Distribution and Clinical Impact of Human Respiratory Syncytial Virus Genotypes in Hospitalized Children over 2 Winter Seasons

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Sequencing studies of the glycoprotein G gene were performed in human respiratory syncytial virus (hRSV) strains detected by reverse-transcription polymerase chain reaction directly from nasopharyngeal aspirates of hospitalized children ≤3 years old over 2 winters. Clinical data were compared between 106 children infected with group A hRSV (96 GA2 genotypes) and 94 children infected with hRSV group B (62 GB3 genotypes). A severity index was defined by assigning 1 point each for the use of >30% supplemental oxygen, admission to an intensive-care unit, and duration of hospital stay of >5 days. Group A and genotype GA2 strains were associated with greater severity of hRSV disease than were group B strains.

Human respiratory syncytial virus (hRSV) is the leading cause of respiratory-tract infections resulting in hospitalization in young children [1]. Two major hRSV groups, A and B have been described on the basis of antigenic and molecular studies [2]. Genetic studies of 2 variable regions within the hRSV G glycoprotein from isolates around the world led to identification of several lineages within each group. Group A strains have been classified into GA1–GA7 [3, 4] and SAA1 [5] genotypes, whereas group B strains have been divided into GB1–GB4 [3] and SAB1–SAB3 [5] genotypes.

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Comparison of clinical effects caused by these different groups and genotypes has been hampered by other factors affecting the severity of hRSV infection in children, such as prematurity, the presence of underlying conditions, and young age [6]. However, these factors (with the exception of young age) cannot explain disease severity in otherwise healthy children born at term, who constitute more than one-half of all hospitalizations for hRSV infection [7].

In the present study, we examined the molecular epidemiology of hRSV infections over 2 consecutive seasons in Quebec City, Canada. We also compared the clinical characteristics of hRSV disease caused by group A and B strains and their associated genotypes in hospitalized children who were also tested for a panel of respiratory viruses.

Subjects, materials, and methods. Children ≤3 years old who were hospitalized with acute respiratory-tract infections at the Centre Hospitalier Universitaire de Québec during the winters of 2001–2002 and 2002–2003 and from whom a nasopharyngeal aspirate was collected on admission were eligible for enrollment in the study. After signed consent was obtained from parents at admission, a research nurse recorded demographic and clinical data on a standardized questionnaire. The study was approved by the Centre Hospitalier Universitaire de Québec Research Ethics Board.

On the basis of variables reported in previous studies [8–10], a severity index was defined a priori by assigning 1 point to each of the following: use of >30% supplemental oxygen, admission to an intensive-care unit, and duration of hospital stay of >5 days. Because 52% of subjects had a severity index of 0, the last was dichotomized as 0 and ≥1 for the purpose of statistical analysis.

All nasopharyngeal aspirate samples were initially tested by real-time reverse-transcription polymerase chain reaction (RT-PCR) for hRSV, influenza A and B viruses, and human metapneumovirus as reported elsewhere [11] and by a commercially available EIA (RSV TestPack; Abbott Laboratories) for detection of the hRSV antigen. Samples were cultured for known respiratory viruses at the request of the treating physician for 356 (79%) of 448 samples. The remainder of the specimens was frozen at −80°C until subsequent hRSV genotypic studies.

Sequencing studies of the hRSV glycoprotein G gene used the original clinical sample. In brief, viral RNA was extracted from 200 μL of nasopharyngeal aspirate using the QIAamp Viral RNA Mini Kit (Qiagen). For phylogenetic analysis, nucleotide sequences were obtained from 2 variable regions of the hRSV G gene using the primers reported by Peret et al. [3].

Table 1. Comparison of demographic and clinical characteristics between human respiratory syncytial virus (hRSV) groups and genotypes in hospitalized children with acute respiratory-tract infections.

