Protocol for Pediatric Cohort Study of Dengue Transmission in Nicaragua

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1. Title

Pediatric Cohort Study of Dengue Transmission in Nicaragua

2. Nature and Purpose

Dengue is the most important mosquito-borne viral disease affecting humans, caused by the four serotypes of dengue virus (DENV). Dengue fever (DF) and dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) have emerged as major public health problems, particularly in Southeast Asia and Latin America. According to the World Health Organization, an estimated 50 million cases of DF and 250,000-500,000 cases of DHF/DSS occur annually, with 3 billion people in over 100 countries at risk for infection. To date, no antiviral therapy or vaccine exists and case management relies on supportive treatment only. Dengue control relies on management of the Aedes aegypti and Ae. albopictus mosquito populations. An efficient, tetravalent vaccine would dramatically improve the fate of thousands of children and adults whose lives are affected by this disease, as would antivirals that could reduce the occurrence or severity of symptomatic dengue. Cohorts need to be established in different areas of the world, and the appropriate field site infrastructure must be created in preparation for trials of candidate tetravalent vaccines and drugs currently in the pipeline. In addition, such studies allow numerous critical questions to be addressed regarding the protective and potentially detrimental elements of the immune response to DENV, as well as issues concerning DENV fitness and evolution.

Since the incidence of dengue has increased dramatically in the Americas in the last two decades, and Managua, Nicaragua, has experienced annual epidemics of considerable magnitude but relatively manageable proportions, we chose this city as an ideal site for a cohort study. In 2004, a Pediatric Dengue Vaccine Initiative-sponsored (PDVI) cohort was successfully established in District II of Managua, Nicaragua, and has been several times since then.

Chikungunya virus (CHIKV) is a re-emerging, mosquito-transmitted arthritogenic alphavirus that causes both endemic and explosive epidemics of debilitating rheumatic disease. In October 2013, the first autochthonous cases of chikungunya fever occurred on the Caribbean Island of St. Martin. In 2014, the virus has spread throughout the Caribbean, Central America, including Nicaragua, and to a lesser extent

to South America and the United States. CHIKV-induced disease is commonly characterized by fever, and intense pain and inflammation in muscles, joints, and tendons. In May 2015, the Pan American Health Organization/World Health Organization published an alert describing the first outbreak of Zika fever in continental South America (specifically in Northeastern Brazil). Zika fever is caused by Zika virus (ZIKV), is similar in clinical presentation to dengue and chikungunya, and is transmitted by the same Aedes mosquitoes as dengue and chikungunya. ZIKV started circulating in Nicaragua in January 2016, in particular in Managua, were the catchment area of the study is located. Because the clinical presentation of the three diseases is very similar, the introduction CHIKV and ZIKV in the study population will likely increase the number of dengue-like illnesses in our cohort study and confound characterization of the epidemiology and clinical presentation of DENV infections. Thus, it is important to test for CHIKV and ZIKV infection in the cohort.

This study will involve the following collaborating institutions:

Centro de Salud Sócrates Flores Vivas (HCSFV), Managua, is a Ministry of Health health center that serves as the primary site for the pediatric dengue cohort study and provides healthcare to the study participants. At the Health Center Dr. Guillermina Kuan, a licensed physician and head of the Health Center, will be responsible for overseeing participant care.

Hospital Infantil Manuel de Jesus Rivera (HIMJR), Managua, is the national pediatric reference hospital. It is the best-equipped pediatric facility in the country. All cases of severe dengue in the greater metropolitan area of Managua are referred to the HIMJR. All suspected dengue cases from the cohort presenting with signs of alarm are referred to study physicians at the HIMJR.

National Virology Laboratory, Centro Nacional de Diagnóstico y Referencia, Ministry of Health, Managua, performs all serological and virological tests for dengue diagnosis and will process the samples collected as part of this study. Dr. Angel Balmaseda is the Head of the National Virology Laboratory and the Major Foreign Collaborator (Foreign Prinicipal Investigator) of the site. Dr. Balmaseda is responsible for the study site in Nicaragua.

The Sustainable Sciences Institute (SSI) oversees the conduction of the study at the three institution mentioned above. SSI is a non-profit organization that has an agreement with the Nicaraguan Ministry of Health to administer collaborative research projects such as this study. SSI relies on the Nicaraguan Ministry of Health's IRB approval, as registered in the OHRP database (FWA00009658).

Dr. Aubree Gordon is an Assistant Professor in the Department of Epidemiology, University of Michigan, Ann Arbor. Dr. Gordon will analyze data generated by the study and contribute to design study instruments (e.g. questionnaires). Dr. Gordon specializes in the epidemiologic features of infectious diseases in tropical regions. She has been working with the Nicaraguan Ministry of Health and Dr. Harris for the past 10 years. She has completed CITI online human subjects training, and the NIH human subjects course. Dr. Gordon has taught a responsible conduct of research course, meeting NIH requirements, at UC Berkeley.

