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Rubella epidemic in Vietnam: Characteristic of rubella virus genes from pregnant women and their fetuses/newborns with congenital rubella syndrome

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ABSTRACT

Background: Rubella remains poorly controlled in Southeast Asia, including Vietnam.
Objectives: The aim of this study was to characterize rubella virus spread in Vietnam during 2011–2012.
Study design: Amniotic fluid, throat swab and placenta samples were collected from 130 patients (110 cases from pregnant women with suspected rubella and 20 cases from fetuses/newborns). Viral RNA was obtained directly from clinical specimens, amplified by PCR, and then the E1 gene containing 739 nucleotides recommended by the WHO to identify the viral genotypes was sequenced.
Results: By screening with real-time PCR, viral RNA was detectable in amniotic fluids from 103 out of 110 (93.6%) pregnant women with suspected rubella and in the throat swabs from all of 20 (100%) fetuses/newborns. In addition, viral RNA was also detected in the placenta from all cases of fetuses/newborns. All of 20 fetuses/newborns presented with congenital cataract. Twenty-four strains with the E1 gene were obtained by PCR. Using phylogenetic analysis with rubella reference sequences, all of the strains were found to be genotype 2B. Interestingly, 94% (30/32) of Vietnamese strains, including 9 strains from the database, formed an independent cluster within the genotype 2B suggesting that indigenous viruses are prevalent in this region.
Conclusions: Rubella virus identified in Vietnam belonged to the genotype 2B. Importantly, the infection rate of rubella virus in fetuses/newborns was 100% and all of them had congenital cataract. Our results indicate an establishment of rubella prevention in this area is an urgent task in order to improve maternal and child health.

1. Background

Rubella is an acute infectious disease that normally has a mild clinical course. However, infections during pregnancy, especially before week 12 of gestation, can cause severe birth defects known as congenital rubella syndrome (CRS).[1] Clinical signs of CRS include cataract, glaucoma, heart disease, loss of hearing, and pigmentary retinopathy. Therefore, it is very important to control the rubella in order to improve maternal and child health worldwide.

Rubella virus (RV) has no host other than humans and is thought to consist of a single serotype. However, information on the epidemiologic characteristics of the virus, such as antigenic variation, virulence, and phylogenetic relationship between circulating strains, is in short supply. In fact, there have been only a very few epidemiologic studies conducted in Southeast Asia.[2] For the prevention of CRS, rubella-containing vaccine has been used in vaccination programs worldwide, but not in many countries in the Asian continents.

The RV contains three structural polypeptides: envelope polypeptides E1 and E2 and capsid polypeptide (C). It has a single-stranded, positive-sense RNA of 9762 nucleotides as its genome. The 5′-terminal two-thirds of the genome encode the nonstructural polypeptides in a single open-reading frame, and the 3′-terminal one-third encodes the structural polypeptides in a single open-reading frame in the sequence of 5′-C-E2-E1-3′. The E1 glycoprotein is considered immunodominant in the humoral response induced against the structural proteins and contains neutralizing and hemagglutinating determinants.[3–7] A 739-nucleotide

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(nt) region within the E1 gene (nt 8731–9469) is accepted as the minimum amount of sequence information required for molecular epidemiological purposes and genotypes.[8,9] Therefore, we focused on the E1 gene for the molecular based epidemiologic study in RV sequences from Vietnamese patients. Although information about genotypic distribution is available for some countries, especially those pursuing elimination, data on the genomic characterization of RV are lacking in many countries, particularly in developing countries including Vietnam.

2. Objectives

The aim of this study was to carry out genomic characterization of RV detected in pregnant women and fetuses/newborns with CRS in Vietnam during the period from 2011 to 2012.

3. Study design

Clinical samples. Samples obtained from 110 pregnant women who were clinically suspected rubella (18–40 years old), 10 abortion fetus (19–25 weeks of age) and 10 newborns from suspected disease pregnant women. All pregnant women patients lived in Ho Chi Minh City and its environs in Vietnam. Clinical specimens were collected during the period from 2011 to 2012 and kept frozen at −80 °C until analyzed. Clinical specimens consisted of an amniotic fluid, throat swab (all from fetuses/newborns) and placenta obtained from patients at the Hung Vuong Hospital, Ho Chi Minh City, Vietnam. Samples obtained from 5 cases of fetuses/newborns overlap with their mothers tested in this study.

Determination of antibody to RV. RV-specific IgM antibody was determined in cord blood using the Elecsys Rubella IgM kit (Roche, Indianapolis, IN, USA).

Isolation of virus by tissue culture. To isolate viruses by tissue culture, amniotic fluids from 4 cases were inoculated into vero cells. The vero cells inoculated with clinical specimens were cultured at 37 °C for 7 days. The supernatant and cultured cells were used to obtain RV genome by PCR with the following methods.

