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ORIGINAL ARTICLE

Genetic diversity in the G protein gene of group A human respiratory syncytial viruses circulating in Riyadh, Saudi Arabia

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Abstract Human respiratory syncytial virus (HRSV) is a frequent cause of hospitalization and mortality in children worldwide. The molecular epidemiology and circulation pattern of HRSV in Saudi Arabia is mostly uncharted. In the current study, the genetic variability and phylogenetic relationships of HRSV type A strains circulating in Riyadh Province were explored. Nasopharyngeal aspirates were collected from hospitalized children with acute respiratory symptoms during the winter-spring seasons of 2007/08 and 2008/09. Among 175 samples analyzed, 39 (22.3 %) were positive for HRSV by one-step RT-PCR (59 % type A and 41 % type B). Propagation of positive samples in HEp-2 cells permitted the recovery of the first Saudi HRSV isolates. Genetic variability among Saudi HRSV-A strains was evaluated by sequence analysis of the complete attachment (G) protein gene. The nucleotide sequence was compared to representatives of the previously identified HRSV-A genotypes. Sequence and phylogenetic analysis showed that the strains examined in this study were very closely related at both the nucleotide and amino acid level, and all of them are clustered in the GA2 genotype (and mostly belonged to the NA-1 subtype). A total of 23 mutation sites, 14 of which resulted in an amino acid change, were recorded only in Saudi strains. This is the first report on genetic diversity of HRSV-A strains in Saudi Arabia. Further analysis of strains on a geographical and temporal basis is needed to fully understand HRSV-A circulation patterns in Saudi Arabia.

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Introduction

Human respiratory syncytial virus (HRSV) is the single most important cause of serious respiratory tract infection in infants and young children worldwide. During the first year of life, more than 50 % of infants get infected with HRSV, and virtually all children experience at least one infection by the age of 2 years [1]. The primary infection in children is mostly significant, associated with lower respiratory tract involvement, and may require hospitalization [2]. Since HRSV induces only partial immune protection, reinfection can occur throughout life, particularly in patients with underlying illness, such as immunosuppression and cardiopulmonary diseases, and in the elderly [3].

HRSV is classified as the prototype member of genus *Pneumovirus* within the family *Paramyxoviridae* [4]. HRSV isolates were divided into two major groups, A and B, according to their antigenic (differential reactivity with monoclonal antibodies) and genetic (sequence diversity of several viral genes) characteristics [5, 6]. Epidemiological studies have shown co-circulation of both groups in epidemics, with a predominance of a single (mostly A) group, and a change in this predominance every one to three

epidemics [7, 8]. Genetic diversity among group A and B viruses has permitted further classification of isolates into distinct genotypes. To date, eight genotypes have been established for HRSV group A (designated GA1-7 and SAA1) [9, 10]. Recently, two GA2 variants (NA1 and NA2) and two novel genotypes (CB-A and ON-1) have been described [11–13]. Although different genotypes may co-circulate in the same epidemic and geographical region, phylogenetically similar viruses can be isolated from distant countries at the same time. This behavior suggests that viruses belonging to different genotypes tend to cluster on a temporal rather than a geographical basis [6, 14].

The G protein is a major surface glycoprotein of HRSV that mediates virus attachment to cell receptors [15]. The mature protein is composed of a short cytoplasmic tail of 38 amino acids (AAs), a membrane anchor domain of 29 AAs, and a long C-terminal ectodomain of 232-233 AAs. The ectodomain is typically formed by two hypervariable regions flanking a highly conserved domain (AA 164-176), which is thought to be the receptor-binding site [16]. Extensive sequence analysis of HRSV genes demonstrated that G is the most variable protein between and within the two groups (diversity up to 54 % and 20 %, respectively). Genetic variability of the G protein is mostly attributed to the second hypervariable region located near the C-terminal end of the protein. This region has been used in many studies for phylogenetic analysis of HRSV strains of both groups [8, 9, 17].

In Saudi Arabia, HRSV has been identified as the leading cause of lower respiratory tract infection in hospitalized children (19.3-45.4 %) [18–23]. Differentiation of circulating strains into groups and genotypes has not been achieved except in one study that described co-circulation of both groups with a slight predominance of group A [19]. There is a significant shortage of data on molecular epidemiology and genetic diversity of HRSV strains circulating in Saudi Arabia and the entire Middle East region. Therefore, the current study was designed to study the prevalence of HRSV in Riyadh Province of Saudi Arabia throughout two epidemic seasons and to analyze the circulation pattern, molecular characteristics, and phylogenetic relationship of Saudi HRSV-A strains.

Materials and methods

Virus stock and reference antibodies

Standard HRSV group A (A2 strain) was grown in HEp-2 cells, which were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), 2 mM L-glutamine and 1× penicillin-streptomycin (Gibco, Grand island, NY). The virus stock titer was determined by infectivity assay and was expressed as 50 % tissue culture infective dose ($TCID_{50}$). This stock was used for optimization and as a positive control in RT-PCR, ELISA, immunofluorescent and neutralization assays. Reference polyclonal antibodies against HRSV were obtained from Euroimmune (Luebech, Germany) to be used in characterization of Saudi virus isolates.