The following institutions are collaborating on investigations that do NOT involve human subjects research, but rather analysis of coded samples withOUT personal identifiers in order to meet the scientific objectives of the grants/study.

Dr. Mark Loeb at McMaster University in Canada has extensive experience in host genetic studies, and has just finished one contract with NIH examining host susceptibility or resistance to West Nile virus infection. We will collaborate with Dr. Loeb and a network he has put together in the Americas as well as Asia to examine host genetic contributions to severe disease as well as protection against dengue virus as part of a dengue host genetics contract from NIH that was recently awarded.

Dr. Perera, Rushika at the Colorado State University (CSU) is an expert in the field of RNA Virology. She will use the metabolomics platform at CSU to identify new biomarkers for dengue diagnosis and prognosis. This analysis will be performed using serum as well as non invasive samples such as urine and saliva.

Dr. Miriam Merad at the Icahn School of Medicine at Mount Sinai in New York is recognized for her studies on the mechanisms that regulate the development and function of innate myeloid cells including dendritic cells and macrophages. Dr. Sacha Gnjatic, also at the Icahn School of Medicine at Mount Sinai, is an expert in the identification of T cell epitopes and the mapping of T cell responses to specific antigens. Together, they will characterize immune signatures in dengue virus infections, including innate immune response and competence and T cell and dendritic cell function.

Dr. Eric Schadt at the Icahn School of Medicine at Mount Sinai in New York has published extensively on and pioneered research in integrating diverse types of large scale, high dimensional data to construct system-level, predictive models of complex phenotypes like common human diseases. Dr. Jun Zhu, also at the Icahn School of Medicine at Mount Sinai is an expert in Bioinformatics. Together they will coordinate data analysis generated by various collaborators of the Dengue Human Immunology Project Consortium (DHIPC) and develop models to identify signatures that define immune response and categories/fingerprints/profiles that correlate with infection outcome.

Dr. Andrew Kasarskis at the Icahn School of Medicine at Mount Sinai in New York is an expert in large-scale biology and application of systems biology to biomedical research. His group will be in charge of data management and dissemination from/to collaborators of the Dengue Human Immunology Project Consortium (DHIPC).

Specific Aims

The overall aim of the project is to maintain a pediatric cohort in Nicaragua to characterize the natural history of dengue transmission and the range of clinical manifestations over time and to establish a potential trial site for antiviral therapies or a safe tetravalent dengue vaccine.
The specific aims of this project are to:

A. Prospectively determine the age- and serotype-specific incidence of symptomatic and asymptomatic dengue virus infection in children at highest risk of primary or secondary infection.
B. Characterize symptoms and disease spectrum and provide appropriate medical attention to all suspected symptomatic dengue cases within the study population.
C. Isolate representative samples of circulating dengue viruses and maintain a biobank of viruses.
D. Maintain a biobank of clinical specimens (serum/plasma, white blood cells, host DNA, urine and saliva) from symptomatic and asymptomatic infected persons linked to clinical information for use by collaborators.
E. Guarantee good clinical and laboratory practices through rigorous quality control.

3. Subjects

All participants will live in District II, Managua, Nicaragua, the catchment area for the Health Center Socrates Flores Vivas (HCSFV). No discrimination will take place in terms of race or gender; however, the age of study participants will be limited to children ages 2 to 14 years old as children have the highest burden of dengue. The subjects may be of three vulnerable populations: the illiterate, the poor, and children.

The current cohort size is 3,452 participants aged 2-14 years. The study maintains ~200-300 children in each 1-year age group. We estimate that this sample size should yield 270-1155 infections per year, assuming an annual incidence of DENV infection of 7-30% (based on prior observed annual incidence rates in this and neighboring districts in Managua since 2001). Estimating that 10-20% of the infections will be symptomatic; this would result in 27-231 symptomatic dengue cases. Of these, ~15% (4-35 children) would require hospitalization for dengue, although we expect 2- to 3-fold more hospitalizations due to other diseases with similar clinical presentations. This cohort size ensures that even in years of relatively low transmission, a minimum number of symptomatic cases will be obtained.

To maintain the cohort age structure and adjust for participants who age out of the cohort study, we plan to enroll up to 300 2-year old annually. Moreover, based on our study data from August 2004 to June 2010, we anticipate an annual loss to follow-up of ~6%. Thus, to maintain the cohort size and compensate for loss to follow-up, we plan to enroll up to 200 children aged 3 to 11.

