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Genetic characterization of human bocavirus among children with severe acute respiratory infection in China

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Summary *Objectives:* To investigate the genetic character of Human bocavirus (HBoV) among children with severe acute respiratory infection (SARI) in China.
Methods: We screened 993 respiratory samples for HBoV by PCR among hospitalized children with SARI between September 2007 and March 2014. Four of HBoV1 samples were selected for complete genomes analysis by next-generation sequencing.
Results: The results show that 200 (20.1%) out of 993 samples were HBoV-positive, most of these HBoV belong to HBoV1 subtype (n = 197), HBoV2 (n = 1) and HBoV3 (n = 2) were also detected. Fifty (5.04%) of 993 SARI patient were detected as HBoV-positive only. Four HBoV1 genomes in this study were conserved and showed no significant difference among the nucleotide diversity from different regions. Analyses of evolutionary rates showed that NS1 exhibited the highest degree of conservation while the VP1 gene exhibited the fastest rate of evolution at 4.20×10^{-4} substitutions/site/year. The nucleotide deletions and substitutions occurred in NP1 and VP1 represented novel molecular signatures enabling subtype differentiation between HBoVs.

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Conclusions: We described some new characteristics in the epidemiology of HBoV among children with SARI, these data will significantly expand the current knowledge of HBoV epidemic and genomic characterization among children with SARI.

Introduction

Human bocavirus1−4 (HBoV) represents a novel pathogen associated with gastrointestinal and respiratory tract illnesses.[1−3] According to the latest ICTV classification of parvovirus, HBoV1 and HBoV3 belonged to *Primate bocaparvovirus* 1 species; HBoV2 and HBoV4 are part of *P. bocaparvovirus* 2 species.[4] The genome of HBoV is ∼5.3 kb in length, and is divided into four partially overlapping genes, namely NS1, NP1, VP1, and VP2, VP2 is totally included within VP1.[5,6] While the prototype HBoV (HBoV1) were first discovered from nasopharyngeal samples,[7] three additional viruses (HBoV2−4) have since been discovered in stool specimens, classified based upon their close phylogenetic relationship with HBoV1.[8,9]

HBoV1 is the most commonly reported genotype and occurring primarily in pediatric patients with respiratory tract infection, but also gastrointestinal symptoms are often observed.[10,11] In contrast, HBoV2 are preferentially detected in stool samples and appear to be more strongly associated with enteric disease, HBoV3 and HBoV4 are occasionally detected in faeces and too rare for any associations.[2] Since the discovery of HBoVs, numerous epidemiological surveillance efforts examining HBoV prevalence in children have been performed across multiple regions, comprising Thailand, United States, France, Jordan and Brazil.[10−14]

Severe acute respiratory infection (SARI) is among the leading causes of morbidity and mortality among children globally.[15] HBoV infection in children has been reported associated with respiratory tract infection, a few cases reported that HBoV as the cause of SARI among Children.[16−18] However, prolonged shed periods of HBoV and high co-infection detection resulted in the on-going debate of the HBoV as the agent of SARI[19]; In addition, the comprehensive research of HBoV genome among children with SARI were limited, especially in China. To better understand the molecular epidemiology and characterization of HBoV genome in children with SARI, we investigated the prevalence of HBoV among 993 inpatient children with SARI in China. Analyses of genome characterization were also performed.

Methods

Study subjects and sample collection

From Sep 2007 to Mar 2014, a total of 993 nasopharyngeal aspirates (NPAs) or induced sputum (IS) were randomly collected from hospitalized children with SARI in Beijing (n = 259), Shanghai (n = 441) and Zhejiang (n = 293) area. The case of SARI was defined according to the World Health Organization case definition for all hospitalized children in whom the onset of illness occurred within seven days of admission. Most of the patients had received clinical diagnosis of respiratory tract infection, including pneumonia, acute bronchitis/bronchiolitis, asthma exacerbation and acute pharyngitis. The most common respiratory symptoms included fever (temperature ≥38 °C), cough, sore throat, shortness of breath, vomit, dyspnea and so on.[15] In addition, none of the samples come from the patients in Pediatric intensive care unit (PICU) and most of the children have no co-morbidities, such as heart and liver diseases. All the samples were collected by medical professionals and placed in a tube containing of viral transportation medium and stored at −80 °C. This project was approved by the Research Ethics Committee of Beijing Children's Hospital, Children's Hospital of Fudan University, Wenzhou Medical College, and the Institutional Review Board at the China CDC. Written informed consent was obtained on the participants' behalf from their parents or guardians.

Molecular typing of HBoV and co-infection detection

Viral nucleic acids were extracted from virus transport medium by the QIAamp MinElute Virus Spin Kit (QIAGEN, Germany), according to the instructions provided by the manufacturer. As previously described,[8] partial VP1/VP2 gene fragment was amplified by nested PCR to screen and type HBoV infection. The first round-PCR primers were F1 (5′-CGCCGTGGCTCCTGCTCT-3′) and R1 (5′-TGTTCGCCATCACAAAAGATGTG-3′) with 609 bp product, the second-round PCR primers were F2 (5′-GGCTCCTGCTCTAGGAAATAAAGAG-3′) and R2 (5′-CCTGCTGTTAGGTCGTTGTTGTATGT-3′) with 576 bp PCR product. Positive products were cloned into pMDT-18 vector and sequenced by ABI 3730xl automated sequencer. HBoV co-infection with other respiratory viruses, including HRSV, HRV/EV, HAdV, HMPV, HPIV1−4, influenza A/B virus and HCoVs (-OC43, -229E, -NL63, -HKU1), was also screened as described previously.[20,21]

Sequencing and phylogenetic analysis of HBoV1 genome

Four samples of HBoV1 infection only in this study were used for complete genomes sequencing by next-generation sequencing. Samples were pretreated as previously,[22] and the amplified DNA was used as a template for Illumina Hiseq 2500 sequencing, paired-end reads (2 × 125 bp reads) were assembled into contigs by CLC genomic workbench.

To analyze genetic variation of HBoV detected, nucleotide sequences were compared to strains available from GenBank. Nucleotide sequence alignment was conducted through MAFFT version 5.[23] Phylogenetic and molecular evolutionary analyses were constructed by Neighbor-Joining Method using MEGA 5.0[24] with the bootstrap value of 1000.

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