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Research paper

Tracing the emerging genotypes of human respiratory syncytial virus in Beijing by evolution analysis of the attachment glycoprotein (G) gene

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ABSTRACT

Background: Emerging human respiratory syncytial virus (HRSV) genotypes, such as ON1 and BA9, are becoming the dominant genotypes prevailing worldwide. *Objective* To trace the emerging HRSV genotypes in Beijing.

Methods: HRSV-positive specimens as determined by direct immunofluorescence, collected from children diagnosed with bronchiolitis from July 2006 to June 2016, were typed by real-time PCR, then genotyped by phylogenetic analyses of the full attachment glycoprotein (G) gene. A Bayesian skyline plot was constructed to analyze the population dynamics for identified HRSV strains, and selective pressure was analyzed.

Results: The previous dominant HRSV A genotype, NA1, was replaced by ON1 in 2014. BA9 was the dominant HRSV B genotype for the duration of the study. The time to the most recent common ancestor (tMRCA) for HRSV A is since the 1943–1944 season; for the genotypes NA1 and ON1, since the 1999–2000 season and 2010–2011 season, respectively. The tMRCA for HRSV B is since the 1956–1957 season; for the genotypes BA and BA9, from the 1998–1999 season and 2005–2006 season, respectively. The mean evolutionary rate of HRSV A (3.65×10^{-3}) was faster than those of HRSV B (3.11×10^{-3}), and the genotypes NA1 (2.01×10^{-3}) and ON1 (1.66×10^{-3}). The estimated effective population size (EPS) infected by HRSV A changed significantly from 2012 to 2013, which is consistent with the detection of ON1. Most positive selection sites were concentrated in the second highly variable region (HVR2) of the G gene.

Conclusions: Over the 10-year period from 2006 to 2016, the dominant genotypes in Beijing were NA1, ON1, and BA9. The HRSV strains in Beijing may have their own unique phylogenetic characteristics.

1. Introduction

Globally, human respiratory syncytial virus (HRSV) is a common cause of acute lower respiratory infection (ALRI) and a major cause of hospital admissions in young children (Shi et al., 2017). Despite the very large volume of collected data on the HRSV replication mechanisms, pathology, and community transmission, there are relatively few therapeutic options available to prevent or treat HRSV infection (Griffiths et al., 2017).

HRSV can be divided into subtypes A and B according to antigenicity difference (Anderson et al., 1985; Gimenez et al., 1986; Agoti et al., 2014); these subtype groups have been confirmed by nucleotide sequence analysis (Johnson et al., 1987). The RNA-dependent replication cycle of HRSV is significant because it is error prone, and there is no proofreading mechanism. This allows for the rapid generation of single nucleotide polymorphisms (SNPs) and other mutations that allow for changes in virus virulence, escape from current herd immunity, and avoidance of potential future antiviral agents or vaccines (Eshaghi et al., 2012). Among the 10 genes in the HRSV genome, which encode 11 proteins, the attachment protein G gene is the most variable. Its extracellular region contains two mucin-like regions named highly variable regions HVR1 and HVR2, which are separated by a 13-amino

Abbreviations: AdV, human adenovirus; ALRI, acute lower respiratory infection; BSP, Bayesian skyline plot; DFA, direct immunofluorescence; EPS, effective sample size; FEL, fixed-effects likelihood; FITC, fluorescein isothiocyanate; Flu, influenza virus; FUBAR, fast unconstrained Bayesian approximation; GTR, general time reversible; HRSV, human respiratory syncytial virus; HVR, highly variable region; IFEL, internal branch fixed-effects likelihood; MCC, maximum clade credibility; MCMC, Markov chain Monte Carlo; MEME, mixed effects model for episodic diversifying selection; NPA, nasopharyngeal aspirate; PIV, parainfluenza virus; SLAC, single likelihood ancestor counting; SNP, single nucleotide polymorphism; tMRCA, the time to the most recent common ancestor

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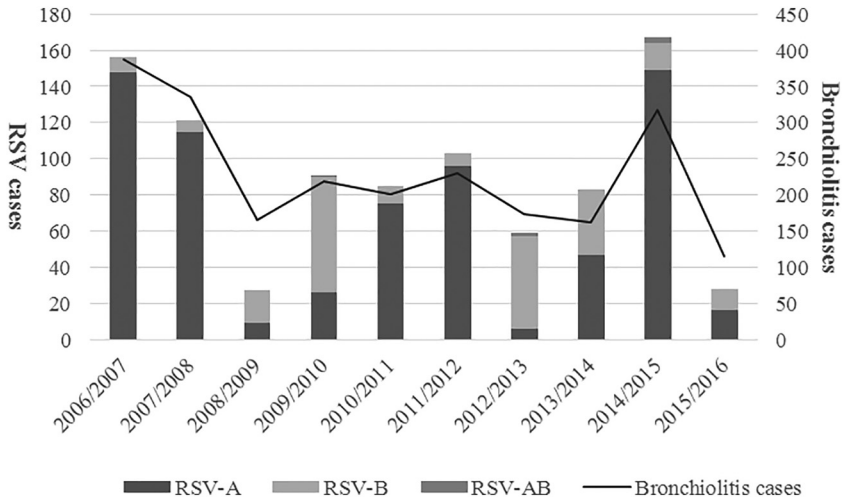

Fig. 1. Distribution of the two HRSV subtypes in children with bronchiolitis in Beijing from 2006 to 2016.
The corresponding specific values are provided in Table S2.

acid highly conserved region (aa 163–189) (Johnson et al., 1987). Based on analyses of the nucleotide sequences of HVR2, the subtypes HRSV A and B are further divided into a variety of genotypes. Several novel genotypes, such as ON1, which has a 72-nucleotide duplication, and BA9, which has a 60-nucleotide duplication in the HVR2 of G gene, are becoming the dominant genotypes prevailing worldwide (Deng et al., 2006; Choudhary et al., 2013; Cui et al., 2013; Hirano et al., 2014; Ábrego et al., 2017).

