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COMPARISON BETWEEN ECTODOMAIN AND G2 REGION OF G GLYCOPROTEIN FOR GENOTYPING OF HRSV

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SHORT COMMUNICATION

ABSTRACT

The Human Respiratory Syncytial Virus (HRSV), isolated in 1955, is the main cause of hospitalization of babies and infants with respiratory illness. Several studies have been conducted worldwide aiming the development of a safe and effective vaccine against HRSV. The G2 region of glycoprotein G is used as genotyping default. In the present study, we performed a phylogenetic analysis of G protein and a comparative study between G2 region and ectodomain of attachment glycoprotein. Fifty-three nasal swab samples from children less than 5 years old and presenting symptoms of acute respiratory illness, assisted at the University Hospital (UH) of University of Sao Paulo (USP) in 2004, were submitted to sequencing by PCR and compared with GenBank sequences. We concluded that the G2 region is adequate for HRSV genotyping.

Key words: Human Respiratory Syncytial Virus; G Glycoprotein; G2 Region; Phylogenetic Analysis

The *Human respiratory syncytial virus* (HRSV) is an enveloped nonsegmented negative-strand RNA virus (family *Paramyxoviridae*, genus *Pneumovirus*). HRSV is the main cause of respiratory infections leading to infant hospitalization (5,14). Acute respiratory infections (ARIs) are the commonest cause of morbidity and mortality in children worldwide, and are responsible for about 30% of deaths in developing countries (12). Outbreaks of respiratory infections caused by HRSV occur yearly and previous infection do not protect against new infections although reinfections with HRSV are usually less severe (9,10). The two major groups of HRSV are classified into group A strains with GA1-GA7 (21,22) and SAA1 (31) genotypes, and group B strains with GB1-GB4 (21) and SAB1-SAB3 (31) genotypes.

Most studies concerning genetic variability and evolution of attachment G protein are based on HRSV A strains (8,24,37), and there is little information available about the genetic diversity and molecular evolution of HRSV B strains (15,27,29). The predominance of HRSV A over HRSV B viruses has been attributed to the higher variability among the HRSV A strains (21,31).

According to Purcell and Fergie (23), the comparison of clinical effects and the different groups and genotypes are hampered by other factors that affect the severity of infection by HRSV, such as prematurity, age of the children, and presence of other pre-existing conditions.

The present study aimed to sequence the glycoprotein G gene and compare the complete sequencing of ectodomain of gene G with the G2 region, in order to identify the existence of differences in HRSV genotyping. Such information provides correct identification of HRSV genotypes, thus contributing to the development of a secure vaccine. Despite isolated more than 50 years ago, the virus is still considered an important agent of respiratory infections and hospital internments. In this scenario, a new vaccine could create new and more efficient options for prevention and treatment of infections caused by HRSV.

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Target

Sequences of 53 Brazilian samples and 89 sequences acquired from GenBank (access number in footnote) were used.

Primers

Oligonucleotide primers previously described for amplifying the ectodomain of G glycoprotein of HRSV were used (21,24,36).

PCR

For amplification assays for glycoprotein G, 10 µl of cDNA were distributed in 0.2 ml tubes containing 10x PCR buffer, 2.5 mM of $MgCl_2$, 1.25 mM of each dNTP, 50 pM of each set primers, 1.25U Taq DNA polymerase (Applied Biosystems) and Ultra pure water for 100 µl. Thermo cycling was programmed for 96ºC for 2 min, 40 cycles of 94ºC for 1 min, 57ºC for 1 min, and 72ºC for 1 min for cDNA/DNA amplification; and 7 min at 72ºC for final amplicon extension.

Sequence Reaction

Sequencing assays were performed separately with 2 µl of ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit (Big Dye version 3.1-Applied Biosystems), and 10-30 ng of PCR product (glycoprotein G), distributed in 0.2 ml tubes containing 5x Save Money buffer, 3.2 pM of each set primers and Ultra pure water for 10 µl. Thermo cycling was programmed for 96ºC for 1 min, 25 cycles of 96ºC for 15 sec, 50ºC for 15 sec, and 60ºC for 4 min for DNA extension.

Purification of Sequence Reaction

The sequenced products were purified by precipitation with acetate and ethanol, to remove excess of salts, distributed in the plate of 96 wells containing 10 µl of formamide Hi-Di (Applied Biosystems), denatured at 90ºC for 3 min, and cooled on ice. Sequence analysis was performed on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) using a 50cm capillary with POP-6™ polymer.

