

recombination may lead to better ways to inhibit infections and develop new antiviral treatments.

#### **A31 A large-scale screening for hepaciviruses in African rodents**

M. Bletsa,<sup>1,\*</sup> B. Vrancken,<sup>1</sup> S. Gryseels,<sup>2</sup> H. Leirs,<sup>2</sup> J. Göyü de Bellocq,<sup>3</sup> A. Laudisoit,<sup>4</sup> E. Wollants,<sup>1</sup> M. Van Ranst,<sup>1</sup> J.F. Drexler,<sup>5</sup> P. Lemey,<sup>1</sup>

<sup>1</sup>Clinical and Epidemiological Virology, Department of Microbiology and Immunology, KU Leuven, Rega Institute for Medical Research, Leuven, Belgium, <sup>2</sup>Department of Biology, Evolutionary Ecology Group, University of Antwerp, Antwerp, Belgium, <sup>3</sup>Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Studenec, Czech Republic, <sup>4</sup>Ecology, Evolution and Genomics of Infectious Disease Research Group, Institute of Integrative Biology, University of Liverpool, Liverpool, UK and <sup>5</sup>Institute of Virology, University of Bonn Medical Centre, Bonn, Germany

Hepatitis C virus (HCV) is considered to be a major public health problem, infecting >3% of the human population and causing acute and chronic liver disease. To date, the zoonotic origins of HCV remain elusive, since no animal population has been identified with closely related hepaciviruses. Inspired by recent findings of divergent hepaciviruses in rodents, we have screened a comprehensive set of African rodent samples that have been collected through various collaborations. Screening was performed by employing a highly sensitive nested PCR assay directed against the NS3 protease-helicase gene. From the 2,361 samples that were screened, 74 were found positive for the presence of hepaciviruses. At this stage, we focus on generating longer stretches of genomic information based on previously described protocols that make use of host rRNA depletion and a simple viral enrichment methodology followed by NGS approaches. By generating and analyzing sequence data from these samples we aim to perform in-depth phylogenetic and phylogeographic analyses gaining valuable insight into the evolutionary origins and epidemic emergence of HCV. Emphasis will be put on the identification of novel hepacivirus lineages more closely related to HCV, as well as the examination of host-specific adaptation and geographic structuring of these viruses.

#### **A32 Search for viral integration insertion sites into the human genome—strategy matters**

T.M. Zorec,<sup>1</sup> L. Hošnjak,<sup>1</sup> B. Kocjan,<sup>1</sup> N. Gale,<sup>2</sup> N. Zidar,<sup>2</sup> P. Strojjan,<sup>3</sup> M. Poljak,<sup>1</sup>

<sup>1</sup>Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia, <sup>2</sup>Institute of Pathology, Faculty of Medicine, University of Ljubljana, Slovenia and <sup>3</sup>Department of Radiation Oncology, Institute of Oncology, Ljubljana, Slovenia

During one of our previous works we investigated the integration of a low-risk human papillomavirus' (HPV) genetic material into the human genome, we aimed to elucidate whether the integration event(s) had helped facilitate the manifestation of cancer in an individual with a HPV11-positive sinonasal squamous cell carcinoma. To elucidate viral integration sites, whole-genome sequencing (WGS) was applied to the tissue sample of sinonasal carcinoma. We employed three different analysis strategies – data pipelines with different topologies that should intuitively all yield qualitatively the same result with minor variations. One of the pipeline topologies was adopted after Li et al. (2013) with slight modifications, whereas the other two were simple meta-algorithms that should generally answer the same question. Although the bulk of the breakpoints yielded through the three different topologies did overlap to some degree, a great deal of breakpoints varied greatly between the approaches.