Source of clinical samples and ethics statement

Nasopharyngeal aspirate (NPA) samples were collected from 175 hospitalized patients at King Khalid University Hospital (KKUH) in Riyadh, Saudi Arabia, from February till May 2008 and from October 2008 till March 2009. The study subjects included infants and young children less than 3 years old and of both sexes. All patients were suffering from acute respiratory symptoms including rhinorrhea, cough, dyspnea and fever. Clinical samples were obtained, under protocols approved by the Ethical Committee of King Saud University, after getting an informed written consent from parents/guardians of the patients. After collection, NPAs were immediately added to 2 ml of viral transport medium (DMEM supplemented with 2× penicillin-streptomycin) and transported on ice to the Virology Research Laboratory, College of Science, King Saud University, for further examination.

Detection and typing of HRSV in clinical samples by RT-PCR

Extraction of viral RNA from clinical samples was performed using a QIAamp Viral RNA Extraction Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. HRSV was detected in the extracted RNA using a OneStep RT-PCR Kit (QIAGEN) and the primers HRSV-U-F (5′-GGAACAAGTTGTTGAGGTTTATGAA TATGC-3′) and HRSV-U-R (5′-CTTCTGCTGTCAAGT CTAGTACACTGTAGT-3′) [24]. The RT-PCR reaction was carried out in a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, CA) using the following cycling conditions: reverse transcription at 50 °C for 30 min; PCR activation at 95 °C for 15 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 2 min; and a final extension step of 72 °C for 10 min. Positive samples were further identified as group A or B by amplification of type-specific fragments, using the same kit and cycling profile, with the primers HRSV-A-F (5′-GATGTTACGGTGGGGAGTCT-3′) and HRSV-A-R (5′-GTACACTGTAGTTAATCACA-3′) for group A viruses and HRSV-B-F (5′-AATGCTAA GATGGGGAGTT-3′) and HRSV-B-R (5′-GAAATTG AGTTAATGACAG-3′) for group B viruses [25]. For all reactions, specific PCR products were visualized in a 1 %

agarose gel stained with ethidium bromide and compared to a 100-bp DNA ladder (QIAGEN).

Virus isolation and characterization

All clinical samples that showed positive reactivity in RT-PCR were propagated in HEp-2 cells for virus isolation. Aliquots of NPAs were diluted in serum-free medium supplemented with 250 U penicillin and 0.25 mg streptomycin per mL (Gibco) and were incubated on HEp-2 cell monolayers for 90 min at 37 °C for virus adsorption. After two washing steps with sterile PBS, cells were maintained in DMEM supplemented with 1 % FBS at 37 °C and 5 % CO_2 for 7 days, with daily observation for cytopathic effect (CPE). Four successive passages were carried out before the samples were considered negative by virus isolation. Saudi HRSV isolates were further characterized by immunofluorescence, ELISA and virus neutralization assay using standard HRSV polyclonal antibodies as described elsewhere [26, 27].

Nucleotide sequencing

The full-length sequence of the G protein gene of HRSV type A in positive clinical samples was obtained for detailed sequence and phylogenetic analysis of Saudi strains. The sequencing protocol involved amplification of two overlapping fragments that cover the entire G gene sequence, using FideliTaq™ RT-PCR Master Mix Plus (GE Healthcare, Buckinghamshire, UK) and the following primers: HRSV-G-F1 (5′-CTCGAGTCAACACATAGC ATTC-3′) and HRSV-G-R1 (5′-GTTGGATTGTTGCT GCATATG-3′) for the first fragment, and HRSV-G-F2 (5′-CAAGATGCAACAAGC CAGATC-3′) and HRSV-G-R2 (5′-ACTGCACTGCATGTTGATTG-3′) for the second fragment. The PCR products were purified using Illustra™ GFX PCR DNA and a Gel Band Purification Kit (GE Healthcare). The purified products were sequenced on both strands with the relevant forward and reverse primers at GeneArt (Regensburg, Germany) using an autosequencer (ABI 3730xl DNA analyzer; Applied Biosystems, Foster City, CA). DNA sequences were edited and assembled using the BioEdit program, version 7.0 (Ibis Biosciences, Carlsbad, CA). Full-length G gene sequences analyzed in this study have been deposited in GenBank with accession numbers JF714705, JF714706 and JX131637 to JX131645.

Sequence data analysis

Multiple sequence alignments were performed, between Saudi strains and a collective pool of international strains that were previously assigned to distinct genotypes (Fig. 1), using the Clustal W algorithm of the MegAlign program, Lasergene software, version 3.18 (DNAStar, Madison, WI). Divergence analysis, identification of mutation sites, and prediction of amino acid changes were carried out using EditSeq and MegAlign programs, Lasergene software (DNAStar Inc., Madison, WI). Potential N- and O-linked glycosylation sites were identified using Net-N-glyc 1.0 (http://www.cbs.dtu.dk/services/NetNGlyc) and Net-O-glyc 3.1 (http://www.cbs.dtu.dk/services/NetOGlyc), respectively.

Phylogenetic analysis

Phylogenetic analysis was performed using a 264-nucleotide fragment of the second hypervariable region of the G gene. The tree was constructed by the neighbor-joining method using MEGA software, version 5.1 [28]. The accuracy of the tree topology was evaluated by bootstrapping of 1,000 replicates. Only bootstrap values exceeding 50 % are shown at the branch nodes. The phylogram includes 41 international strains that represent all HRSV type A genotypes. For the purpose of studying the relationship of Saudi strains with other strains circulating in their geographical proximity, four strains from Qatar that had not yet been assigned to a specific genotype were also included in the phylogram.

