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Phylogenetic Analysis of Nucleoprotein (*N*) Gene of Measles Viruses Prevalent in Okinawa, Japan, during 2003 - 2007

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Outbreaks of measles still occur every 5 to 7 years in Japan (1,2). In Okinawa Prefecture, two large measles outbreaks occurred during 1998 - 2001 (3), and both were attributed to the population's low immunity level (about 60 to 70%). Unfortunately, these outbreaks resulted in the deaths of 9 children. Therefore, to achieve a measles-free population, the Okinawa Prefecture Office and the Okinawa Medical Association have aggressively promoted measles immunizations, and to date have covered more than 95% of the children in the prefecture. In addition, to find all patients with measles, the prefecture office enforced sentinel surveillance systems, including laboratory confirmation of measles. As a result, the number of patients with measles rapidly decreased in the prefecture. However, a small number of patients with measles, fewer than 20, have been reported. To better understand the molecular epidemiology of the recently prevalent measles viruses (MVs), we performed sequence and phylogenetic analyses of their nucleoprotein (*N*) genes.

Throat swabs and blood samples were collected and served as test specimens in this study. All measles patients develop typical clinical symptoms: high fever, cough, conjunctivitis, Koplik's spots on the buccal mucosa, and a rash initially on the face, torso, upper neck, and back, spreading eventually to the hands and feet. Virus RNA was extracted from the samples using a QIAamp Viral RNA Mini kit (Qiagen, Valencia, Calif., USA) and suspended in DNase/RNase-free water. Following RNA extraction, reverse transcriptase-polymerase chain reaction (PCR) and nested PCR were performed as previously described (4). Amplicons were purified using a QIAquick PCR Purification kit (Qiagen), and nucleotide sequences were determined by direct sequencing (4). The nucleotide sequences of the partial *N* gene of MVs (385 bp) were analyzed phylogenetically using Molecular Evolutionary Genetics Analysis (MEGA) software version 4 (5). Evolutionary distances were estimated using Kimura's two-parameter method, and phylogenetic trees were constructed using the neighbor-

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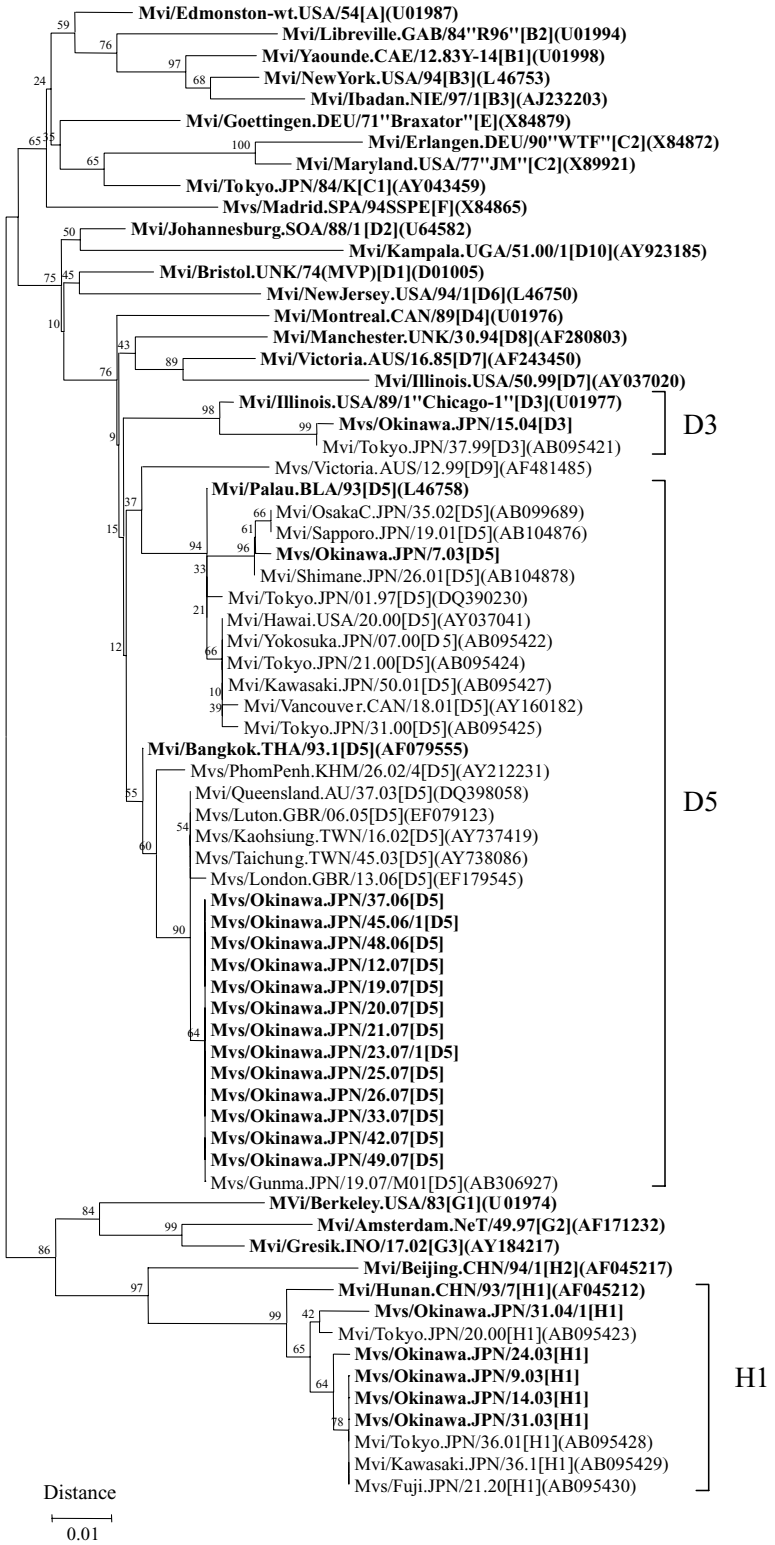

