

# Molecular Characterization of an Adenovirus 3-16 Intertypic Recombinant Isolated in Argentina from an Infant Hospitalized with Acute Respiratory Infection<sup>∇</sup>

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**An intertypic recombinant adenovirus with a serotype 3-like hexon gene and a serotype 16-like fiber (99% identical to that of the prototype strain of human adenovirus 16 [HAdV-16], Ch79) was isolated in Argentina from an infant admitted to the hospital with acute respiratory disease. Consistent with the results of molecular characterization, strain Arg827/04 was designated H3-F16.**

## CASE REPORT

In January 2004, a 3-month-old male infant with a diagnosis of myoclonic epilepsy and severe developmental delay was admitted to the Juan P. Garrahan Hospital in Buenos Aires, Argentina, with fever, conjunctivitis, and bronchiolitis (disseminated rales in both lung fields, wheezing, and hypoxemia). The chest X ray showed atelectasis of the right upper lobe, which disappeared in 2 days. The patient was treated with amoxicillin and nebulized albuterol. A nasopharyngeal aspirate (NPA) collected on admission was positive for human adenovirus (HAdV) by indirect immunofluorescence. He was discharged after 13 days and followed up in the neurology department. His medical record shows two readmissions to the hospital over the following 10 months, with similar respiratory symptoms. However, blood samples taken during these episodes were negative for bacterial infection, and the new NPAs were negative for respiratory viruses. After discharge, his follow-up was discontinued.

Total DNA extracted from the NPA with a QIAamp DNA blood minikit (Qiagen, Valencia, CA) tested positive for subspecies B1 adenovirus by previously described real-time-PCR-based procedures (4). Virus isolation was performed on A549 cells by following standard protocols at the CEMIC Virology Laboratory in Buenos Aires, Argentina. The adenovirus isolate, designated Arg827/04, was shipped in dry ice to the Lovelace Respiratory Research Institute in New Mexico for further characterization. For detailed molecular characterization of the virus by restriction enzyme analysis (REA) and sequencing, the isolate was passaged once on A549 cells in a 25-cm<sup>2</sup> flask and subsequently amplified in a 75-cm<sup>2</sup> flask.

Intracellular viral DNA was extracted by the method developed by Shinagawa et al. (15), with modifications as previously described (8). The seven hypervariable regions of the hexon gene (HVR1-7) and the fiber gene were amplified by PCR. Sequences were generated from both strands at the DNA Research Services core facility, University of New Mexico Health Sciences Center. Primers used for amplification and sequencing are listed in Table 1. Sequence data were edited and analyzed using Lasergene software (DNASTar, Inc., Madison, WI) and deposited in the National Center for Biotechnology Information (NCBI)/GenBank database under accession no. GU014392 for the hexon gene and GU004377 for the fiber gene. Hexon and fiber sequences were compared with adenovirus sequences available from the NCBI database using the Basic Local Alignment Search Tool (BLAST) program optimized for highly similar sequences (Mega BLAST). The hexon gene exhibited 99% sequence identity with the intermediate variant 3-16 H16 strain SC8 (also known as the San Carlos agent; accession no. X76552) and 97% sequence identity with both the prototype strain GB of HAdV-3 (AB330084) and several field strains of HAdV-3 isolated in North America and Asia (AY599836 and EF494650, among others). By following the molecular typing approach developed by Sarantis and colleagues (14) and Lu and Erdman (10), the serotype identity of the isolate was determined to be HAdV-3-like. The fiber gene of strain Arg827/04 was identified as HAdV-16-like based on its 99% sequence identity with the fiber gene of the prototype strain of HAdV-16 (Ch79). Interestingly, the fiber gene sequence also showed 98% and 97% sequence identity with the fiber genes of the recently sequenced simian adenoviruses (SAdV) SAdV-35.2 and SAdV-35.1, respectively (13) (GenBank accession no. FJ025910 and FJ025912).

For genomic characterization and genome type identification by REA, 1 μg of viral DNA was initially digested with restriction endonuclease BamHI according to the manufacturer's recommendations (Promega, Madison, WI) and further characterized by digestion with BglII, HindIII, SmaI, and XhoI. DNA fragments were analyzed by horizontal agarose gel electrophoresis as previously described (8). Genomic DNAs of the

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TABLE 1. Primers used to amplify and sequence the hexon and fiber genes of Arg827/04

Gene	Direction of primer <sup>a</sup>	Primer (5'→3')	Use
<b>Hexon, HVR1 to -7</b>			
Hex1	F	GTACTCTGAACAGCATCG	Amplification and sequencing
Hex 6	R	AATAGGCAGTTCATCC	
HVR7	F	CTGATGTACTACAACAGCACTGGCAACATGGG	Sequencing
HVR7	R	GCGTTGCGGTGGTGGTTAAATGGGTTTAC	
Internal HVR1 to -6	F	AACATATCAGCCAGAGC	
Internal HVR1 to -6	R	ATGATTCTTCTCCAACCTTG	
<b>Fiber</b>			
SpB fiber	F	CTACCAGCAGCACCTC	Amplification and sequencing
SpB fiber	R	TAAAGCTGTGCGTGG	
Internal fiber	F	TCCATTAACAATTCCTCC	Sequencing
Internal fiber	R	GGAGGAATTGTTAATGGA	

<sup>a</sup> F, forward; R, reverse.

prototype strains of HAdV-3 (GB) and HAdV-16 (Ch79) were digested as a reference for comparison. Restriction profiles visualized by UV transillumination at 303 nm after staining with ethidium bromide and photographed in a Gel Doc imager (Bio-Rad, Temecula, CA) are shown in Fig. 1.