Results

Prevalence of HRSV in Riyadh

A total of 175 NPA samples were obtained from hospitalized children with acute respiratory tract infection in Riyadh during two winter-spring seasons: 2007/08 and 2008/09. Of these samples, 39 (22.3 %) were positive for HRSV, of which 23 (59 %) belonged to group A and 16 (41 %) belonged to group B. The predominance of HRSV type A viruses was observed in both seasons, with a prevalence of 58.8 % and 59.1 %, respectively. Although HRSV infection was recognized in all age groups included in the study (1 month to 3 years), the majority of infected patients (56.4 %) were infants younger than 6 months old. This observation was consistent for infection with both group A and group B, with a median age of 6 and 5 months, respectively. The infection rate was also more prominent in male patients than females (16.3 % and 8.6 % for males and 8.5 % and 7 % for females in groups A and B, respectively).

Genetic analysis of Saudi HRSV type A strains

Sequence diversity among Saudi strains

Genetic variability of the different HRSV type A Saudi strains was evaluated by sequence comparison of complete G gene

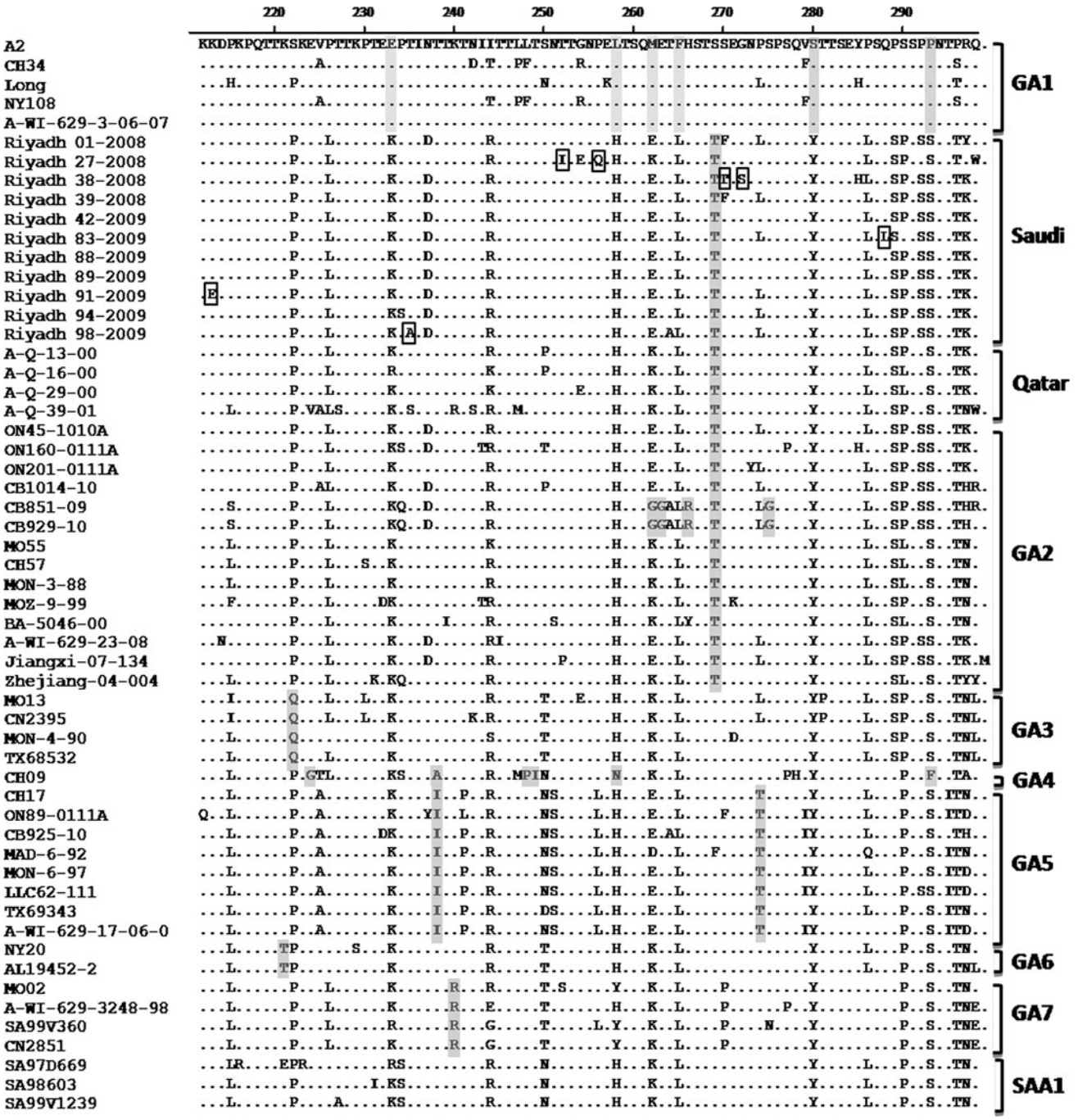

Fig. 1 Alignment of deduced amino acid sequences of the C-terminal fragment of the second hypervariable region (last 87 AAs) of the HRSV group A G protein. The alignment was performed using the Clustal W method in the MegAlign program (DNASTAR). The prototype strain A2 is shown as the consensus sequence. Identical residues are indicated by dots, while sequence variation is identified in single-letter code. Residues recognized only in specific Saudi strains are shown in boxes, while genotype-specific residues are shaded. Genotypes as well as Saudi and Qatari strains are indicated in brackets on the right side

sequences. In general, a high degree of sequence identity was revealed, with overall homology ranging from 96.2-100 % and 93.2-100 % at the nucleotide and amino acid level, respectively. The majority of the sequence variation was observed in the C-terminal fragment of the second hypervariable region of the G gene, where heterogeneity reached 7.2 % for nucleotides and 13.9 % for amino acids. Among the 23 Saudi HRSV type A strains, only 10 (five from each season) had unique G gene sequences. Therefore, these strains were utilized in further genetic and phylogenetic analyses.