#### **A33 The cervico-vaginale microbiota in chlamydia trachomatis notified women: a case-control study at the sexually transmitted infection outpatient clinic in Amsterdam**

Charlotte van der Veer,<sup>1</sup> S.M. Bruisten,<sup>1</sup> J.J. van der Helm,<sup>2</sup> H.J.C. de Vries,<sup>2,3</sup> R. van Houdt,<sup>4</sup>

<sup>1</sup>Public Health Laboratory, Public Health Service Amsterdam, Amsterdam, The Netherlands, <sup>2</sup>Public Health Service Amsterdam, Sexually transmitted infections outpatient clinic, Amsterdam, The Netherlands, <sup>3</sup>Department of Dermatology, Amsterdam Medical Centre, University of Amsterdam, Amsterdam, The Netherlands and <sup>4</sup>Department of Medical Microbiology and Infection Prevention, VU University Medical Centre, Amsterdam, The Netherlands

Increasing evidence suggests that the cervico-vaginal microbiome (CVM) plays an important role in acquiring sexually transmitted infections (STIs). Here we studied the CVM in women exposed to *Chlamydia trachomatis* (Ct). We included 98 women who were notified by Ct-positive sex partners via contact-tracing at the STI outpatient clinic in Amsterdam, the Netherlands. Cervico-vaginal samples and clinical data were collected for all women. CVM compositions were characterized by sequencing of the V3/V4 region of the 16srRNA gene using the Illumina MiSeq platform. High quality reads were assigned to operational taxonomic units and classified using a vaginal reference package. Hierarchical clustering delineated CVM clusters based on microbial relative abundances. Possible determinants for acquiring Ct were analyzed using multivariable logistic regression. The CVM was characterized for 93 women, of whom 52 were Ct positive and 41 Ct negative. We identified three major CVM clusters. Clustered CVM predominantly comprised either diverse anaerobic bacteria ( $n=39$ ; 42%), *Lactobacillus iners* ( $n=32$ ; 34%) or *Lactobacillus crispatus* ( $n=22$ ; 24%). In multivariable analysis, we found that the CVM was significantly associated with *C. trachomatis* infection (OR = 4.2 (95% confidence interval, CI: 1.2–15.4) for women with diverse anaerobic CVM and OR = 4.4 (CI: 1.3–15.6), for women with *L. iners*-dominated CVM, compared to women with *L. crispatus*-dominated CVM), as was younger age (OR = 3.1, CI: 1.1–8.7, for those  $\leq 21$  years old) and reporting a steady sex partner (OR = 3.6, CI: 1.4–9.4). Women who tested positive for *Chlamydia trachomatis* infection after having been contact-traced by a chlamydia positive partner were more likely to have CVM dominated by *L. iners* or by diverse anaerobic bacteria, than by *L. crispatus*.

#### **A34 Automated profiling of the human virome from raw metagenomic data**

Yumna Moosa,<sup>1,2</sup> Michael Vilsker,<sup>3</sup> Ewout Vanden Eyden,<sup>4</sup> Vagner Fonseca,<sup>5</sup> Sam Nooij,<sup>6</sup> Koen Deforche,<sup>3</sup> Tulio de Oliveira,<sup>1,2</sup>

<sup>1</sup>Nelson R Mandela School of Medicine College of Health Sciences, University of KwaZulu-Natal (UKZN), South Africa, <sup>2</sup>Centre for the AIDS Programme of Research in South Africa (CAPRISA), UKZN, South Africa, <sup>3</sup>Emweb, Herent, Belgium, <sup>4</sup>Rega Instituut, KU Leuven, Belgium, <sup>5</sup>Universidade do Estado da Bahia (UNEB), Brazil and <sup>6</sup>Rijksinstituut voor Volksgezondheid en Milieu (RIVM), The Netherlands

Viruses influence human health as conventional pathogens, as modulators of gene expression and through their involvement in complex host-microbiome interactions. Next generation sequencing (NGS) has enabled us to explore the role of the microbiome in human health and disease. Metagenomic sequencing should allow us to profile all biological elements in a clinical sample in an unbiased, hypothesis-free way. However viruses display much greater variation than all other elements, and the existing tools and methods for virus identification and

