A14 Estimating time since HIV infection using next-generation sequencing data: A unique tool to help understand HIV prevention among high-risk young women in Ukraine

Francois Cholette,[1] Christina Daniuk,[1] Emma Lee,[1] Rupert Capina,[1] Eve Cheuk,[2] Marissa Becker,[2] Sharmistha Mishra,[3] Hui Ting Ma,[3] Olga Balakireva,[4] Daria Pavlova,[4] and Paul Sandstrom[1]

[1]National HIV and Retrovirology Laboratories, JC Wilt Infectious Diseases Research Centre, Public Health Agency of Canada, Winnipeg, Manitoba, Canada, [2]Community Health Sciences, Center for Global Public Health, University of Manitoba, Winnipeg, Manitoba, Canada, [3]Li Ka Shing Knowledge Institute, St. Michael's Hospital, Toronto, Ontario, Canada and [4]Ukrainian Institute for Social Research after Oleksandr Yaremenko, Kiev, Ukraine

The transitions study examines HIV risk among adolescent girls and young women through their sexual life course from first sex, to past and current engagement in casual sex, transactional sex, and, for some, formal sex work (FSW). Understanding the timing of HIV infection and the circumstances around early infection in young females is critical to HIV prevention interventions. We inferred time since HIV infection using next-generation sequencing (NGS) of the HIV *pol* gene isolated from cross-sectional samples among high-risk young women in Dnipro, Ukraine. Dried blood spots were collected on Whatman 903 cards from young women aged 14–24 engaged in casual sex ($n = 894$), transactional sex ($n = 464$), and FSW ($n = 452$). The HIV *pol* gene was sequenced using an in-house NGS HIV drug resistance mutation genotyping assay. Time since HIV infection was inferred using an online tool as described by Puller et al. (2017) freely available at https://hiv.biozentrum.unibas.ch/ETI/. The mean estimated time since HIV infection (ETI) for participants engaged in casual sex, transactional sex, and FSW is 1.98, 1.84, and 3.01 years, respectively. ETI was used to determine the duration of HIV infection for each participant and compared to the number of sexually active years prior to FSW. Among FSW, 61 per cent of participants were infected with HIV prior to entry into sex work. In general, ETI from NGS data suggests that FSWs were infected with HIV before entry into FSW. Expansion of targeted prevention programs beyond FSW could play an important role in mitigating HIV transmission at the population level.

A15 Archived ART resistance in the latent reservoir of virally suppressed Ugandans

A. A. Capoferri,[1] B. A. Lynch,[1] J. L. Prodger,[2,3] S. J. Reynolds,[1,4,5] J. Kasule,[5] D. Serwadda,[5,6] S. Lamers,[7] C. Martens,[8] T. C. Quinn,[1,3,4] and A. D. Redd[1,4]

[1]Division of Infectious Diseases, Johns Hopkins University School of Medicine, Baltimore, MD, USA, [2]Department of Microbiology and Immunology, Western University, London, Canada, [3]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA, [4]Laboratory of Immunoregulation, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA, [5]Rakai Health Sciences Program, Kalisizo, Uganda, [6]Makerere University, Kampala, Uganda, [7]Bioinfo Experts Inc., Thibodaux, LA, USA and [8]Genomics Unit, Research Technologies Branch, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA

The increased access of antiretroviral therapy (ART) has drastically improved the health of infected individuals. However, increased levels of ART resistance globally threaten ART effectiveness. Resistance monitoring is currently limited to viremic individuals or prior to ART initiation. The archival nature of the HIV latent reservoir (LR) of virally suppressed patients allows examination for the persistence of ART-resistant latent viral variants. Whole blood samples were collected longitudinally in Rakai, Uganda, from 70 virally suppressed HIV-1 infected individuals. The quantitative viral outgrowth assay was performed to measure the frequency of replication-competent latently infected resting-memory CD4 + (rCD4) T-cells. RNA was extracted from HIV p24-positive outgrowth supernatant, and the reverse transcriptase (RT) region was sequenced using a validated site-specific next-generation sequencing assay (Illumina, San Diego, CA). Consensus sequences containing >2.5 per cent of the total raw amplicons of each outgrowth well were analyzed for ART drug resistance mutations using the Stanford Database. The presence of clonal sequence is expressed as both percent clonality and Shannon Entropy. Replication-competent virus was cultured from 52/70 (74.3%) individuals, of which, RT-pol sequence data were obtained from 49/52 (94.3%) individuals. The presence of ART-resistant virus was found in the LR from one individual on second-line therapy that included a protease inhibitor. There were 20 and 44 total prominent consensus sequences from all wells at years 1 and 3 of follow-up, respectively. ART-resistant mutations for both RT-inhibitor drug classes were found in 30 per cent and 27.3 per cent of the total prominent consensus sequences of this one individual from years 1 and 3, respectively. The major ART resistance profile in this individual included: M184V, Y188L, K191E, and G190A. The percentage of total outgrowth that was clonal (percent clonality) increased from year 1 to year 3 (38.1–81.8%) and Shannon Entropy decreased (0.722–0.576). The presence of archived replication-competent ART-resistant virus in the LR was found in only one individual. There were two ART-resistant prominent consensus sequences isolated at year 3 that were not sampled 2 years earlier. The persistence of resistant, intact replication-competent proviral sequences in the LR of this individual seem to be supported by clonal expansion.

