

## LETTER TO THE EDITOR

## Molecular epidemiology of human respiratory syncytial virus in Iran

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Human respiratory syncytial virus (HRSV) is the main cause of acute respiratory infection in infants and young children in terms of effect and prevalence (1). It is responsible for 27–96% of hospitalized patients infected by viruses in developing countries (2). In the late fall and winter, HRSV causes bronchiolitis and pneumonia in infants during the first months of life (1). The HRSV is recognized as an important pathogen in the immunocompromised patients, individuals with cardiopulmonary disease, and in the elderly (3).

HRSV (the family *Paramyxoviridae*, the genus *Pneumovirus*) contains the non-segmented negative-strand RNA that encodes 11 viral proteins, e.g. 3 transmembrane glycoproteins (G, F, SH), 3 matrix proteins (M, M2-1, M2-2), 3 proteins associated with nucleocapsid (N, P, L), and 2 nonstructural protein (NS1, NS2) (4). The F and G glycoproteins are important antigens that induce a protective immune response (5). According to the reactions of monoclonal antibodies against the G glycoprotein, there have been recognized two major antigenic subgroups A and B of HRSV (4). Sequencing studies have shown that this glycoprotein is the most divergent protein within the two HRSV subgroups (6). The G protein contains two hypervariable regions in ectodomain that are separated by a conserved 13-amino-acid motif (7). The second hypervariable region (A subgroup, nt 649-918; B subgroup, nt 652-921) in the G protein has been used for

the study of molecular epidemiology of HRSV (8). This is the first study of molecular epidemiology of HRSV in Iran that investigates genotypes for second hypervariable region of the G protein by RT-PCR. Molecular epidemiology of HRSV has provided important information about the clinical and epidemiological features of HRSV genotypes and could be useful in terms of the proper diagnosis and design of an appropriate vaccine.

We tested 72 throat swabs collected from children aged 5 years and less suffering from acute respiratory symptoms during the season 2007-2008. Throat swabs were stored at -80°C until used. During this season the first HRSV infection was detected on November 5, 2007 and the last one on March 28, 2008. RNA was extracted directly from the swabs with high pure nucleic extraction kit<sup>TM</sup> (Roche Diagnostics). cDNA was synthesized by using of random primers and Moloney murine leukemia virus (both Fermentas) at 37°C for 45 mins. External PCR was carried out with a subgroup A-specific forward primer and subgroup B-specific forward primer (8). For the hemi-nested PCR, primer nRSAG and primer nRSBG were used as the forward primers for subgroup A and B, respectively (9). In the external and hemi-nested PCR, primer F<sub>1</sub> was used as a reverse primer for both subgroups A and B (8). For the amplification HRSV G protein gene, cDNA product was added to the master mixture containing optimized buffer, deoxynucleotide triphosphates, MgCl<sub>2</sub>, Taq DNA polymerase (Fermentas). Amplification conditions consisted of 2 mins at 95°C, 30 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 2 mins, and a final extension at 72°C for 7 mins. The RT-PCR

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**Abbreviation:** HRSV = human respiratory syncytial virus

