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Genetic variation of human respiratory syncytial virus among children with fever and respiratory symptoms in Shanghai, China, from 2009 to 2012

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ABSTRACT

Human respiratory syncytial virus (HRSV) of genus Pneumovirus is one of the most common pathogens causing severe acute lower respiratory tract infection in infants and children. No information on the genotype distribution of HRSV is available in East China (e.g. Shanghai). From August 2009 to December 2012, 2407 nasopharyngeal swabs were collected from outpatient children with fever and respiratory symptoms in Shanghai. HRSV infection was determined using a multiplex RT-PCR assay. The second hypervariable region (HVR2) of G protein gene of HRSV was amplified and sequenced from HRSV positive samples. Genotypes were characterized by phylogenetic analyses. Of 2407 nasopharyngeal samples, 184 (7.6%) were tested as HRSV positive. From 160 positive subjects with sufficient nasopharyngeal samples, 69 HVR2 sequences were obtained by RT-PCR and sequencing. Three HRSV epidemic seasons were observed from August 2009 to December 2012, and an extreme outbreak of HRSV occurred in the 2009–2010 epidemic season. A genotype shift of predominant HRSV strains from B group in the 2009–2010 epidemic season to group A in the subsequent epidemic seasons was observed. Ten HRSV genotypes, including four group A genotypes NA1, NA3, NA4, and ON1, and six group B genotypes BA9, BA10, SAB4, CB1, BAc, and BA?, were detected in Shanghai. Seven genotypes (NA1, BA9–10, SAB4, CB1, BAc and BA?) were found in the 2009–2010 epidemic season. The co-circulation of multiple genotypes was associated with the extreme outbreak of HRSV among children with fever and respiratory symptoms in the 2009–2010 epidemic season.

1. Background

Human respiratory syncytial virus (HRSV) of genus Pneumovirus is one of the most common pathogens causing severe acute lower respiratory tract infection in infants and children, accounting for 15–40% pneumonia or bronchiolitis in children (Rudan et al., 2008). HRSV is a negative single-strand RNA virus with a genome of about 15 kilobases that encodes 11 proteins (Collins and Melero, 2011). The attachment glycoprotein (G protein) responsible for HRSV infection is the most variable protein (Teng et al., 2001). It contains two hypervariable regions (HVRs). The second HVR of G protein has the highest degree of divergence that can reflect overall gene variability, and is commonly used for genotyping HRSV (Galiano et al., 2005). According to genetic variability of HRV2, two genetically distinct HRSV groups A and B have been divided (Mufson et al., 1985). Groups A and B were further subdivided into 11 (ON1, GA1–GA7, SAA1, NA1, and NA2) and 20 (GB1–GB4, BA1–BA10, SAB1–SAB4, URU1, and URU2) genotypes (Arnott et al., 2011; Eshaghi et al., 2012).

One main feature of HRSV epidemic is that the predominant genotypes can shift among different epidemic seasons (Peret et al., 2000; Zhang et al., 2010b). The molecular epidemiological characteristics of HRSV were described previously in Southwestern and Northwestern China (Cui et al., 2013b; Qin et al., 2013; Zhang et al., 2010a,b). However, less information on the genotype distribution of HRSV is available in East China. HRSV infection is often associated with acute respiratory tract infections (ARTIs). There is less information available for HRSV infection among children with fever and respiratory symptoms. Here we report the molecular epidemiological characteristics of HRSV among children with fever and respiratory symptoms in Shanghai, China, from 2009 to 2012.

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2. Material and methods

2.1. Study subjects and sample collection

From August 2009 to December 2012, 2407 nasopharyngeal swabs were collected from outpatient children with fever and respiratory symptoms in Shanghai Nanxiang Hospital, a district-level general hospital in Shanghai, China. Nanxiang Hospital is the biggest and best general hospital in Jiading district of Shanghai, and majority of patients in this hospital are from suburbs and rural area of Shanghai. Demographic information including symptom, age, gender and diagnosis were recorded for each patient. The study was approved by the Ethics committee of Shanghai Nanxiang Hospital and informed content was obtained from each child's parent or guardian. These samples were transported to laboratory in virus transport media (hank's buffer, BSA, HEPES and antibiotics) immediately and divided into three aliquot parts. One aliquot was used to extract RNA and the others were stored at −80 °C for future use.

2.2. RNA extraction and detection of HRSV infection by a multiplex RT-PCR

Total RNA was extracted from each sample using a QIAamp Viral RNA Mini Kit (Qiagen, Germany). Extracted RNA was tested for the viruses associated with ARTIs using a multiplex RT-PCR assay modified from ref (Wang et al., 2009), which can detect simultaneously 17 respiratory viruses (including HRSV) under five tubes. The detail protocol is available on request from the authors. For each HRSV positive sample determined by the multiplex RT-PCR assay, another RT-PCR was performed to amplify the C terminus region (i.e. HVR2 region) of G gene of HRSV using previously described primers (Zhang et al., 2010b). All RT-PCR reactions were performed using the QIAGEN One Step RT-PCR Kit (Qiagen, Germany). Amplified products were subjected to direct sequencing.

