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Genotyping of Mumps Virus Strains Detected in Kobe City from 1991 to 2012

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Mumps is an acute infectious viral disease characterized by inflammation of the salivary glands. Complications associated with this disease include aseptic meningitis, deafness, encephalitis, orchitis, and oophoritis (1). The mumps virus (MuV) belongs to the genus *Rubulavirus* of the family *Paramyxoviridae* (2). Based on genetic variability in small hydrophobic (SH) genes, MuVs have been currently confirmed to have 12 genotypes, designated as A to N (excluding E and M) (3). Molecular characterization of MuV is an important component of mumps surveillance because it can facilitate the identification of the transmission route of the virus as well as distinguish between wild-type and vaccine strains.

In Japan, large mumps epidemics have recurred at intervals of 4–5 years (4). In Kobe City, Japan, we conducted a survey of MuV infection and genotyped MuV strains from 1991 to 2012. Clinical specimens were collected from patients with infectious parotitis, aseptic meningitis, and encephalitis during routine surveillance for infectious agents. All samples were transferred to

the Kobe Institute of Health for laboratory diagnosis.

Clinical samples of saliva, throat swabs, or cerebrospinal fluid (CSF) were inoculated with Vero-E6 or FL cells to recover viruses. MuV isolation was confirmed by a direct immunofluorescence test using an antibody specific for MuV (ViroStat, Portland, ME, USA). Some specimens were directly analyzed by nested RT-PCR, as recommended by Jin et al. (5). MuV was detected in 138 parotitis and 6 aseptic meningitis cases and 1 encephalitis case (Table 1). In this study, the entire SH gene comprising 316 nucleotides was sequenced from a total of 85 MuV strains and genotyped according to previously reported methods (Table 1) (3,5,6). The breakdown is 81 recovered viruses (76 parotitis cases, 4 aseptic meningitis cases and 1 encephalitis case) and 4 MuV genes by RT-PCR of the SH region (2 parotitis cases, 2 aseptic meningitis cases). These 85 analyzed patients consisted of 44 males and 41 females, with a median age of 6 years (range, 1–36 years). Of these 85 patients, 16 (18.8%) were vaccinated, while 69

Table 1. Mumps virus detection and genotype, 1991–2012 (by year, diagnosis, and specimen)

Year of sampling	Clinical diagnosis	No. of analyzed cases ¹⁾	Specimen	Genotype					No. of detected cases
				B	F	G1	G2	H	
1991	Aseptic meningitis after implementation of vaccination	1	CSF	1					1
1996	Infectious parotitis	1	Throat swab	1					1
1998	Aseptic meningitis	1	CSF		1				1
2001	Aseptic meningitis	1	CSF	1					1
2004	Infectious parotitis	5	Saliva, 4; throat swab, 1				5		6
2006	Infectious parotitis	31	Saliva				31		86
2009	Encephalitis	1	CSF				1		1
2010	Infectious parotitis	36	Saliva, 35; throat swab, 1			6	14	16	40
2011	Aseptic meningitis after implementation of vaccination	1	CSF	1					1
2011	Aseptic meningitis	1	Throat swab			1			1
2011	Infectious parotitis	3	Saliva				3		3
2012	Aseptic meningitis	1	Saliva			1			1
2012	Infectious parotitis	2	Saliva			2			2
Total		85		4	1	10	54	16	145

¹⁾: For detection of 2006 were from the same region, part of the detected strains were analyzed. In the other years, strain of storage were analyzed.

CSF, cerebrospinal fluid.

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Fig. 1. Phylogenetic tree based on the 316 nucleotides of the SH gene of mumps virus. A total of 85 strains from Kobe and previously published sequences were examined. ●, mumps sequence obtained in the present study. Naming of mumps viruses is MuVi(s)/Kobe.JPN/sample number.year[genotype]. MuVs, sequence derived from RNA extracted from clinical material. MuVi, sequence derived from RNA extracted from mumps virus isolate in cell culture.

were either not vaccinated or had an unknown or undocumented vaccination status.

The gene sequences were deposited with DNA Data Bank of Japan (DDBJ) (accession no. AB904527–AB904611).

A phylogenetic tree of the 316 nucleotides of the SH gene was constructed using the neighbor-joining method from ClustalW (version 2.1) of DDBJ. Fig. 1 illustrates the phylogenetic relationships between strains sequenced in this study and strains previously reported. In our analysis, genotype G was divided into 2 subgenotypes, G1 and G2, which was also proposed by Palacios et al. (7). A total of 4, 1, 10, 54, and 16 strains were classified as genotypes B, F, G1, G2, and H, respectively (Table 1).

