Short Communication

Molecular Epidemiology of Human Metapneumovirus from 2005 to 2011 in Fukui, Japan

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SUMMARY: To investigate the molecular epidemiology of human metapneumovirus (HMPV) infections in acute respiratory infections (ARI), we performed genetic analysis of the F gene in HMPV from patients with ARI in Fukui Prefecture from August 2005 to July 2011. HMPV was detected in 53 of 741 nasopharyngeal swabs (7.2%). Phylogenetic analysis helped us assign 31 strains to subgroup A2, 1 strain to subgroup B1, and 21 strains to subgroup B2. The prevalence of HMPV was peaked between January and June. A high degree of nucleotide identity was seen among subgroup A2 strains (95.6–100%) and subgroup B2 strains (97.5–100%). In addition, no positively selected sites (substitutions) were found in the F gene in these HMPV strains. The results suggest that the prevalent HMPV strains in Fukui were associated with various ARI in Japan during the investigation period.

Human metapneumovirus (HMPV), which belongs to the family Paramyxoviridae, genus Metapneumovirus, is an important cause of acute respiratory infections (ARI) in humans (1). Higher morbidity is observed in young children, the elderly, and immunocompromised people (2,3). Indeed, HMPV infection in some infants and the elderly may result in severe ARI such as bronchitis and bronchopneumonia (4,5).

HMPV is classified into two genotypes (A and B) and four subgroups (A1, A2, B1, and B2) by phylogenetic analysis (6,7). It has been suggested that these genotypes circulate in variable proportions in some areas (8,9). Although information available for HMPV molecular epidemiology has been gradually accumulated, the epidemiology of HMPV remains unclear (4,10). In this study, we performed genetic analysis of the fusion (F) gene of HMPV from patients with ARI in Fukui Prefecture from August 2005 to July 2011 to investigate the molecular epidemiology of HMPV infections.

Throat swab samples were collected from 741 patients (age range, 0–100 years; mean ± standard deviation [SD], 14.6 ± 29.2 years) with ARI at clinics collaborating with the local health authority of Fukui Prefecture in the surveillance of viral diseases in Japan. Informed consent was obtained from the subjects or assent was obtained from their parents for the donation of throat swab samples. The study comprised 584 patients aged 0–5 (1.46 ± 1.32 years), 33 patients aged 6–12 (7.85 ± 2.03 years), and 124 patients aged 13–100 years (76.7 ± 17.5 years). Of the 741 patients, 170 were diagnosed with upper respiratory infections (URI) and 396 with lower respiratory infections (LRI), including bronchitis and pneumonia.

We tried to detect influenza virus, human rhinovirus, enteroviruses, respiratory syncytial virus, and adenoviruses using reverse transcriptase-polymerase chain reaction (RT-PCR) and cell culture methods (Vero E6, RD, MDCK, and HEp-2 cells) (11–14). Viral nucleic acid was extracted from the samples using the MiniElute Virus Spin Kit or EZ1 Virus Mini Kit v2.0 (QIAGEN, Valencia, Calif., USA) and suspended in DNase/RNase-free water. After RNA extraction, nested RT-PCR was performed as previously described (4,15). Amplicons were purified using a QIAquick PCR Purification Kit (QIAGEN) and the nucleotide sequences were determined by direct sequencing (4,15). Sequence data were registered under the accession nos. AB716360–AB716412 at GenBank. Next, we performed phylogenetic analysis based on partial F gene (position: 3783–4099 of strain NL/1/99) of the most frequently analyzed HMPV strains using Molecular Evolutionary Genetics Analysis (MEGA) software version 4 (16). Evolutionary distances were estimated using Kimura's two-parameter method, and a phylogenetic tree was constructed using the neighbor-joining method (17,18). The reliability of the tree was estimated using 1,000 bootstrap replications. In addition, we calculated the pairwise distances for the present strains to assess the frequency distribution of HMPV genotypes A and B, as previously described (6,7,19). Furthermore, positive selections were identified using DATAMONKEY (http://www.datamonkey.org) (20). We used the following four methods to estimate the synonymous (dS) and nonsynonymous (dN) rates at every codon in the alignment: single likelihood ancestor counting (SLAC); fixed effects likelihood (FEL); and internal fixed effects likeli-
Table 1. Subject data in this study

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of samples</th>
<th>HMPV positive</th>
<th>Diagnosis reported in HMPV positive patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>/subgroup, no. of patients</td>
<td>URI</td>
</tr>
<tr>
<td>2005</td>
<td>20</td>
<td>1/A2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/B2</td>
<td>1</td>
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<tr>
<td>2006</td>
<td>155</td>
<td>6/A2</td>
<td>5</td>
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<tr>
<td></td>
<td></td>
<td>5/B2</td>
<td>3</td>
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<tr>
<td>2007</td>
<td>144</td>
<td>8/A2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/B1</td>
<td>1</td>
</tr>
<tr>
<td>2008</td>
<td>214</td>
<td>4/B2</td>
<td>1</td>
</tr>
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</tr>
<tr>
<td>2011</td>
<td>48</td>
<td>1/A2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10/A2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/B2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>741</td>
<td>53</td>
<td>10</td>
</tr>
</tbody>
</table>

1): Unknown fever 3, conjunctivitis 1, gastroenteritis 1, unknown 2.

URI, upper respiratory infection; ILI, influenza like illness.