Variable	hRSV group		hRSV genotype	
	A (n = 104)	B (n = 79)	GA2 (n = 96)	GB3 (n = 62)
Male sex	52.9	58.2	50.0	58.1
Age				
0–2 months	26.0	41.8[a]	26.0	35.5
3–5 months	11.5	12.7	11.5	14.5
6–11 months	27.9	25.3	26.0	27.4
12–23 months	24.0	13.9	26.0	16.1
24–35 months	10.6	6.3	10.4	6.5
Prematurity (<37 weeks)	10.6	11.4	11.5	9.7
Underlying condition	8.7	3.8	8.3	4.8
Mixed infections	5.8	11.4	5.2	14.5
Signs and symptoms on admission				
Cough	98.1	100.0	97.9	100.0
Wheezing	96.1	97.5	95.8	96.8
Fever	82.7	57.0[a,b]	83.3	60.0[a,b]
Diarrhea	30.8	16.5[a]	30.2	14.5[a]
Heart rate/min, median[c]	157	156[b]	159	156.5[b]
Respiratory rate/min, median[c]	52	52	52	52
Leukocyte count, median, $\times 10^9$ leukocytes/L[c]	10.5	11.1	10.2	10.8
C reactive protein level, median, mg/mL[c]	17	10	17	8.5[a]
Intensive-care unit admission	12.5	8.9	11.5	6.5
Use of >30% supplemental oxygen	42.3	24.1[a,b]	42.7	25.8[a]
Duration of hospital stay of >5 days	26.0	27.9	26.0	29.0
Final diagnosis				
Pneumonia	28.9	19.0	31.2	17.7
Bronchiolitis	97.1	90.0[b]	96.9	91.9
Otitis	48.1	44.3	47.9	45.2
Upper respiratory-tract infection	5.8	6.3	5.2	4.8
Severity index[d]				
0	44.2	59.5[b]	42.7	56.4
1	40.4	25.3	42.7	29.0
2	5.8	10.1	6.2	11.3
3	9.6	5.1	8.3	3.2

NOTE. Data are percent of children in group, unless otherwise indicated.

[a] $P < .05$ in univariate analysis, proportions compared with χ^2 or Fisher's exact tests and continuous variables compared with the Wilcoxon rank sum test.

[b] $P < .05$ in logistic regression, adjusted for age (<3 or ⩾3 months), prematurity, underlying conditions, and mixed infection.

[c] Comparison of top quartile vs. 3 lower quartiles.

[d] Dichotomized (0 and ⩾1) for the logistic regression, adjusted for the variables listed above.

Nucleotide sequences were entered into a multiple alignment generated by Clustal W software (version 1.83) and corrected through final visual inspection with the SeqLab application (Wisconsin package version 10.3; Accelrys). Phylogenetic analysis was first performed for the first variable G region (277 nt) with sequences from all hRSV strains using distance methods with PAUP software (version 4.0b10; Sinauer Associates). Kimura 2 parameters were used for the distance method using the neighbor-joining algorithm. The topological accuracy of the tree was estimated by the bootstrap method with 500 replicates.

A second phylogenetic tree was constructed by comparing sequences of the second variable region of the G gene (270 nt). Representative strains from our study corresponding to each cluster found in the first variable region (6 in group A and 13 in group B), as well as previously defined hRSV genotypes from GenBank, were included in the analysis. A genotype was defined as a cluster of sequences with bootstrap values of 70%–100% and a p distance of ⩽0.07 [5].

Categorical variables were compared by χ^2 or Fisher's exact test, and continuous variables were compared by the Wilcoxon rank sum test. Logistic regression was used to examine the association of hRSV groups and genotypes with clinical data and