A16 Next-generation sequencing to detect transmitted drug resistance mutations in Romanian people who inject drugs

Marius Surleac, Simona Paraschiv, Ionelia Nicolae, Leontina Banica, Ovidiu Vlaicu, Raluca Jipa, Adrian Abagiu, and Dan Otelea

[1]Molecular Diagnostics Laboratory, National Institute for Infectious Diseases "Matei Bals", Bucharest, Romania and [2]Clinical Department, National Institute for Infectious Diseases "Matei Bals", Bucharest, Romania

Romania has faced an HIV outbreak among people who inject drugs (PWID) since 2011. The introduction of so-called 'legal highs' (amphetamine-type stimulants) on the drug market a few years prior contributed substantially to this outbreak. Next-generation sequencing (NGS) provides the possibility to detect drug resistance mutations with higher sensitivity than Sanger sequencing. The aim of this study was to search for transmitted drug resistance (TDR) mutations in strains from PWID recently diagnosed with HIV infection by parallel use of Sanger sequencing and NGS. The study was conducted on strains from 34 PWID diagnosed with HIV infection between 2016 and 2017. Sequencing was performed for the *pol* (PR, RT, and INT) and *env* (V2-V3 loop) regions. Sanger sequencing was performed with the commercial ViroseqTMHIV-1 Genotyping system (Abbott Laboratories) and with an in-house protocol for the *env* gene. NGS was performed in the same genomic regions using Nextera DNA Library Preparation Kit (Illumina) and the Miseq instrument (Illumina). NGS data were processed for error correction, read mapping, and detection of drug resistance mutations with HIV-1 Deepchek analysis software. Geno2pheno algorithm was used for viral tropism prediction and the WHO 2009 list for TDRM analysis. By using NGS, we detected seven cases (20.6%) of TDR in PWID and only two cases (5.8%) with standard sequencing. The TDR mutations detected by NGS were K103N, K101EN, Y181C, T215S in RT gene, I54V and M46L in PR, and none in INT. Two NNRTI mutations (K103N and K101EN) were detected in the same sample. Most of the TDR identified were present in the minority population (between 1% and 2% of the total reads) explaining the higher sensitivity of NGS method compared with standard sequencing. No significant differences were observed between these two methods when tropism prediction was analyzed. The majority of the viruses circulating in this group were R5-tropic. All strains showed more resistance mutations when analyzed by deep sequencing than by Sanger sequencing and more than previously observed in other risk groups. NGS proved to be a sensitive tool to detect TDR in newly infected PWID.

A17 The effect of intra-host evolution of HIV-2 capsid on disease progression

M. T. Boswell,[1] A. Palm,[2] S. Karlson,[2] F. Månsson,[2] H. Norrgren,[2] M. Jansson,[2] P. Medstrand,[2] S. Rowland-Jones,[1] and J. Esbjornsson[2]

[1]Nuffield Department of Medicine, University of Oxford, Oxford, UK and [2]Department of Laboratory Medicine, Faculty of Medicine, Lund University, Lund, Sweden

