were analyzed by polymerase chain reaction techniques for detection of the fragments of ORF K1 of HHV-8, which were then genotyped and analyzed regarding the genetic variability. Our study described 106 positive cases for HHV-8 in the saliva from 751 AIDS patients without previous KS. In addition, we performed a phylogenetic analysis of HHV-8 in 34 of the 106 AIDS patients without KS and in 33 of the 37 patients with active KS. The distribution of HHV-8 genotypes A, B, C, and F in AIDS individuals was indistinguishable by comparing non-KS and KS groups, as well as regarding ethnicity. Considering the KS group, genotype B was associated with better prognosis of KS tumor. Interestingly, we found a particular profile of diversity within clade C and two recombinant patterns of HHV-8 in the saliva of AIDS individuals without KS. We emphasize the need to achieve standard genotyping protocol for ORF K1 amplification, thus allowing for substantial detection of HHV-8 variants. Our findings can shed light on the role of HHV-8 variability in the pathogenesis of AIDS-KS. Our perspective is study polymorphisms and phylogenetic inferences in HHV-8 sequences encoding microRNA.

A51 Rubella genotype 1H is still circulating in Turkey

Tulay Yalcinkaya

Virology Laboratory, Public Health Institution of Turkey, Sihhiye-06100, Ankara

Rubella virus, the sole member of the *Rubivirus* genus in the Togaviridae family, is a positive-strand RNA virus. Based on phylogenetic analysis of sequences of the structural coding protein, two virus clades including a total of thirteen genotypes have been identified. Infection with rubella virus generally leads to mild disease with symptoms that include rash and low fever. In pregnancy, however, rubella infection can cause miscarriages and serial birth defects including hearing, vision, mental and heart impairment, which are collectively known as congenital rubella syndrome (CRS). CRS occurs in up to 85 per cent of children born to women with rubella infection during the first trimester of pregnancy. In addition, CRS can lead to neonatal deaths in up to 30 per cent of cases. Laboratory investigation plays an important role in both diagnosis and surveillance of rubella and CRS, since clinical diagnosis is unreliable and up to 50 per cent of infections are estimated to be subclinical. Because phylogenetic analysis of rubella virus genotypes can help determine whether circulating strains result from endemic transmissions or importations, laboratory surveillance for rubella also includes the molecular characterisation of viruses. Rubella genotype 1H was detected in a seven-year-old patient's urine specimen in 2016 (GenBank accession number KY048160). There are only three previous genotype 1H sequences from Turkey which were collected in 2001. No sequences are available from countries bordering Turkey (except for one 2B from Iran). Other 1H sequences are mostly from Russia and Belarus and none have been detected since 2008. The sequences of the recent isolate and three previous isolates cluster as a separate branch of genotype 1H. It seems likely that this lineage of 1H has been circulating in the country (and perhaps bordering countries) during the last fifteen years.

A52 Ebola virus phylogenetic analysis during the 2014–2016 West African outbreak

Shannon L. M. Whitmer and Ute Ströher

Viral Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA

Following the conclusion of active Ebolavirus disease (EVD) transmission within West Africa, sporadic EVD-cases continued to re-emerge outside of the expected viral incubation period. Epidemiological evidence suggested that these cases represented sexual transmission from persistently infected, asymptomatic EVD survivors. To address these questions, we directly sequenced EBOV from clinical specimens collected during acute and persistent infection from individuals associated with these re-emerged EVD cases. This sequence analysis was used in conjunction with on-the-ground epidemiological tracing to identify transmission chains and potential routes of infection. Due to a lack of knowledge regarding the effect of persistence on Ebola viral sequences, we were unable to support or refute whether these re-emerged cases represented evidence of transmission from EVD survivors, despite extensive phylogenetic analysis. To address this knowledge gap, we also sequenced Ebola virus directly from the semen of EVD survivors ('SAVS'—semen-acquired viral sequences) and identified molecular characteristics associated with viral persistence. Through extensive use of phylogenetic software and models, we identified that a subset of SAVS exhibited evidence of a slowed or acute-like substitution rates, de novo U-to-T hyper-editing and a moderate change in evolutionary pressure within the viral glycoprotein. Altogether, our data illustrate that phylogenetic analysis and evolutionary hypothesis testing can yield important insights into disease transmission networks and the mechanisms of viral replication.