There have been 7,455 total participants since the study's inception. We expect this number to increase to a maximum of 8,100 total participants to include children who will be recruited from November 2014 to August 31, 2015.

From 2016 to 2020, we plan to continue enrolling ~500 participants annually (up to 300 2-year old to maintain the cohort age structure and up to 200 participants aged 3 to 11 to compensate loss to follow-up) for 5 years (2016-2020), thus increasing study sample size from 8,100 to 10,600.
The subjects may be of three vulnerable populations: the illiterate, the poor, and children. This study consists entirely of children, as children bear the greatest burden of dengue in Nicaragua. The purpose of this study is to maintain a pediatric cohort to determine the transmission of the disease and its clinical manifestations so therefore the population must be children. This study will also include the

poor and illiterate as they represent a part of the general population in Managua and have a high burden of dengue disease.

4. Recruitment

New participants will be enrolled beginning January 2011, while those that are currently active will be re-enrolled in November and December 2010. Participant re-enrollment will initially be based on obtaining consent from current participants at the time of their medical consultations at the HCSFV in late 2010, while those who are to be newly enrolled will be recruited in early 2011. Those active participants who do not visit the health center during this time will be enrolled through home visits. Recruitment of new 2- and 3-year olds in 2011, and 2-year olds yearly thereafter will occur either through door-to-door home visits in the catchment area of the HCSFV or at the health center through promotion of the study to parents of 2-year olds who attend the health center for medical consultation. The appropriately-trained HCSFV nurse will provide information to parents, one-on-one or in small groups, and will answer questions regarding the study. The discussion will begin with a preliminary explanation of the study, and the consent form will be reviewed and read out loud together, providing a more detailed explanation of all of the aspects of the study.

5. Screening Procedures

Inclusion Criteria:

To participate in the study: 1) informed consent must be obtained from the child's parent or legal guardian, 2) children aged 6 and over must give verbal assent to participate, 3) children in rented households must have lived in the neighborhood for at least 2 years and children in owner-occupied households must have lived in the neighborhood for at least 6 months, 4) families must intend to remain in the neighborhood for the length of the study, 5) participants must have a vaccination card in order to confirm age and residence, or, if a vaccination card cannot be presented, participants must have a pre-existing medical chart at the HCSFV, and 6) if a child is sick (fever and/or chronic illness), he/she must be examined at the HCSFV to determine eligibility for participation in the cohort.

Exclusion Criteria:

Children will be excluded from the study if: 1) their parent or guardian fails to give informed consent, 2) they decline to give verbal assent if aged 6 and over, 3) they do not meet minimum residency requirements, 4) they have a chronic illness that puts them at risk for serious health complications, 5) their parents are not willing to bring them to the HCSFV for their medical attention during the study period, or 6) they do not meet or are unable to adequately document meeting the appropriate age of enrollment.

6. Study Procedures

Eligibility for the study is determined by the study criteria, after which the informed consent process will take place. These steps will occur in either of the following ways: 1) at the time of the child's routine medical visit to the HCSFV or 2) through a home visit. Demographic data and information regarding each participant's health history will be obtained at the time of enrollment and should take approximately 15 minutes. The house of each participant will be geo-referenced using satellite

technology.

Annual Sample Collection

Each year in March/April, a study nurse will make contact with the participant and parent to collect a sample of blood from each participant. This contact will either occur at the health center or during a visit to the participant's home. A 7cc blood sample will be collected at this time, and the process should take approximately 5 minutes per child. The nurse will also ask questions about the participant's health, including whether the participant has been ill, has attended the HCSFV, or has traveled out of Managua. Within at least a week but no later than a month of the participant's annual sample collection, study participants will be expected to attend the HCSFV to (a) receive results from the Complete Blood Count (CBC) test performed on the blood sample, (b) make and receive a photo ID card, if the participant does not already have one, (c) scan the participant's fingerprint to facilitate future registration and identification, if such a scan is not already on file. A physician will be available to explain the laboratory results and make a follow-up appointment if necessary. This process generally takes 15 minutes or less per child.

Continuous Procedures over the Duration of the Study

Attending the HCSFV for illness

All children who report to the HCSFV will be channeled through the reception mechanism at the health center's Admission desk. All children will be asked if they are participants in the study, and if so will be asked to provide a means for rapid identification with either their study ID card or a fingerprint scan, both of which are linked to a study code by which participant's medical charts are organized. The parent and child will then be directed to the clinic for consultation with a physician (available 24h/day for ambulatory care). A standard form will be completed for all medical consults.