Sequence Analysis

The sequence analysis was carried out using PCR products with previously described primers (21,24,36). The phylogenetic trees were constructed using the PAUP*4.0 program, version Beta, Sinauer Associates, Incorporation, for Power Macintosh and Unix (28) using the Neighborn joining algorithm and heuristic search. Bootstrap values were calculated with 100 replicates (6).

Phylogenetic trees

The genetic analysis with PAUP* are shown in Fig. 1.

Genotyping of HRSV

Out of 53 sequenced samples, 18 (34%) were GA2, 27 (51%) were GA5, 3 (5.5%) were SAB1, 4 (7.5%) were SAB3 and 1 (2%) was GB3 with insertion of 60 nucleotides.

The alignments of glycoprotein G ectodomain and G2 region indicated that the G2 region is sufficient enough for genotyping of HRSV. The analysed samples did not show many differences from the genotypes described by Peret *et al*., (21,22) and Venter *et al.* (31). The values of bootstrap were superior to 70% (calculated with 100 rejoinders) for the verification of the sustentation branches in topologies of the gotten trees (6). As already extensively observed in the literature (1,11,13,19,21,22, 25,34,35), the groups A and B co-circulate in outbreaks with the predominance of one over the other. The phylogenetic analysis showed that groups A and B co-circulated in São Paulo city with predominance of group A (4,16,32) in the studied period. The found genotypes GA2 and GA5 (group A), SAB1 and SAB3 (group B), and a additional single sample presenting repetitive insertion of 60nt to GB3 genotypes (related as BA-like) have also been found in recent studies (2,3,7,38).

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RESUMO

Comparação entre o ectodomínio e a região G2 da glicoproteína G para genotipagem de HRSV

O vírus respiratório sincicial humano (HRSV), isolado em 1955, é a principal causa da hospitalização de bebês e crianças pequenas com sintomas de doença respiratória. No mundo inteiro, vários estudos para o desenvolvimento de uma vacina segura e eficiente contra o HRSV têm tido alta prioridade. A região G2 da glicoproteína G é usada como padrão para genotipagem do HRSV. Neste estudo, foi realizada a análise filogenética da glicoproteína G e o estudo comparativo entre a região G2 e o ectodomínio dessa glicoproteína. Cinquenta e três amostras de *swab* nasal de crianças com menos de cinco anos de idade, apresentando doença respiratória aguda, atendidas no Hospital Universitário (HU) da Universidade de São Paulo durante o ano de 2004, foram submetidas a sequenciamento por PCR e comparadas com seqüências do *GenBank*. A região G2 mostrou ser adequada para a genotipagem do HRSV.

Palavras chave: Vírus Respiratório Sincicial Humano; Glicoproteína G; Região G2; Análise Filogenética

