Short communication

Displacement of predominant respiratory syncytial virus genotypes in Malaysia between 1989 and 2011

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From 1989 to 2011 in Kuala Lumpur, Malaysia, multiple genotypes from both respiratory syncytial virus (RSV) subgroups were found co-circulating each year. RSV-A subgroup predominated in 12 out of 17 years with the remaining years predominated by RSV-B subgroup. Local RSV strains exhibited temporal clustering with RSV strains reported in previous epidemiological studies. Every few years, the existing predominant genotype was replaced by a new one. The RSV-A genotypes GA2, GA5 and GA7 were replaced by NA1 and NA2, while BA became the predominant RSV-B genotype. A unique local cluster, BA12, was seen in 2009, and the recently-described ON1 genotype with 72-nt duplication emerged in 2011. Our findings will have important implications for future vaccine intervention.

1. Introduction

Respiratory syncytial virus (RSV) is the most common virus causing respiratory infections in children in Malaysia, a tropical country in Southeast Asia (Khor et al., 2012). RSV cases occur throughout the year with peak activity between September and December (Khor et al., 2012). RSV can be categorized into subgroup A and subgroup B, based on reactivity to monoclonal antibodies targeting epitopes on G (attachment) protein and F (fusion) protein (Mufson et al., 1985). The G gene encodes a transmembrane protein with two hypervariable regions, and a 270-nucleotide segment of the second hypervariable (carboxyl terminus) region is commonly used for genotyping (Peret et al., 2000; Reiche and Schweiger, 2009; Shobugawa et al., 2009; Venter et al., 2002). Both RSV subgroups can be further categorized into genotypes. Currently, there are 11 genotypes in RSV subgroup A (GA1 – GA7, SA1, NA1, NA2 and ON1) and 17 genotypes in RSV subgroup B (GB1 – GB12, SAB1 – SAB4 and BA) (Arnott et al., 2011; Eshaghi et al., 2012; Peret et al., 2000; Shobugawa et al., 2009; Trento et al., 2003; Venter et al., 2001; Zlateva et al., 2005). The BA genotype can be further classified into clusters of BA1 – BA11 (Baek et al., 2012; Dapat et al., 2010). In the present study, we describe the changing molecular epidemiology of RSV strains collected over a 23-year period in Malaysia, from where data is limited.

University Malaya Medical Centre is a 900-bed teaching hospital in Kuala Lumpur, Malaysia. Nasopharyngeal aspirates were collected from children with suspected respiratory infection at clinicians’ discretion, and tested for RSV using immunofluorescence and viral isolation as previously described (Khor et al., 2012). From laboratory records, 2004 RSV cases were diagnosed between 1989 and 2011. Up to 15 samples were randomly selected from each year for this study. RNA was extracted using QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). The second hypervariable region of the G gene was amplified with forward (5’-TCACCTTGAAGTGTICAATGTA-3’) and reverse (5’-GGCAACTCCATTGT-TATTGTG-3’) primers (Jalal et al., 2007) using Access RT-PCR System (Promega, Madison, USA). After reverse transcription, the mixtures were denatured at 95 °C for 2 min, followed by 40 cycles of 94 °C for 45 s, 50 °C for 45 s, and 68 °C for 1 min, followed by a final extension at 68 °C for 5 min in a Veriti Thermal Cycler (Applied Biosystems, Carlsbad, USA). PCR products were purified using QIAquick Gel Extraction Kit (Qiagen). Cycle sequencing was performed using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) with a 3730xl DNA Analyzer (Applied Biosystems). Sequences were edited and aligned using Geneious v5.0.3 (Biomatters, Auckland, New Zealand). The best substitution model was determined using jModelTest v0.1.1 (Posada, 2008). Phylogenetic trees were constructed using maximum likelihood with 1000 bootstrap reiterations using MEGA v5.05 (Tamura et al., 2011). Nucleotide and amino acid similarities and amino acid alignments were generated using GeneDoc v2.7 (Nicholas et al., 2000).
Fig. 1. Phylogenetic analysis of RSV in Kuala Lumpur, Malaysia (1982–2011). Phylogenetic tree of Malaysian and reference RSV subgroup A strains. The 18537 strain (GenBank accession number M17213) from RSV subgroup B was used as the outgroup (A). Phylogenetic tree of Malaysian and reference RSV subgroup B strains. The Long strain (GenBank accession number AY911262) from RSV subgroup A was used as the outgroup (B). Malaysian sequences from this study are labeled with (d). Sequences are named as accession number_country of origin_strain name_year of isolation. Reference strains are listed in Supplementary Table 1. The best substitution models for both phylogenetic trees were determined using jModelTest v0.1.1. Both phylogenetic trees were constructed using the maximum likelihood algorithm with the general time reversible + G (GTR + G) substitution model running in MEGA 5.05. The phylogenetic trees were supported with 1000 bootstrap reiterations.
There were seven RSV-A genotypes detected: GA2 (reference sequences of known genotypes (Supplementary Table 1). Different years (1989, 1999, 2002, 2003 and 2009). Dominant subgroup in 12 years, while RSV-B predominated in 5 year, while RSV-B was not detected in 6 years. RSV-A was the predominant genotype up until the early 2000s (Arnott et al., 2011; Reiche and Schweiger, 2009; Shobugawa et al., 2009; Venter et al., 2002), before being replaced in recent years by NA1 and NA2 (Arnott et al., 2011; Eshaghi et al., 2012; Shobugawa et al., 2009). A recent study from China reported GA2 as the predominant RSV genotype in 2006–2009 (Zhang et al., 2010), which showed that the BA genotype (formerly GB13) has become the predominant RSV-B genotype since 2007, except for 2009 when BA12 was also present. The BA genotypes replaced the predominant RSV-B genotype SAB3 from 2000 onwards.

There is conflicting data on whether RSV evolution is temporally or geographically related (Choi and Lee, 2000; Eshaghi et al., 2012; Garcia et al., 1994; Kuroiwa et al., 2005). Studies from Europe and Asia have also shown that GA2, GA5, and/or GA7 were the predominant RSV-A genotypes up until the early 2000s (Arnott et al., 2011; Reiche and Schweiger, 2009; Shobugawa et al., 2009; Venter et al., 2002), before being replaced in recent years by NA1 and NA2 (Arnott et al., 2011; Eshaghi et al., 2012; Shobugawa et al., 2009). A recent study from China reported GA2 as the predominant RSV genotype in 2006–2009 (Zhang et al., 2010), which our reanalysis showed could be classified as NA1 (data not shown).

Our study also showed the localized nature of RSV. This may result in temporal clusters that dominated, such as our BA12 cluster, which comprised 8/14 (57.1%) of RSV sampled in 2009, while only 2 other similar strains were reported elsewhere, in Cambodia and USA (Arnott et al., 2011; Rebuffo-Scheer et al., 2011). There may also be unusual local cases which occur sporadically, such as the ON1 genotype detected here in 2011, a year after it was first described in Ontario (Eshaghi et al., 2012). The Malaysian ON1 strain, MY-2444006-11, showed 100% nucleotide similarity with the Ontario strain ON67-1210A in a 334 nt overlapping region, including the same 72 nt duplication in the second hypervariable region.

It is important to monitor the circulating RSV strains in different geographical regions, particularly in developing tropical countries where data is relatively lacking. In Malaysia, new RSV genotypes emerge to replace older circulating genotypes every few years, with occurrence of locally-specific clusters. It will be interesting to examine the association of clinical severity with these different genotypes in the future. These findings have implications for future planning of potential vaccines which are effective in all regions (Murata, 2009).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2012.12.017.

References