A53 Systematic application of metagenomics NGS to identify and sequence viral pathogens in infections of the central nervous system

Anne Piantadosi,[1] Shibani Mukerji,[2] Simon Ye,[3] Jacob Lemieux,[1] Lisa Friemark,[3] Daniel Park,[3] Gordon Adams,[1] Michael Leone,[2] Marcia Goldberg,[1] Tracey Cho,[2] Eric Rosenberg,[1] and Pardis Sabeti[3]

[1]Division of Infectious Disease, Department of Medicine, Massachusetts General Hospital, Boston, MA 02114, USA, [2]Department of Neurology, Massachusetts General Hospital, Boston, MA 02114, USA and [3]Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA

Unbiased metagenomics sequencing allows the detection of any potential pathogen in a sample using a single methodology. This technique has been used to successfully identify pathogens in case reports of patients with central nervous system (CNS) infection. Metagenomics sequencing also provides genomic information that can be used to classify pathogens and perform studies of molecular epidemiology, especially for viruses, which have small genomes amenable to full-genome sequencing. Here, we apply metagenomics sequencing to detect and sequence viruses in a prospective cohort of patients with CNS infection. We enroll patients with both known (control) and unknown or suspected CNS infection and obtain samples of cerebrospinal fluid. We perform unbiased library construction from both RNA and DNA, followed by deep sequencing and metagenomics analysis. In patients with known infections, we have successfully sequenced Herpes Simplex Virus (HSV)-1 in two cases and HSV-2 in one case, obtaining partial genomes that allowed species classification. We have also successfully sequenced enterovirus in two cases, obtaining full-length viral genomes that allowed strain classification and phylogenetic analysis. In one patient with unknown infection, we identified Powassan virus, an emerging tick-borne flavivirus that causes encephalitis in the Northeastern United States. In that case, our NGS results were obtained three weeks earlier than routine clinical testing by serology, highlighting the potential application of this method for rapid diagnosis of infection. As work in progress, we are currently sequencing the full viral genome, which will be the first Powassan virus genome sequenced directly from a clinical sample. This will allow phylogenetic comparison

to genomes isolated from tick vectors in order to assess whether there are viral characteristics associated with human infection. Overall, our results highlight the utility of metagenomics NGS to identify and study the molecular epidemiology of viruses that cause CNS infection.

A54 Viral metagenomics: Relative viral enrichment and detection limits in clinical serum and faeces

Dennis Schmitz,[1,2] Jeroen Cremer,[1] Harry Vennema,[1] Annelies Kroneman,[1] and Marion Koopmans[1,2]

[1]National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands and [2]Erasmus Medical Centre (EMC), Rotterdam, The Netherlands

Any etiological agent containing nucleic acids should be identifiable using random next-generation sequencing (NGS) of human clinical materials. Unlike PCR based and baiting strategies, random NGS metagenomics is able to identify unknown and genetically drifted agents without relying on prior knowledge. This makes it suitable for the identification of RNA-viruses, which naturally drift due to their error-prone RNA-dependent RNA polymerase. However, NGS applied to virome investigation (viral metagenomics) presents biological and technical barriers. Viruses often cannot be cultured or isolated, therefore, low viral load samples are common. Furthermore, during NGS, all nucleic acid molecules compete for limited sequencing capacity (whether viral or non-viral) and the costs of NGS increases proportional with required sequencing depth. Therefore, to efficiently sequence low viral load samples, a protocol has to be developed that enriches viruses/viral nucleic acid. We first focused on single stranded RNA-viruses in serum and faeces. Different stages of the protocol were tested in the process from RNA-virus positive sample to dsDNA input for NGS: centrifugation, filtration, endonuclease treatment, RNA extraction, reverse transcription, second strand synthesis and library preparation. Different combinations of these methods were applied to human faeces and serum and assessed using qPCR. Subsequently, the optimal method was applied to Chikungunya virus positive serum and norovirus positive faeces ranging from Ct eight and eleven up to Ct 35 and 30, respectively. Lastly, these samples were sequenced using an Illumina MiSeq (PE300, $\sim 10^6$ reads/sample) and analyzed to determine detection limits. Our method reliably generates full (>95 per cent) viral genomes up to Ct 26 in both serum and faeces, while allowing identification of viral agent up to Ct 30. Viral metagenomics proved its merit by also identifying sapovirus, coxsackievirus, parechovirus, and picobirnavirus in faeces. The coxsackievirus and parechovirus were confirmed using qPCR with a Ct of 28 and 29.18, respectively. The identified sapovirus could not be confirmed using our diagnostic qPCR, although NGS data coverage indicated a high viral load. Further analysis of this sapovirus showed many mutations in the qPCR primer binding site, explaining the negative result in our diagnostic assay. These results emphasize the power and promise of viral metagenomics.