The human immunodeficiency virus type 2 (HIV-2) is an important cause of acquired immune deficiency syndrome (AIDS) in West Africa. The virus started circulating in humans around 1938 and has spread predominantly within West Africa with an estimated 1–2 million people being infected today. Compared with the

pandemic HIV-1, HIV-2 infected people have longer AIDS-free survival times, higher CD4+ counts and lower risk of vertical and horizontal transmission. Approximately 35 per cent of HIV-2 infected individuals are classified as so-called long-term non-progressors with undetectable viral loads and limited disease progression after 10 years of follow-up. It has been shown that HIV-2 is more sensitive to the host restriction factor TRIM5α when compared with HIV-1, and this has been linked to conformational changes in the retroviral capsid. TRIM5α binds at the interface between three capsid hexamers, initiates early uncoating and proteasomal degradation. TRIM5 genotype has shown only modest effects on HIV-1 disease outcomes. HIV-2 capsid sequences bearing a specific poly-proline motif have been associated with lower viral loads and presentation of protective HLA I-restricted epitopes. The major aims of this study were to (1) determine HIV-2 capsid intra-host evolutionary rates and (2) identify residues that are affected by positive selection and that can be linked to HIV-2 viral load and disease progression in conjunction with TRIM5 genotype. The Guinea-Bissau Police cohort is unique, with decades of relatively frequent follow-up. One hundred and sixty-five patients were included for genotyping of TRIM5, 62 females and 103 males. Median age at enrolment was 52.6 years (range 30–87) and 7.9 per cent of patients had a CD4 percentage < 15 per cent at enrolment. Six of these individuals were included for amplification of HIV-2 capsid from longitudinally collected samples. Viral RNA was extracted from stored blood plasma samples and capsid of the circulating viral quasispecies was amplified, cloned, and sequenced, as previously described. Bayesian analysis will be used to determine intra-host evolutionary rates, dN/dS ratios and how these parameters associate with disease progression and TRIM5 genotype.

A18 HIV-2 molecular epidemiology in Spain: 30 years since the first case

S. Requena,[1] E. Caballero,[2] A. B. Lozano,[3] R. Tellez,[4] L. Morano,[5] M. C. Nieto,[6] N. Margall,[7] V. Soriano,[8] and C. de Mendoza[1] on behalf of the HIV-2 Spanish Study Group

[1]Instituto de investigación sanitaria Puerta de Hierro-Segovia de Arana (Health Research Institute Puerta de Hierro-Segovia de Arana), Majadahonda, Madrid, Spain, [2]Hospital Universitario Vall d'Hebron (Universitary Hospital Vall d'Hebron), Barcelona, Spain, [3]Hospital de Poniente, Almería, Spain, [4]Hospital Universitario Fundación Jiménez Díaz (Universitary Hospital Fundación Jiménez Díaz), Madrid, Spain, [5]Hospital Álvaro Cunqueiro, Pontevedra, Spain, [6]Hospital Universitario de Basurto (Basurto Universitary Hospital), Bilbao, Spain, [7]Hospital de la Santa Creu i Sant Pau, Barcelona, Spain and [8]Hospital Universitario La Paz (Universitary Hospital La Paz), Madrid, Spain

HIV-2 is a retrovirus that mainly infects West Africans. In Europe, HIV-2 has been circulating since the 1980s, and more recent immigration has contributed to its spread. Excluding HIV-2 in all HIV seropositive individuals precludes misinterpretation of viral loads (VL) and antiretroviral (ART) choices. Surveillance registers enables tracking of epidemic spread and identification of major contributors, allowing the establishment of convenient preventive measures. The HIV-2 Spanish study group was founded in 1989. Since then, blood specimens from HIV-2 carriers have been collected. More than 40 Spanish hospitals are part of the group and provide clinical and epidemiological data. Records for each HIV-2 patient include country of origin, gender, age, transmission category, monitoring of HIV-2 VL, CD4 counts, drug resistance, and HIV-2 subtype. Up to December 2017, 354 HIV-2+ individuals were identified in Spain and incidence (15–20/year) has been stable within the last decade. At diagnosis, mean age was 44.6 years and 63 per cent were male. The majority were Africans (78%), whereas 16.5 per cent were native Spaniards. 78.2 per cent acquired HIV-2 by heterosexual contact. HIV-2 subtyping using the HIV2EU tool was performed in 126 subjects: 86 Africans and 27 native Spaniards. The subtype distribution was as follows: 108 (85.7%) HIV-2 subtype A and 18 (14.3%) B. Africans and Spaniards were mostly infected with subtype A (87.2% and 77.8%, respectively). HIV-2 subtype B was found in six native Spaniards (22.2%; 6/27), five patients from Equatorial Guinea (71.4%; 5/7), four from Senegal (18.1%; 4/22), two from Ivory Coast (100%; 2/2), and one from Burkina Faso (50%; 1/2). Using phylogenetic analyses, two clusters were identified among homosexual Spanish men (subtype A: 8 men and subtype B: 2 men with viral isolates related to Malian and Senegalese isolates). Before starting ART, CD4 count mean values for subtype A and B were 378 and 357, respectively. Corresponding VL values were 2.63 and 2.32 HIV-2 RNA log copies/ml, respectively.

