Short Communication

Genotyping of Mumps Virus Detected in Yokohama City from 1999 to 2010

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SUMMARY: A survey of mumps infections from 1999 to 2010 was conducted in Yokohama City, Japan, and 17 cases—including 4 cases of aseptic meningitis—were positive for mumps virus (MuV). Based on the phylogenetic analysis of the small hydrophobic gene of the MuV genome, 3, 2, and 12 of the isolates were classified into genotypes B, L, and G, respectively. The results were supported by phylogenetic analysis of hemagglutinin-neuraminidase genes. The 3 isolates of genotype B, obtained in 2000, 2004, and 2007, were closely related to indigenous lineages and vaccine strains in Japan. Two isolates obtained from 1999 to 2000 were assigned to genotype L. Twelve isolates obtained from 2000 to 2010 were classified into genotype G, in which 8 isolates obtained from 2000 to 2006 and 4 isolates obtained in 2010 were closely related to MuVi/Gloucester.GBR/32.96 and MuVi/London.GBR/0.03, respectively. Precise analyses of nucleotide sequences suggested that the 4 viruses isolated in 2010 were not directly derived from the evolution of MuV existing before 2006 in the Yokohama area.

Mumps is an acute infectious viral disease characterized by enlargement of the parotid and salivary glands. Although up to one-third of mumps infections are subclinical, serious complications including pancreatitis, orchitis, deafness, aseptic meningitis, and encephalitis may occur (1). The mumps virus (MuV) is a member of the genus Rubulavirus of the Paramyxoviridae family. This is an enveloped virus with a 15.3-kb non-segmented, single-stranded, negative-sense RNA genome encoding 7 proteins: fusion (F) protein, hemagglutinin-neuraminidase (HN) surface glycoprotein, putative membrane-associated small hydrophobic (SH) protein, and 4 core proteins—nucleoprotein (NP), phosphoprotein (P), matrix (M) protein, and large (L) protein (2). The SH gene in the genome is 316 nucleotides in length and encodes a product of 57 amino acid residues. This is known to be the most variable gene in the entire genome, and is therefore used as a marker to analyze the phylogenetic relations of this virus. Phylogenetic reconstruction studies of different isolates of wild-type mumps viruses have been conducted according to the criteria proposed using the 316 nucleotides of the entire SH gene sequences of the reference strains recommended by the World Health Organization (1). These studies revealed the existence of 12 genotypes designated A to N (excluding E and M). The HN gene is 1793 nucleotides in length and encodes a product of 582 amino acid residues. The HN protein represents the surface envelope glycoprotein, which contains scattered neutralizing epitopes and putative glycosylation sites. It is suggested that the region of HN gene should be used additionally when an ambiguous result is generated by analysis of the SH gene or a new lineage is suspected (1). Here, we performed a survey of MuV infection and genotyping of the MuV in the Yokohama City area, Japan, over the 12-year period from 1999 to 2010.

The surveillance of MuV infection was conducted on specimens from 8 pediatric clinics and 3 hospitals, and 17 cases were positive for MuV among the specimens examined (Table 1). Clinical diagnoses of 13 of the cases revealed typical MuV infectious disease characterized by enlargement of the parotid and salivary glands. One of the 13 cases exhibited typical mumps symptoms and was complicated by aseptic meningitis. The remaining 4 cases with suspected MuV infection included 1 case of respiratory illness and 3 cases of aseptic meningitis. The cerebrospinal fluid (CSF) specimens from the 4 cases of aseptic meningitis were negative for enteroviruses and herpes simplex virus but positive for MuV. The 17 patients consisted of 11 males and 6 females ranging in age from less than 1 year up to 12 years, with an average age of around 6 years old. Vaccination status of the patients was unknown in all but 4 cases because of the absence of records; 3 patients were vaccinated and 1 had no vaccination.

Extracts of 17 clinical samples of throat swabs and CSF from patients were inoculated onto Vero cells to recover viruses. The recovered viruses were confirmed to be MuV by neutralization test using the MuV NT kit (Denka Seiken Co., Niigata, Japan) in 13 cases, followed by sequence analysis of both SH and HN regions (3). The 4 specimens from which the virus failed to propagate in Vero cells were directly analyzed for MuV genes by RT-PCR of the SH region and partial HN gene sequence (3). Since genotyping based on sequence variations of SH and HN genes has been established, we analyzed the SH and HN regions of the obtained MuV samples and genotyping was conducted. The nucleotide sequences were deposited in GenBank with the accession numbers listed in Table 1. The obtained data were used.