Relationship to different HRSV type A genotypes

To compare the Saudi strains to the different genotypes circulating across the globe, 45G gene sequences representing strains of all genotypes were included in this study. Significant sequence similarity was observed with strains of NA1 and GA2 genotypes and those recovered from Qatar (95.7-98.6 % for nucleotides and 93-98 % for amino acids). The Saudi strains were more distantly related to those of the CB-A genotype, with an average of 94.7-97.6 % and 91.8-96.5 % sequence identity at the nucleotide and amino acid level, respectively. The GA1, GA3-7 and SAA1 genotype sequences showed a considerable degree of divergence from those of the Saudi strains at both levels (91-93.4 % sequence identity for nucleotides and 86-90.6 % for amino acids).

Characteristic changes in amino acids

Analysis of the deduced amino acid sequence of the Saudi strains revealed a predicted protein length of 297 or 298 amino acids. The latter was identified in only two strains, Riyadh 1/2008 and Riyadh 27/2008. Differences in G protein length were frequently observed among strains of most genotypes including GA1, GA2, NA1, GA6 and GA7, due to mutations in the first stop codon (Fig. 1). Multiple sequence alignment of G gene sequence identified 23 mutations specific to one or another of the Saudi strains, among which 14 resulted in amino acid changes. Riyadh 27/2008 holds a significant number of these specific determinants, including 8 nucleotides (0.9 %) and 6 amino acids (1.52 %) (Table 1).

For detailed analysis of amino acid variation among different genotypes, the C-terminal fragment of the second hypervariable region of the G protein (the last 86-87 residues) was compared (Fig. 1). The unavailability of representative strains to genotypes GA3, GA6 and SAA1 hindered the use of the complete gene for this purpose. Due to the extreme variability of the aligned region, only 21 (24.1 %) amino acids were identified as well conserved in all genotypes. Certain amino acids were recognized to be specific to individual genotypes, including 233E, 258L, 262M, 265F, 280S and 293P for GA1; 269T for GA2; 222Q for GA3; 224G, 238A, 248P, 249I, 258N and 293F for GA4; 238I and 274T for GA5; 221T for GA6; 240R for GA7 and 262G, 263G, 266R, and 275G for CB-A.

Heterogeneity in the glycosylation profile

Glycosylation patterns of the G proteins of Saudi strains were predicted and compared to those of other strains belonging to different genotypes. The number of putative N-linked glycosylation sites generally ranges from 3 to 5 in all international strains analyzed in this study. However, the Saudi strains have a broader range that extends from two sites in Riyadh 83/2009 to six sites in Riyadh 27/2008. Two N-glycosylation sites (residues 103 and 251) are well conserved among all Saudi strains. These two residues appear to be stable in all HRSV-A strains with few exceptions (mostly among the GA5 genotype). Two other sites were identified in the majority of Saudi strains, including residues 135 (except in Riyadh 83/2009) and 294 (except in Riyadh 1/2008 and Riyadh 83/2009). Although these sites are generally common in HRSV-A strains, their existence is variable among strains of the same genotype(s). Riyadh 27/2008 strain is exceptional, harboring two more N-glycosylation sites: residue 237, which is frequently identified in members of all genotypes (particularly GA-1), and residue 100, which is specific to this strain and was not recorded before. No evidence for a genotype-specific N-glycosylation pattern was found, except for the glycosylation site at residue 250, which is specific to the GA-5 genotype only.

In contrast, the G protein contains a large number of serine and threonine residues that are potential acceptors for O-linked carbohydrate side chains. The number and position of O-glycosylation sites varies widely among HRSV-A strains (range: 26-59) as determined by Net-O-glyc 3.1 (G-score: 0.5-0.8). Saudi strains are generally heavily O-linked glycosylated (range: 43-59), and the majority of sites (no=32) are conserved.

Phylogenetic analysis

In order to place Saudi HRSV-A strains in the context of previous genotypic classification, phylogenetic analyses was performed utilizing the last 264 nucleotides of the gene (Fig. 2). The phylogenetic tree showed that all Saudi strains were clustered in genotype GA2 with strains isolated from Argentina, Canada, China, Korea, Mozambique, Qatar and the USA. The GA2 genotype appears to contain strains belonging to the NA-1 and CB-A groups, and both may be considered subtypes rather than genotypes. The majority of Saudi strains belonged to NA-1 subtype, while only Riyadh 27/2008 was located outside this cluster, along with the Qatar strains identified in 2000-2001.