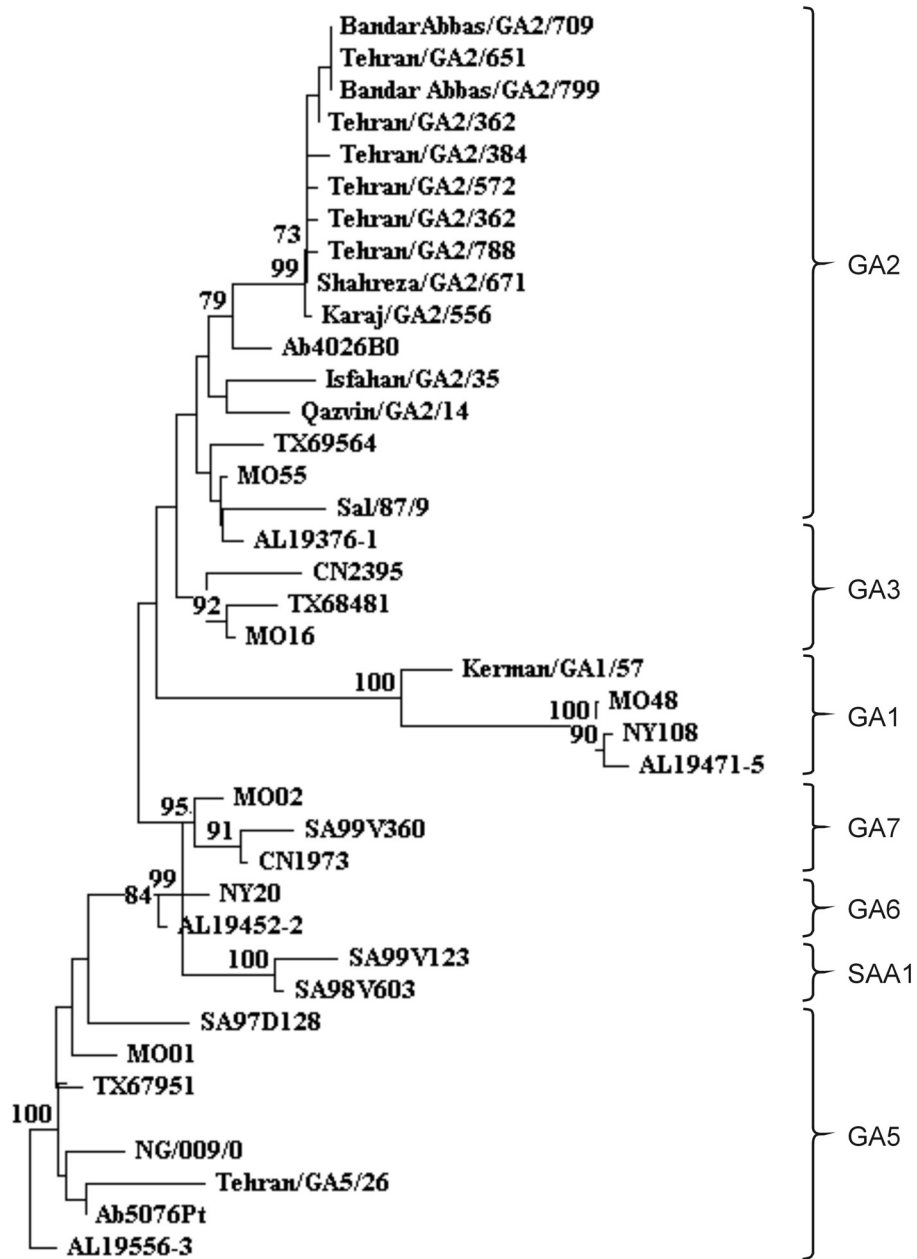


Fig.1.

Phylogenetic tree of Iranian HRSV strains based on the second hypervariable region of G protein gene  
The numbers at the nodes indicate bootstrap confidence obtained from 1,000 replicates. The genotypes are indicated on the right.

product was separated by electrophoresis on a 1.5% agarose gel and visualized under UV light. For the hemi-nested PCR, 5 µl of external PCR product was used with the same conditions. The hemi-nested amplicons were 400 bp for the both subgroups A and B viruses. Purified PCR products of the G protein gene were sequenced in the forward and reverse directions. The nucleotide sequences of the second hypervariable region of the G protein gene were aligned with HRSV sequences from the Genbank database by using the CLUSTAL X (version 1.83). Phylogenetic analysis was performed using neighbor-joining method in MEGA software (version 3). All HRSV sequence data were submitted to the GenBank database under the Acc. Nos. GQ259155 and GU339396-GU339408.

Of the 72 throat swabs tested, 14 (19.44%) were positive for HRSV. This finding was similar to that found in developed and developing countries: 21% in Austria (10), 16.2% in Germany (11), 18.4% in Malaysia (12) and 25.46% in Jordan (13).

All HRSV-positive samples clustered in three genotypes of subgroup A: 12 strains (85.71%) in genotype GA2, 1 strain (7.1%) in genotype GA1, and 1 strain (7.1%) in genotype GA5 (Fig.1). This study revealed that multiple genotypes of subgroup A co-circulated during the season 2007–2008 in Iran. Also, our results showed that genotype GA2 was the predominant genotype of isolates obtained from several cities (Tehran, Isfahan, Karaj, Qazvin, Bandar Abbas, and Shahreza). Thus, we speculated that this genotype might be the predominant one in this season. Remarkably, we detected no strain of subgroup B, what may be associated with several reasons. It has been assumed that viruses of subgroup B may cause less severe disease and as a result, they may be recognized to a lesser extent. Another possibility was that infants infected with the viruses of subgroup B produced a higher level of antibodies to the glycoprotein G in comparison with infants infected with the viruses of subgroup A. Moreover, the infection with B strains may produce a long-lasting group-specific immunity (14).

All respiratory samples were collected from children under 5 years having acute respiratory symptoms. Remarkably, 9 HRSV-positive samples (64.2%) were detected in children aged 1 year or less. Hence, our study and studies of others revealed that the HRSV is a major viral agent causing respiratory infections in infants and young children (1).

Outbreaks of HRSV occur each winter in temperate climates and during rainy season in tropical countries (15). Our study showed a temperature-dependent pattern of HRSV prevalence in Iran, since all HRSV-positive cases

were detected from November 2007 to March 2008. Also, the majority of HRSV-positive samples (35.71%) were detected in January 2008.

In conclusion, we propose to perform an in-depth study of molecular epidemiology of HRSV over longer period of time. The outcome could be valuable for a better understanding of the subgroup prevalence and genotype distribution patterns of HRSV infections.

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