

Genotypes of respiratory syncytial virus group B identified in Uruguay

Brief Report

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Summary. Genotypes of *Human respiratory syncytial virus* (HRSV) of group B from Uruguay were assigned to strains isolated during 1999 and 2001 outbreaks and others formerly reported isolated in the period 1989–1996. The nucleotide sequences of the C-terminal portion of the G protein were compared to sequences representative of previously defined HRSV genotypes. Most Uruguayan strains clustered into five of the previously identified genotypes. Nine isolates clustered in two genotypes named URU1 and URU2 which were not described up to present. Two of the analyzed sequences isolated in 2001 have a six nucleotide duplication that is discussed in terms of HRSV variability.

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Human respiratory syncytial virus is the single most important cause of severe lower respiratory tract infections in infants and young children world-wide [6]; it became an important pathogen for the elderly and immunocompromised adults [8]. *Human respiratory syncytial virus* (HRSV) is classified within the family *Paramyxoviridae*, with a non segmented negative stranded RNA that encodes for eleven proteins.

Two antigenic groups A and B, have been identified by their reactivity with panels of monoclonal antibodies [1, 18] which correlated with different genetic groups [7]. The highest genetic diversity reported for the G attachment protein occurs between and within both groups A and B [17, 24]. Genetic variability of HRSV G glycoproteins from isolates of both groups has been studied in great detail around the world, identifying several variants or lineages within each group, which have been assigned to different genotypes [15, 19, 20, 28, 29].

Both groups A and B circulate concurrently in each epidemic period, but as a rule, group A viruses are isolated more frequently than viruses from antigenic group B [3]. In Uruguay, this pattern was also confirmed examining the distribution of both groups over seventeen epidemic years (1985–2001), where a high prevalence of A strains occurred in cycles of up to four consecutive years followed by a single intervening year in which B strains were dominant [22, 2].

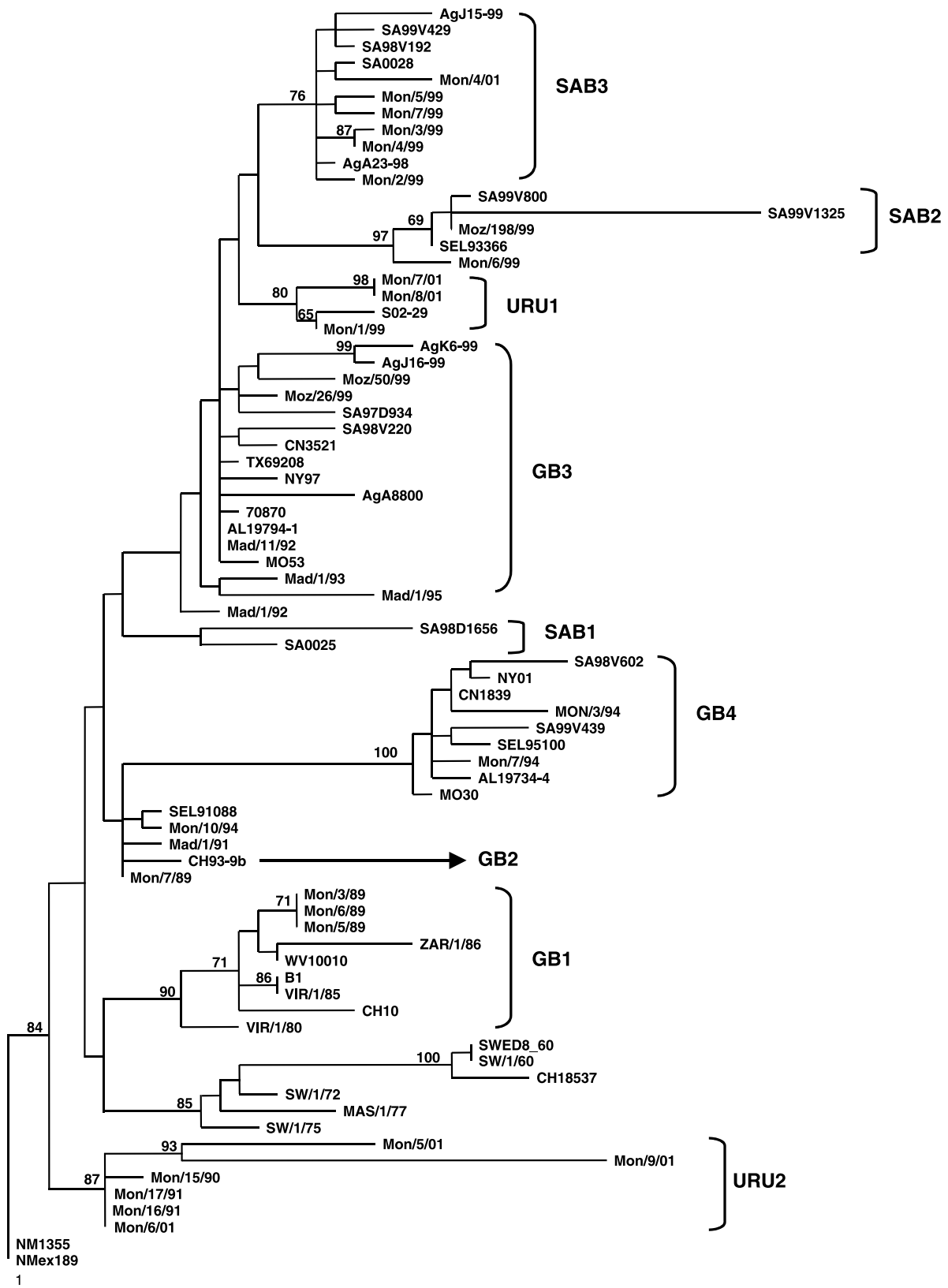
The antigenic and genetic variability of HRSV group A isolated during 1987–2001 in Uruguay has been reported previously [9, 10]. In these studies several evolutionary lineages were identified among isolates, and most of them were assigned to previously reported genotypes: GA3, GA5, GA1 and GA2. Genetic diversity of group B strains (1989–1996) isolated in Uruguay has been reported [16], however, up to present no information about genotypes assignment of the group B strains circulating in Uruguay has been informed.

