Japanese Journal of Infectious Diseases

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Received: April 10, 2019. Accepted: May 14, 2019
Published online: May 31, 2019
DOI:10.7883/yoken.JJID.2019.124

Advance Publication articles have been accepted by JJID but have not been copyedited or formatted for publication.
Predominant detection of the subgroup A2b human metapneumovirus strain with 111-nucleotide duplication in Yokohama City, Japan in 2018

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Running head: Epidemiology of HMPV

Key words: Human metapneumovirus / G gene / Nucleotide duplication / surveillance

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Summary

Human metapneumovirus (HMPV) has been a major causative agent of acute respiratory infections in humans. Recently two types of variant A2b subtype HMPV strains possessing 111- or 180-nucleotide duplication (nt-dup) in the G gene (HMPV A2b_{180nt-dup} and HMPV A2b_{111nt-dup}, respectively) were detected in Japan, Spain, Vietnam, and China. Surveillance for infectious agents in Yokohama City, Japan revealed that the HMPV_{111nt-dup} strain became predominant in Yokohama City in 2018. In contrast, no classical HMPV A2b strain was detected after 2017. These data suggest a beneficial role of the 111nt-dup in the G gene for the transmission of HMPV.

Main text

Human metapneumovirus (HMPV), a member of the family Pneumoviridae, is a major causative agent of acute respiratory infections (ARI), especially in young children, older adults, and patients with underlying diseases such as cardiopulmonary diseases and diabetes (1-3). The virus was first detected in 2001 (4), and seroepidemiological studies have demonstrated that HMPV had been circulating worldwide for more than 60 years (4). HMPV has a nonsegmented negative sense RNA genome that contains eight genes in the order, 3'-N-P-M-F-M2-SH-G-L-5', and encodes nine viral proteins, including three
surface F (fusion), SH (small hydrophobic), and G (glycol-) proteins. Based on the antigenicity difference, HMPV are divided into two groups, A and B (5). Each group is further divided into two subgroups (the subgroups A1 and A2 in group A, and the subgroups B1 and B2 in group B) (6). Detailed phylogenetic analysis of HMPV strains shows two distinct lineages, A2a and A2b, in the subgroup A2 (7). Recently, unique variant HMPV strains possessing a 180- or 111-nucleotide duplication (nt-dup) in the G gene have been detected in Japan, Spain, Vietnam, and China (GeneBank accession number MF462419.1) (8-10). These variants belong to the subgroup A2b and are called HMPV A2b180nt-dup and HMPVA2b111nt-dup, respectively (8, 10). This study reports surveillance data of HMPV strains detected in Yokohama City, Japan between 2013 and 2018.

According to the National Epidemiological Surveillance of Infectious Diseases (NESID), instituted by the Infectious Diseases Control Law in Japan, clinical specimens (throat swabs and nasal secretions) were collected from patients with upper or lower acute respiratory infections in Yokohama City. Collected samples were tested for HMPV by RT-PCR as previously described (10). The samples positive for HMPV were further analyzed, and the nucleotide sequence of the HMPV G gene was determined as previously described (9). The sequence data were deposited in the DDBJ/EMBL/GenBank.
nucleotide sequence database under accession numbers LC192170-LC192253, LC270124, LC275891, LC275892, LC316180, LC337921-LC337941, LC360498, LC466037-LC466056, and LC466061. Phylogenetic analysis was performed by using MAFFT (11) and MEGA (12) software.