Fig. 1. Seasonal variations in HMPV infection.

hood (IFEL). SLAC, FEL, and IFEL methods detect sites under selection at external branches of the phylogenetic tree, and the IFEL method investigates sites along the internal branches. Positive selection (dN > dS) was determined by a P-value of < 0.1 (SLAC, FEL, IFEL).

A summary of patient and viral data is shown in Table 1. HMPV was detected in 53 of 741 (7.2%) patients with ARI during the investigation period. The age of the 53 HMPV-positive patients was 7.1 ± 18.4 years. Of the 53 detected, 48 (90.6%) were found in samples from patients under 5 years of age. No other viruses were detected in these patients. Notably, over 60% of the patients were clinically diagnosed with wheezy bronchitis.

In this study, HMPV was found to be prevalent from January to June (Fig. 1). Previous studies have suggested that HMPV is prevalent during the winter-spring season in many countries, including Japan (10). Indeed, Mizuta et al. showed that the peak season for HMPV is from winter to spring (between January and May) and the low season is during fall (around September and October) (10). Thus, the findings of the present study are in agreement with those of the previous study (10).

The phylogenetic tree based on the partial nucleotide sequence of the partial F gene (317 bp) is shown in Fig. 2. Of the 53 HMPV strains, 31 and 22 were classified as genotypes A and B, respectively. All strains belonging to genotype A were assigned to subgroup A2 and 1 strain belonging to genotype B was assigned to subgroup B1, while the other 21 strains were assigned to subgroup B2. The nucleotide identity among the present strains was 83.9–100%. Although there were some differences, a high degree of nucleotide identity was seen among subgroup A2 strains (95.6–100%) and subgroup B2 strains (97.5–100%), as indicated in a previous report (21). The pairwise distance values among the present strains belonging to subgroup A2 were 0.019 ± 0.013 and those of subgroup B2 were 0.014 ± 0.008.

Pairwise distance values based on the nucleotide sequences among the present strains were relatively low (<0.02). In addition, we did not observe any evidence of positive selection. These results suggest that a highly conserved partial F coding region in HMPV was prevalent in Fukui Prefecture, Japan. In addition, we found that most of the nucleotide substitutions of the F gene were synonymous in the present strains. Based on these data, the dN/dS ratios of all substitutions were synonymous. Thus, no positively selected sites were found in the present strains. There were 30 common sites under negative selection as identified by SLAC, FEL, and IFEL methods. To our best knowledge, it is not known if these negatively selected sites are involved in immune responses and viral functions. However, F protein is the major antigenic determinant of protection (22). Therefore, we consider these negatively selected sites as potential players in viral functions, host immunity, and vaccine development.

A previous longitudinal epidemiological study suggested that subgroups A2 and B2 were predominant in Yamagata Prefecture, Japan, from 2004 to 2009 (10). In
Fig. 2. Phylogenetic tree constructed on the basis of partial sequences of human metapneumovirus partial F gene (317 bp). Distances were calculated using Kimura’s two-parameter method, and the tree was plotted using the neighbor-joining method. Numbers above the branches are bootstrap probabilities (%). Reference strains were NL/00/1 (AF371337), CAN99-81 (AY145294), JP/02/10190 (AB113377), JP/03/11015 (AB113372), JPS03-180 (AY330902), CAN97-83 (AY145296), NL/17/00 (AY304360), JTY06-1 (AB503857), JP/03/37085 (AB19485), JP/03/11011 (AB113371), JP/03/20036 (AE126612), JTY06-2 (EU127918), JTY06-3 (EU127919), JP/03/37092 (AB119486), JP/03/11030 (AB119489), JP/03/11054 (AB119491), JPS03-240 (AY330905), O0601 (EF589610), JPS03-187 (AY330903), JPS03-178 (AY330901), Yamaguchi09-15 (AB533251), Yamaguchi09-09 (AB533245), Yamaguchi09-09 (AB533249), NL/1/99 (AY304361), Yamaguchi09-17 (AB533244), JPS02-76 (AY330908), JPS03-194 (AY330904), JP/03/10036 (AB126608), JTY06-1 (EU127917), JP/03/10023 (AB126608), JP/03/20034 (AB119493), CAN98-75 (AY297748), JP/03/13016 (AB126607), NL/1/94 (AY304362), and Yamaguchi09-15 (AB533243). Avian metapneumovirus type C (AMPV-C [AY579780]) was also included as an outgroup.
addition, these subgroups have been found to be predominant in other studies (4,21). Our results appear to be compatible with these previous studies (4,10,21). Furthermore, we performed partial F gene analysis for subgroups A2, B1, and B2, and found a high level of sequence identity (95.6–100%). These results were almost identical to a previous report (93.5–97.6%) (23). Our data support the theory that F protein is relatively well conserved.

In this study, we did not perform bacteriological examinations by culture-based methods or PCR detection of major respiratory pathogens. Therefore, we could not exclude co-infection with respiratory pathogens.

In conclusion, HMPV strains detected in samples from patients with ARI during 2005–2011 in Fukui Prefecture, Japan showed a high degree of genetic identity. Our results indicated that subgroups A2 and B2 were predominant. These HMPV strains might be associated with various ARI in Japan during the investigation period. However, additional epidemiological studies are needed to gain a better understanding of HMPV in other areas of Japan.

Acknowledgments This work was supported in part by a Grant-in-Aid for Research on Emerging and Re-emerging Infectious Diseases, Labour and Welfare Programs from the Ministry of Health, Labour and Welfare of Japan.

Conflict of interest None to declare.

REFERENCES