Suspected dengue cases

The study aims to obtain clinical information and isolate the virus from participants with dengue. As dengue has a wide range of clinical manifestations, especially in children (where not all symptoms may be reported), the criteria used will be broader than the MOH definition of a clinical case of dengue. If a patient is suspected of dengue or a fever of unknown origin (FUO), an acute phase serum sample will be obtained, as is routinely done by the MOH for suspected dengue cases. Any virus obtained will be stored for future studies. Specifically, MOH guidelines specify collection of 5ml of blood on day 1 of illness for laboratory analysis for diagnosis of dengue, and ~ 1ml of blood daily for (CBC) clinical management, as ordered by the physician. The remainder will be used for research purposes as part of this study; thus, no additional blood is drawn from suspected dengue cases during the acute phase than that mandated by MOH norms. It is difficult to specify the exact amount remaining since it depends on how much serum is obtained from the sample after separation from blood cells and how is needed for the different diagnostic assays, which can vary depending on whether repetitions are necessary. A 5-ml convalescent sample will be collected 14-21 days post-illness onset as part of this study because it is necessary for serological diagnosis of dengue (levels of anti-DENV antibodies in paired acute- and

convalescent-phase samples are analyzed in parallel), although it is not part of the MOH norms for primary care. Additionally, non-invasive samples will be collected from suspected dengue cases on the initial visit to the Health Center. Up to 10 ml of saliva will be collected specifically for this study, aliquotted and stored. As urine is already collected as part of the MOH guidelines at the Health Center for patients suspected of dengue, no additional urine collection will be necessary. Up to 10 ml of urine will be separated from the rest of the sample and refrigerated until further processing and storage.

Moreover, suspected dengue cases (or a subset of those) will be screened for DENV infection by rapid test and/or RT-PCR. A second 5-ml blood sample will be collected on day 4-6 post-illness from participants with confirmed dengue. As a rule, we will draw the blood together with the daily CBC sampling whenever possible in order to minimize discomfort and risk for the participant. Please refer to the attachment "Blood Collection during the Study" for a summary of blood collection over the duration of the study, and a comparison of MOH norms and study procedures.

Additionally, during periods when Chikungunya virus (CHIKV) and Zika virus (ZIKV) circulate, suspected dengue cases will be tested for CHIKV and ZIKV (by RT-PCR, serology, and/or other diagnostic test) using the acute and/or convalescent samples collected for DENV testing.

For clinical management of suspected dengue cases, the Nicaraguan MOH traditionally collects a 5ml acute sample for serological testing and surveillance purposes, as well as daily blood samples of 1ml for CBC analysis with platelet count during the first week of symptoms, as prescribed by the physician, as mentioned above. This allows for appropriate management of dengue cases, since falling platelet counts (leading to thrombocytopenia, or $\leq$100,000 platelets/mm^3) and rising hematocrit (to $\geq$20% of baseline value) are signs of the potentially fatal DHF/DSS. Platelet counts and hematocrit determination are routinely performed by all MOH centers whenever possible during the acute phase of the illness. Daily clinical and laboratory review will continue until the fever and altered laboratory results subside, the child is transferred to the hospital, or a cause of illness other than dengue is confirmed. If the suspected dengue/FUO case requires hospitalization, the study will arrange for transfer from the HCSFV to the national pediatric reference Hospital Infantil Manuel de Jesus Rivera ("La Mascota"), where the usual criteria for hospitalization will be applied to the study participant. The child will be accompanied by study personnel to the hospital until one of the study doctors at the hospital checks him/her. The study will cover the costs of the extra laboratory tests that will help the hospital doctors treat the child. These tests will be performed daily and will include an X-ray or sonogram (for detection of pleural effusion, a sign of plasma leakage) and collection of 5ml of blood, as per MOH and hospital norms. Again, the remainder will be used for study purposes. Blood is normally taken daily when children are in the hospital for dengue, so this does not entail additional blood draws.

Suspected Zika cases presenting without fever or history of fever

Participants suspected of having Zika but presenting without fever or history of fever will follow the same sampling procedures as those suspected of dengue (see above) if they have specifically consented for this (see Consent Form Part D). Briefly, 5 ml of acute and convalescent blood samples, and up to 10

ml of saliva and urine will be collected. These samples will be used for diagnosis of ZIKV, DENV and CHIKV, and to study the immune response against these infections.

Confirmed Zika cases
Additional samples will be collected from participants with a laboratory-confirmed ZIKV infection if they have specifically consented for this (see Consent Form Part D). A second 5-ml blood sample will be collected on day 4-6 post-illness from these participants, as for DENV-positive participants (see above). As a rule, we will draw the blood together with a CBC sampling whenever possible in order to minimize discomfort and risk for the participant. This sample will be used to study the immune response against ZIKV infections. Moreover, up to 10 ml of saliva and up to 10 ml of urine will be collected, aliquotted and stored in the convalescent phase of the disease (approximately 14-21 days post-illness onset). These samples will be used to develop and/or validate new, non-invasive diagnostic tests for ZIKV.