Discussion

HRSV is a highly dynamic virus that has a widespread global dissemination and the ability to cause repeated outbreaks in communities. Epidemiological and evolutionary studies have indicated that HRSV outbreaks are community based and mostly correlate with temporal rather than spatial factors [29, 30]. Although numerous

Table 1 Nucleotide and amino acid substitutions specific to HRSV type A Saudi strains

HRSV type A Saudi strain	Nucleotide mutation	Corresponding amino acid change
Riyadh 1/2008	A567G	I189M
	C795T*	
	C837T*	
Riyadh 27/2008	A241G	N81D
	C328A	Q110K
	C376T	P126S
	A587G	K196R
	C684T*	
	C755T*	T252I*
	C767A*	P256Q*
	A828C*	
Riyadh 38/2008	C305T	
	A420T	Q140H
	T808A*	S270T*
	G814A*	G272S*
Riyadh 39/2008	A222G	
Riyadh 42/2008	A425C	Q142P
Riyadh 83/2009	A863T*	Q288L*
Riyadh 91/2009	C236T	
	A637G*	K213E*
Riyadh 98/2009	C477A	
	C657A*	
	A704G*	T235A*

* The nucleotide or amino acid change is located in the C-terminal fragment (last 264 nucleotides/87 amino acids) of the second hypervariable region of the G gene/protein

investigations have been conducted to elucidate the molecular epidemiology and circulation patterns of HRSV all around the world [9, 11], only a single study was done in the Middle East region [31], and none in Saudi Arabia.

In the current study, the prevalence of HRSV in Saudi Arabia from February 2008 to March 2009 was studied in the community of Riyadh. Among 175 NPAs examined, HRSV was detected in 22.3 % of the specimens. This finding correlates well with most of the studies conducted in Saudi Arabia [18, 19, 21, 23, 32], in Middle East countries [31–33] and internationally [11, 34]. Both group A and B HRSV were identified in positive samples during the entire study period, with a predominance of group A viruses (59 %). A higher incidence of group A HRSV was commonly observed in most communities and in most epidemic episodes [8, 10]. It has been proposed that this predominance may be caused by the long-lasting group-specific immunity induced by group B viruses and the ability of group A strains to provoke a more recognizable clinical picture [35].

Ten Saudi HRSV-A strains with unique G gene sequences were selected for sequence and phylogenetic analysis. These strains appear to be genetically similar, and all of them clustered with genotype GA2. Phylogenetic analysis also suggested that the NA-1 and CB-A clusters cannot be separated as distinct genotypes rather than variants belonging to the GA2 genotype. Most of the Saudi strains (except Riyadh 27/2008) were grouped within the subtype NA-1. Previous studies have demonstrated that genotype GA2 and its subtypes (particularly NA-1) are the most geographically distributed. They represent the dominant genotypes identified in most epidemics worldwide: Canada, 98.2 % [12]; China, 97 % [8]; Croatia, 82.9 % [36]; Korea, 96.4 % [11]; and Japan, 100 % [37]. Furthermore, GA2 is the sole genotype that can persist in communities for long periods without being replaced by other genotypes: 20 years in Sweden [38], 13 years in the USA [39], 6 years in Argentina [40]. Taking all of this evidence together, this may explain why all Saudi HRSV-A strains in this study belonged to the GA2 genotype. Since the trend of co-circulation of different genotypes in single epidemics is well established [9], we cannot exclude the existence of other genotypes in Saudi Arabia. The strains described in this analysis represent only the community of Riyadh during a limited period of time (two winter seasons). In addition, they were obtained from hospitalized children with severe disease, and no other age and disease groups were investigated.

Due to the lack of similar phylogenetic studies in the Middle East region, the exact relationships between strains circulating in Saudi Arabia and those in neighboring countries cannot be established. For this reason, four G gene sequences that represent HRSV-A strains isolated in Qatar at 2000 and 2001 were included in our analysis. Although all Qatar strains appear to cluster within the genotype GA2, they tend to form a separate branch from the NA-1 and CB-A subtypes. There is no concrete association between the Qatari and Saudi strains (except for Riyadh 27/2008); perhaps a result of time divergence. All of the isolates that showed close similarity to Saudi strains were from 2007-2011, while those related to Qatari strains were mostly from 1998-2004 (Fig. 2). Recently, analysis of 16 HRSV-A strains isolated in Iran during the year 2009 showed a marked predominance of the GA1 genotype (93.75 %) [31]. These findings support the suggestion that HRSV strains tend to aggregate on a temporal rather than geographical basis.

Analysis of the predicted amino acid sequence of the G protein in Saudi HRSV-A strains identified 14 Saudi-specific amino acids, among which five and nine were located in the first and second hypervariable region, respectively (Table 1). The correlation between the position of these substitutions and the epitope structure of the G protein