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Table 1. Mumps virus strains isolated from 1999 to 2010 in the Yokohama City, clinical features of patients, origins of specimens, and dates of sampling

<table>
<thead>
<tr>
<th>No.</th>
<th>MuV strain</th>
<th>Date of sampling</th>
<th>Specimen</th>
<th>Age (yr)</th>
<th>Gender</th>
<th>Diagnosis</th>
<th>Vaccination history</th>
<th>Genotype</th>
<th>Accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MuVi/Yokohama.JPN/38.99</td>
<td>Sep-99</td>
<td>ts</td>
<td>4</td>
<td>M</td>
<td>Mumps</td>
<td>Unknown</td>
<td>L</td>
<td>AB699697</td>
</tr>
<tr>
<td>2</td>
<td>MuVi/Yokohama.JPN/19.00</td>
<td>May-00</td>
<td>ts</td>
<td>5</td>
<td>F</td>
<td>Cervical lymphadenopathy</td>
<td>Unknown</td>
<td>G</td>
<td>AB699698</td>
</tr>
<tr>
<td>3</td>
<td>MuVi/Yokohama.JPN/25.00</td>
<td>Jun-00</td>
<td>ts</td>
<td>9</td>
<td>F</td>
<td>Mumps</td>
<td>Unknown</td>
<td>B</td>
<td>AB699699</td>
</tr>
<tr>
<td>4</td>
<td>MuVi/Yokohama.JPN/42.00</td>
<td>Oct-00</td>
<td>ts</td>
<td>3</td>
<td>M</td>
<td>Mumps</td>
<td>Unknown</td>
<td>L</td>
<td>AB699700</td>
</tr>
<tr>
<td>5</td>
<td>MuVi/Yokohama.JPN/4.01</td>
<td>Jan-01</td>
<td>ts</td>
<td>Unknown</td>
<td>M</td>
<td>Mumps</td>
<td>Unknown</td>
<td>G</td>
<td>AB699701</td>
</tr>
<tr>
<td>6</td>
<td>MuVi/Yokohama.JPN/15.02</td>
<td>Apr-02</td>
<td>ts</td>
<td>&lt;1</td>
<td>M</td>
<td>respiratory illness</td>
<td>Unknown</td>
<td>G</td>
<td>AB699702</td>
</tr>
<tr>
<td>7</td>
<td>MuVi/Yokohama.JPN/28.02</td>
<td>Jul-02</td>
<td>ts</td>
<td>7</td>
<td>F</td>
<td>Mumps</td>
<td>Unknown</td>
<td>G</td>
<td>AB699703</td>
</tr>
<tr>
<td>8</td>
<td>MuVi/Yokohama.JPN/33.02</td>
<td>Aug-02</td>
<td>ts</td>
<td>3</td>
<td>F</td>
<td>Mumps</td>
<td>Unknown</td>
<td>G</td>
<td>AB699704</td>
</tr>
<tr>
<td>9</td>
<td>MuVs/Yokohama.JPN/39.04</td>
<td>Oct-04</td>
<td>CSF</td>
<td>3</td>
<td>M</td>
<td>Aseptic meningitis</td>
<td>Unknown</td>
<td>B</td>
<td>AB744089</td>
</tr>
<tr>
<td>10</td>
<td>MuVi/Yokohama.JPN/38.05</td>
<td>Sep-05</td>
<td>ts</td>
<td>3</td>
<td>M</td>
<td>Mumps</td>
<td>Unknown</td>
<td>G</td>
<td>AB699705</td>
</tr>
<tr>
<td>11</td>
<td>MuVs/Yokohama.JPN/44.05</td>
<td>Nov-05</td>
<td>CSF</td>
<td>8</td>
<td>M</td>
<td>Aseptic meningitis</td>
<td>Previously vaccinated</td>
<td>G</td>
<td>AB744090</td>
</tr>
<tr>
<td>12</td>
<td>MuVi/Yokohama.JPN/29.06</td>
<td>Jul-06</td>
<td>CSF</td>
<td>6</td>
<td>F</td>
<td>Mumps</td>
<td>Aseptic meningitis</td>
<td>Unknown</td>
<td>G</td>
</tr>
<tr>
<td>13</td>
<td>MuVs/Yokohama.JPN/27.07</td>
<td>Jul-07</td>
<td>CSF</td>
<td>1</td>
<td>M</td>
<td>Aseptic meningitis</td>
<td>Unknown</td>
<td>B</td>
<td>AB744091</td>
</tr>
<tr>
<td>14</td>
<td>MuVs/Yokohama.JPN/35.10</td>
<td>Sep-10</td>
<td>ts</td>
<td>11</td>
<td>M</td>
<td>parotiditis</td>
<td>Previously vaccinated</td>
<td>G</td>
<td>AB699707</td>
</tr>
<tr>
<td>15</td>
<td>MuVi/Yokohama.JPN/37.10</td>
<td>Sep-10</td>
<td>ts</td>
<td>12</td>
<td>M</td>
<td>parotiditis</td>
<td>Previously vaccinated</td>
<td>G</td>
<td>AB699708</td>
</tr>
<tr>
<td>16</td>
<td>MuVi/Yokohama.JPN/45.10</td>
<td>Nov-10</td>
<td>ts</td>
<td>6</td>
<td>M</td>
<td>Mumps</td>
<td>Unknown</td>
<td>G</td>
<td>AB699709</td>
</tr>
<tr>
<td>17</td>
<td>MuVs/Yokohama.JPN/52.10</td>
<td>Dec-10</td>
<td>ts</td>
<td>8</td>
<td>F</td>
<td>Mumps</td>
<td>Unvaccinated</td>
<td>G</td>
<td>AB699710</td>
</tr>
</tbody>
</table>