In this study, we assign genotypes to HRSV group B isolates obtained during 1999 and 2001, and to those previously reported and isolated between 1989–1996 from Uruguay [16]. HRSV sequences from the rest of the world available at the GenBank were compared in order to analyze the phylogenetic relationships and to assign Uruguayan group B strains to reported genotypes. Two kinds of findings have been identified: i) two genotypes not previously described (URU1 and URU2), and ii) strains with six nucleotide insertions isolated in the same year.

Nasopharyngeal aspirates (NPAs) were collected at the Pereira Rossell Children's Hospital (Montevideo, Uruguay) from hospitalized children under 5 years of age. Samples that were positive for HRSV by immunofluorescence were selected. Sub-typing of HRSV group B was done through an ELISA assay (according to the technique described in Frabasile et al. [9]) with group specific monoclonal antibodies kindly supplied by Dr. Melero, from Instituto Carlos III of Spain. Reverse transcription was carried out with a negative sense primer that contains an oligo-dT tail LG3(–) 5'-GGCCCGGGAAGCTTTTTTTTTTTTTTTT-3'; which has an *Ava* I site (underlined) for cloning purposes, and PCR amplification using the primers LG3(–) and LG5(+) 5'-GGATCCCGGGGCAAATGCAAACATGTCC-3', which has an *Ava* I site (underlined) and the first 20 nucleotides of the G protein from the Long strain. Nucleotide sequencing was done using fluorescent dye-terminators on an ABI 377 sequencer (Perkin Elmer-Applied Biosystem) with the primer GB496(+) 5'-GATGATTACCATTTTGAAGTGTTC-3'.

Fig. 1. Maximum parsimony phylogenetic tree of HRSV group B isolates (last 270 nucleotides of the second variable region of the G glycoprotein gene). Inner numbers represent bootstrap proportion in support to the adjacent node (500 replicates). Genotypes are indicated in brackets. Reference sequences were downloaded from the GenBank and included for comparison: From United States (TX, Texas; AL, Alabama; NY, New York; MO, Missouri; CH, Rochester, New York); Canada (CN) [19]; SA, Soweto, South Africa [28]; Ag, Agincourt, South Africa [29]; Mozambique (Moz) [21]; B1 [13]; WV, West Virginia, SWED 8–60 [23]; Sel, Seoul, [4]; Vir, Virginia; NMex, New Mexico; Mas, Massachusetts; SW, Sweden; Mon, Montevideo; Mad, Madrid; Zar, Zaragoza [16]; S, Sapporo, Japan (Accession number: AB161413); 70870, [26]

HRSV group B diversity



Sequences of seven HRSV viruses from the 1999 outbreak and six from the 2001 outbreak, were determined. For comparison, 63 previously reported HRSV group B sequences from Uruguay [16] and the rest of the world were downloaded from GenBank, and were aligned using CLUSTALX 1.8 [11]. Phylogenetic analyses were done under maximum parsimony (MP) and distance based (not shown) criterion (Fig. 1). These analyses were performed with PAUP* 4.0b4a [25].

Both, MP and distance based phylogenies displayed very similar topologies, and resembled the previously reported for group B isolates [5, 16]. As noted earlier [16] viruses isolated in different places and in different epidemic periods grouped together (Fig. 1 e.g. Vir/1/80 and Mon/6/89), whereas viruses from the same epidemic and place were located in plural branches (Fig. 1 e.g. Mon/4/01, Mon/7/01 and Mon/6/01) confirming the notion that RSV can spread world-wide. Unlike HRSV group A, where strains place in two main branches and several sub branches [9, 10, 20, 28] group B isolates do not follow this pattern.