If a ZIKV-positive participant develops neurological symptoms, the study will arrange for transfer from the HCSFV to the national pediatric reference Hospital Infantil Manuel de Jesus Rivera ("La Mascota"), where the participant will be assessed by a study neurologist. If the participant requires hospitalization for his/her neurological symptoms, the study neurologist will follow the hospitalization. The results of the neurological assessment and of the hospitalization follow-up, as well as the results of any specific testing conducted, will be recorded in the participant's clinical file. This information will be accessible to the physicians providing care to the participant at the HCSFV.

Screening of cases of fever with a known focus (diagnosis other than dengue or undifferentiated fever)
To ensure that the HCSFV is successfully diagnosing febrile illnesses and screening for dengue, a percentage of cases of fever with an identified focus will be screened for dengue. During the period of high dengue transmission, permission will be requested from parents to collect an acute sample for dengue testing from children who have blood taken for CBC by doctor's orders (totaling 3ml), as well as from children with diagnoses of respiratory infections or urinary tract infections (UTI), justified as "Búsqueda Activa" ("active search") as directed by the MOH (without requiring additional consent). This is expected to yield acute blood specimens from an average of 60% of cases with a differential diagnosis. During the rest of the year, permission will be requested from parents to collect an acute sample (totaling 3 ml) for dengue testing from children who have blood taken for CBC by doctor's order (an average of ~25%).

Other contact from the study and HCSFV
To evaluate compliance with the study procedure of early presentation to the HCSFV when ill and to promote Health Center attendance, study nurses will visit the houses of study participants. A full-scale undertaking where over 80% of houses are visited will occur at least once per year to evaluate participant compliance and satisfaction. At these visits, a brief questionnaire will be administered regarding the child's health and attendance to the HCSFV over the previous several months.

Additional Informed Consent Processes

An informed consent process that requests permission to store clinical information, and blood samples for future studies will occur (Consent Form Part B) at the time of enrollment, as well as an additional informed consent process that requests permission to store and use DNA from new participants (Consent Form Part C). No additional samples will be taken to obtain the DNA.

Additionally, parents/guardians of participants enrolled before February 2016 and suspected of having Zika without fever or history of fever, as well as parents/guardians of participants enrolled before February 2016 who test positive for ZIKV infection will be asked to undergo an additional informed consent process for permission to collect and use the additional samples described above (Consent Form Part D). An assent script will be read to participants six years of age and older, and their agreement will be obtained before any samples are taken.

For all new enrollments after February 2016, the additional consent process for the collection and use of these additional samples (Consent Form Part D) will take place together with the other aspects of the consent (Consent Form Parts A to C). Parents/guardians of participants who undergo this additional consent process at enrollment will not need to be re-consented when their child is suspected of having Zika without fever or history of fever, nor when their child tests positive for ZIKV infection.

Please note that the description of the quantity of blood, which is often stated in terms of teaspoons and tablespoons in the United States, is perceived in Nicaragua as confusing, with the potential of having the participant think that they might be consuming something. The use of "ml or cc" to describe the volume is more culturally acceptable. It is a term understood by lay people, as it is common for people to use liquid medicines for injections.

Procedures External to Direct Patient Participation

Procedures pertaining to viral RNA and blood samples

After serum/plasma from the blood specimens have been separated for serological, virological, and blood chemistry testing, the remainder will be separated to collect PBMCs and aliquoted and stored at -70°C or liquid nitrogen until further processing. Any virus isolated will be stored as first-passage aliquots at -70°C and liquid nitrogen for future use in pathogenesis studies. Dengue virus (DENV) RNA spanning the entire length of the viral genome will be amplified from acute phase sera, a subset of PBMCs, and isolated viruses. Sera and PBMCs will be stored in aliquots at -70°C and liquid nitrogen, respectively, for use in evaluation and validation of new diagnostic tests (such as RVPs, NS1 capture ELISAs, and type- and epitope-specific assays), measurement of neutralizing and enhancing anti-DENV antibodies and DENV-specific serum avidity, evaluation of the host immune response (e.g., microarray and proteomic studies, B and T cell responses *ex vivo*), and additional and as yet undefined investigations. The sample collected on day 4-6 of illness will be used to study acute-phase immunological responses; in particular cell-mediated immunity. A small aliquot of the blood sample (0.8ml) will be stored in Trizol or the equivalent for further RNA extraction and gene expression analysis.