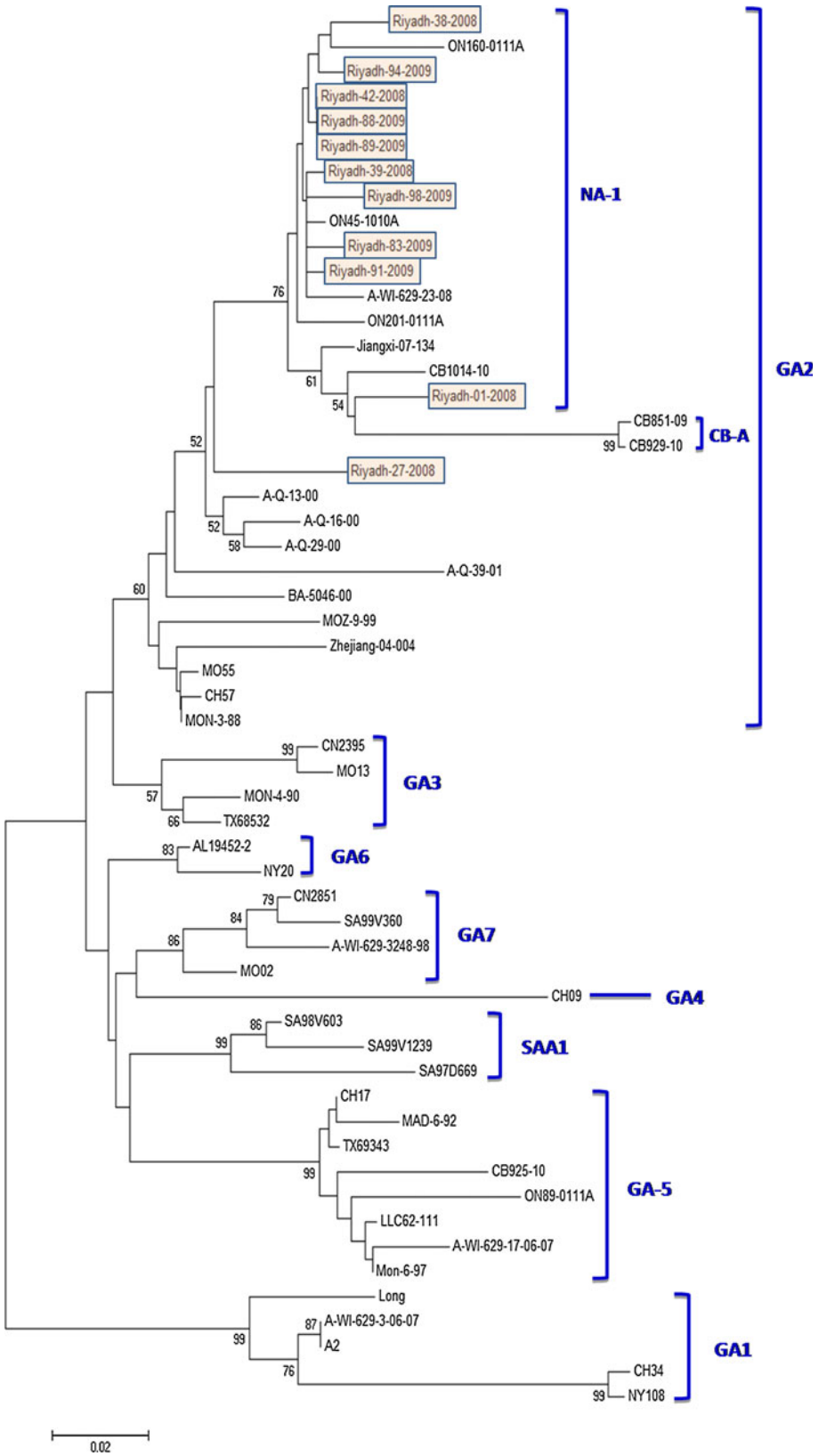

Fig. 2 Phylogenetic analysis of HRSV group A strains based on the C-terminal fragment of the second hypervariable region of G gene. Multiple sequence alignment was performed using Clustal W, and phylograms were generated by the MEGA 5.1 program using the neighbor-joining method. Numbers at branch nodes correspond to bootstrap values that indicate the stability of the branch over 1000 replicates. Only bootstrap value greater than 50% are displayed. Saudi strains are indicated by shaded boxes and designated according to city-sample number-year. Genotypes/subtypes are shown in brackets at the right side. The scale bar at the bottom indicates the number of nucleotide changes per site

suggested a potential effect on two antigenic sites: First, a linear B cell epitope located between residues 196 and 204, which may be affected by the substitution I189M of Riyadh 27/2008 strain [41], and second, epitope 68G, which occupies the region between residues 205 and 256 [42]. In the Saudi strains, the latter contained several substitutions

that could possibly alter its reactivity to monoclonal antibodies: T252I and P256Q in the Riyadh 27/2008 strain, K213E in the Riyadh 91/2009 strain, and T253A of Riyadh 98/2009 strain. The accumulated data suggest that variation in this region is to be expected in clinical isolates, but this variability rarely affects the viability of the virus [8]. However, further investigations are required to fully understand the possible contribution of these mutations to virus immunogenicity and reinfection.

The G protein is also characterized by an abundance of N- and O-linked glycan side chains. The exact number and position of glycosylation sites is highly variable among HRSV-A strains. These variations play an important role in virus antigenicity, as they affect the epitope expression and mediate evasion of the host immune response [43, 44]. The glycosylation profile of Saudi HRSV-A strains shows some exceptional features: (1) the presence of the lowest and highest number of N-glycosylation sites among all strains identified worldwide (two and six sites for Riyadh 83/2008 and 27/2009, respectively); (2) the existence of a novel N-glycosylation site that was not recorded before at residue 100 of the Riyadh 27/2008 strain; (3) the lowest number (n=43) of O-glycosylation sites worldwide, in Riyadh 27/2008, although this strain has the most N-glycosylation sites. This wide spectrum of variation in the glycosylation pattern of Saudi HRSV-A strains may have a deep impact on virus infectivity and immunogenicity *in vivo* despite their close phylogenetic relationship.

In conclusion, the genetic variability of HRSV-A strains circulating in Riyadh Province during two winter-spring seasons was investigated using sequence and phylogenetic analysis of the G protein gene. The data presented here demonstrate that Saudi strains belong to the GA2 genotype and have specific features related to amino acid substitutions and glycosylation patterns. More descriptive studies, including retrospective and community-based investigations are needed for a complete understanding of the epidemiology of HRSV-A in Saudi Arabia.

