over a decade, our understanding of its origin and dissemination patterns is limited. In this study, we employed a Bayesian phylogeographic approach to reconstruct the spatio-temporal dispersion pattern of this clade in Afghanistan and Iran for the first time. We performed a secondary data analysis on eligible HIV-1 CRF35_AD (gag and pol) sequences available in the Los Alamos HIV database (432 sequences available from Iran, 16 sequences available from Afghanistan, and a single CRF35_AD-like pol sequence available from USA). Sequences were excluded prior to analysis if they showed evidence of incorrect subtype assignment, frameshift, or drug resistance mutations, and/or stop codon positions. Subtype assignment was confirmed by maximum likelihood phylogenetic analysis. In order to reconstruct the spatio-temporal history of CRF35_AD, we used discrete Bayesian phylogeographic model in BEAST v1.8.1. Between-country viral dispersion rates were tested with Bayesian Stochastic Search Variable Selection method as implemented in SPREAD v1.0.7, and were considered as significant when Bayes factor values were >3. We checked the robustness of the key parameter estimates through a sensitivity analysis, using different priors and data subsets. The findings suggested that CRF35_AD sequences were genetically similar to parental sequences from Kenya and Uganda, and to a set of subtype A1 sequences available from Afghan refugees living in Pakistan. Our results also showed that across all phylogenies, Afghan and Iranian CRF35_AD sequences formed a monophyletic cluster (posterior clade credibility> 0.9). The divergence date of this cluster was estimated to be between 1990 and 1992. Within this cluster, a bidirectional dispersal of the virus was observed across Afghanistan and Iran. We could not clearly identify if Afghanistan or Iran first established or received this epidemic, as the root location of this cluster could not be robustly estimated. Three CRF35_AD sequences from Afghan refugees living in Pakistan nested among Afghan and Iranian CRF35_AD branches. However, the CRF35_AD-like sequence available from USA diverged independently from Kenyan subtype A1 sequences, suggesting that it may not be a true CRF35_AD lineage. The CRF35_AD viruses from Afghanistan, Iran, and Afghan refugees living in Pakistan seem to constitute a single epidemic, with multiple genetic exchanges among these populations. The date of onset for this epidemic (1990–1992) coincides with the rise of heroin production in Afghanistan (1970s). This highlights the potential role of drug trafficking in epidemic ignition in this region. Mass migration of Afghan refugees and illegal workers to Iran may be other possible contributors to among-country virus transmission.

A23 Large phylogenetic clusters highlight the HIV-1 epidemic in Canadian at risk populations

Rupert Capina,[1] Francois Cholette,[1] Eric Enns,[1] Christina Daniuk,[1] Tracy Taylor,[1] James Brooks,[1] P. Richard Harrigan,[2] and Paul Sandstrom[1]

[1]National Microbiology Laboratory at JC Wilt Infectious Diseases Research Centre, Winnipeg, MB, Canada and [2]BC Centre for Excellence in HIV/AIDS, Vancouver, BC, Canada

Since 1998, Canadian provinces and the Public Health Agency of Canada collaborated to monitor the country's HIV epidemic to form the Canadian HIV Strain and Drug Resistance Surveillance Program (SDR). The program traditionally reported incidence rates and prevalence of subtypes and drug resistance among newly diagnosed and not yet treated individuals. However, modern methods in phylogenetics have not yet been implemented in SDR. Here, we attempt to further characterize the HIV-1epidemic in Canadian populations experiencing high diagnosis rates by using phylogenetic clustering to deduce transmission dynamics. HIV-1 pol sequencing and genotyping was conducted on specimens submitted to the SDR program from treatment-naive individuals newly diagnosed with HIV from 2004 to 2013. REGAv3 and COMET were used for subtyping and Sierra, the Stanford Algorithm web service, was used for drug resistance genotyping. Phylogenetic clusters were inferred using patristic distances generated from bootstrap resampled trees (FastTree2). Statistical analyses were done using R. After quality filtering, 1,009 specimens were successfully sequenced and genotyped. Of those, 907 (89.9 per cent) formed a cluster of two or more. Overall, fifty-six clusters were inferred: three clusters ($n > 100$), six clusters ($n = 29–74$), seven clusters ($n = 8–15$), thirteen clusters ($n = 3–7$), and twenty-seven clusters ($n = 2$). We investigated predictors of clustering and found that people who self-reported inject drugs significantly clustered ($P = 0.006$), independent of other epidemiological variables such as age, sex, geographical regions, and risk behaviors. Phylogenetic clustering is a valuable tool to enhance HIV surveillance and monitoring efforts. Timely identification and investigation of clusters can inform focused prevention interventions. Effective use of HIV drug resistance genotype data for public health action will require revising information flows of the current provincial surveillance system building upon recommended clinical laboratory testing practices.