The analyzed sequences were genotyped by comparison with sequences previously assigned to specific genotypes throughout the world [19, 20, 28, 29] (Fig. 1).

Uruguayan group B isolates clustered into seven groups (Fig. 1). Of these, five correspond to already identified genotypes: GB1, GB2, GB4, SAB2 and SAB3. Genotypes GB1, GB4, SAB2 and SAB3 displayed significant bootstrap statistics, with values of 76–100%. Although two Uruguayan isolates (Mon/10/94 and Mon/7/89) are located near GB2 genotype, this could not be supported by a significant bootstrap value as was seen in previous reports [20, 28, 29].

As was predicted by Peret et al. [20] and Venter et al. [28] some strains could not accommodate to the current classification. Thus, some isolates from Uruguay were grouped in two different clusters from the reported genotypes and were named URU1 and URU2. These two new groups contain viruses isolated in Uruguay in 1990, 1991, 1999 and 2001; however URU1 includes one strain isolated in Japan. The criterion to define these two groups as different genotypes from previously reported was the same used by Venter et al. [28] who considered arbitrarily a genotype as the sequences clustered together with bootstrap values of 70–100%. The Uruguayan genotypes URU1 and URU2 described here had bootstrap values of 80% and 85% respectively. The second criterion used by Venter et al. [28], a p-distance of 0.07 or less between all members of the same cluster, was also taken into account. The URU1 genotype representatives fulfill this criterion (Table 1a). In URU2 genotype the majority of the strains display values less than 0.07, except for one of them which shows p-distance values ranging from 0.087 to 0.115 (Table 1b); however it clustered in this genotype supported by a significant bootstrap value (Fig. 1). A BLAST search was performed and showed that Mon/1/99 sequence from URU1 genotype, appeared closely related to a strain isolated in Japan (S02-29, accession number: AB161413). This strain displays a p-distance value ranging from 0.012 to 0.032 with the other members of the group and clusters into this genotype with a bootstrap value of 80%. The sequence most closely related to Mon/7/01 and Mon/8/01 (70870, [26]) clustered separately to URU1 genotype in the phylogenetic analysis. No sequences could be identified at the GenBank that clustered into URU2 genotype.

HRSV group B diversity

Table 1. Intragenotypic p-distance values of URU1 (A) and URU2 (B) genotypes, calculated with MEGA2 [14]. Intragenotypic p-distance values are the proportion of nucleotide sites at which the two sequences compared are different

	Mon/7/01	Mon/8/01	S02-29		
a) URU1					
Mon/7/01					
Mon/8/01	0.000				
S02-29	0.032	0.032			
Mon/1/99	0.020	0.020	0.012		
	Mon/15/90	Mon/6/01	Mon/16/91	Mon/17/91	Mon/5/01
b) URU2					
Mon/15/90					
Mon/6/01	0.008				
Mon/16/91	0.008	0.000			
Mon/17/91	0.008	0.000	0.000		
Mon/5/01	0.060	0.056	0.056	0.056	
Mon/9/01	0.095	0.087	0.087	0.087	0.115

One of the identified genotypes (URU1) has one strain isolated in 1999 (Mon/1/99) and two in 2001 (Mon/7/01 and Mon/8/01). It is interesting to report that the two viruses isolated in 2001 have an insertion of six nucleotides in the C-terminal third of the G protein gene, starting after residue 694. The extra sequence in the Uruguayan strains is an insertion of six nucleotides (AAG AAA) between the 689–694 duplicated residues. The repeated segment is translated in a duplication of the amino acids KE from the 225 and 226 residues respectively. Besides the extra sequence, the two Uruguayan strains had identical sequences in the sequenced segment. Also in 2001 a virus isolated in Buenos Aires, Argentina had exactly the same nucleotide duplication in the same position (Monica Galiano; personal communication).

Very few reports with nucleotide duplication in the HRSV G protein gene have been reported until now. Only viruses with a three nucleotide insertion [12, 23] and two inserted triplets [10] were seen. Recently a major change introduced in the G protein of the HRSV, by a sixty nucleotide duplication was identified in three strains isolated in Buenos Aires, Argentina [27].

The duplication observed in the Uruguayan strains may reinforce the idea previously reported by Trento et al. [27], about the proneness of the HRSV polymerase to copy repeatedly part of sequence of the G protein gene, as another mechanism to generate diversity in respiratory syncytial viruses.

Note: Nucleotide sequence data reported are available in the GenBank databases under the accession numbers: AY488794-AY488806.

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