Consistent with the results of molecular characterization, strain Arg827/04 was designated H3-F16, reflecting its identity as an intertypic recombinant with an HAdV-3-like hexon (H3) and an HAdV-16-like fiber (F16). Recombination is a well-recognized mechanism of adenovirus evolution resulting in novel intraspecific genomic variants. Homologous recombination is possible between closely related serotypes, and cross-over sites are confined to regions of high sequence homology (2, 11). Serologically intermediate adenovirus strains exhibiting antigenic determinants (or genomic sequences) of one se-

rotype in the hexon protein and of another in the fiber polypeptide occur in nature and have been described for the species B and D HAdVs (1, 6, 7, 16). Of interest and relevance to this study is the intermediate strain 3-16, known as the San Carlos (SC) agent, isolated in 1959 during an outbreak of infectious hepatitis in the San Carlos Apache Indian reservation in Eastern Arizona (5). This strain exhibited characteristics of HAdV-3 by neutralization (although it differed from the prototype strain of serotype 3, GB, in its homologous- and heterologous-virus neutralization titers) and characteristics of HAdV-16 by hemagglutination inhibition. Years later, the San Carlos strain (isolate SC8) was characterized by REA by Li and Wadell (9) and identified as corresponding to genome type 3p1, with 3p-like BamHI, BglII, HindIII, SmaI, and XhoI restriction fragment profiles. The REA data for the recombinant Argentine 3-16 virus show clear evidence of a completely different genomic backbone with unique BglII, SmaI, and XhoI profiles and an HAdV-16p-like BamHI profile (Fig. 1). As described above, the sequence of the hexon gene of Arg827/04 spanning nucleotides 301 to 1515 was compared to the corresponding available sequence for SC8 (GenBank accession no. X76552) using ClustalW implemented in Lasergene and found to be 99.4% identical at the nucleotide level and 99.3% identical at the amino acid level.

No restriction sites for the endonucleases used in our analysis are predicted within the fiber gene sequence, indicating that the differences between SC8 and Arg827/04 map to other regions of the viral genome. Based on the few reports published in the literature, HAdV-16 and intertypic recombinants involving HAdV-16 appear to be rarely associated with cases of acute respiratory disease requiring hospitalization (3, 12). In addition, their infrequent detection during surveillance studies of HAdV-associated respiratory disease suggests that these viruses may have a restricted geographic area of circulation and/or not be particularly virulent.

The value of sequencing the seven hypervariable regions of the hexon gene and the fiber gene for the correct identification and designation of recombinant adenovirus strains is exemplified by our data. Despite the fact that strain Arg827/04 displayed an HAdV-16p-like BamHI profile, the hexon sequence unequivocally defined its serotype identity as HAdV-3. According to the genome type denomination system proposed by

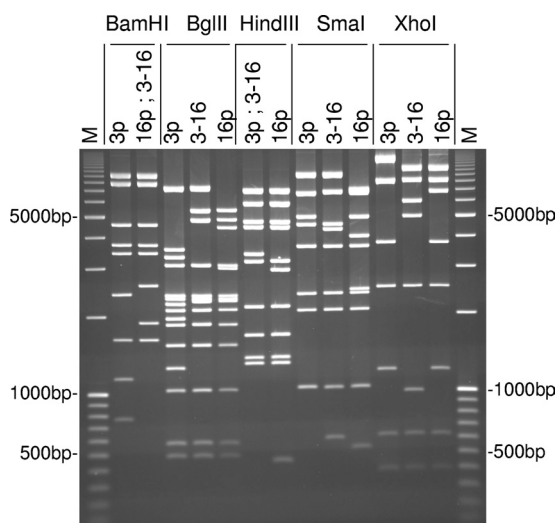


FIG. 1. Restriction enzyme analysis of genomic Arg827/04 DNA in comparison with HAdV-3p strain GB and HAdV-16p strain Ch79. BamHI profiles for HAdV-16p and the 3-16 intertypic recombinant Arg827/04 exhibited identical band patterns, which are illustrated in a single lane. HindIII profiles for HAdV-3p and the 3-16 intertypic recombinant Arg827/04 exhibited identical band patterns that were also illustrated in a single lane. M, 1-kbp plus 100-bp molecular markers.

Li and Wadell, and consistent with the handling of previously characterized subspecies B1 recombinants such as HAdV-7h (7), this genome should be designated a novel HAdV-3 genomic variant. We strongly recommend the generation and use of hexon and fiber identity information for the designation of all adenovirus strains for epidemiology studies. We also recommend the use of the candidate nomenclature H to denote hexon identity and F instead of H to denote fiber identity, since not all HAdV fibers exhibit hemagglutinating activity (7).

Appropriate informed consent was obtained from the guardians of the patient described in this report. The study was approved by the Institutional Ethics and Review Committees of the Hospital de Pediatría Juan P. Garrahan and CEMIC University Hospital, Buenos Aires, Argentina. Because the clinical specimen and demographics data were deidentified prior to study, the protocol was exempt from internal board review at the Lovelace Respiratory Research Institute.

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