A24 Role of phylogenetic analysis in epidemiological case definitions during an outbreak of HIV-1 in people who inject drugs in Ireland

L. Dunford,[1] A. Waters,[1] M. Neary,[1] J. Dean,[1] C. Giese,[2] D. Igoe,[2] C. Hurley,[3] K. O'Donnell,[2] M. Fitzgerald,[3] and C. De Gascun,[1,4] the HIV Epidemiology Subgroup

[1]National Virus Reference Laboratory, University College Dublin, Dublin, Ireland, [2]HSE Health Protection Surveillance Centre, Dublin, Ireland, [3]Department of Public Health, Dublin, Ireland and [4]School of Medicine, University College Dublin, Dublin, Ireland

In 2015, an upsurge in acute HIV-1 subtype B infections was observed in Ireland among people who inject drugs, the majority of whom was homeless. The epidemiological investigation identified a significant association with injection of a new cathinone derivative, colloquially known as 'snow blow'. Concatenated HIV-1 polymerase and protease partial gene sequences (881 nucleotides; $n = 48$) were aligned with all subtype B patient sequences analysed at the NVRL from 2000 to 2015 ($n = 918$) and appropriate reference sequences using Bioedit v7.05. A neighbour-joining guide tree was constructed under a Kimura-2-Parameter model. Directed by this initial analysis, a maximum likelihood tree was constructed with a smaller number of more related Irish reference sequences ($n = 274$) under a HKY model of evolution and a gamma distribution. Statistical support was provided by bootstrapping with 1,000 replicates. Trees were constructed using PAUP* software version 4.0 beta10 and Mega version 7. The sequences from cases under investigation clustered within larger transmission networks of Irish people who inject drugs. More refined phylogenetic analyses confirmed that 79 per cent of the cases fell into four distinct clusters; cluster 1 ($n = 16$), cluster 2 ($n = 16$), cluster 3 ($n = 3$), and cluster 4 ($n = 3$), with high bootstrap support for each cluster (>75 per cent). There were ten outliers which branched outside the four clusters. The phylogenetic analysis largely supported the epidemiological investigation and the majority of epidemiologically linked cases were found to be contained within the same genetic clusters. In addition, this analysis identified two further possible cases and also eliminated three more 'cases'

from the outbreak. The present study highlighted the integral role that real-time phylogenetic analyses can play alongside extensive epidemiological investigations, assisting the clarification of epidemiological case definitions in temporal transmission network in a HIV outbreak investigation.

A25 Analysis of non-structural genes subtype A1 HIV-1 circulating in Russia

K. Gromov,[1] A. Murzakova,[2] D. Kireev,[2] and E. Kazennova[1]

[1]N.F. Gamaleya Federal Research Centre for Epidemiology and Microbiology of the Ministry of Health of the Russian Federation, Moscow, Russian Federation and [2]Central Research Institute of Epidemiology of The Federal Service on Customers' Rights Protection and Human Well-being Surveillance, Moscow, Russian Federation

The HIV infection epidemiological situation in Russia is characterized by the predominance of a unique genetic variant of subtype A1 HIV-1 named AFSU. This variant has significant differences from other subtype A1 HIV variants in the genes coding for structural proteins Gag, Pol, and Env, but no analysis of the non-structural genes was carried out. These genes may have a significant influence on the rate of viral replication and transmission, playing major role in the pathogenesis of HIV virus and interaction with the human immune system. The aim of this work was to find out if the differences in *vif*, *vpr*, *vpu*, *tat*, *rev*, and nef *genes* of AFSU variant from other subtype A1 variants exist. NGS methodology was used for the analysis of the viruses in blood plasma samples obtained from HIV-infected patients in different regions of Russia, previously identified as AFSU. We received forty-seven complete genome sequences using the MiSeq (Illumina, USA); additionally fifty-four complete genome sequences of subtype A1 HIV-1 were extracted from Genbank. All sequences were divided into fragments corresponding to vif, vpr, vpu, tat, rev, and nef genes. All sequences were subjected to phylogenetic analysis using MEGA 6.0 program. Phylogenetic analysis of vif, vpr, vpu, tat, and rev genes has shown that all AFSU samples formed a sub-cluster inside the subtype A1 cluster formed by other A1 nucleotide sequences. The *nef* gene sequences did not form any clusters irrespectively of the mode of phylogeny estimation. The results of the phylogenetic analysis showed that AFSU HIV-1 non-structural genes *vif*, *vpr*, *vpu*, *tat*, and *rev* have differences from other subtype A1 HIV-1 variants. The *nef* gene sequences did not show any phylogenetic differences. The information will be used as a background for further investigations of the epidemiological and biological characteristics of the HIV1 viruses prevailing in Russia.