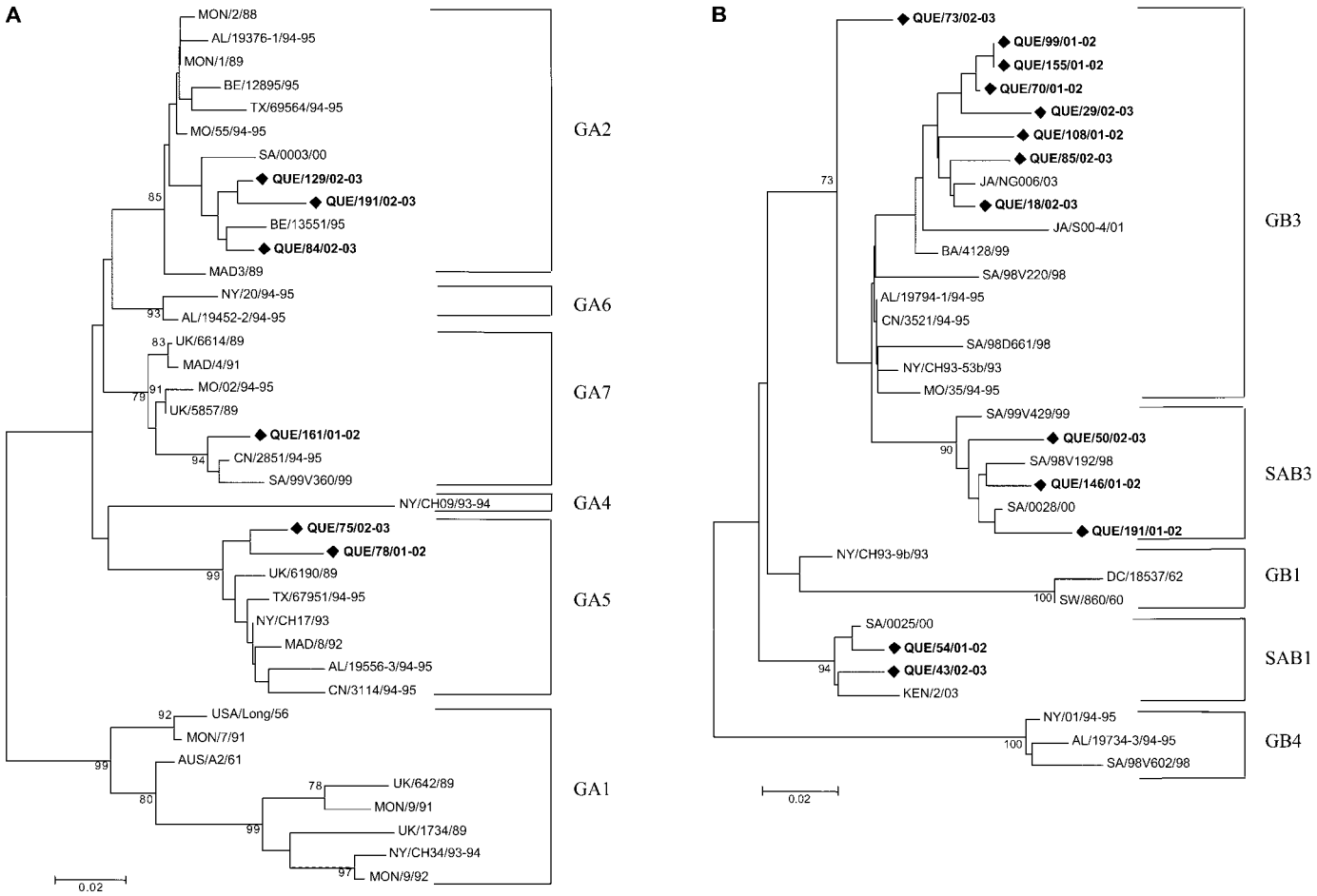

Figure 1. Group A *(A)* and B *(B)* human respiratory syncytial virus (hRSV) G gene phylogenetic relationships of sequences obtained from the second variable region (nt 649–918 of prototype strain A2 for group A and nt 652–921 of prototype strain 18537 for group B [2]) of hRSV strains isolated in Quebec (*black diamonds,* in bold type) and those available in GenBank. Viruses are identified by the geographic location (Quebec [QUE], Montevideo [MON], Alabama [AL], Belgium [BE], Texas [TX], Missouri [MO], South Africa [SA], Madrid [MAD], New York [NY], United Kingdom [UK], Canada [CN], United States [USA], Australia [AUS], Japan [JA], Buenos Aires [BA], Kenya [KEN], Sweden [SW]), followed by no. designation and year of isolation. The prototypes AUS/A2/61 (GenBank accession no. U50362) and USA/Long/56 (GenBank accession no. M17212) for group A and SW/860/60 (GenBank accession no. M55633) and USA/18537/62 (GenBank accession no. M17213) for group B are also shown. Nucleotide sequence alignments of either group A or group B sequences were used to create the neighbor-joining trees in the figure. The scales represent 0.02 substitutions per base per indicated horizontal distance. The numbers present at some of the internal nodes of the trees represent the number of bootstrap replicates. Only significant bootstrap replicate numbers with values of >70% are shown. Tentative genotype assignments are based on those of Peret et al. [3, 4] and Venter et al. [5].

The figure is available in its entirety in the online edition of the *Journal of Infectious Diseases*.

Figure 2. Group A *(A)* and B *(B)* human respiratory syncytial virus G gene phylogenetic relationships of sequences obtained from the first variable region (nt 258–544 of prototype strain A2 for group A and nt 226–544 of prototype strain 18537 for group B [2]) of 200 strains from hospitalized children in Quebec City. The figure and legend are available in their entirety in the online edition of the *Journal of Infectious Diseases*.

