

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, Sıhhiye-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,<sup>1</sup> Shibani Mukerji,<sup>2</sup> Simon Ye,<sup>3</sup> Jacob Lemieux,<sup>1</sup> Lisa Friemark,<sup>3</sup> Daniel Park,<sup>3</sup> Gordon Adams,<sup>1</sup> Michael Leone,<sup>2</sup> Marcia Goldberg,<sup>1</sup> Tracey Cho,<sup>2</sup> Eric Rosenberg,<sup>1</sup> and Pardis Sabeti<sup>3</sup>

<sup>1</sup>Division of Infectious Disease, Department of Medicine, Massachusetts General Hospital, Boston, MA 02114, USA, <sup>2</sup>Department of Neurology, Massachusetts General Hospital, Boston, MA 02114, USA and <sup>3</sup>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