discovery are not effective enough. We have developed a bioinformatics pipeline to identify and classify all known viruses present in a metagenomic sample. Viral NGS reads are identified using a protein-based alignment method, DIAMOND, which is substantially faster than the standard BLAST method, and more reliable for viruses. These reads are automatically assembled into contigs using SPAdes, a *de novo* assembler. The contigs are then used to classify the virus at species level using a pan-viral typing tool based on all available taxonomic reference sequences from the International Committee on Taxonomy of Viruses (ICTV) database. This bioinformatics pipeline is Java-encoded and will include an easy-to-use web interface that is fit-for-purpose for researchers or clinicians. This tool can assemble viral contigs from paired-end reads generated by an Illumina MiSeq sequencer. So far 1865 viruses can be identified at species level resolution and 10 viruses (chikungunya virus, dengue virus, HBV, HCV, HHV8, HIV-1, HPV, HTLV-1, YFV, and Zika virus) at the genotype level. A web version of the pan-viral typing tool is already available and a web version with extended NGS functionality is currently being evaluated. Eliminating the need for virus-specific laboratory techniques, or targeted sequence capture, means a virome can be profiled in the context of its non-viral microbiome. Preliminary findings suggest our tool offers greater functionality than existing alternatives, with greater sensitivity to known viruses (including bacteriophages), automatic assembly and good quality phylogenetic analyses. A systematic comparison is underway.

#### **A35** Viral evolution and innate immune responses during acute HIV-1 infection and their association with disease pathogenesis

A.S. Hassan,<sup>1,\*</sup> J. Hare,<sup>2</sup> G. Kamini,<sup>3</sup> L.M. Yindom,<sup>4</sup> A. Kamali,<sup>5</sup> E. Karita,<sup>6</sup> W. Kilemba,<sup>6</sup> M.A. Price,<sup>7,8</sup> P. Borrow,<sup>4</sup> P. Bjorkman,<sup>9</sup> J. Albert,<sup>10</sup> P. Kaleebu,<sup>5</sup> S. Allan,<sup>6,11</sup> P. Fast,<sup>7</sup> E. Hunter,<sup>6,11</sup> J. Gilmour,<sup>2</sup> T. Ndung'u,<sup>3</sup> S. Rowland-Jones,<sup>4</sup> E.J. Sanders,<sup>1,4</sup> J. Esbjornsson,<sup>4,9,10</sup>

<sup>1</sup>KEMRI/Wellcome Trust Research Programme, Kilifi, Kenya, <sup>2</sup>IAVI Human Immunology Laboratory, London, UK, <sup>3</sup>Kwazulu-Natal Research Institute for Tuberculosis and HIV, Durban, South Africa, <sup>4</sup>Nuffield Department of Medicine, University of Oxford, UK, <sup>5</sup>Medical Research Council/Uganda Virus Research Institute (MRC/UgRI), Uganda, <sup>6</sup>Rwanda and Lusaka, Rwanda/Zambia HIV Research Group (RZHRG) Kigali, Zambia, <sup>7</sup>IAVI, New York, NY, USA, <sup>8</sup>UCSF Department of Epidemiology and Biostatistics, San Francisco, CA, USA, <sup>9</sup>Department of laboratory medicine, Lund University, Sweden, <sup>10</sup>Department of Microbiology Tumor and Cell Biology, Karolinska Institute, Sweden and <sup>11</sup>Emory Vaccine Center Emory University, Atlanta, USA

The rate of HIV-1 disease progression varies widely between individuals. This has been attributed to a combination of virological and immunological events during acute HIV infection (AHI). However, the exact mechanisms explaining the relationship between HIV-1 diversity, evolutionary dynamics and host immune responses, and their effect on disease pathogenesis remain unclear. We aim to dissect HIV-1 viral diversity, evolutionary dynamics and select parts of the innate immune responses observed during AHI, and elucidate virus-host mechanisms involved in the regulation of HIV-1 disease pathogenesis during the acute and chronic stages of infection. A retrospective longitudinal study design from well-characterized AHI cohorts will be used. Archived samples from about 122 patients with AHI (defined as HIV-1 antibody negative and RNA or p24 antigen positive) from Europe (Sweden [ $n=32$ ]) and Africa (Kenya [ $n=32$ ], Rwanda [ $n=14$ ], Uganda [ $n=13$ ], Zambia [ $n=15$ ], and South Africa [ $n=16$ ]) will be included. Each patient will contribute plasma samples from four serial time points (<14, 30 [+/- 15], 90 [+/- 30] and 360 [+/- 180] days post estimated date of infection, EDI) collected prior to treatment initiation. HIV-1 env