Procedures pertaining to participant DNA
For patients and their parents/guardians who consent to genetic studies, DNA will be preserved in its crude cellular form until time of extraction. The DNA samples will be retained by the primary investigators (project coordinators) and stored at the University of California, Berkeley, or in the CNDR in Managua, Nicaragua. DNA samples will be used for investigation of genomic single nucleotide polymorphisms (SNPs), HLA loci, and killer immunoglobulin receptor (KIR) polymorphisms in relation to susceptibility/resistance to severe dengue in future collaborative genetic studies.

DNA amplified from dengue viral RNA, DENV RNA, and crude or extracted human genomic DNA will be sent from Nicaragua to collaborators via UC Berkeley (see list of sites below). This is for processing but not for long term storage. Clinical information in databases that contain NO personal identifiers will be sent to collaborators to accompany the samples. All host nucleic acids sent to collaborators will be returned to UC Berkeley or destroyed after genetic analysis is completed.

Procedures pertaining to saliva and urine samples
Urine and saliva samples will be refrigerated immediately after collection. Then they will be aliquotted and stored at -70°C. Coded samples with all personal identifiers unlinked will be sent to Colorado State University for metabolomics analysis.

Sample storage
Long-term storage will only occur at the University of California, Berkeley, and the CNDR in Managua Nicaragua. As mentioned in Section 3a, the following collaborating institutions will participate in the analysis and processing of coded samples without personal identifiers in order to meet the scientific objectives of the grants/study:

The Broad Institute in Cambridge, Massachusetts; Integral Molecular in Philadelphia, Pennsylvania; Dr. David Relman's Laboratory at Stanford University School of Medicine; the DeRisi Laboratory in the Department of Biochemistry, University of California, San Francisco; Dr. Munir Alam's Laboratory in the Human Vaccine Institute at Duke University Medical Center; Dr. James Crowe's Laboratory in the Department of Pediatrics, Microbiology and Immunology at the Vanderbilt University Medical Center, Dr. Andrew Fire's Laboratory at Stanford University; Dr. Philip Armstrong at the Connecticut Agricultural Experiment Station; Dr. Laura Kramer, Director of the Arbovirus Laboratories at the Wadsworth Center at the New York State Dept Health in Albany, NY; Dr. Theodore Pierson at the Laboratory for Infectious Diseases at the NIH; Dr. Wei-Kung Wang's laboratory in the Department of Tropical Medicine, Medical Microbiology and Pharmacology in the John A. Burns School of Medicine at the University of Hawaii at Manoa; Dr. Mark Loeb at McMaster University in Canada; Dr. Michael Bamshad's laboratory at the University of Washington (Seattle) and Dr. Barry Beaty at Colorado State University. DNA may be analyzed at the Broad Institute and in Dr. Bamshad and/or Dr. Loeb's laboratory.

Contact with study participants will be primarily through personnel (physicians, nurses, and reception area staff) of the HCSFV. Study nurses will perform the house visits. House visits will be performed as described below for annual sample collection and to collect data on study attendance. Participants will

generally receive no more than 2 house visits per year. The exception to this will be when a home visit becomes necessary for medical follow-up. Home visits generally are of 15 minutes or less duration, and the process of drawing blood should take approximately 5 minutes per child.

7. Risks

The risks associated with blood draws include potential complications such as pain, bleeding and bruising at the venipuncture site; in very rare instances, infection may occur. There is no anticipated risk for the collection of urine and saliva. There is minimal risk that patient confidentiality could be violated. The repercussions of this risk are minimal since there is no social stigma associated with dengue virus infection. There is no anticipated legal risk associated with the study.

Measures to minimize risk/discomfort

To minimize blood draw risks, all blood draws will be venous collections performed by professional health care personnel. The use of stringent aseptic technique and post-venipuncture pressure to the site should minimize bleeding and infectious complications. No risk is anticipated for the collection of urine and saliva. Nevertheless, urine and saliva collection will be performed by trained study personnel and will follow international and HCSFV guidelines. All information obtained from the subjects will be confidential. To ensure this, all samples will be labeled with a study code, and clinical information and test results will be kept in databases without personal identifiers. The separate password-guarded database linking the study code to personal identifiers will only be accessible to study coordinators and key personnel. With these measures in place, it is unlikely that confidentiality will be breached.

8. Benefits

The benefits to subjects for participation in this study are as follows:

1. The study participant will contribute to the research effort to improve control, prevention and knowledge of dengue.
2. The study participant will receive educational information about dengue and its prevention and control.
3. The study participant will receive annual results of a complete blood chemistry, including platelets, that can identify anemia and other illnesses.
4. The study participant will have access to study physicians 24 hours a day, 7 days a week at the HCSFV regardless of the cause of illness.
5. The study participant will have access to transportation 24 hours a day, 7 days a week from the health center to other centers of medical attention in cases of emergency.
6. The study participant will have access to timely laboratory results as study personnel at the health center's clinical laboratory is available 24 hours a day, 7 days a week. In other health centers in Managua, clinical laboratories are only open during working hours.

