Sequence analysis of the G gene of hRSVA ON1 genotype from Egyptian children with acute respiratory tract infections

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Abstract

Human respiratory syncytial virus causes severe lower respiratory tract infection in neonates and children. Genotype ON1, with duplication of 72-nt in the G gene, was first detected in Canada and then recorded in other countries. In the current study, we describe the first detection of the ON1 genotype among children in Egypt in 2014/2015. Sequence analysis of the full-attachment G gene revealed that the majority of the strains examined were related to the ON1 genotype and only one sample related to N1 genotype. The Egyptian ON1 strains showed unique non-silent mutations in addition to variable mutations near the antigenic sites in comparison to the original ON1 ancestor strain. Continuous surveillance of hRSV regionally and globally is needed to understand the evolutionary mechanisms and strategies adopted by hRSV and their inducers for better adaption to the host.

Human respiratory syncytial virus continues to cause severe lower respiratory tract infection in children. It is among the main causes of hospitalization of infants and young children with pneumonia. In adults, although the disease is asymptomatic, moderate to severe upper respiratory tract signs may occur [1]. hRSV is related to the genus Orthopneumovirus of the family Pneumoviridae. Two groups of hRSV exist: RSVA and RSVB, based on their reactions with monoclonal antibodies [2]. The viral genome encodes 11 proteins, including G- and F- proteins as the major surface proteins. These two proteins are involved in cell receptor binding [3] and fusion to the cell membrane [4], respectively. Neutralizing antibodies are secreted against both proteins [5]. Based on G protein genetic variability, the hRSVA group is subdivided into 15 genotypes [GA1–7, SAA1, NA1–4 CB-A, and ON1–2] [6, 7], while the hRSVB group is subdivided into 24 genotypes [SAB1–4, BA1–12, GB1–4, GB5/CB1, CBB and URU1–2] [8, 9].

The ON1 genotype was first detected in 2010, in Canada. It was suggested to have evolved from the NA1 genotype, which was rapidly replaced by a tandem repeat of 72 nucleotides in the 3rd hypervariable region of the G gene [7].

The G protein is a type II surface glycoprotein of approximately 300 amino acids in length, and with heavy N- and O-linked glycosylatation. However, the amino acid sequence positions of potential glycosylation sites are poorly conserved [10].

In the current study, sequence variation in the hRSV G gene from children with severe lower respiratory tract infection in Egypt were screened. The phylogenetic analysis of the G gene was compared to various globally selected hRSV strains.

One hundred and ninety-nine nasopharyngeal swabs were collected from young children suffering from lower respiratory tract infection between August 2014 and April 2015 from Aboelreesh Hospital, Cairo University, Giza, Egypt. All patients were under five years of age. Nasopharyngeal swabs were centrifuged at 5000 r.p.m. at 4 °C for 5 min, and viral RNA was extracted from 250 µl of the clarified supernatant fluid using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA).

Real-time reverse transcriptase polymerase chain reaction was performed on RNA samples. cDNA synthesis was performed on individual samples using cDNA Synthesis Premix (Seegene, Korea). Respiratory virus detection kits A and B (AnplexTM II RV16 detection; Seegene, South Korea) were used to screen for the existence of adenovirus,
influenza A virus, influenza B virus, HPIV1-4, HRV, hRSVA, hRSV B, HBoV, hMPV, HCoV-229E, HCoV-NL63, HCoV-OC43 and enterovirus, according to the manufacturer’s instructions. A reaction volume of 20 µl was used containing: 8 µl cDNA, 5 µl 4×RV primer and 5 µl 4× master mix with the CFX96 real-time PCR detection system (Bio-Rad, USA). The following conditions applied: 4 min at 50 °C and 15 min at 95 °C, followed by 50 cycles of 95 °C for 30 s, 60 °C for 1 min and 72 °C for 30 s. At the end of the chain reactions, a CMTA was performed by cooling to 55 °C then incubation for 30 s, and then heating from 55 to 85 °C. Fluorescence signals were measured continuously during temperature increases, and melting peaks were recorded and analysed.

Verso 1-Step RT-PCR kit ReddyMix, with ThermoPrime Taq, was used to amplify the full-length G gene according to the manufacturer’s instructions. For amplification of the hRSVA, primers SHA TCGAGTCAACATAGCATTC and F1 CAACCTCATTGTATTTGCC were used while SHB CATAGATTCTACCGATTGC and F1 CAAC TCCATTGTATTTGCC were used for amplification of hRSVB as previously described [11]. PCR products were purified with the QIAquick gel extraction Kit (Qiagen) followed by commercial direct gene sequencing (BigDye Terminator Cycle Sequencing) using the primers used for gene amplification. The nucleotide sequences were processed and trimmed by MEGA 5.2 freeware and submitted to GenBank to obtain accession numbers of the nucleotide sequences (KY865196–KY865210). The sequences thus obtained were aligned with selected published sequences from different hRSVA genotypes.