Determination of RV gene by real-time PCR. For screening of RV RNA determination, the real-time PCR was used. All primers were designed from E1 gene of rubella virus. The sequences for primers and probe used for the real-time PCR were 5′-CAT CTG GAA TGG CAC ACA GC-3′ (rubella_tqF, sense, nt 8476–8495) and 5′-CTA CAA GCA GTA CCA CCC CAC-3′ (rubella_tqR, antisense, nt 8601–8581), and FAM/BHQ1-fluorescence labeled probe (5′-FAM-TGC ACC TTC TGG GCT GTC AAC GC-BHQ1-3′ (rubella_tqPr, sense, nt 8501–8523). Nucleotide position is based on rubella virus vaccine strain wistar RA 27/3 (accession # FJ211587). Briefly, total RNA was extracted from clinical specimens and cultured cells using the RNA extraction kit (^{NK}RNAPREP kit, Nam Khoa Biotek Co., Ho Chi Minh City, Vietnam). Viral cDNA was synthesized with mixture of random primer and oligo(dT) primer using iScript reverse transcriptase (Bio-Rad Laboratories, CA, USA) with the following condition: 25 °C, 5 min, 42 °C, 30 min and 85 °C, 5 min. Five microliters of cDNA product was placed in the real-time PCR buffer containing Platinum Taq (Invitrogen, Carlsbad, CA, USA) and amplified with the following condition: 95 °C, 3 min 30 s then 50 cycles consisting of 94 °C, 30 s and 60 °C, 1 min. The sensitivity of this real-time PCR method was 270 copies of RV/ml.

Detection of RV gene by nested RT-PCR and sequence. For sequencing and genotyping of the RV gene, using the amniotic fluid and the throat swab, nested RT-PCR was carried out with primers designed from the E1 gene of RV. A 908-bp fragment was amplified by the primer combination of RV8633F/RV9540R in the E1 gene containing the WHO-recommended sequence window (739 bp; nt 8731–9469). The sequences of all primers used in this study are listed in Table 1. As shown in Table 1, we used method 1 which can yield a 908-bp single fragment by PCR. Alternatively, method 2 consisting of two fragments (485 bp and 596 bp, respectively) was also used when method 1 was not successful. Viral RNA was heated to 95 °C for 1 min then cooled on ice immediately before adding pre-mixture for cDNA synthesis. The cDNA was synthesized by the same method as mentioned above. Five microliters of cDNA was placed in PCR buffer containing Platinum Taq and 360 GC Enhancer (20% v/v; Applied Biosystems, Foster city, CA, USA) due to the RV genome having an extremely high GC-rich sequence. Amplification conditions included pre-incubation at 95 °C, 5 min, followed by 40 cycles consisting of 94 °C, 30 s, 60 °C, 30 s and 72 °C, 1 min for the 1st round PCR and 94 °C, 30 s, 65 °C, 30 s and 72 °C, 1 min for the 2nd round PCR.

Amplicons were analyzed by electrophoresis on 1% agarose gels staining with ethidium bromide and recovered using the promega Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA). The amplicons were subjected to direct sequencing using the ABI PRISM™ Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems), on a capillary sequencer model 3130 (Applied Biosystems).

Characterization of RV gene by phylogenetic analysis. For phylogenetic analysis, obtained nucleotide sequences were multiple aligned with CLUSTAL W, version 1.81. The distance matrix of the nucleotide substitutions among each sequence was estimated by the eight-parameter method[10] and phylogenetic trees were constructed by the neighbor-joining method[11] from the matrix. These procedures were computed with Phylo_win, version 1.2[12] on a DEC alpha 2000 server, and the trees were drawn with TreeView, version 1.5.2.[13] 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 recovered in this study, 17 reference strains recommended by WHO and 32 strains obtained from database were used as reference strains of known genotypes.

Accession numbers submitted to database. Nucleotide sequence data of RV sequences from Vietnamese patients are available in the DDBJ/EMBL/GenBank databases under the accession numbers AB706298–AB706308 and AB745027–AB745039 for RV E1 gene.

4. Results

By screening with real-time PCR, RV RNA was detectable in amniotic fluids from 103 out of 110 (93.6%) pregnant women and throat swabs in all of 20 (100%) fetuses/newborns. Virus-specific IgM antibody was positive in cord blood in 19 of 20 (95%) fetuses/newborns tested. In addition, RV RNA was detected in the placenta tissues in all cases of fetuses/newborns. At gross examination, all of 20 fetuses/newborns presented with congenital cataract. None of the pregnant women in this study had an obvious history of vaccination to rubella.

Among RV RNA-positive cases, sufficient amplicons in the E1 gene (908 bp) covering the WHO recommended region were obtained in 24 cases (17 samples of amniotic fluid from pregnant women and 7 samples of throat swabs from fetuses/newborns) by nested RT-PCR and then sequenced. Virus strains identified were named according to the WHO systematic nomenclature for RV.[9]

Vietnamese RV strains recovered in this study were analyzed, in comparison with the WHO reference strains and strains from the database to cover all genotypes. The nt difference between the Vietnamese strains ranged from 0.2% to 2.1%. The mean divergence within all Vietnamese viral sequences was 2.2% and 6.1% relative to the WHO 2B reference strains.

The phylogenetic tree showed all Vietnamese strains sequenced in the present study belonged to the genotype 2B group with the