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Virological Analysis of a Regional Mumps Outbreak in the Northern Island of Japan—Mumps Virus Genotyping and Clinical Description

Kazuki Okajima[1]*, Kenichi Iseki[1], Shin Koyano[2], Atsushi Kato[3], and Hiroshi Azuma[2]

[1]Fukagawa City Hospital, Fukagawa 074-0006;
[2]Asahikawa Medical University, Asahikawa 078-8510; and
[3]National Institute of Infectious Diseases, Tokyo 208-0011, Japan

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Mumps is an acute, contagious, vaccine-preventable disease caused by mumps virus (MuV) and is typically characterized by swelling of either or both parotid glands. A mumps epidemic occurs every 3–5 years in non-vaccinated populations, and humans are the only natural hosts for MuV, which is transmitted by the respiratory route and replicates primarily in the upper respiratory mucosal epithelium. Primary replication leads to viremia and triggers secondary viral replication (1). Parotitis is not, however, a necessary component of mumps symptoms. MuV can display neurovirulence. MuV can also enter the cerebrospinal fluid (CSF) through the choroid plexus, leading to a form of aseptic meningitis in up to approximately 10% of cases. More serious complications, such as deafness and encephalitis, occur less frequently and up to 30% of infections are asymptomatic or display only non-specific respiratory symptoms. Furthermore, reinfection with mumps has been confirmed in serological studies (2,3) and a significant portion of adult sudden sensorineural deafness is reportedly related to MuV infection (4).

In Japan, 5 mumps vaccines (genotype B) are currently licensed, and single dose is distributed on a voluntary basis (5), with a coverage rate of approximately 23% (6). However, herd immunity level is insufficient to prevent epidemics; therefore, mumps outbreaks in children repeat every several years.

MuV is a member of the genus *Rubulavirus* of the family *Paramyxoviridae*. Eight proteins, the nucleocapsid (N), V, phospho (P), matrix (M), fusion (F), small hydrophobic (SH), hemagglutinin-neuraminidase (HN), and large (L) proteins, are coded by 7 genes. The SH gene sequence is highly variable and is, therefore, used for MuV genotyping (7–9).

Fukagawa city (coordinates: 43°43′N, 142°2′E) is a relatively isolated rural region in Hokkaido, Japan, with a population of approximately 23,000. From April to August 2010, a total of 212 patients were diagnosed with mumps in the pediatric department of our hospital. In January 2011, 3 additional patients were diagnosed; the age range of these 215 patients was 1–19 years, and they had no significant medical history of immunodeficiency, malignancy, or developmental delay. Diagnosis was made on the basis of typical clinical manifestations and/or laboratory test results of serological study or amylase in the serum or urine. Seventeen patients were hospitalized; of these, 10 were diagnosed with meningitis, 3 with orchitis, and 1 with hepatitis. Two unrelated patients developed audiometry-con-