All the samples examined were collected from children suffering from severe respiratory infections in Egypt in 2014–2015. Respiratory syncytial virus was found in 77/223: 37 strongly positive (2 hRSVB and 35 hRSVA) and 40 weakly positive (11 hRSVB and 29 hRSVA) (data not shown). Among the 35 hRSVA samples, mixed infection was found in 12/38, 6/38 were found to contain hRSVA/HRV infection and 6/38 were positive for hRSVA with one of the following: HBoV, adenovirus, PIV-4, influenza B virus, human enterovirus and HVoV-OC43 (data not shown).

Full-length G gene amplification was successful in 15/35 hRSVA samples, but failed completely with hRSVB (0/2). A phylogenetic tree based on the nucleotide sequence of G is shown in Fig. 1. Most of the currently characterized hRSVA strains (14/15; 93.3 %) were grouped with the novel ON1 genotype (Fig. 1) that was first detected in Canada [7], while a single strain was grouped within strains related to the N1 genotype. The current study records the first report of the ON1 genotype in Egypt. Globally, genotype ON1 has frequently been reported in many countries and it seems that it is the most commonly detected genotype in group A hRSV [12].

Strains related to the ON1 genotype are characterized by the presence of a 72 nt insertion in the C-terminal end of the G gene, with subsequent duplication of amino acids (QEETLH STTSEYLPSQVYTTTS) flanking positions 261–283 and 285–307 (Fig. 2). This duplication was found conserved in RSVA/Eg/BSU-3/2014, RSVA/Eg/BSU-9/2014, RSVA/Eg/BSU-19/2015 and RSVA/Eg/BSU-21/2015. R295K amino acid substitution was detected in three strains: RSVA/Eg/BSU-1/2014, RSVA/Eg/BSU-4/2014 and RSVA/Eg/BSU-8/2014. The remainder of the ON1 Egyptian strains showed multiple amino acid substitutions in the duplication regions (Fig. 2). Four amino acid substitutions in the original Canadian viruses, including L274P, L298P, Y304H and L310P, occurred in 6, 5 and 4 out of the 14 Egyptian ON1 strains, respectively (Fig. 2). These substitutions refer to the same positions within the parent and the resulting duplication region; thus, this event is considered noteworthy because the adjacent region (aa 265–273) is a reported antigenic site [13]. The P310L (P286L in viruses without the duplication) amino acid substitution has been associated with abrogation of reaction of peptides to convalescent-phase human serum [14] and was recorded in 11 out of the 15 Egyptian strains.

Furthermore, changes were observed in the region that would change the potential N-glycosylation profile from the original Canadian viruses. Some of these changes would cause the loss of a site (e.g. T137P, T320A in RSVA/Eg/BSU-5/2014 and RSVA/Eg/BSU-28/2015) while others would cause site gains (e.g. S100N in 8/14 Egyptian ON1 isolates). Of particular interest is the existence of accumulated signature coding changes in the epitope regions of the G protein, in addition to the existence of amino acid changes in both copies of the duplicated motif of the ON1 genotype (Fig. 2).

Botteosso et al. [15] recorded a total of 29 amino acid sites that were found to be putatively positively selected in hRSVA [15]. These sites were found to be epitopes in escape-mutants either screened with specific monoclonal antibodies (residue numbers 226, 237, 265, 274, 275, 284, 286 and 290) [16–18] or in naturally isolated strains (residue numbers: 215, 225, 226, 265, 280 and 293) [14, 16, 19]. All the Egyptian strains and the original ON1 strain were found to possess P215, although most of the group A genotypes were found to possess L215 [15]. Meanwhile, two unique amino acid substitutions were detected among Egyptian strains: Thr 253 Lys (except Eg/BSU-29/2015 that possesses Thr 253) and Phe 265 Leu. The latter was found to be an epitope described in escape-mutant strains [16].

Glu 262 Lys was detected in a single Egyptian sample (RSVA/A/Eg/BSU-7/2014) belonging to the ON1 genotype (Fig. 2). This amino acid substitution was found in genotypes GA2 and NA4 (data not shown), but these genotypes were not detected in Egypt. This amino acid substitution was recently detected in Chinese ONA1 strains, with no evidence to date of biological significance [20].

The present study revealed a high detection rate of hRSVA in-patients infants hospitalized with lower respiratory tract infections. Two genotypes were found, ON1 and N1, with a
Fig. 1. Phylogenetic tree of hRSVA and reference sequences of identified genotypes. The phylogenetic tree for the G gene of hRSVA was constructed using the maximum-likelihood method with 1000 bootstrap replicates, using MEGA 5.2 software. hRSV strains from Egypt are denoted in blue. Reference strains representing different genotypes were obtained from the GenBank database and included in the tree.
Fig. 2. Deduced amino acid sequences of the G protein of Egyptian hRSVA strains in comparison to reference ON1 and N1 genotypes. Alignment of RSVA genotypes N1 and ON1. Dots indicate identical residues. Red bold type denotes putatively positively selected amino acid residues. N-glycosylation sites are underlined. Boxed frames indicate the 23 amino acid-duplicated region in ON1 strains.
predominance of genotype A ON1. It is important to screen the evolution of hRSV by screen sequence variation of the G gene of the virus. Positively selected sites were detected in the Egyptian strains and two unique amino acid substitutions were detected.

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Conflicts of interest
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References