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Conflicts of interest The authors declare that they have no conflicts of interest.

References

1. Collins PL, Crowe JE (2007) Respiratory syncytial virus and metapneumovirus. In: Knipe DM, Howley PM, Griffin DE, Martin MA, Lamb RA, Roizman B, Straus SE (eds) Fields virology, 5th edn. Lippincott Williams and Wilkins, Philadelphia, pp 1601–1646
2. Glezen WP, Taber LH, Frank AL, Juliuss AK (1986) Risk of primary infection and reinfection with respiratory syncytial virus. Am J Dis Child 140:543–546
3. Falsey AR, Walsh EE (2000) Respiratory syncytial virus infection in adults. Clin Microbiol Rev 13:371–384
4. King AM, Adams MJ, Carstens EB, Lefkowitz EJ (2012) Virus taxonomy and nomenclature of viruses. Ninth report of the International Committee on taxonomy of viruses. Elsevier-Academic Press, Amsterdam
5. Anderson LJ, Hierholzer JC, Tsou C, Hendry RM, Fernie BF, Stone Y, McIntosh K (1985) Antigenic characterization of respiratory syncytial virus strains with monoclonal antibodies. J Infect Dis 151:626–633
6. Sullender W (2000) Respiratory syncytial virus genetic and antigenic diversity. Clin Microbiol Rev 13:1–15
7. Carballal G, Videla C, Sequeira MD, Mistchenko A, Requeijo PV, Arbiza J (2000) Respiratory syncytial virus: changes in prevalence of subgroups A and B among Argentinian children, 1990-1996. J Med Virol 61:275–279
8. Zhang Y, Xu W, Shen K, Xie Z, Sun L, Lu Q, Liu C, Liang G, Beeler JA, Anderson LJ (2007) Genetic variability of group A and B human respiratory syncytial viruses isolated from 3 provinces in China. Arch Virol 152:1425–1434
9. Peret TC, Hall CB, Hammond GW, Piedra PA, Storch GA, Sullender WM, Tsou C, Anderson LJ (2000) Circulation patterns of group A and B human respiratory syncytial virus genotypes in 5 communities in North America. J Infect Dis 181:1891–1896
10. Venter M, Madhi SA, Tiemessen CT, Schoub BD (2001) Genetic diversity and molecular epidemiology of respiratory syncytial virus over four consecutive seasons in South Africa: identification of new subgroup A and B genotypes. J Gen Virol 82:2117–2424
11. Baek YH, Choi EH, Song MS, Pascua PN, Kwon HI, Park SJ, Lee JH, Woo SI, Ahn BH, Han HS, Hahn YS, Shin KS, Jang HL, Kim SY, Choi YK (2012) Prevalence and genetic characterization of respiratory syncytial virus (RSV) in hospitalized children in Korea. Arch Virol 157:1039–1050
12. Eshaghi A, Duvvuri VR, Lai R, Nadarajah JT, Li A, Patel SN, Low DE, Gubbay JB (2012) Genetic variability of human respiratory syncytial virus A strains circulating in Ontario: a novel genotype with a 72 nucleotide G gene duplication. PLoS One 7:e32807
13. Shobugawa Y, Saito R, Sano Y, Zaraket H, Suzuki Y, Kumaki A, Dapat I, Oguma T, Yamaguchi M, Suzuki H (2009) Emerging genotypes of human respiratory syncytial virus subgroup A among patients in Japan. J Clin Microbiol 47:2475–2482
14. Choi EH, Lee HJ (2000) Genetic diversity and molecular epidemiology of the G protein of subgroups A and B of respiratory syncytial viruses isolated over 9 consecutive epidemics in Korea. J Infect Dis 181:1547–1556
15. Levine S, Klaiber-Franco R, Paradiso PR (1987) Demonstration that glycoprotein G is the attachment protein of respiratory syncytial virus. J Gen Virol 68:2521–2524
16. Johnson PR, Spriggs MK, Olmsted RA, Collins PL (1987) The G glycoprotein of human respiratory syncytial viruses of subgroups A and B: extensive sequence divergence between antigenically related proteins. Proc Natl Acad Sci USA 84:5625–5629
17. Kuroiwa Y, Nagai K, Okita L, Yui I, Kase T, Nakayama T, Tsutsumi H (2005) A phylogenetic study of human respiratory syncytial viruses group A and B strains isolated in two cities in Japan from 1980-2002. J Med Virol 76:241–247
18. al-Hajjar S, Akhter J, Al-Jumaah S, Qadri H (1998) Respiratory viruses in children attending a major referral centre in Saudi Arabia. Ann Trop Paediatr 18:87–92
19. Al-Majhdi FN, Al-Jarallah A, Elaeed M, Latif A, Gissmann L, Amer HM (2009) Prevalence of respiratory syncytial virus