The genotypes of strains are also described. GenBank accession numbers of each strain are indicated.

1): Virus from these specimens could not be propagated on Vero cells.
ts, throat swab; CSF, cerebrospinal fluid; M, male; F, female.

Two isolates, MuVi/Yokohama.JPN/38.99 and MuVi/Yokohama.JPN/42.00, obtained from 1999 to 2000, were assigned to genotype L by phylogenetic analysis of SH genes (Table 1 and Fig. 1). They were found to be similar to the 8 reported cases obtained from 2000 to 2001 by comparing the SH region; MuVi/Yokohama.JPN/38.99 differs from MuVi/Tokyo.JPN/6.01 (AB105480) in only one base at nucleotide position 282, and the nucleotide sequence of MuVi/Yokohama.JPN/42.00 was identical to that of the reference strain (4).

Twelve isolates obtained from 2000 to 2010 were classified into genotype G by phylogenetic analysis of the SH genes: MuVi/Yokohama.JPN/25.00, MuVs/Yokohama.JPN/39.04, and MuVs/Yokohama.JPN/27.07, obtained in 2000, 2004, and 2007, respectively, were closely related to indigenous lineages and a vaccine strain in Japan. The SH gene sequence in MuVs/Yokohama.JPN/39.04 was identical to MuVi/Hoshino.JPN/0.81 (previous name Hoshino strain) (AB003414), a vaccine strain used in Japan. MuVs/Yokohama.JPN/27.07 was identical to MuVi/Matsuyama.JPN/0.84 (Matsuyama strain) (D90233). MuVi/Yokohama.JPN/25.00 was closely related to the previously identified MuVi/Ehime.JPN/0.90s (941 strain) (AB115991) with 5 nucleotide differences out of 316 nucleotides in the SH domain (the positions of the altered nucleotide sequence are 113, 134, 136, 148, and 291), and to MuVi/Himeji.JPN/24.00 (JQ945269) with 8 nucleotide differences (at 113, 134, 136, 159, 174, 247, 268, and 291).
Fig. 1. Phylogenetic relationships between mumps virus strains obtained in the present study and previously published sequences based on the entire SH gene. Phylogenetic analyses based on the 316 nucleotides of the SH gene aligned using MEGA 4.0.2. Genotype A sequences were used as the out group. The bar indicates nucleotide substitutions per site.

- Mumps virus strains obtained in the present study,
- Reference strains proposed by WHO (Ref. 1). Genotype A–N.