2.3. HRSV genotyping and phylogenetic analysis

Nucleotide sequences obtained in this study were aligned with HRSV genotype reference sequences reported previously using Muscle program implemented in MEGA 5.05. Genotype reference sequences were retrieved from GenBank according to strain name and/or GenBank accession numbers reported in previous studies. The phylogenetic trees were constructed using the neighbor-joining method implemented in MEGA 5.05. The evolutionary distances used to reconstruct phylogeny was calculated based on Kimura 2-parameter model and the reliability of each branch was assessed with 1000 bootstrap replicates. HRSV genotyping of Shanghai sequences were determined according to the phylogenetic trees. When the Shanghai sequences are unable to closely cluster with any reference sequences, but form an independent cluster with a high bootstrap support (>85), they are defined as new genotypes.

2.4. Nucleotide sequence accession numbers

The HVR2 sequences of HRSV reported in this article are available in GenBank under accession numbers of KJ658764–KJ658832.

3. Results and discussion

3.1. HRSV seasonal distribution and patient characteristic

From 2407 samples from children with fever and respiratory symptoms, 184 (7.6%) samples were detected to HRSV positive. The HRSV prevalence among children with fever and respiratory symptoms was substantially lower than among children with symptoms of acute respiratory tract infections (ARTIs) (Zhang et al., 2010a,b). Clinical manifestations of 154 HRSV infected cases with available information are shown in Table 1. The ratio of boys to girls was 1.2:1. Vast majority of patients (90.3%) were between one and 10 years old, and the most common symptoms observed were fever, coughing and runny nose (Table 1).

HRSV epidemics exhibit distinct seasonal variation with high HRSV infection rates among children with fever and respiratory symptoms during the winter to spring months (December to February) (Supporting Fig. S1), well consistent with previous observations on children with ARTIS (Zhang et al., 2010a,b). The prevalence of HRSV among children with fever and respiratory symptoms in Shanghai was divided into three epidemic seasons by two three-month intervals from May to July in 2010 and 2011, during which no HRSV infection was found. The sample numbers are almost similar for each month except January to June 2012. Half (50%) of HRSV cases appeared from August 2009 to April 2010 with a peak at January 2010, indicating that the 2009–2010 epidemic season experienced an extreme HRSV outbreak among children with fever and respiratory symptoms (Fig. 1A).

3.2. Genotype shift in HRSV prevalence

Among 184 HRSV positive samples, only 160 samples had enough amounts to support further experiments for amplification of HVR2 fragment. From the 160 HRSV positive samples, 69 HVR2 sequences were obtained. The failure in the amplification of viral genomic fragments in other samples may be due to low viral load of these samples or the variations in primer binding sites. Phylogenetic analysis with the HVRs reference sequences retrieved from GenBank reveals that 23 were HRSV A group and 46 were B group (Fig. 2). HRSV B strains were predominant in the 2009–2010 epidemic season, accounting for 91.5%. The predominant strains were changed from group B in the 2009–2010 epidemic season to group A (100% and 81.3%, respectively) in the 2010–2011 and 2011–2012 epidemic seasons (Fig. 1B). The genotype shift trend of HRSV was consistent with that in other regions of China, where predominant strains were shifted from group A in 2006 to group B in 2009, and further to group A in 2011 (Qin et al., 2013; Zhang et al., 2010b).

Of 23 HRSV A strains, vast majority (19/23, 82.6%) clustered with the reference sequences of NA1 genotype, indicating majority of A strains were NA1 genotype. One strain clustered with ON1 reference strains with high bootstrap value support (88%) (Fig. 2A), and carried the ON1-specific 24-amino acid repeat (Supporting Fig. S2A). Interestingly, one and two Shanghai strains clustered together with the NA3 and NA4 strains with high bootstrap support (97–100%). The NA3 and NA4 genotypes were two new genotypes recently identified in Beijing, the capital of China (Cui et al., 2013b). Their nomenclatures were because they clustered outside of NA2 strain and had distinct amino acid sequence characteristics from NA1 and NA2 (Fig. 2A). Compared with other NA and ON1 strains, NA3 strains have five genotype-specific amino acids at sites 255, 270, 280, 283 and 310, and NA4 strains have nine genotype-specific amino acids at sites 226, 239, 242, 244, 250, 262, 274, 316, and 321, as well as an additional residue (Tyr) at the C-terminal (Supporting Fig. S2A).

Among 46 Shanghai HRSV B strains, 34 belonged to genotype BA that has a unique 20-amino acid repeat in the C-terminal region of G protein, accounting for 73.9%; 11 belonged to CB1 and one was SAB4 strain (Fig. 2B). The CB1 genotype was a new HRSV genotype identified in Beijing. The CB1 strains exhibit different amino acid sequence characteristics from GB2 stains (Supporting Fig. S2B). BA strains were further divided into ten genotypes, BA1–BA10. Of these BA strains, 21 were identified as BA9 and one was BA10 since they clustered within the clades of corresponding genotype