A26 Probing the compartmentalization of HIV-1 in the central nervous system through its neutralization properties

K. Stefic,[1,2] A. Chaillon,[3] M. Bouvin-Pley,[1] A. Moreau,[1] M. Braibant,[1] F. Bastides,[4] G. Gras,[4] L. Bernard,[4] and F. Barin[1,2]

[1]Université François Rabelais, Inserm U966, Tours, France, [2]Laboratoire de Bactériologie-Virologie, CHU Bretonneau, Tours, France, [3]Department of Medicine, University of California San Diego, La Jolla, CA, USA and [4]Médecine Interne et Maladies Infectieuses, CHU Bretonneau, Tours, France

Compartmentalization of HIV-1 has been observed in the cerebrospinal fluid (CSF) of patients at different clinical stages. Compartment specific modifications have been frequently described in the variable loops and the glycosylation sites of the envelope glycoproteins, a known mechanism to escape neutralizing antibodies (NAb). Considering the low permeability of the blood–brain barrier, we wondered if a lower NAb selective pressure in the central nervous system (CNS) could favor the evolution of NAb-sensitive viruses in this compartment. Single-genome amplification (SGA) was used to sequence near full-length HIV-1 envelope variants (453 sequences) from paired CSF and blood plasma samples of nine subjects infected by HIV variants of different clades and suffering from neurologic syndromes. Dynamics of viral evolution were evaluated with a Bayesian coalescent approach for individuals with longitudinal samples ($n = 4$). For six subjects, pseudotyped viruses expressing envelope glycoproteins variants representative of the quasispecies present in each compartment were generated, and their sensitivity to autologous neutralization, broadly neutralizing antibodies (bNAbs) and entry inhibitors was assessed. Significant compartmentalization of HIV populations between blood and CSF were detected in five out of nine subjects by all tests ($P < 0.01$). Bayesian analyses revealed independent evolution of CSF viral populations for extended periods of time (up to eight years for one patient). There was no difference in sensitivity to autologous neutralization between blood- and CSF-variants, even for subjects with compartmentalization. However, we observed major differences of sensitivity to sCD4 or to at least one bNAb targeting either the N160-V1V2 site, the N332-V3 site or the CD4bs, between blood- and CSF-variants in all cases. Our data show that selective pressure by autologous NAb is not the main driver of HIV evolution in the CNS. Given that each of the conserved neutralizing epitopes is associated to a specific property for cell entry, our data suggest that functional properties of the envelope are responsible for compartmentalization. Considering the possible migration from CSF to blood, the CNS could be a reservoir of bNAb-resistant viruses, an observation that should be considered for immunotherapeutic approaches.

A27 Exploring novel mechanisms of HIV-2 mutagenesis

M. E. Meissner,[1,2,3] S. Baliga,[1] M. Roth,[1] J. Baller,[3,4] and L. M. Mansky[1,2]

[1]Institute for Molecular Virology, Madison, WI, USA, [2]Molecular, Cellular, Developmental Biology, and Genetics Graduate Program, Saint-Paul, MN, USA, [3]Bioinformatics and Computational Biology Graduate Program, Minneapolis, MN, USA, [4]Minnesota Supercomputing Institute, Minneapolis, MN, USA and [5]University of Minnesota, Minneapolis, MN, USA

Over thirty-six million individuals are infected with HIV worldwide. Nearly 95 per cent of these individuals are infected with HIV type 1 (HIV-1), which has a high rate of viral mutation that helps drive immune evasion, disease progression, and rapid emergence of drug resistance. HIV type 2 (HIV-2) accounts for fewer than two million infections overall, remains primarily restricted to West Africa, and exhibits a significantly attenuated disease phenotype compared to HIV-1, characterized by lower rates of transmissibility and a slower progression to AIDS. HIV-2 has recently been found to have a significantly lower rate of mutation compared with HIV-1, which may be related to the differences in viral disease progression and persistence. Although the main driver of HIV mutagenesis is the low fidelity of the virally encoded reverse transcriptase, host factors may contribute to the mutation rate as well. The host protein SAMHD1 has been previously shown to restrict HIV-1 infection in myeloid lineage cells by depletion of dNTP pools through a triphosphohydrolase activity. In addition to inhibiting reverse transcription, this disruption of cellular dNTP levels may contribute to misincorporation of nucleotides and result in mutation of the virus. Here, we propose the use of NGS to explore the role of SAMHD1 on HIV-1 and HIV-2 mutagenesis. Using HIV-1 and HIV-2 Vpx-viruses (which are sensitive to SAMHD1 restriction), we will use