risk of having a severity index of ⩾1. Variables with a significance level of ⩽.1 in univariate analysis and potential confounding factors were entered into the initial multivariable model. The final multivariate regression model included age (dichotomized as <3 months and ⩾3 months), prematurity, underlying conditions, and the presence of mixed infections. Analyses were conducted separately for the severity index and clinical data listed in table 1 and for hRSV groups A and B and genotypes GA2 and GB3. $P < .05$ was considered to be statistically significant. GenBank accession numbers of the representative sequences obtained in the present study are AY927376–AY927413.

Results. During the 2 seasons, 448 patients with acute respiratory-tract infections were recruited. A total of 331 (73.9%) had at least one respiratory virus detected by PCR, viral culture, or antigen detection, which consisted of 249 (55.6%) hRSV, 61 (13.6%) influenza A or B viruses, 32 (7.1%) human metapneumovirus, 14 (3.1%) adenoviruses, 11 (2.5%) parainfluenza viruses, and 35 (7.8%) mixed infections. hRSV was detected by RT-PCR in 228 (50.9%) children.

Sequencing of the first variable region of the hRSV G gene was performed for 200 (87.7%) of 228 hRSV-infected subjects for whom sufficient nasopharyngeal aspirate was available, and group assignments were made for all sequences. hRSV group A was detected in 106 patients (53%), whereas group B was found in 94 patients (47%).

The second phylogenetic tree, constructed with the sequenced second variable region of the G gene of representative strains selected by their distribution in the branching order (figure 1), virtually replicated the topology of the phylogenetic trees obtained with sequences from the first variable region of the G gene (determined for all strains) (figure 2). Then, we applied the obtained representative genotype attribution to the total cohort. Thus, of the 106 group A viruses, 98 segregated into the GA2 genotype, 7 into the GA5 genotype, and 1 into the GA7 genotype. Of the 94 subgroup B viruses, 76 segregated into the GB3 genotype, 14 into the SAB3 genotype, and 4 into the SAB1 genotype.

For the 2 seasons, the proportions of group A and B isolates were nearly equal: 106 (53%) and 94 (47%), respectively. However, 80% of all strains in the first season were of group B, whereas 80% of all isolates in the second season were of group A. Genotype GB3 was predominant in the first year (74% of all isolates), and genotype GA2 was predominant in the second year (75% of all isolates).