A19 The origin of acute HCV in HIV-infected men who have sex with men in the Netherlands

S. Popping,[1] C. Boucher,[1] G. Verjans,[1] B. Rijnders,[2] and D. van de Vijver[1]

[1]Department of Viroscience, Erasmus Medical Center, Rotterdam, The Netherlands and [2]Department of Internal Medicine and Infectious Diseases, Eramsus Medical Center, Rotterdam, The Netherlands

In recent years, several outbreaks of hepatitis C virus (HCV) infections have been observed among HIV-infected men who have sex with men (MSM) in Europe. In the Netherlands, high incidence rates of 10/1,000 person-years are reported. In this analysis, we describe whether acute HCV is linked to specific transmission networks. A total of 50 Dutch HIV-infected MSM, diagnosed with acute HCV genotype 1a between 2013 and 2014, were included. Target enrichment for viral nucleic acid separation and deep sequencing were used to recover whole HCV genomes. Phylogenetic trees were constructed by use of the maximum likelihood method. Pairwise distance matrices were generated by use of the Kimura-2 parameter distance estimation method. The consistency of the phylogenetic clustering was tested by bootstrap analysis with 1,000 replicates. A cluster was defined as having a genetic distance of at most 1.5 per cent and bootstrap values of 100 per cent. The most recent common ancestor was estimated with a coalescent-based model with a Bayesian statistical framework. Four transmission clusters were identified that included a total of 38 patients (76% of the total). The clusters were indicative of recent outbreaks, as highlighted by small genetic distances and a most recent common ancestor after the year 2000, when the first cases of HCV infection in HIV-infected MSM were reported. The HCV epidemic among HIV-infected MSM is a young epidemic, with most of the acute infections linked within the four major transmission networks. Prevention strategies identifying and targeting these transmission networks can potentially curb the epidemic.

A20 Sample preparation for whole-genome next-generation sequencing (NGS) of hepatitis C virus (HCV) routine RNA samples

Julia Hillung,[1,2] María Alma Bracho,[2,3,4] Javier Pons Tamarit,[3] and Fernando González-Candelas[1,2,3,4]

[1]Instituto de Biología Integrativa y de Sistemas (I2SysBio), CSIC-Universidad de Valencia, Valencia, Spain, [2]Unidad Mixta 'Infección y Salud Pública' FISABIO-Salud Pública, Valencia, Spain, [3]Fundación para el Fomento de la Investigación Sanitario y Biomédica de la Comunidad Valenciana (FISABIO), Valencia, Spain and [4]CIBER en Epidemiología y Salud Pública, Spain

Next-generation sequencing (NGS) is a technique that can capture the variability of viral populations in transmission studies. The conventional sample preparation for NGS, based on amplicons, is a potential source of errors, derived from the variable affinity of specific primers for different viral variants and from irregular DNA polymerase efficiency. In this context, we propose a more reliable method for viral whole genome sample preparation, starting from nucleic acids obtained and stored with conventional procedures. Our goal was to obtain complete hepatitis C virus (HCV) genome sequences to subsequently perform extensive phylogenetic analyses. Additionally, we aimed to test the effectiveness of nuclease treatment used to remove contaminating host DNA. Nucleic acids were obtained from almost cell-free blood plasma of HCV-infected patients. As a source for Illumina library preparation, double-stranded cDNA was generated using random primers. The HCV genome was not amplified before library preparation, avoiding possible biases derived from unequal copying. To get rid of possible host contaminants in the samples, a DNase treatment step was added. Libraries were paired-end sequenced on the Illumina platform using MiSeq reagent kit v3. After conservative filtering of contaminant human reads by alignment with the human reference genome using Burrows-Wheeler Aligner (BWA), the remaining reads were mapped to the