sequences determined by single genome sequencing (SGS), with 20 SGS clones from each time point, will be generated. In addition, a selected panel of innate immune markers will be profiled using the Meso Scale Discovery (MSD) electro-chemiluminescence-based platform and/or ELISAs. A multi-dimensional Bayesian framework of hierarchical phylogenetic models (HPM) will be applied, allowing for both fixed and random effects prior specifications to test for differences associated between and within patient group parameters, and where all measured virus-host parameters will be considered simultaneously. In addition, evolutionary parameters in different stages of the disease i.e. acute and chronic phases, will also be measured and accounted for in the HPM by addition of the epoch modeling approach to quantify the relationships between viral parameters, innate responses and their effect on disease pathogenesis. The proposed study is likely to constitute one of the largest virus-host dataset of longitudinally collected data of both virus sequences (covering a wide range of HIV-1 subtypes) and innate immune markers to date. The results of the proposed analyses will increase our understanding of HIV-1 pathogenesis and may have implications for therapeutic and prophylactic vaccine design.

#### **A36** Prevalence of HIV-1 subtypes in Slovenia with an emphasis on molecular and phylogenetic investigation of subtype A

J. Mlakar,<sup>1,\*</sup> Maja M. Lunar,<sup>1</sup> A.B. Abecasis,<sup>2</sup> A.-M. Vandamme,<sup>2,3</sup> J. Tomazič,<sup>4</sup> T.D. Vovko,<sup>4</sup> B. Pečavar,<sup>4</sup> G. Volčanšek,<sup>4</sup> M. Poljak,<sup>1</sup>

<sup>1</sup>Faculty of Medicine, Institute of Microbiology and Immunology, University of Ljubljana, Ljubljana, Slovenia, <sup>2</sup>Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical Universidade Nova de Lisboa, Lisbon, Portugal, <sup>3</sup>Clinical and Epidemiological Virology, Rega Institute for Medical Research, K. U. Leuven, Leuven, Belgium and <sup>4</sup>Department of Infectious Diseases, University Medical Center Ljubljana, Ljubljana, Slovenia

In Slovenia, a small country in Central Europe, less than 1 per 1,000 inhabitants are estimated to be infected with HIV-1. As in most of the Central and Western European countries, the majority of patients diagnosed with HIV-1 are infected with subtype B. However, due to migration, other subtypes can become more prevalent in the country. The aim of this study was to determine HIV-1 subtypes circulating in Slovenia and to further examine the molecular epidemiology of subtype A. A total of 367 Slovenian HIV-1 sequences were included in the study, representing 58% of all patients diagnosed in Slovenia until the end of the year 2013. Subtype was assigned by employing different HIV subtyping tools coupled with Maximum likelihood phylogenetic analysis. The latter was performed to examine the molecular epidemiology of subtype A as well. Identified clusters of Slovenian subtype A sequences were further analyzed for the determination of the time of the most recent common ancestor (tMRCA) by using Monte Carlo Markov chain (MCMC) method available in BEAST 2.1.3 software. We determined the prevalence of subtype B to be 85.3%, while subtype A was the most prevalent non-B subtype found in 18 patients (4.9%), followed by CRF02\_AG (2.4%), subtype C (1.1%), subtypes D, G and CRF01\_AE (0.8% each) and subtypes F1 and CRF22\_01A1 (0.3% each). Subtypes could not be assigned to 12 sequences (3.3%). The phylogenetic tree obtained by ML analysis of the subtype A and subtype A related recombinants revealed that Slovenian sequences were part of 6 major international clusters. Two clusters consisting only of Slovenian sequences were identified and thus additional MCMC analysis was employed. Results of a Slovenian cluster of 4 subtype A sequences showed a posterior probability value of 1 and a tMRCA between the years 1985 and 2008, with a mean in the year 2001. In conclusion, in a Central