**MuVi/Niigata.JPN/34.02 (02–34 strain) (AB116017) and MuVi/Yamaguchi.JPN/0.00 (Yamaguchi99/JPN.00 strain) (AB105482) (Fig. 1). Among the 12 isolates of genotype G shown in Table 2, the 8 isolates obtained from 2000 to 2006 showed high degrees of identity to the reference strain MuVi/Gloucester.GBR/32.96 (AF280799), with less than 1.9% difference in nucleotide sequence (5–6 nucleotides out of 316 nucleotides). They also showed the highest degree of identity to MuVi/Niigata.JPN/34.02 strain (5–6 nucleotides out of 316 nucleotides).**

**Comparison of the MuVi/Gloucester.GBR/32.96 with all 8 isolates obtained until 2006 showed the presence of common variations in the nucleotide sequence of the SH region at positions 134, 136, 146, 160, and 193 (Table 2). Among the variations, those at positions 136, 160, and 193 gave non-synonymous mutations, while those at 134 and 146 gave synonymous mutations. In contrast, 4 isolates obtained in 2010—MuVs/Yokohama.JPN/35.10, MuVi/Yokohama.JPN/37.10, MuVi/Yokohama.JPN/45.10, and MuVi/Yokohama.JPN/52.10—showed the highest degrees of identity (only 2 nucleotide differences out of 316 nucleotides) to the sequence of MuVi/London.GBR/0.03 (GBR03–1720536 strain) (EU606236), but they were distantly related to MuVi/Gloucester.GBR/32.96 (10 nucleotides out of 316 nucleotides). Mutations commonly observed in the same region in the 2010 Yokohama isolates were at nucleotide positions 68, 71, 134, 144, 146, 149, 166, 232, and 291. Among these, mutations at positions 144 and 166 were non-synonymous. These results suggest that 4 isolates obtained in 2010 were not directly derived from evolution of those present before 2006 in the Yokohama area. To further analyze the genotype in more detail, we focused on the HN gene. The HN protein plays an important role in the initial process of viral infection by attaching to the cellular sialic acid receptors, and then
the HN protein is involved cooperatively in the next infection step of membrane fusion and viral penetration. This protein is the major target for the humoral immune response upon MuV infection (5,6). The HN gene comprises 1749 nucleotides that code for 582 amino acid residues, including the neutralizing epitopes and the putative glycosylation site. Therefore, we examined the diversity of HN genes of the isolates obtained from the clinical specimens, as well as MuV recovered from Vero cell culture. Phylogenetic analysis of HN genes supported the genotype classification obtained by analysis of SH genes: 3 isolates of genotype B, 2 isolates of genotype L, and 12 isolates of genotype G (Fig. 2). In 4 clinical specimens (sources of MuVs/Yokohama.JPN/39.04, MuVs/Yokohama.JPN/44.05, MuVs/Yokohama.JPN/27.07, MuVs/Yokohama.JPN/35.10), the 447 nucleotide sequences of the N-terminal region of the HN gene were directly analyzed. In case of the other samples, MuVs were recovered from cultured Vero cells, and the 1749 base coding region of the HN gene was analyzed. On analysis of the HN region, the genotype B isolate MuVs/Yokohama.JPN/25.00 showed 98.0% identity (35 nucleotide differences) to the Hoshino strain, and this was highest degree of identity (98.7% identified). The 3 isolates of 2010 showed 21 nucleotide differences (99.0%–99.4% identity). Similarity of nucleotide differences (98.8% identity). Commonly observed differences in the nucleotide sequence from that of MuVs/Glou- cester.GBR/32.96 (7). Asn at this site constitutes a putative glycosylation signal, Asn-Ala-Thr, with 8 other putative glycosylation sites, Asn-X-Thr and Asn-X-Ser, for glycosylation in many isolates of genotype G, except MuVs/Gloucester. However, preliminary studies demonstrated that no substantial differences in the neutralizing activity were detected, suggesting that Pro at position 353 has little effect on immunogenicity (data not shown). No significant change was reported in the neutralizing test antibody titers against genotype L strains using 7 post-vaccination sera with the Hoshino strain (genotype B) (4). By comparison with the HN region of MuVs/Gloucester.GBR/32.96, the 7 isolates obtained from 2000 to 2006 in the Yokohama area showed 10–18 nucleotide differences (99.0%–99.4% identity). Similarly, the 3 isolates of 2010 showed 21 nucleotide differences (98.8% identity). Commonly observed differences in the nucleotide sequence from that of MuVs/Gloucester.GBR/32.96 in the 7 isolates of 2000 to 2006 were located at nucleotide positions 12, 35, 210, 723, 1083, 1188, 1194, 1303, and 1704 in the HN region. Common differences seen in the 3 isolates of 2010 were located at nucleotide positions 35, 62, 136, 190, 375, 375, 405, 438, 498, 639, 669, 723, 794, 999, 1083, 1299, 1303, and 1692, of which 4 sites (35, 723, 1083, and 1303) were common to the 7 isolates of 2000 to 2006. However, comparison with MuVs/Sheffield.GBR/1.05 (JQ946046), and MuVs/New York.USA/1.10 (JX287389), indicated substitutions at these 4 sites, sug- gesting that these changes are specific to MuVs/Gloucester.GBR/32.96. Mutation in the HN gene at nucleotide position 35, which is one of the above 4 sites, from adenine to guanine caused a non-synonymous change from Asn to Ser at amino acid position 12 in many isolates of genotype G, except MuVs/Gloucester. GBR/32.96 (7). Asn at this site constitutes a putative glycosylation signal, Asn-Ala-Thr, with 8 other putative sites, Asn-X-Thr and Asn-X-Ser, for glycosylation in the HN protein (8). Twelve isolates from the Yokohama
Fig. 2. Phylogenetic relationships between mumps virus strains obtained in the present study and previously published sequences based on the entire HN gene. Phylogenetic analyses based on the 1749 nucleotides of the HN gene aligned using MEGA 4.0.2. Genotype A sequences were used as the out group. The bar indicates nucleotide substitutions per site.