9. Compensation

All study participants will be given school supplies or the equivalent valued at ~US$1.50. This gift is used instead of monetary compensation in order to avoid undue influence to participate, as this is a vulnerable population. The study gift will be given to the parent each year after the blood draw.

Compensation in the form of small gifts appropriate for this population have been chosen in order to avoid undue influence of monetary compensation in the study population, which is largely poor.

10. Confidentiality

Individually identifiable information will be collected from participants, including names, parents' names, birth dates, addresses, and GPS coordinates of the household. These data will be stored on computer and will be accessible to study personnel at the HCSFV on a per-need basis. Such persons include data entry personnel, admissions personnel, and persons supervising and managing study data. All computers with participants' data will be password-protected (see below). The participants' code will be accessible to study personnel who may provide medical attention to the participant, and who are shown the study ID card. No individual identities will be used in any reports or publications resulting from this study.

Physicians, nurses, and HCSFV staff who work on the study will have access to personal identifiable information on clinical records and charts as necessary for providing medical care. Other than these clinical staff, only the Data Managers, Site and Study Coordinators and PI will have access to identifiable information. While participant names will appear on clinical forms, which are source materials and part of the participant's medical records, names and other identifiable information will not be present in study databases where clinical, laboratory and demographic information collected from the patient will be stored for research purposes. The GPS data will be used to produce maps for use in the field. All such maps will be collected at the end of all field-work and stored in a locked file cabinet. Laboratory technicians at the CNDR, will have access to the specimens. Tubes and vials will be labeled with a barcode sticker, such that clinical specimens sent to the CNDR for processing and storage will not carry patient names or identifiable information.

At the time of enrollment, contact information for the participants will be obtained by an interviewer and entered into a password-protected database of identifiable information and kept in a locked office. Each study participant will have a medical chart at the HCSFV. They will be accessed by medical personnel and limited study personnel, and will be kept in a locked room per Ministry of Health norms. Surveys and consent forms are kept separately in a locked file cabinet in a locked office.

Each subject will have a unique identification code, and all specimens will be labeled only with this code. The key to this code will be kept in the password-guarded master study database. The study coordinators and key personnel will be the only ones with access to the master database. Access to the database containing identifiable information will be strictly controlled. All devices and computers with identifiable data will be password-protected. Databases with identifiable data will also be password-protected. All passwords will meet the CPHS definition of a secure password (i.e. passwords of 10 characters or more which contain at least one of each of the following: upper-case letter, lower-case letter, number and symbol).

Secure data encryption will be used whenever identifiable information is: 1) stored in a networked

computer/device; 2) stored in mobile devices (smart phones, laptops, tablet computers) which are not permanently stored in a secure location; and 3) transmitted via email or internet. No identifiable information will be stored in the cloud. All encryption passwords will meet the CPHS definition of a secure password.

Encoded databases, which do not contain identifiable information, will be created and used for all analyses. Collaborating research groups and institutions will be sent coded samples with all personal identifiers unlinked; this includes any researchers performing genetic studies. No collaborating research group or institution will have access to the database which relates the study code to personal identifying information.

As a part of enrollment, participants will be asked for permission to store samples and use samples/data in other studies. Samples/data will be kept for those who granted permission for storage and future use. Data collection instruments are included as a part of the child's medical record. Therefore, the data collection instruments will not be destroyed by study staff. Moreover, as per UCOP data retention policies, all data on participants will be stored for at least 7 years after they reach age of maturity (18 years of age).

Specimens are marked only with the study code. The key to this code will be kept in an encrypted and password-guarded master study database. The study coordinators and key personnel will be the only ones with access to the master database. As a part of enrollment, participants will be asked for permission to use samples/data in other studies. The link between data and identifiable information will be destroyed 7 years following study completion for all those who did not grant permission for future use.

Identifiable data may be transmitted via email or internet. In these cases, secure data encryption is used. All encryption passwords meet the CPHS definition of a secure password. No identifiable information is stored in the cloud.

Appropriate measures will be taken to protect subject's privacy. Medical consults will be conducted in dedicated rooms in the study health center, only accessible to the participant, his/her parent/guardian, and medical personnel. Interviews (at enrollment and during annual sample collection) will be conducted in the waiting area of the health center or at the participant's home. In the waiting area of the health center, interviews will be conducted as discretely as possible (in close contact with the interviewer).

11. Informed Consent

A consent form will be used to consent new participants. Study personnel will introduce the study and recruit participants. One-on-one or in small groups, information regarding the study will be provided to the adult(s) living in the same household as the case or in close proximity. The discussion will begin with a preliminary explanation of the study, and the consent form will be reviewed together, providing a more detailed explanation of all of the aspects of the study. Only participants and children whose

parents have consented to their participation and children over age 5 who have given assent will be permitted to enter the study. Among children, assent will be sought from subjects over 5 years of age, and training for informed consent will include details on only obtaining guardian consent from the appropriate person for participants under 18 years of age.