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          1
13.10     AAGAATGAAT CTCATGGGGT CGTAACGTCT CGTGACCCTG CCGTTGCACT ATGCCGGCGA
14a.10    ---------- ---------- ---------- ---------- ---------- ----------
14b.10    ---------- ---------- ---------- ---------- ---------- ----------
14c.10    ---------- ---------- ---------- ---------- ---------- ----------
16.10     ---------- A--------- ---------- ---------- ---------- ----------
18.10     ---------- ---------- ---------- ---------- ---------- ----------
30.10     ---------- ---------- ---------- ---------- ---------- ----------
32.10     ---------- ---------- ---------- ---------- ---------- ----------
06.11     ---------- ---------- ---------- ---------- ---------- ----------
         61
13.10     TCCAACCTCC GTTATACCTC ACATTTCTAT TGCTAATTCT TCTTTATCTG ATCATAACTT
14a.10    ---------- ---------- ---------- ---------- ---------- ----------
14b.10    ---------- ---------- ---------- ---------- ---------- ----------
14c.10    ---------- ---------- ---------- ---------- ---------- ----------
16.10     ---------- C--------- ---------- ---------- ---C-C---- ----------
18.10     ---------- ---------- ---------- ---------- ---------- ----------
30.10     ---------- ---------- ---------- ---------- ---------- ----------
32.10     ---------- ---------- --G------- ---------- ---C------ ----------
06.11     ---------- ---------- ---------- ---------- ---------- ----------
        121
13.10     TGTATGTCTG GATTACATTA ACCATCACTT ATAAGACTGT GGTGCGACAT GCAGCACTGT
14a.10    ---------- ---------- ---------- ---------- ---------- ----------
14b.10    ---------- ---------- ---------- ---------- ---------- ----------
14c.10    ---------- ---------- ---------- ---------- ---------- ----------
16.10     ---------- --------C- ---------- ---------- ---------- ----------
18.10     ---------- ---------- ---------- ---------- ---------- ----------
30.10     ---------- ---------- ---------- ---------- ---------- ----------
32.10     ---------- ---C---CC- --------CC -C-------- ---------- ----------
06.11     ---------- ---------- ---------- ---------- ---------- ----------
        181
13.10     ACCAGAGATC CTACTTTCAC TGGAGTTTCG ATCACTCACC CTAGGAAGAT CTCCAGTTAG
14a.10    ---------- ---------- ---------- ---------- ---------- ----------
14b.10    ---------- ---------- ---------- ---------- ---------- ----------
14c.10    ---------- ---------- ---------- ---------- ---------- ----------
16.10     ---------- --T------- ---------- ---------- ---------- ----------
18.10     ---------- ---------- ---------- ---------- ---------- ----------
30.10     ---------- ---------- ---------- ---------- ---------- ----------
32.10     ---------- ---------- ---------- ---------- ---------- ----------
06.11     ---------- ---------- ---------- ---------- ---------- ----------
        241
13.10     GACAAGTCCC AATCCATCAT GCAAGAACAA TCTGCATTTG AATAATGCCG TTCAATCATG
14a.10    ---------- ---------- ---------- ---------- ---------- ----------
14b.10    ---------- ---------- ---------- ---------- ---------- ----------
14c.10    ---------- ---------- ---------- ---------- ---------- ----------
16.10     ---------- ---------- ---------- --C------- ---------- ----------
18.10     ---------- ---------- ---------- ---------- ---------- ----------
30.10     ---------- ---------- ---------- ---------- ---------- ----------
32.10     ---------- ---------- ---------- ---------- ---------- ----------
06.11     ---------- ---------- ---------- ---------- ---------- --A-------
        301
13.10     AGACATAAAG AAAAAA
14a.10    ---------- ------
14b.10    ---------- ------
14c.10    ---------- ------
16.10     ---------- ------
18.10     ---------- ------
30.10     ---------- ------
32.10     ---------- -G----
06.11     ---------- ------
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Fig. 1. Nucleotide sequences of SH gene detected in this study in chronological order. Left column shows week and year part of strain names. From top to bottom, AB725762.1, AB725761.1, AB725764.1, AB725766.1, AB725763.1, AB725760.1, AB725759.1, AB725765.1, and AB725767.1. Numbers above row denote nucleotide position. Underlined strains are not identical to index sequence.

*Corresponding author: Present address: Department of Pediatrics, Asahikawa Medical University, Midorigaoka E 2Jo, Asahikawa 078-8510, Japan. Tel: +81-166-68-2481, Fax: +81-166-68-2489, E-mail: okaji5p@asahikawa-med.ac.jp

firmed unilateral hearing loss as a sequela. One of these patients who developed hearing loss was positive for meningitis despite a vaccine history 9 years prior to the episode. According to this patient's medical record, Torii strain vaccine was administrated. On admission, anti-mumps IgG and IgM titers were measured using an EIA mumps kit (Denka Seiken, Tokyo, Japan), in which the MuV Enders strain was used as an antigen. The IgG titer (reference range: positive at a titer of 4 or more) was 13.7, and the IgM titer (reference range: positive at a titer of 1.2 or more) was below the detectable limit. The other patient was not vaccinated but did not show clinical signs of meningitis. Because 2 patients with hearing loss were observed, virological analysis of several stored samples (13 CSF's and 3 throat swabs) was performed to discern any underlying factors. All analyses were anonymously performed and complaint with the national guidelines for clinical research.

Nucleic acid detection and genotyping of MuV were performed as described previously (7). In brief, using a standard technique, the SH gene was RT-PCR amplified from CSF or throat swab samples after RNA isolation (Roche Diagnostics, Tokyo, Japan), using the primer pair "tcaagtagtgtcgatgatctc" and "aggtggcatt gtctgacattg" corresponding to the nucleotides 6130–6150 and 6656–6636, respectively, of NC_002200.1. A specific 526-bp band was amplified and sequenced. Multiple sequence alignment and phylogenetic analysis by the neighbor-joining method were performed using GENETYX-MAC (GENETYX Co., Tokyo, Japan). Nine of 13 CSF samples were successfully analyzed (GenBank accession numbers, AB725759.1–67.1), but none of the throat swab samples were suitable for analysis.

The six samples collected in April, May, and August 2010 had identical sequences, while the other 3 samples collected in April, and August 2010 and January 2011 revealed slightly different sequences (Fig. 1). The latter sequences were detected at either the beginning or end of the outbreak. All of these sequences were determined as genotype G by phylogenetic analysis (Fig. 2). None of the samples from the 3 vaccinated patients were successfully analyzed due to amplification failure. Nonetheless, our success rate was comparable with that of previous reports (9,10).