From January 2013 to December 2018, 2,392 clinical specimens were collected (the data of 1,306 samples from January 2013 to June 2016 were reported previously (9)) and 163 specimens (6.8 %) were positive for HMPV. The entire nucleotide sequence of the open reading frame (ORF) of the G protein was successfully determined for 153 HMPV strains of the 163 positive samples. Phylogenetic analysis of the nucleotide sequence of the G gene demonstrated that 6, 69, 35, and 43 strains were classified into subgroups A2a, A2b, B1, and B2, respectively (Table 1). No HMPV subgroup A1 strain was detected during this study period. Among the 69 HMPV subgroup A2b strains, 16 and 24 strains had the 180nt-dup and 111nt-dup, respectively (Table 1). HMPV A2b111nt-dup strains were first detected in 2017 (10) and became the most prevalent strain in 2018 in Yokohama City (Table 1). In contrast, no HMPV A2b strain without the nt-dup (the classical HMPV A2b strain) has been detected since 2017. Phylogenetic analysis demonstrated that the HMPV A2b180nt-dup and HMPV A2b111nt-dup strains detected in Yokohama City were clustered with HMPV A2b180nt-dup strains detected in Spain and
China (the cluster is shown with an asterisk in Fig. 1). The cluster also contained the classical HMPV A2b strains detected in Yokohama City and Spain, suggesting that the 180- and 111-nt dup events in the G gene occurred in recent years. Furthermore, all 24 HMPV\textsubscript{111nt-dup} strains detected in Yokohama City formed a unique subcluster (the double asterisk in Fig. 1).

Because the routine HMPV surveillance data were obtained by PCR, no evidence was obtained for infectious viruses with the 111nt-dup. Fifteen clinical samples, which were positive for HMPV A2b\textsubscript{111nt-dup} strains, were selected for virus isolation. VeroE6 cells constitutively expressing the transmembrane protease, serine 2 (VeroE6/TMPRSS2) (13), were used. From these 15 clinical samples, 11 HMPV strains were isolated and nucleotide sequence analysis demonstrated that these strains indeed possessed the 111nt-dup in the G gene.

Human respiratory syncytial virus (RSV) is another member of the family Pneumoviridae and causes severe ARI in infants. Nucleotide duplication events in the G gene have also been reported in RSV (14). Although the duplication event shows little clinical impact on RSV infections (15), RSV strains with the nucleotide duplication in the G gene are currently the dominant strains globally (16). Our study demonstrated that the HMPV strains possessing the nucleotide duplication in the G gene is now a predominant

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strain, as observed for RSV. These data suggest a beneficial role of the nucleotide duplication in the G gene for the subgroup A2b HMPV transmission.

This study was performed with the approval of the ethics committee of the Yokohama City Institute of Public Health and the National Institute of Infectious Diseases (approval number: #873).

We thank all participants, patients and staff members of the clinics and hospitals for their contributions. We are also grateful to all members of the Yokohama City Institute of Public Health for their technical support and dedicated assistance.

**Conflict of interest**

None to declare
References


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Figure legend

Figure 1

A phylogenetic tree was constructed using the G gene sequences of 69 A2b HMPV strains detected in Yokohama City, Japan and 47 strains obtained from the NCBI nucleotide sequence database. The tree was rooted with A2a HMPV strain HMPV/Yokohama.JPN/P7877/2015 and tested with bootstrapping (100 replicates). HMPV A2b111nt-dup and HMPV A2b180nt-dup strains are indicated by filled circles and diamonds, respectively. The cluster containing HMPV A2b111nt-dup and HMPV A2b111nt-dup strains is indicated by a bracket with an asterisk. The subcluster containing only HMPV A2b111nt-dup strains is indicated by a bracket with double asterisks.
### Table

**Table 1**

Numbers of HMPV strains detected in Yokohama City between January 2013 and December 2018 and their subgroup classification

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
<th>2017</th>
<th>2018</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2a</td>
<td>-</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>A2b</td>
<td>-</td>
<td>11</td>
<td>3</td>
<td>12</td>
<td>3</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>111</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td>180</td>
<td>0</td>
<td>3</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>B1</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>9</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>B2</td>
<td>-</td>
<td>15</td>
<td>6</td>
<td>1</td>
<td>15</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>15</td>
<td>25</td>
<td>29</td>
<td>14</td>
<td>38</td>
<td>153</td>
</tr>
</tbody>
</table>

*a nucleotide duplication in the G gene*