The consent will be explained verbally, and a document, written in Spanish, will be presented to the potential participant. Additional consent processes will be conducted for the option to allow use of DNA for study related purposes. The interviewer will also sign the consent form as a witness/study representative. An unsigned copy of the consent form will serve as an information sheet describing the study procedures, risks, benefits, and contact information, and will be given to the study participant. Verbal assent will be obtained from all children aged 6 and older following parental consent.

The informed consent and assent processes will involve reading the consent document (see attachment) aloud to all potential participants; those who are not able to write will be asked to sign their name with their fingerprint. Some subjects may belong to lower socio-economic classes. As there are no financial incentives associated with this study, we do not think that this study involves any coercion; therefore, we do not feel we are putting this population at greater risk. Training for informed consent will include details on only obtaining guardian consent from the appropriate person.

<u>For assent waiver for subjects 5 years of age and younger</u>
The capability of children 5 years of age and younger to understand the study objectives and procedures is so limited that we consider that they can't reasonably consulted.

An assent script will be read to participants six years of age and older, and their agreement must be obtained before any samples are taken. The person reading the assent script will document that verbal assent was given in the participant's record. Those participants who do not agree to the terms of this consent will not be withdrawn from the primary study.

An informed consent document written in Spanish will be read to the prospective recruit by an interviewer trained in this process. The interviewer will ask for a signature on the consent form from the parent/guardian after ascertaining that he/she understood the nature of the study. Participants who are illiterate will be asked to mark the signature line with a thumbprint and a witness will sign, as is required by Nicaraguan law and clinical practice norms for illiterate persons. The interviewer will also sign the consent form as a witness/study representative. A signed copy of the consent form will serve as an information sheet describing the study procedures, risks, benefits, and contact information, and will be given to the study participant.

<u>For consent part D</u>
Parents/guardians of participants enrolled before February 2016 and suspected of having Zika without fever or history of fever, as well as parents/guardians of participants enrolled before February 2016 who test positive for ZIKV infection will be asked to undergo an additional informed consent process for permission to collect and use additional samples (Consent Form Part D). An assent script will be read to

participants six years of age and older, and their agreement will be obtained before any samples are taken.

For all new enrollments after February 2016, the additional consent process for the collection and use of these additional samples (Consent Form Part D) will take place together with the other aspects of the consent (Consent Form Parts A to C). Parents/guardians of participants who undergo this additional consent process at enrollment will not need to be re-consented when their child is suspected of having Zika without fever or history of fever, nor when their child tests positive for ZIKV infection.

<u>For assent script part D</u>
Assent will be sought from participants aged 6 years and older.
Participants enrolled before February 2016 and suspected of having Zika without fever or history of fever, as well as participants enrolled before February 2016 who test positive for ZIKV infection, will be asked to undergo an additional assent process for permission to collect additional samples. This additional assent process will be carried out as described for the main part of the study. For all new enrollments after February, the additional assent process will take place together with the assent for the main study.

12. Management of Adverse Events/Reporting

Severe disease or death is a potential outcome of dengue virus infection, especially if a child presents to the health center late in the course of DHF or DSS. As this is a community-based study, the probability of a death occurring in study participants is low, and multiple measures have been put into effect to encourage participants to present in the first few days of fever to minimize this risk. All participants will receive the best medical care available in Nicaragua. If a non-serious adverse event, such as a local infection, occurs as a result of a blood draw or any other study procedure, the participant will be treated at the HCSFV or if necessary at the HIMJR without any charge to the participant. The study will provide reimbursement (50 cordobas) per trip for any necessary medical visits. Any compensation for the adverse event will follow the University of California policy. If a serious adverse event occurs, the study investigators are familiar with the University of California policy and will follow the policy.

In the event of any adverse event, whether related to study participation or not, the study personal will notify both CPHS and the Nicaraguan IRB within 48 hours of the event or when study personnel become aware of the event. This notification may initially come through phone communication within 7 days, but in all cases a written report will be filed in the native language and to CPHS within 14 days of the adverse event. If there is a breach of confidentiality via unauthorized computer access or the loss of a computer, we will notify CPHS and the Nicaraguan IRB immediately.

If a non-serious adverse event occurs as a result of any study procedure, the participant will be treated at the HCSFV without any charge to the participant. The study will provide reimbursement (50 cordobas) per trip for any necessary medical visits. Any compensation for the adverse event will follow the UC policy.

13. Contact Information:

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