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Continued circulation of a single genotype of dengue virus serotype 2 in the Philippines

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ABSTRACT

Objective: To obtain descriptive information of behavioral pattern in Chinese school-aged children with cleft lip and palate. **Methods:** A total of 93 cleft lip and palate patients between the age of 6-11 year-old and treated at West China Stomatology Hospital were selected. And another 100 unaffected controls, matched for age and gender, were recruited randomly from a common primary school in Chengdu. Chart review of medical records was used to obtain psychosocial checklists. Scores were compared with published norms and controls to evaluate the risk of problems, separately for three diagnostic groups. **Results:** The patients group had lower scores of social and academic competencies, especially those with facial deformity or speech problem. No difference was found in the aspect of activity competency. All patients showed elevations in behavior problems. But the type of behavior problems varied in different genders. **Conclusions:** Chinese school-aged children with cleft lip and palate are at raised risk for social and academic difficulties. Specific pattern of behavior problems displays differently depending on gender of the patient.

1. Introduction

Dengue is the fastest emerging infection transmitted by *Aedes* mosquitoes and currently poses arboviral threat to human health. All four serotypes of the positive stranded viral pathogen are prevalent in the (sub) tropical regions of the world and infect 50-100 million individual annually[1-5]. The maximum burden is borne by countries of the Asia Pacific Region. Among the estimated 2.5 billion people at risk globally, more than 70% (about 1.8 billion) reside in Asia Pacific countries. Its epidemiology is rapidly evolving, with increased frequency of outbreaks and expansion to new geographical areas that were not affected previously[3,6]. DENV causes a wide spectrum of clinical manifestations in humans ranging from a flu-like illness, known as dengue fever, to the more severe dengue hemorrhagic fever (DHF) and dengue shock syndrome[7].

According to WHO[8], dengue fever and dengue hemorrhagic fever are prevalent in all regions of the Philippines, with epidemics occurring every 3-4 years. Urban centres such as Metro Manila, Cebu and Davao are the areas with the highest morbidity and mortality rates.

Dengue occurs throughout the year, with rates increasing one-to-two months after the onset of the rainy season in June. The main vector responsible for dengue transmission in the Philippines is *Aedes aegypti*, which is predominant in urban areas, but *Aedes albopictus* may be a secondary rural vector[6].

The first known outbreak of DHF was reported in 1954. Since then, DHF has been epidemic mainly in Metro Manila and other urban and semiurban areas of the country[6,9]. All four dengue virus serotypes are present in the Philippines, although DENV 1, DENV 2 and DENV 3 are predominant. DENV 2 has been the most commonly isolated serotype in outbreaks/epidemics from 1995-2001. DENV 1 and 4 were predominant in 2004 while all four serotypes were isolated

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from 2005 to present throughout each year[6,8].

The evolutionary relationships between several isolates of the same virus become evident only by comparing nucleotide sequences that share a common ancestry, from which data can be generated quantitatively by phylogenetic trees. Many studies have made comparative analyses of nucleotide and amino acid sequences of short segments of specific gene regions to study the molecular epidemiology and evolution of the dengue virus strains characterizing them into genotypes. It is therefore useful to do genotypic characterization in monitoring the eventual appearance of genetic changes in dengue viruses, identifying the circulating genotype in a certain area and detecting the introduction of new genotypes[8-15].

This study aims to determine the genetic variability of DENV 2 since the last report in 2005 by Salda *et al*, using the *C-prM* gene junction of isolates during the 2008-2010 outbreaks. Klungthong *et al* (2008) also identified the gene target as one of the best target genes for molecular genotyping of DENV 2.

2. Materials and methods

2.1. Sample collection

De-identified serum samples positive for dengue infections were obtained from a tertiary hospital and were transported to the National Institutes of Health, University of the Philippines-Manila for analysis. The study protocol was approved by the Institutional Review Board, NIH, UP Manila. These samples were collected during outbreaks from 2008 to 2010.

2.2. RNA extraction

Viral RNA was extracted and purified from 140 μL of serum sample using QIAamp Viral RNA kit (Qiagen, Germany), following manufacturer's recommendations. Extracted RNA was eluted in 60 μL sterile elution buffer and stored at -80 ℃ until processed.

2.3. RT-PCR and sequencing

The identification of dengue virus from serum samples was carried out following the RT-PCR protocol of Chien *et al*[16] for dengue detection and serotyping. Briefly, 3 μL of extracted RNA was subjected to reverse transcription and PCR using primers (mD1 and D2) located at the junction region of the capsid and pre-membrane genes (*C-prM*). PCR products were sent to Macrogen Korea for sequencing. DNA sequencing was performed using an ABI PRISM BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, FisterCity, CA) on an automated sequencer (ABI PRISM 3100 model; Applied Biosystems). The *C-prM* gene was sequenced using the primers, mD1 and D2.

2.4. Phylogenetic analysis

Sequence alignments were performed using the ClustalW function of MEGA 4 (Molecular Evolutionary Genetics Analysis) software[17]. Comparisons were made with DENV-3 reference strains available from the Genbank (Table 1). Phylogenetic trees were constructed by the neighbour-joining method and maximum parsimony with MEGA 4. The reliability of neighbour-joined trees was estimated by bootstrap analysis.

Table 1

Description of Philippine DENV 2 isolates sequenced.

Virus ID No.	GenBank accession No.	Year of isolation
8-40	JQ217382	2008
8-60	JQ217383	2008
9-7	JQ217384	2009
9-34	JQ217385	2009
9-52	JQ217386	2009
9-58	JQ217387	2009
9-69	JQ217388	2009
9-71	JQ217389	2009
10-16	JQ217390	2010
10-20	JQ217391	2010
10-133	JQ217392	2010
10-159	JQ217394	2010
10-173	JQ217393	2010
10-176	JQ217395	2010
10-177	JQ217400	2010
10-188	JQ217399	2010
10-227	JQ217398	2010
10-229	JQ217397	2010
10-230	JQ217396	2010

3. Results

Nineteen DENV 2 isolates were identified from nucleotide sequences generated from the *C-prM* gene junction (Table 1). All the sequences were aligned with the homologous regions (nt 204-588) of the prototype DENV 2 isolate New Guninea C (GenBank: M29095).

3.1. Phylogenetic analysis

To investigate the viral genotype of DENV 2 isolates in the Philippines, 385 bp of the *C-prM* gene junction of the 19 DENV 2 isolates were analyzed in this study. Eleven global isolates of known genotypes were also aligned with the Philippine isolates (Table 2). Prototype sequences for DENV 1 [GenBank: AB074761], DENV 3 [GenBank: M93130], and DENV 4 C [GenBank: AF326576] were used as outgroups for the phylogenetic tree construction. A dendogram was drawn based on the pairwise comparison of nucleotide sequence of *C-prM* gene junction. The dendogram was inferred using the Neighbor-Joining method and bootstrap was calculated from 1 000 replicates. The dendogram based on the *C-prM*