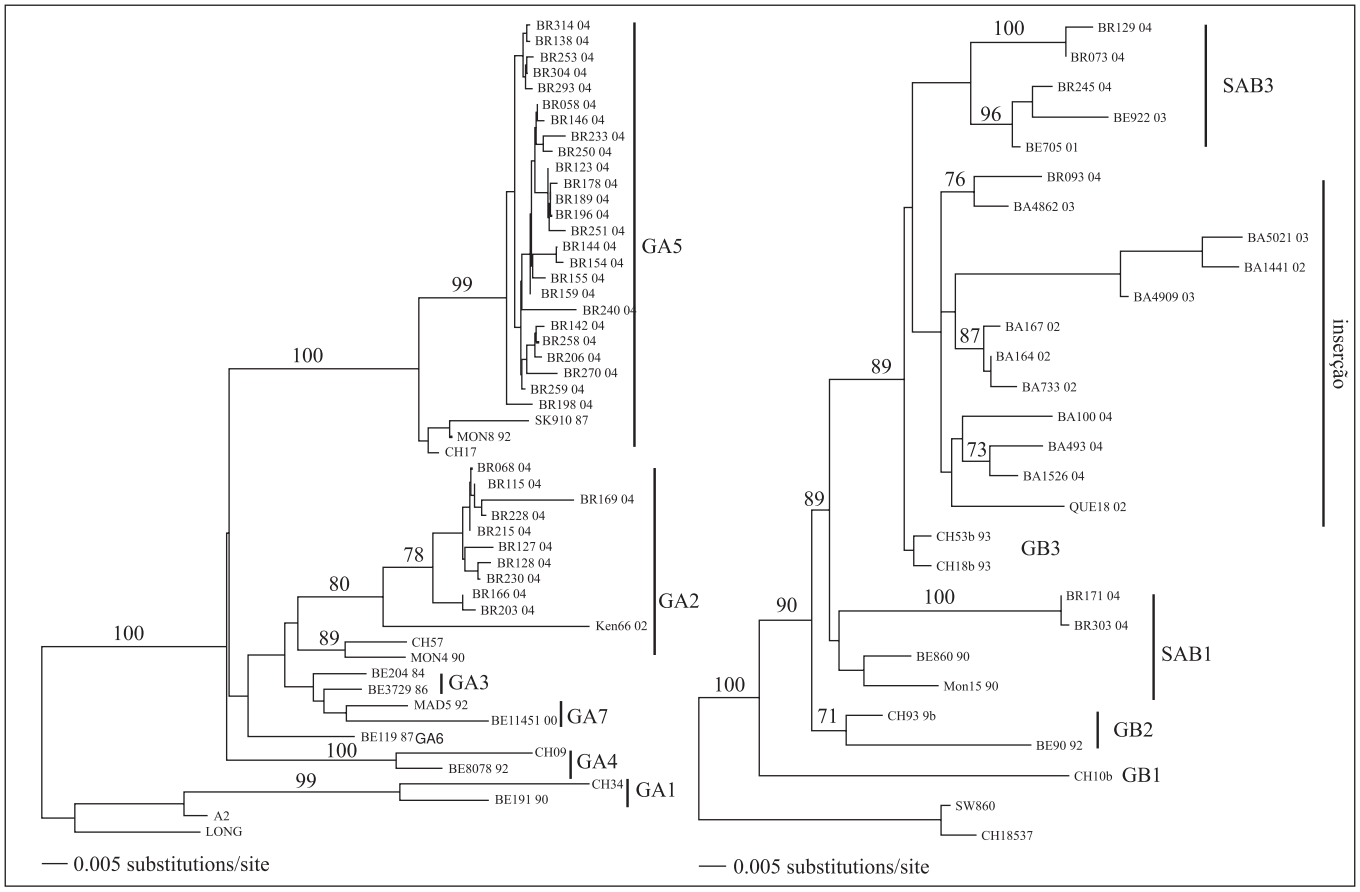

Figure 1. Ecdomain of group A and group B. 7Analized by distance method (Tamura Ney Model) with gamma distribuition (2.6559). *Heuristic search* find trees.

Footnote: Access number of GenBank X03149, M55633, M17212, M17213, AF065250-AF065255, AF065257, AF065258, AF193304, AY343620, AY343622-AY343626, AY343631, AY343633, AY343640, AY343644-AY343647, AY343650, AY343652- AF343660, AY343554, AY343556-AY343558, AY343561, AY343568, AY343572, AY343573, AY343577, AY343581-AY343584, AY343587, AY343590, AY343592, AY343594, AY343596, AY343597, AY524573, AY524575, AY524581, AY524584, AY524585, AY524593, AY524595, AY524606, AY524607, AY524620, AY524632, AY524635, AY524644, AY524648, AY524652, AY524660, AY524663, Z33412, Z33415, Z33417, Z33419, Z33420, Z33426, Z33430, AY660668, AY660669, AY660671, AY660674, AY660675, AY660677-AY660679, AY660681, AY660683, AY660684, AY751204, AY751206, AY751207, AY751211-AY751213, AY751215, AY751217-AY751219, AY751220, AY751222-AY751224, AY751226, AY751228-AY751233, AY751235, AY751236-AY751239, AY751241-AY751245, AY751247, AY751248, AY751264, AY751087, AY751089, AY751092- AY751094, AY751103, AY751105, AY751107, AY751111, AY7511117-AY751120, AY751122- AY751125, AY751131, AY751136, AY751144, AY751145, AY751148, AY751149, AY751151, AY751153, AY751157- AY751159, AY751162, AY751163, AY751169, AY751172- AY751174, AY751176, AY751179-AY751182, AY751184, AY751186, AY751187, AY751189, AY751191, AY751194-AY751198, AY333361, AY773291, DQ227367, DQ227369, DQ227374, DQ227381, DQ227391, DQ227395

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