A55 Foot-and-mouth disease virus undergoes abundant viral genomic changes at distinct stages of infection of cattle

I. Fish,[1,2] C. Stenfeldt,[1,2,3] S. J. Pauszek,[1] B. P. Brito,[1,2] E. J. Hartwig,[1] G. Smoliga,[1] L. L. Rodriguez,[1] and J. Arzt[1]

[1]Foreign Animal Disease Research Unit, United States Department of Agriculture, Plum Island Animal Disease Center, Agricultural Research Service, Greenport, NY, USA, [2]Oak Ridge Institute for Science and Education, PIADC Research Participation Program, Oak Ridge, TN, USA and [3]Department of Veterinary Population Medicine, University of Minnesota, Twin Cities, MN, USA

The rapid evolution of pathogenic RNA viruses presents a major challenge for scientists and others fighting to control transmission, predict and prevent future epidemics. Foot-and-mouth disease virus (FMDV), the picornavirus responsible for the eponymous disease, is one of the costliest livestock pathogens across much of the globe. Understanding how the virus changes over time both within hosts and through chains of transmission is of central importance for vaccine development, vaccination and quarantine strategies and international trade regulations. Cloven-hoofed animals including swine, cattle, and other domesticated and wild bovids are susceptible to the disease. Importantly, cattle and buffalo can be long-term carriers of the virus with the role of these animals in transmission being an active subject of research. Recent publications examining the full-length FMDV genome have begun to explain the complexities of the quasispecies and its behavior through transmission events and within hosts. Several of our lab's recent publications have addressed the question of which factors are responsible for inducing the carrier state. Our current endeavors build upon these concepts with detailed genomic study of FMDV in experimentally infected cattle through the acute and persistent phases of infection. Beginning with a heterogeneous inoculum mirroring the diversity that might be seen in an intensive farm outbreak, we have followed the progression of consensus genomes in twelve steers through different stages of disease including incubation, clinical disease, and the post-acute carrier state. In this study, we have documented convergent novel mutations at the canonical host cell entry RGD motif. We also characterized divergent minority genomes that, through powerful selective sweeps, became dominant at distinct points of infection. This study included both vaccinated and unvaccinated animals, with protection correlating with different patterns of viral evolution, notably at major antigenic sites. This is the first study to evaluate the full consensus genome of FMDV at distinct stages of infection, thus revealing significant micro-evolutionary events that can be of substantial benefit to disease control strategies and epidemiological modeling. The next stage of this work, supported by preliminary NGS data, will incorporate quasispecies-level analysis, elucidating the dynamic selective and population pressures during viral infection.

A56 Evolutionary analyses of foot-and-mouth disease virus in Southeast Asia using whole-genome sequences

Barbara Brito,[1,2] Steven J. Pauszek,[1] Ethan J. Hartwig,[1] George R. Smoliga,[1] Le T. Vu,[3] Pham P. Vu,[3] Carolina Stenfeldt,[1,2] Luis L. Rodriguez,[1] Donald P. King,[4] Nick J. Knowles,[4] Kasia Bachanek-Bankowska,[4] Ngo T. Long,[3] H. Dung,[5] and Jonathan Arzt[1]

[1]Foreign Animal Disease Research Unit, Plum Island Animal Disease Center, ARS, USDA, NY, USA, [2]Oak Ridge Institute for Science and Education, PIADC Research Participation Program, Oak Ridge, TN, USA, [3]Department of Animal Health, Ministry of Agriculture and Rural Development, Regional Animal Health Office No 6, Ho Chi Minh City, Vietnam and [4]The Pirbright Institute, Pirbright, UK

Foot-and-mouth disease (FMD) is one of the most important diseases of livestock worldwide. The causative agent, FMD virus (FMDV) is an aphthovirus from the Picornaviridae. The FMDV ORF is translated as a single polyprotein that codes for four structural proteins and eight non-structural proteins. Molecular epidemiology and evolution of FMDV have been traditionally studied using the sequence coding for VP1 (639 nt), the capsid protein containing most relevant antigenic domains. Although full-genome sequencing of this virus is not used as a routine diagnostic or surveillance tool, the availability of full-genome sequences in public repositories has increased over recent years.