- Mumps virus strains obtained in the present study.
- Reference strains proposed by WHO (Ref. 1). Genotype A–N.
- Only 447 nucleotide sequence of the N-terminal of the HN region was used for this analysis.

As described above, the epidemiological study in the Yokohama area from 1999 to 2010 indicated that genotypes B, G, and L were the major types during the 2 years from 1999 to 2000, but genotype G dominated after 2001. Analyses of nucleotide sequences, as well as predicted amino acid sequences, of the SH and HN regions of the isolates of genotype G from 2000 to 2006 and 2010 suggest that the isolates of genotype G in 2010 were not derived from mutation of a prototype in Japan, but were more likely derived from outside Japan. It may be worth mentioning that during the endemic of G genotype for the 10 years from 2000 to 2010, genotype B strains were isolated from 2 cases of aseptic meningitis. The nucleotide sequence of MuVs/Yokohama.JPN/39.04 was identical to that of the Hoshino strain, as determined by the analysis of 824 nucleotides spanning from the entire SH region to the N-terminal HN gene product. Although the vaccination history of this patient was not known (Table 1), these results suggest that development of aseptic meningitis was caused by vaccination or alternatively that the patient may have developed a MuV infection from a vaccinated individual (9). However, it cannot be concluded that MuVs/Yokohama.JPN/39.04 was derived from the Hoshino vaccine
strain based on the present results alone. Analysis of other regions of the genome, as well as detailed epidemiological analysis of MuV spread in the Yokohama area, would be necessary in order to come to a definitive conclusion. On the other hand, the nucleotide sequence of MuVs/Yokohama.JPN/27.07 showed significant differences (1.5%; 12 nucleotides out of 824 nucleotides) from both the Hoshino and the Miyahara vaccine strains, but matched completely with the Torii vaccine strain in the 316 nucleotides of the SH region (10). This suggests that MuVs/Yokohama.JPN/27.07 is derived from the Torii vaccine strain. However, this conclusion cannot be finalized, since there is no record of the vaccination history of the patient, and only the nucleotide sequence of the SH region was compared with that of the Torii strain. It is important to obtain detailed information such as comparison with other gene(s) to reach a conclusion. Sporadic isolation of subtype B in the Yokohama area raises concerns regarding the possibility of the spread of the vaccine strain used for vaccination. From this viewpoint, periodic monitoring of the MuV genotype is important along with analysis of geographic and temporal distributions.

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Conflict of interest None to declare.

REFERENCES