infection in Riyadh during the winter season 2007-2008 and different risk factors impact. Inter J Virol 5:154–163
20. Al-Shehri MA, Sadeq A, Quli K (2005) Bronchiolitis in Abha, Southwest Saudi Arabia: viral etiology and predictors for hospital admission. West Afr J Med 24:299–304
21. Bakir TM, Halawani M, Ramia S (1998) Viral aetiology and epidemiology of acute respiratory infections in hospitalized Saudi children. J Trop Pediatr 44:100–103
22. Meqdam M, Subaih SH (2006) Rapid detection and clinical features of infants and young children with acute lower respiratory tract infection due to respiratory syncytial virus. FEMS Immunol Med Microbiol 47:129–133
23. Shier MK, Badr MA (2005) Respiratory syncytial virus infection in patients visiting King Khaled university hospital. Minoufia Med J 18:11–18
24. Cane P, Pringle C (1991) Respiratory syncytial virus heterogeneity during an epidemic: analysis by limited nucleotide sequencing (SH gene) and restriction mapping (N gene). J Gen Virol 72:349–357
25. Yamada A, Lam L-Y, Tam JS (2004) Typing and subtyping of influenza viruses and respiratory syncytial viruses by multiplex RT-PCR. Int Congr Ser 1263:381–385
26. Hughes JH, Daniel RM, Vincent V (1988) Detection of respiratory syncytial virus in clinical specimens by viral culture, direct and indirect immunofluorescence, and enzyme immunoassay. J Clin Microbiol 26:588–591
27. Rossiter PB, Jessett DM, Taylor WP (1985) Microneutralisation systems for use with different strains of peste des petits ruminants virus and rinderpest virus. Trop Anim Health Prod 17:75–81
28. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739
29. Cane P, Pringle C (1995) Evolution of subgroup A respiratory syncytial virus: evidence for progressive accumulation of amino acid changes in the attachment protein. J Virol 69:2918–2925
30. Peret TC, Hall CB, Schnabel KC, Golub JA, Anderson LJ (1998) Circulation patterns of genetically distinct group A and B strains of human respiratory syncytial virus in a community. J Gen Virol 79:2221–2229
31. Faghihloo E, Salimi V, Rezaeil F, Naseril M, Mamishi S, Mahmoodi M, Mokhtari-Azad T (2011) Genetic diversity in the G protein gene of human respiratory syncytial virus among Iranian children with acute respiratory symptoms. Iran J Pediatr 21:58–64
32. Meqdam MM, Nasrallah GK (2000) Enhanced detection of respiratory syncytial virus by shell vial in children hospitalised with respiratory illnesses in northern Jordan. J Med Virol 62: 518–523
33. Fattouh AM, Mansi YA, El-Anany MG, El-Kholy AA, El-Karaksy HM (2011) Acute lower respiratory tract infection due to respiratory syncytial virus in a group of Egyptian children under 5 years of age. Ital J Pediatr 37:14
34. Rajala MS, Sullender WM, Prasad AK, Dar L, Broor S (2003) Genetic variability among Group A and B respiratory syncytial virus isolates from a large referral hospital in New Delhi, India. J Clin Microbiol 41:2311–2316
35. Hall CB, Walsh EE, Schnabel KC, Long CE, McConnochie KM, Hildreth SW, Anderson LJ (1990) Occurrence of groups A and B of respiratory syncytial virus over 15 years: associated epidemiologic and clinical characteristics in hospitalized and ambulatory children. J Infect Dis 162:1283–1290
36. Forcic D, Ivancic-Jelecki J, Mlinaric-Galinovic G, Vojnovic G, Babic-Erceg A, Tabain I (2012) A study of the genetic variability of human respiratory syncytial virus in Croatia, 2006-2008. J Med Virol 84:1985–1992
37. Nakamura M, Itokazu K, Taira K, Kawaki T, Kudaka J, Nidaira M, Okano S, Koja Y, Tamanaha K, Kimura H, Noda M (2009) Genotypic and phylogenetic analysis of the G gene of respiratory syncytial virus isolates in Okinawa, Japan, 2008. Jpn J Infect Dis 62:326–327
38. Ostlund MR, Lindell AT, Stenler S, Riedel HM, Wirgart BZ, Grillner L (2008) Molecular epidemiology and genetic variability of respiratory syncytial virus (RSV) in Stockholm, 2002-2003. J Med Virol 80:159–167
39. Rebuffo-Scheer C, Bose M, He J, Khaja S, Ulatowski M, Beck ET, Fan J, Kumar S, Nelson MI, Henrickson KJ (2011) Whole genome sequencing and evolutionary analysis of human respiratory syncytial virus A and B from Milwaukee, WI 1998-2010. PLoS One 6:e25468
40. Viegas M, Mistchenko AS (2005) Molecular epidemiology of human respiratory syncytial virus subgroup A over a six-year period (1999-2004) in Argentina. J Med Virol 77:302–310
41. Plotnicky-Gilquin H, Goetsch L, Huss T, Champion T, Beck A, Haeuw JF, Nguyen TN, Bonnefoy JY, Corvaia N, Power UF (1999) Identification of multiple protective epitopes (protectopes) in the central conserved domain of a prototype human respiratory syncytial virus G protein. J Virol 73:5637–5645
42. Rueda P, Delgado T, Portela A, Melero JA, Garcia-Barreno B (1991) Premature stop codons in the G glycoprotein of human respiratory syncytial viruses resistant to neutralization by monoclonal antibodies. J Virol 65:3374–3378
43. Melero JA, Garcia-Barreno B, Martinez I, Pringle CR, Cane PA (1997) Antigenic structure, evolution and immunobiology of human respiratory syncytial virus attachment (G) protein. J Gen Virol 78:2411–2418
44. Palomo CP, Cane A, Melero JA (2000) Evaluation of the antibody specificities of human convalescent-phase sera against the attachment (G) protein of human respiratory syncytial virus: influence of strain variation and carbohydrate side chains. J Med Virol 60:468–474