Fig. 1. Phylogenetic tree constructed based on the nucleotide protein (*N*) gene sequences of various strains of the measles virus. Evolutionary distance was calculated using Kimura's two-parameter method, and the tree was plotted using the neighbor-joining method. Numbers at each branch indicate the bootstrap values of the clusters supported by that branch. Numbers in parentheses are GenBank accession numbers. Genotypes of the reference and present strains are indicated in bold.

joining (NJ) method (6). Each tree's reliability was estimated using 1,000 bootstrap replications.

Table 1 summarizes the cases, MV genotypes, and epidemiologic data. Of 20 MV strains, 1, 14, and 5 strains had genotypes D3, D5, and H1, respectively. The strains detected in 2003 had genotypes D5 (1 strain) and H1 (4 strains), and those detected in 2004 had genotypes D3 (1 strain) and H1 (1 strain). In 2005, no MV strains were detected because no patients with measles were reported in the surveillance project. The 13 MV strains detected in 2006 and 2007 had only genotype D5. However, 15 cases were sporadic in the two outbreaks, each of which involved more than 10 patients. Epi-

Table 1. Summary of epidemiologic and measles virus data

Case no.	Month, year	Activity[1)]	Epidemiology-based infection source	Virus	Genotype
1	Feb, 2003	Sporadic case	Unknown	MVs/Okinawa.JPN/7.03	D5
2	Feb, 2003	Sporadic case	Unknown	MVs/Okinawa.JPN/9.03	H1
3	Apr, 2003	Sporadic case	Unknown	MVs/Okinawa.JPN/14.03	H1
4	Jun, 2003	Sporadic case	Kanto area	MVs/Okinawa.JPN/24.03	H1
5	Jul, 2003	Sporadic case	Unknown	MVs/Okinawa.JPN/31.03	H1
6	Apr, 2004	Outbreak: 5 cases	Unknown	MVs/Okinawa.JPN/15.04	D3
7	Jul, 2004	Outbreak: 8 cases	Unknown	MVs/Okinawa.JPN/31.04/1	H1
8	Sep, 2006	Outbreak: 12 cases	Kanto area	MVs/Okinawa.JPN/37.06	D5
9	Nov, 2006	Outbreak: 4 cases	Kanto area	MVs/Okinawa.JPN/45.06/1	D5
10	Dec, 2006	Sporadic case (1)	Kanto area	MVs/Okinawa.JPN/48.06	D5
11	Mar, 2007	Sporadic case	Kanto area	MVs/Okinawa.JPN/12.07	D5
12	May, 2007	Sporadic case	Kanto area	MVs/Okinawa.JPN/19.07	D5
13	May, 2007	Sporadic case (1)	Kyushu area	MVs/Okinawa.JPN/20.07	D5
14	May, 2007	Sporadic case (1)	Kanto area	MVs/Okinawa.JPN/21.07	D5
15	Jul, 2007	Sporadic case	Kanto area	MVs/Okinawa.JPN/23.07/1	D5
16	Jul, 2007	Sporadic case	Kanto area	MVs/Okinawa.JPN/25.07	D5
17	Jul, 2007	Sporadic case	Kanto or Shikoku area	MVs/Okinawa.JPN/26.07	D5
18	Aug, 2007	Sporadic case	Kanto area	MVs/Okinawa.JPN/33.07	D5
19	Oct, 2007	Outbreak: 10 cases	Kanto area	MVs/Okinawa.JPN/42.07	D5
20	Dec, 2007	Sporadic case	Kyushu area	MVs/Okinawa.JPN/49.07	D5

[1)]: For sporadic case, the number of cases that spread is indicated in parentheses.

demiologic investigation showed that most of the patients were high school students and adults who had traveled to other areas of Japan (Kanto, Shikoku, or Kyusyu). Thus, most of the patients might have been infected in areas outside Okinawa Prefecture. We constructed a phylogenetic tree based on the *N* gene of the presently isolated MV strains, including reference strains (Figure 1). All MV genotypes (D3, D5, and H1) were located in the same cluster as the strains detected from other areas of Japan (Sapporo, Gunma, Tokyo, Osaka, and Shimane). In particular, the present strains with genotype D5 were highly homologous. These results suggest that the present strains and other local strains were closely related genetically. Moreover, various MVs with the D5 genotype detected from other areas of Japan in 2007 (Gunma Prefecture and the Tokyo metropolitan area) were highly homologous (7).

Okinawa Prefecture is located in a subtropical area and consists of hundreds of Ryukyu Islands with many famous sightseeing spots; the prefecture draws approximately 6 million visitors per year. At present, only a small number of the prefecture's population may be susceptible to measles because of the regular and widespread measles immunization program. However, since the disease is highly contagious in humans (8,9), up-to-date information on the epidemic status of measles in the prefecture and in Japan as a whole is needed because of its rapid spread from one area to another.

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