

## Results Report

1. Title of Research and Development: "Comprehensive study on virus quasi-species and vascular permeability factors in severe dengue infection in humans for innovative epidemic and clinical managements."
2. Principal Investigator: Futoshi Hasebe (Professor, Center for International Collaborative Research, Nagasaki University, Japan)
3. Counterpart Principal Investigator: Le Thi Quynh Mai (Vice Director, National Institute of Hygiene and Epidemiology, Vietnam), Maria Luisa G. Daroy (Scientist, Research and Biotechnology, St. Luke's Medical Center, Philippines)
4. Results of Research and Development:

### **1) Determination of the virus serotype, genotype and genomic sequences of epidemic DENV strains in the Philippines and Vietnam,**

In 2013, an outbreak associated with DENV serotype-3 (DENV-3) genotype III that was previously not reported in Hai Phong and Ha Tinh district, Central Vietnam, suggesting virus introduction. In Hai Phong, in a patient that presented encephalitic symptoms prior to the development of dengue symptoms, DENV-3 genotype III was isolated from CSF sample of the patient (Minh Huong Phu Ly et. al. J. Clin Virol 2015 Sep;70:93-96). Currently, the virological and biological characteristics of the CSF isolate and serum isolates are being determined by using sequence and virological analyses.

The Philippines counterpart received the allocated grants from fiscal year (FY) 2015. Thereafter, clinical samples were collected from a total of 374 dengue patients. Virus isolation and virological characterization of these samples are currently being performed.

### **2) Analyses of DENV quasispecies and factors associated with vascular permeability to determine the mechanism of severe dengue**

Previously, clinical samples have been obtained from a large number of dengue fever (DF) and dengue hemorrhagic fever (DHF Grade I and II) patients, and analyses including sequence analyses and vascular permeability studies have been performed. However, samples from severe dengue (DS) patients were not available for analyses. Annually, a high number of DS patients were treated at the Children Hospital No. 1 which is located at Ho Chi Minh, Southern Vietnam. Starting from early FY2015, discussions for research collaborations were carried out. Subsequently, ethical committee approval was obtained from the hospital in August. From August until the end of the FY, clinical data and samples (blood, ascites and pleural effusions) from 61 DS patients (including 24 patients with dengue shock syndrome, DSS) were obtained. DENV sequence analyses and transcriptome analyses were performed using blood cells from these patients by next generation sequencer, Ion Proton. In FY2014, amino acid mutations of the NS4B region, which are hypothesized to be associated with host adaptability in mosquito (C6/36) and mammalian cells (Vero) in DENV-1 was determined. Using the limited dilution method, 3 phenotypes of DENV quasispecies was successfully isolated from a single patient. Additionally, by using a plasmid clone with same mutations introduced into the NS4B region, studies to determine the biological functions of the virus protein are currently being performed.

### **3) Determination of DENV antibody neutralizing titer to predict DENV epidemics**

In collaboration with the Preventive Medicine Center, Hanoi, samples from 100 Hanoi residents (healthy individuals) were collected, and the levels of anti-DENV IgM and IgG antibodies were determined by using an in-house ELISA assay. The antibody seroconversion rates, and data on primary and secondary infection among the residents of Hanoi has been obtained for further analyses. The serum samples were sent to Institute of Tropical Medicine, Nagasaki University for further analyses on the levels of neutralizing antibodies by using the FcγR-expressing BHK cells.