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A human adenovirus species B subtype 21a associated with severe pneumonia

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Summary Between 2005 and 2013 six severe pneumonia cases (all requiring mechanical ventilation, two fatal outcomes) caused by human adenovirus type 21 (HAdV-B21) were observed in Germany. So far, HAdV-B21 was mainly associated with non-severe upper and lower respiratory tract infections. However, a few highly virulent HAdV types, e.g. HAdV-B14p1, were previously associated with severe, fatal pneumonia. Complete genomic sequences of the German HAdV-B21 pneumonia isolates formed a single phylogenetic cluster with very high sequence identity (≥99.897%). Compared to the HAdV-B21 prototype (only 99.319% identity), all isolates had a unique 15 amino acid deletion and a 2 amino acid insertion in the RGD loop of the penton base which may affect binding to the secondary receptor on the host cells. Moreover, a recombinant E4 gene region derived of HAdV-B3 was identified by bootscan analysis. Thus, the highly virulent, pneumotropic HAdV-B21 was denominated as subtype 21a. Surprisingly, there was 99.963% identity with agent Y/SIBU97 (only 13.4 kb available in GenBank of the 35.4 kb genome) which was associated with 10 fatalities due to cardiopulmonary failure in Sarawak, Malaysia, in 1997.

In conclusion, a HAdV-B21 subtype (21a) associated with severe pneumonia in Germany was phylogenetically linked to an adenovirus isolated in Malaysia.

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Introduction

Human adenoviruses (HAdV) are non-enveloped DNA viruses with a double-stranded genome of about 35 kb. Currently 67 HAdV types, classified into seven species (A—G), have been identified. Depending on the tropism of the HAdV type, adenoviruses can cause a multitude of diseases. However, almost all HAdV infections are non-life-threatening and self-limiting in immunocompetent hosts, but can disseminate and result in a high lethality in immunosuppressed patients, such as stem cell transplant recipients.[1]

The HAdV-B21 prototype was isolated in 1956 from an eye swab sampled during an investigation of trachoma in Saudi Arabia.[2] Later studies showed an association of HAdV-B21 with upper and lower respiratory tract infections.[3—7] Using immunological methods and characterization by restriction enzyme analysis (REA), several studies described the circulation of multiple HAdV-B21 strains in Europe during the 1960s—1980s in upper and lower respiratory tract infections.[8—10] However, these HAdV-B21 strains do not appear to have been highly virulent and did not cause fatalities.

Probably all HAdV types with a tropism for the respiratory tract (including the most frequently isolated types of species HAdV-C) can be transmitted by droplets and replicate in the upper respiratory tract. However, only four types (type 4 of species HAdV-E, types 3, 7 and 14p1 of species HAdV-B) of the 67 HAdV types are associated with more severe lower respiratory tract infections, e.g. pneumonia and acute respiratory distress syndrome (ARDS) in immunocompetent patients. Severe lower respiratory diseases caused by HAdV types 3, 4 and 7 were associated with military recruits e.g. in US army "boot camps" suggesting crowding and physical exercise in spite of acute respiratory symptoms as essential co-factors that might have promoted epidemic spread and severe disease manifestations.[11—13] An exceptionally high virulence of type 14p1 was presumed because it caused pneumonia outbreaks also in the American civilian population with multiple fatalities. Subsequently it was also isolated from pneumonia patients in Ireland.[14—16]

In 1997 HAdV-B21 (strain "agent Y/SIBU97") was found during an investigation of an enterovirus 71 hand-foot-and-mouth disease outbreak in Sarawak, Malaysia. Agent Y/SIBU97 was detected in five fatal cases (due to cardiac/cardiopulmonary failure) which were negative for enterovirus 71 (and any other pathogen) and in five fatal cases with viral co-infections (enterovirus, HSV-2, dengue). Moreover, agent Y/SIBU97 was the only pathogen detected in five children suffering from acute flaccid paresis.[17,18] Retrospectively, the emergence of a highly virulent subtype of HAdV-B21 in Sarawak can be suspected because the hexon sequence of agent Y/SIBU97 was found to be divergent from the prototype sequence (only 99.34% identity) and the REA of its genome showed different band patterns compared to the HAdV-B21 prototype. Up to now, there were no reports on the circulation of agent Y/SIBU97 or any other lethal HAdV-B21 infection since the 1997 Sarawak outbreak.

In 2008, a fatal case of HAdV-B21 pneumonia was diagnosed in a child in Freiburg, Germany. A partial hexon sequence was found to be 100% identical to agent Y/SIBU97 but a detailed analysis was not feasible then because a virus isolate and deep sequencing techniques were not yet available.

In this study, genomic sequence data of this case and five other German HAdV-B21 pneumonia cases indicated circulation of a highly virulent adenovirus subtype 21a, which is closely related to agent Y/SIBU97.

Materials and methods

Patients and specimen

After occurrence of two fatal pneumonia cases with HADV-B21 (2008 and 2013), we retrospectively searched the digital archive of the German Adenovirus Reference Laboratory ("Konsiliarlabor") for other cases of HAdV-B21 infection in Germany since 2002. Only pneumonia cases were found and designated as LRTI-1 to LRTI-6 according to their occurrence. All identified HADV B21 cases were analyzed by next generation sequencing (NGS) and clinical data was extracted from medical records. This retrospective study was approved by the institutional review board of the Hannover Medical School. A health care worker (contact of the index case LRTI-2) with keratokonjunctivitis was designated as ES-1.

Diagnostic HAdV PCR

A generic, quantitative real-time PCR for HAdV was performed on the clinical patient specimens as described previously.[19,20]

Virus isolation and purification

All clinical isolates and the HAdV-B21 prototype were propagated on HFF-1 (ATCC, SCRC-1041) and A549 (ATCC, CCL-185) cells. HAdV nucleocapsids were purified from cell culture lysates using the Sartorius Vivapure AdenoPACK 20 (Sartorius, Göttingen, Germany). Viral DNA was extracted with the Qiagen blood kit (Qiagen, Hilden, Germany).

Next generation sequencing and bioinformatic data analysis

Library preparation was performed using the Nextera DNA Sample Prep Kit (Illumina, San Diego, CA) according to manufacturer's protocol. Amplified, size-selected libraries were quantified and quality controlled using the Qubit HS assay (Invitrogen, Carlsbad, CA) and the Agilent 2100 Bioanalyzer (Santa Clara, CA) DNA samples were sequenced with an Illumina MiSeq for paired-end 250 bp reads. Sequence assembly and data analysis was performed using the CLC Genomics Workbench version 6.0.4 (Aarhus, Denmark).

Phylogenetic reconstruction and sequence comparison

Nucleotide and amino acid sequences were aligned using the ClustalW algorithm implemented in the BioEdit package