Four strains, MuVi/Kobe.JPN/910244.91, MuVi/Kobe.JPN/960136.96, MuVi/Kobe.JPN/010414.01, and MuVs/Kobe.JPN/110227.11, obtained in 1991, 1996, 2001, and 2011, respectively, were classified as genotype B. The strains MuVi/Kobe.JPN/910244.91 and MuVs/Kobe.JPN/110227.11 were detected in patients who had developed aseptic meningitis after they were vaccinated. The nucleotide sequence of the SH region of both these strains was consistent with that of MuVi_Urabe_AM-9.JPN_wk.67 (AB000388) and MuVi_Hoshino.JPN_vaccine (AB470486), respectively. This finding suggests that the strains MuVi/Kobe.JPN/910244.91 and MuVs/Kobe.JPN/110227.11 are derived from the Urabe and Hoshino vaccine strains, respectively. No sequence variation was detected in the SH region of the MuVi/Kobe.JPN/010414.01 and MuVi_Torii.JPN_vaccine strains (6). Although the source patient was vaccinated, the date of vaccination is unknown. These results suggest that the development of aseptic meningitis is a result of vaccination. However, it cannot be concluded that the MuVi/Kobe.JPN/010414.01 strain was derived from the Torii vaccine strain based on the nucleotide sequence of the SH region alone. It is also possible the vaccine strain used for vaccination has spread. On the other hand, of 316 nucleotides of the SH gene, the nucleotide sequence of the MuVi/Kobe.JPN/960136.96 strain showed differences in 3 nucleotides (1%) compared with that of the MuVi_Himeji89.JPN_wk.00 (JQ945269) strain. This patient was not vaccinated. Wild-type strains of genotype B have not been detected in Kobe City since 2000.

The strain MuVi/Kobe.JPN/980168.98, obtained from an unvaccinated patient in 1998, was classified as genotype F by the phylogenetic tree of SH genes. The SH gene sequence of this strain was compared with that of MuV strains in the GenBank database, and the nucleotide identity score was highest (94%) among the strains detected in various parts of China since 2000 (8). However, the origin of the MuVi/Kobe.JPN/980168.98 strain is unknown from the GenBank database.

Strains of genotype G (G1 and G2) were frequently detected during the study period, and genotype G accounted for 78% of strains detected since 2000. Genotype G has been subdivided into 2 subgenotypes, G1 and G2, by Palacios et al. (7). In Kobe City, only subgenotype G2 strains were circulated from 2004 to 2009, however, both subgenotypes G2 and G1 and genotype H were detected during the 2010 outbreak. Moreover, subgenotypes G1 and G2 were found to coexist in 2011

as well. Ten Kobe strains of subgenotype G1, obtained from 2010 to 2012, were found to be closely related to each other, with a single base nucleotide substitution. These sequences were compared with the MuV SH sequences in the GenBank database, and the nucleotide identity score was highest (99–100%) among strains detected in Guam, Hawaii, and Yokohama (9) in 2010.

Fifty-four strains of subgenotype G2 in the present study were related to each other, with the SH nucleotide sequences similarity of 97–100%. They were also closely related to many Japanese strains detected since 2000 that have been deposited in GenBank. The Kobe strains of subgenotypes G1 and G2 were related to each other, with 94–96% nucleotide similarity.

Sixteen strains of genotype H, which were detected in 2010, were closely related to each other, with a single base nucleotide substitution in the SH gene sequences. They were related to Hong Kong strains detected in 2008 and 2011, with 98% nucleotide similarity. Genotype H has been reported to be found in Japan in 1997 (10), suggesting that this genotype H has been circulating in East Asia and Japan since 2000.

Although mumps is preventable by vaccination, mumps outbreaks within highly vaccinated populations have recently been reported worldwide (11–16). In the present study, the percentages of vaccinated patients with G1, G2, and H, which are the major genotypes detected since 2000, were 10%, 15%, and 25%, respectively. In Japan, strains of genotype B have been used as vaccine seed viruses since the 1980s (4). However, the immunization coverage against mumps in Kobe City is not clear. The effect of a vaccine cannot be addressed by this study alone.

Mumps vaccine has been introduced into routine immunization in 117 countries globally. Japan is the only developed country conducting mumps immunization on a voluntary basis (4). Mumps surveillance, including genotyping, is still more important while considering introduction of routine immunization.

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Conflict of interest None to declare.

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