Subjects with missing information on at least one risk or potentially confounding factor for severe disease (17/200) were excluded, leaving 183 subjects for analysis. Overall, 12 (6.6%) children had an important underlying medical condition (7 congenital heart diseases, 3 chronic pulmonary conditions, 2 immune deficiencies), 20 (10.9%) were born prematurely (<37 weeks of gestation), and 15 (8.2%) had mixed viral infections (12 hRSV and influenza A or B viruses and 3 hRSV and adenovirus). Of the 183 analyzed subjects, 104 (56.8%) had infections with group A viruses, whereas 79 (43.2%) had infections with group B viruses.

In univariate analysis, children infected with group A virus were significantly older than children infected with group B virus ($P = .02$), had more fever ($P < .001$) and diarrhea ($P = .04$), and more frequently required the use of >30% supplemental oxygen ($P = .01$) (table 1). Comparison between 2 most frequent genotypes (GA2 and GB3) showed that children infected with the GA2 genotype had more fever ($P < .001$) and diarrhea ($P = .04$), had a higher level of C-reactive protein ($P = .03$), and more frequently required the use of >30% supplemental oxygen ($P = .03$) (table 1).

In multivariate analysis adjusted for age, prematurity, underlying conditions, and mixed infections, children infected with group A viruses had significantly more fever ($P < .01$), more bronchiolitis ($P = .04$), and a faster heart rate ($P = .04$) and more frequently required the use of >30% supplemental oxygen ($P = .03$) than did those infected with group B viruses (table 1). Children infected with GA2 genotype viruses had significantly more fever ($P < .01$) and a faster heart rate ($P = .02$) than did those infected with GB3 genotype viruses (table 1). The risk of having a severity index of ⩾1 was greater in children infected with group A than with group B viruses (odds ratio [OR], 2.0 [95% confidence interval {CI}, 1.1–3.8]) and with genotype GA2 than group B viruses (OR, 2.2 [95% CI, 1.1–4.1]). GA2 genotype was not associated with a higher risk of having a severity index of ⩾1, compared with GB3 genotype (OR, 1.8 [95% CI, 0.9–3.5]).

Discussion. Our phylogenetic analysis revealed that viruses from group A (GA2, GA5, and GA7) and group B (GB3, SAB1, and SAB3) genotypes circulated in Quebec City, Canada, over 2 consecutive winter seasons. A rapid change in hRSV strains from group B to group A occurred over those 2 seasons. Also, we saw a clear preponderance of one genotype in each season, representing three-quarters of all isolates. This is in concordance with the predominance of GA2 genotype (78% of all isolates) in 2000 in South Africa [5] and of GB3 genotype (83% of all isolates) in 2002–2003 in Japan [12]. Children infected with hRSV group A were significantly older than were those

infected with group B, which is consistent with data by Hall et al. [13]. The greater frequency of fever, more frequent requirement for the use of >30% supplemental oxygen, and faster heart rate seen in those with infection with group A viruses could be related to greater disease severity. Because genotypes GA2 and GB3 represented, respectively, 92% and 78% of group A and B viruses, clinical findings in each study group may in fact represent those of predominant genotypes.

Using a composite severity index and after controlling for multiple confounding variables, we found that group A (and genotype GA2) hRSV strains were associated with more-severe disease. Because the medical staff was unaware of group and genotype results, potential bias leading to a differential misclassification of the components of the severity index is unlikely.

Previous studies that examined the relationship between hRSV groups and disease severity found either no difference or a greater severity of infections due to group A viruses [8, 9, 13, 14]. These discrepancies could be attributed to differences in study design and population, definitions of disease severity, and distribution of confounding factors and genotype shifts from year to year. Although a few studies examined clinical severity associated with specific hRSV genotypes [10, 15], they had limited sample sizes and did not use multivariate methods for analysis.

Characterization of hRSV genotypes directly from clinical samples could have allowed us to avoid the potential introduction of viral mutations. Also, the use of RT-PCR rather than viral culture could have permitted better detection of hRSV. In our study, PCR testing was systematically performed for 4 viruses and cultures were done for 80% of the samples. However, we could have missed some mixed infections with poorly growing viruses.

