu J, et al. Characteristics of neutralizing antibodies to adenovirus capsid proteins in human and animal sera. *Virology* 2013;437:118–23.
- [45] Wigand R, Sehn N, Hierholzer JC, de Jong JC, Adrian T. Immunological and biochemical characterization of human adenoviruses from subgenus B. I. Antigenic relationships. *Arch Virol* 1985;84:63–78.
- [46] Hierholzer JC, Pumarola A. Antigenic characterization of intermediate adenovirus 14–11 strains associated with upper respiratory illness in a military camp. *Infect Immun* 1976;13:354–9.
- [47] Aoki K, Benkö M, Davison AJ, Echavarria M, Erdman DD, Harrach B, et al. Toward an integrated human adenovirus designation system that utilizes molecular and serological data and serves both clinical and fundamental virology. *J Virol* 2011;85:5703–4.
- [48] Sarantis H, Johnson G, Brown M, Petric M, Tellier R. Comprehensive detection and serotyping of human adenoviruses by PCR and sequencing. *J Clin Microbiol* 2004;42:3963–9.
- [49] Lu X, Erdman DD. Molecular typing of human adenoviruses by PCR and sequencing of a partial region of the hexon gene. *Arch Virol* 2006;151:1587–602.
- [50] Madisch I, Harste G, Pommer H, Heim A. Phylogenetic analysis of the main neutralization and hemagglutination determinants of all human adenovirus prototypes as a basis for molecular classification and taxonomy. *J Virol* 2005;79:15265–76.
- [51] Madisch I, Wölfel R, Harste G, Pommer H, Heim A. Molecular identification of adenovirus sequences: a rapid scheme for early typing of human adenoviruses in diagnostic samples of immunocompetent and immunodeficient patients. *J Med Virol* 2006;78:1210–7.
- [52] Walsh MP, Seto J, Jones MS, Chodosh J, Xu W, Seto D. Computational analysis identifies human adenovirus type 55 as a re-emergent acute respiratory disease pathogen. *J Clin Microbiol* 2010;48:991–3.
- [53] Soileau SL, Schneider E, Erdman DD, Lu X, Ryan WD, McAdams RM. Case report: severe disseminated adenovirus infection in a neonate following water birth delivery. *J Med Virol* 2013;85:667–9.