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Original Article

Measles Epidemics Among Children in Vietnam: Genomic Characterization of Virus Responsible for Measles Outbreak in Ho Chi Minh City, 2014

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ABSTRACT

Background: Measles remains poorly controlled in Southeast Asia, including Vietnam.
Objectives: The aim of this study was to characterize genes of virus responsible for a measles outbreak among children in Vietnam in 2014.
Study design: Throat swab samples were collected from 122 pediatric patients with suspected measles. Furthermore, peripheral blood mononuclear cells (PBMCs) from 31 of these cases were also collected. Measles virus (MV) RNA was obtained directly from the clinical specimens, amplified by PCR, and then the N and H genes were sequenced.
Results: MV RNA was detectable in throat swabs from all 122 patients tested. Positive-strand viral RNA, which indicates the intermediate replicative form of MV, was also detected in PBMCs from all 31 cases from whom these cells were collected. One hundred and eighteen strains with the N gene were obtained by RT-PCR and sequenced. Using phylogenetic analysis with measles reference sequences, all of the Vietnamese strains were found to be genotype D8. However, all strains formed a distinct cluster within the genotype D8 group (D8-VNM) suggesting their own lineage. This distinct cluster was supported by a branch with a 99% bootstrap value and 3.3% nucleotide divergence in the N-450 region of the N gene from the D8 reference strain. Notably, all of the D8-VNM variant strains represented unique amino acid sequences consisting of R442, S451 and G452 in the N-450 region of the N gene.
Conclusions: Measles viruses responsible for outbreaks in Southern Vietnam belonged to a genotype D8 variant group which had unique amino acid sequences in the N gene. Our report provides important genomic information about the virus for measles elimination in Southeast Asia.

1. Introduction

Although measles is now a vaccine-preventable disease, it remains one of the leading causes of death in children, especially in resource-poor regions of the world (Moss and Griffin, 2006; Nandy et al., 2006; Wolfson et al., 2009). Therefore, it is very important to control the measles in order to improve child health worldwide. Measles is a highly contagious respiratory viral disease characterized by the appearance of fever and a rash that can be very serious. Although improvements have been made to the control of measles worldwide by the WHO, large-scale outbreaks have recently been observed, particularly in developing countries including those of Southeast Asia (Simons et al., 2012; Perry et al., 2014).

The measles virus (MV) is a single-stranded, negative-sense RNA virus, belonging to the genus *Morbillivirus*, family *Paramyxoviridae*. It consists of 15,894 nucleotides (nt) encoding six structural proteins. Although MV is thought to be serologically monotypic, high genetic variability in the N and H genes has been known. In particular, the 450 nt C-terminus of the N gene is the target area for MV genotyping recommended by the WHO (Expanded Programme on Immunization (EPI), 1998; Anon., 2005, 2006, 2012).

Sniadack et al. reported on large-scale measles epidemics in Vietnam during 2008–2010 (Sniadack et al., 2011). However, detailed molecular-based epidemiologic studies on MV circulating in Vietnam have not yet appeared. Genetic information of the viruses has been used in molecular epidemiologic studies to identify the transmission pathways and the

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route of infection. Therefore, genetic surveillance of the MV has provided a means to measure the success of the measles control program (Liffick et al., 2001).

Here we report on genomic characterization of a large-scale measles outbreak in Vietnam in 2014. In this study, we focused on the N-450 region to characterize the MV circulating in Vietnam and succeeded in determining that the virus has a unique genomic characterization.

2. Materials and Methods

2.1. Patient Samples

Throat swab samples were obtained from 122 pediatric patients (aged from 2 months to 14 years old; 46 cases of less than one year of age and 76 cases older than 1 year; 70 boys and 52 girls) with suspected measles having symptoms of severe rash typical of measles, high fever for 2–3 days, cough, coryza, red eyes and Koplik's spot at the Children Hospital No. 1, Ho Chi Minh City, Vietnam that have occurred between February to March 2014. Furthermore, peripheral blood mononuclear cells (PBMCs) from 31 of these cases were also collected. The clinical samples were collected within 8 days after the onset of rash. All clinical samples were stored frozen at −80 °C until use.

2.2. Ethical Approval

An approval for this study was obtained from the Children Hospital No. 1 Research Ethics Board. Fully informed, written consent was obtained from parents or legal guardians of all of the children patients that participated in this study.

2.3. Separation of PBMCs

PBMCs were separated by Ficoll–Isopaque (Nacalai Tesque, Kyoto, Japan) density-gradient centrifugation, and washed three times with PBS (pH 7.4) to remove free virus in circulating blood. The cell pellet obtained was resuspended in 1 mL of RPMI-1640 media (Life Technologies, Rockville, MD, USA) containing 10% dimethyl sulfoxide and frozen in liquid nitrogen until use for RNA extraction.

2.4. Determination of MV RNA by Real-time PCR

For screening of MV RNA determination, the real-time PCR was used with primers designed from the N gene: 5′-TTATTTGTGGAGTCTCCAGGTC-3′ (MV342F; sense, nt 342–363), 5′-CCTCATCCTCCATGTTGGTAC-3′ (MV486R; antisense, nt 486–466), and FAM-5′-AGAGGATCACCGATGACCCTGACG-3′-BHQ1 (labeled probe; nt 373–396). Nucleotide position is based on measles virus vaccine strain Changchun-47 (accession #: FJ416068).