Mumps outbreaks, even among vaccinated populations, have recently been reported worldwide, including in the UK, the US, the Netherlands, and Canada (10–14). In the present outbreak, we detected MuV genotype G, which has been the predominant genotype commonly detected in recent outbreaks. The majority of MuV strains we detected were identical at the SH

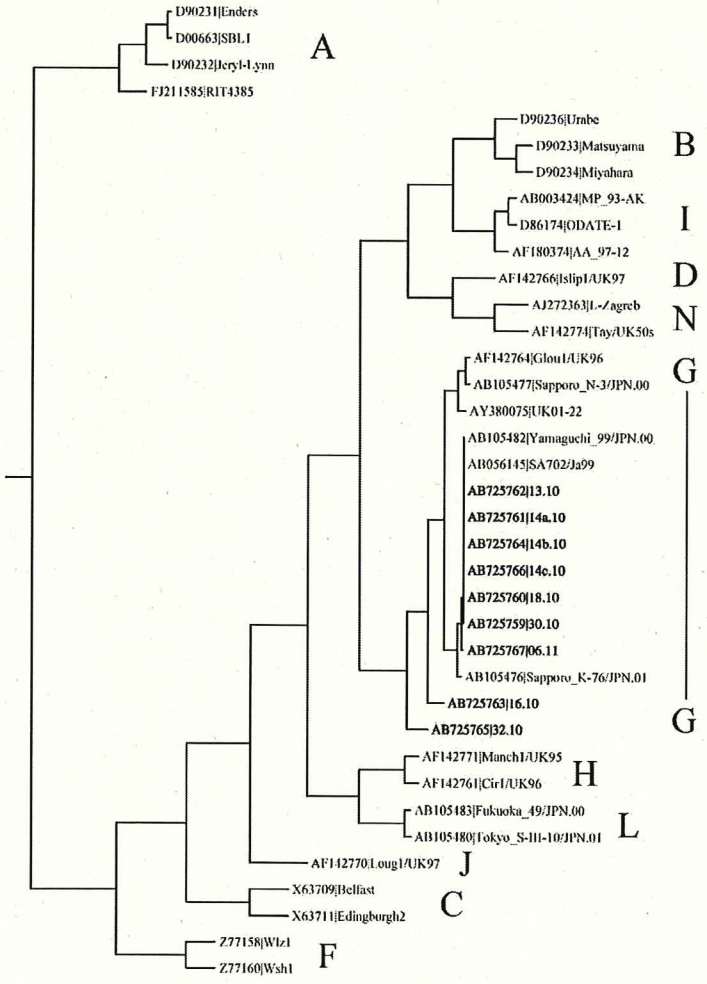

Fig. 2. Phylogenetic analysis of selected MuV strains. Strains detected in this study are in boldface. Capital letters denote MuV genotype.

gene level to those previously detected in geographically and chronologically distant areas of Yamaguchi (AB105482.1) and Saitama (AB056145.1), Japan (15,16). Although not identical, 2 other MuV genotype G strains (AB105476.1–77.1) were isolated in Sapporo, Japan, which is located approximately 100 km from Fukagawa on the same island. One finding of interest is that one strain isolated in the UK (AF142764.1) was phylogenetically similar to the strains we detected.

MuV genotypes H and D were detected in South Korea (17) and the Netherlands (18), respectively. In the present study, only genotype G was detected, with minor variations observed at the beginning and end of the outbreak. A short-time survey of vaccine coverage in the Fukagawa area revealed a rate of approximately 30%, which was slightly higher than the nationwide rate. Almost 30% of patients in this study were vaccinated (data not shown). Overall, the clinical courses of the vaccinated patients were mild, except for the patient who developed hearing loss. The anti-mumps serological study suggested secondary vaccine failure rather than primary vaccine failure.

The incidence of hearing loss in mumps patients is reportedly 0.001%–1.0% (19–21). The rate in the present study was 0.93% (2/215), which was comparable with the highest incidence thus far reported (11). Another study from Japan also reported a high rate of hearing problems. There are several other reported cases of hearing loss after mumps vaccination (20,22,23). We have been unable to conduct a survey to assess sensorineural deafness in this area.

Genotype mismatch between vaccine and epidemic virus strains has been often discussed in association with several outbreaks (24,25), demonstrating that antibodies against a vaccine strain failed to prevent infection by other strains. On the other hand, some publications have reported that antibodies raised against vaccine strains were able to neutralize other genotype strains (26,27). Antibodies raised against the Enders strain (genotype A) can be successfully employed in detection kits. We observed that a MuV genotype B vaccine failed to prevent the manifestations of mumps (genotype G) in a significant portion of a population, although the vaccine was able to ameliorate the clinical course of the disease. Our current mumps vaccine scheme may not be optimal. We were unable to discern any significant factors in either the host or viral side in this mumps outbreak with a high sequela rate. Further elucidation of the virological and epidemiological backgrounds of this occurrence was beyond the scope of this study.

Conflict of interest None to declare.

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