The present study was limited to 2 winter seasons. Herd immunity against previously circulating groups or genotypes and preexisting neutralization titers in mothers of infected children could have altered the severity of disease caused by specific hRSV groups or genotypes. We did not control for host genetic and environmental factors. Finally, more children infected with group B viruses (16%) than those infected with group A viruses (2%) were excluded from the analysis of clinical disease severity because of missing data. However, this is unlikely to have biased results, because the distribution of known confounding factors was similar in analyzed and excluded children.

In conclusion, using a composite severity index and multivariate analysis to control for confounding factors, we found that infections with group A and genotype GA2 viruses were associated with more-severe hRSV diseases than were infections with group B viruses during 2 winter seasons. Additional molecular studies over a longer period of time are needed to better define the role of specific genotypes in hRSV epidemiology and in the related severity of disease, which could affect therapeutic approaches and vaccine development.

References

1. Iwane MK, Edwards KM, Szilagyi PG, et al. Population-based surveillance for hospitalizations associated with respiratory syncytial virus, influenza virus, and parainfluenza viruses among young children. Pediatrics **2004**; 113:1758–64.
2. Johnson PR, Spriggs MK, Olmsted RA, Collins PL. 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 **1987**; 84:5625–9.
3. Peret TC, Hall CB, Schnabel KC, Golub JA, Anderson LJ. Circulation patterns of genetically distinct group A and B strains of human respiratory syncytial virus in a community. J Gen Virol **1998**; 79:2221–9.
4. Peret TCT, Hall CB, Hammond GW, et al. Circulation patterns of group A and B human respiratory syncytial virus genotypes in 5 communities in North America. J Infect Dis **2000**; 181:1891–6.
5. Venter M, Madhi SA, Tiemessen CT, Schoub BD. 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 **2001**; 82:2117–24.
6. Purcell K, Fergie J. Driscoll Children's Hospital respiratory syncytial virus database: risk factors, treatment and hospital course in 3308 infants and young children, 1991 to 2002. Pediatr Infect Dis J **2004**; 23:418–23.
7. Wang EE, Law BJ, Stephens D. Pediatric Investigators Collaborative Network on Infections in Canada (PICNIC) prospective study of risk factors and outcomes in patients hospitalized with respiratory syncytial viral lower respiratory tract infection. J Pediatr **1995**; 126:212–9.
8. McConnochie KM, Hall CB, Walsh EE, Roghmann KJ. Variation in severity of respiratory syncytial virus infections with subtype. J Pediatr **1990**; 117:52–62.
9. Walsh EE, McConnochie KM, Long CE, Hall CB. Severity of respiratory syncytial virus infection is related to virus strain. J Infect Dis **1997**; 175:814–20.
10. Martinello RA, Chen MD, Weibel C, Kahn JS. Correlation between respiratory syncytial virus genotype and severity of illness. J Infect Dis **2002**; 186:839–42.
11. Boivin G, De Serres G, Côté S, et al. Human metapneumovirus infections in hospitalized children. Emerg Infect Dis **2003**; 9:634–40.
12. Sato M, Saito R, Sakai T, et al. Molecular epidemiology of respiratory syncytial virus infections among children with acute respiratory symptoms in a community over three seasons. J Clin Microbiol **2005**; 43:36–40.
13. Hall CB, Walsh EE, Schnabel KC, et al. 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 **1990**; 162:1283–90.
14. McIntosh ED, De Silva LM, Oates RK. Clinical severity of respiratory syncytial virus group A and B infection in Sydney, Australia. Pediatr Infect Dis J **1993**; 12:815–9.
15. Venter M, Collinson M, Schoub BD. Molecular epidemiological analysis of community circulating respiratory syncytial virus in rural South Africa: comparison of viruses and genotypes responsible for different disease manifestations. J Med Virol **2002**; 68:452–61.