Total RNA was extracted from throat swab and PBMC samples using the magnetic bead-based genomic RNA/DNA purification method (^{NK}DNARNAprep-MAGBEAD kit, Nam Khoa-Biotek, Ho Chi Minh, Vietnam). Viral cDNA was synthesized and amplified by the same method as reported previously (Pham et al., 2013). The sensitivity of this assay was 100 copies of MV/mL.

2.5. Detection of MV RNA by RT-PCR and Sequence

The sequences of primers used for RT-PCR to detect MV RNA are as follows: Tag-MV1108F: 5′-gtaaaacgacggccagtGCTATGCCATGGGAGTAGGAGTGG-3′ (sense, nt 1108–1131; tag sequences of M13 universal primer are shown by lowercase letters) and Tag-MV1697R: 5′-tattta ggtgacactatagGGCCTCTCGCACCTAGTCTAG-3′ (antisense, nt 1697–1677; tag sequences of SP6 universal primer are shown by lowercase letters) which can yield a 590-bp amplicon in the N gene containing the WHO-recommended sequence window (N-450 region; nt 1233–1682). Furthermore, an entire sequence of the H gene was also amplified by the nested RT-PCR with the primer combination of MV6963F: 5′-GTGTCTTGGAGGRTTGATAGGGA-3′ (sense, nt 6963–6985) and MV9388R: 5′-CGGTGCTTGATGTTCTGACAC-3′ (antisense, nt 9388–9368) for the outer primer pairs (2426 bases), and MV7081F: 5′-ACATCAAAATCYTATGTAAGGTC-3′ (sense, nt 7081–7103) and MV9292R: 5′-ATCGGGCTATCTAGGTGAAC-3′ (antisense, nt 9292–9273) for the inner primer pairs (2212 bp).

Viral cDNA obtained for the real-time PCR was amplified using QIAGEN Multiplex PCR Kit (Qiagen Inc., Chatsworth, CA, USA). For N gene amplification, PCR conditions included pre-incubation at 95 °C for 15 min, followed by 40 cycles consisting of 95 °C for 30 s, 65 °C for 30 s and 72 °C for 1 min. For H gene amplification, MV cDNA was amplified by the nested PCR with the following conditions: 35 cycles consisting of 95 °C for 30 s, 50 °C for 30 s and 72 °C for 2 min 30 s for the 1st PCR and 95 °C for 30 s, 55 °C for 30 s and 72 °C for 2 min 30 s for the 2nd PCR. Obtained amplicons were analyzed and subjected to direct sequencing by the same method as reported previously (Pham et al., 2013).

2.6. Detection of Positive-strand MV-RNA in PBMCs

To detect positive-strand of MV-RNA, viral cDNA was synthesized with MV-specific antisense primer (MV486R) using iScript reverse transcriptase with the following condition: 25 °C for 5 min, 42 °C for 30 min and 95 °C for 5 min. Obtained viral cDNA was determined by the real-time PCR with the same condition as described above.

2.7. Characterization of MV Gene by Phylogenetic Analysis

For phylogenetic analysis, obtained nucleotide sequences were multiple aligned with CLUSTAL W, version 1.81 as reported previously (Pham et al., 2013). The distance matrix of the nucleotide substitutions among each sequence was estimated by the eight-parameter method and phylogenetic trees were constructed by the neighbor-joining method from the matrix. These procedures were computed with Phylo_win, version 1.2 on a DEC alpha 2000 server, and the trees were drawn with TreeView, version 1.5.2. To confirm the reliability of the pairwise comparison and phylogenetic tree analysis, bootstrap resampling and reconstruction were carried out 1000 times. Bootstrap values greater than 60% were considered supportive of the observed groupings. In addition to our sequences, 28 reference strains recommended by the WHO and 142 strains of genotype D8 obtained from database including Measles Nucleotide Surveillance and GenBank were used as reference strains of known genotypes.

2.8. Accession Numbers Submitted to Database

Nucleotide sequence data of MV strains from 118 Vietnamese patients are available in the DDBJ/EMBL/GenBank databases under the accession numbers AB928085 to AB928202 for N gene and AB968375 to AB968382 for H gene.

3. Results

3.1. Clinical Findings

All pediatric patients with measles were recovered in the course of the transient. Clinical findings of 122 childhood measles patients were

Fig. 1. Phylogenetic tree generated by neighbor-joining analysis of genetic distances in the N-450 region of the N gene based on WHO-recommended sequence window. Tree was constructed with 28 reference strains from all genotypes recommended by WHO (1a) and 142 strains of genotype D8 obtained from database (1b). Because of limited space, the number of strains of D8-VNM isolates has been reduced in Fig. 1b. Vietnamese strains identified in this study are indicated in blue. WHO reference strains are indicated in italics. Bootstrap values of >60% are shown at the branch nodes.