Bronchiolitis, a common lower respiratory tract disease of infancy, seriously threatens pediatric health globally owing to its characteristics of high incidence and young onset age. A pathogenic study of bronchiolitis showed that HRSV is considered the most common pathogen (50–80%) (Meissner, 2016). To trace the emerging genotypes of HRSV, clinical specimens were collected from infants and young children who were diagnosed with bronchiolitis in Beijing during the 10 consecutive years of this study, and the molecular evolution of the HRSV G gene in these samples was explored.

2. Materials and methods

2.1. Clinical specimens

From July 2006 to June 2016, nasopharyngeal aspirates (NPAs) were collected from children with bronchiolitis at the Affiliated Children's Hospital, Capital Institute of Pediatrics for respiratory virus screening. Specimens in 2.5 ml Hank's were vortexed and then centrifuged at 500 ×*g* for 10 min. Cell pellets from all NPA specimens were re-suspended and spotted onto acetone-cleaned slides. Individual monoclonal antibody reagents against HRSV, human adenovirus (AdV), influenza virus (Flu) A and B, and parainfluenza virus (PIV) 1–3, each labeled with fluorescein isothiocyanate (FITC), were used for virus identification by direct immunofluorescence (DFA) (Diagnostic Hybrids, Athens, OH, USA). The supernatants from all NPA specimens were used for RNA extraction.

This study was approved by the Ethics Committee of the Capital Institute of Pediatrics (Approval number: SHERLLM 2015 012).

2.2. RNA extraction and reverse transcription (RT) reaction

RNA extraction from HRSV-positive NPA specimens was performed using Trizol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. The viral RNA was then reverse-transcribed using random hexamer primers and Moloney murine leukemia virus reverse transcriptase (Invitrogen, Carisbad, USA), according to the manufacturer's guidelines.

2.3. PCR for HRSV subtyping and full-length G gene sequencing and amplification

For HRSV subtyping, we performed real-time PCR in a 25-μl reaction mixture containing 2.5 μl of cDNA, 12.5 μl of 2× TaqMan universal PCR master mix (Applied Biosystems, Roche, Branchurg, New Jersey, USA), primers (800 nM), and probes (200 nM) as previously described (Sun et al., 2006). The following primers and probes were used: forward primer HRSV A-S (5′-CAACATTGAGATAGAATCTAGAAAATCCT ACA-3′), reverse primer HRSV A-AS (5′-ATTTGGTTATTACTAATGCY-GCTATACA-3′), and probe HRSV A-probe (5′-Cy5-TGGCTCCAGAATA CAGGCATGACTCTCC-TAMRA-3′) for HRSV A; and forward primer HRSV B—S (5′-CTGAAGATGCAAATCATAAATTCACA-3′), reverse primer HRSV B-AS (5′-TGATATCCAGCATCTTTAAGTATCTTTATA GTG-3′), and probe HRSV B-probe (5′-FAM-AGGTATGTTATATGCTAT GCTCAGGTTAGGAAGGGA-TAMRA-3′) for HRSV B. The assay was performed on a 7500 Real-Time PCR System (Applied Biosystems) under the following conditions: 50 °C for 2 min, 95 °C for 10 min, and 45 cycles of 95 °C for 15 s, 60 °C for 1 min.

For the amplification of full-length G genes, we performed nested PCR using primers S4298 (5′-TGGCCYTAYTTTACACTAATAC-3′) and F164 (5′-GTTATGACACTGGTATAC CAAC-3′) in the first round, producing a 1489-bp fragment, and primers S4373 (5′-ATCTCCATCATG ATTGCAAT-3′) and F5763 (5′-ATAGCCTTTGCTAACTGC AC-3′) in the second round, producing a 1390-bp fragment (Arnottm et al., 2011; Cui et al., 2013). The amplification profile comprised an initial period of denaturation at 94 °C for 5 min, followed by 40 amplification cycles of 94 °C for 30 s, 49 °C (in the first round) or 42 °C (in the second round) for 30 s, and 72 °C for 1 min, and then a final extension at 72 °C for 10 min. The amplicons were analyzed by electrophoresis in a 1.5% (*w/v*) agarose gel. All PCR reactions contained 4 μl (in the first round) or 1 μl (in the second round) of DNA, 0.5 μl of each forward and reverse primer (10 mM), 0.5 μl of Easy Taq DNA Polymerase (TransGen Biotech Co., Ltd., Beijing, China), and 2.5 μl of 10× Easy Taq Buffer and were made up to a final volume of 25 μl with distilled water.

All PCR products were sequenced by Sino Geno Max Co., Ltd., Beijing, China.