European country, where subtype B predominates, the second most common subtype was found to be subtype A. Non-B subtypes were observed in one out of seven patients in Slovenia, a fraction that is not negligible, thus proving importance of surveillance of HIV subtype diversity and corresponding molecular epidemiology of non-B subtypes.

### A37 HIV drug resistance monitoring in children receiving first line antiretroviral therapy at two pediatric hospitals in Ho Chi Minh City

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Drug resistance is the main reason for antiretroviral treatment (ART) failure. Information on the prevalence of pediatric HIV drug resistance (HIVDR) in Vietnam is important to assist in the determination of the optimal ART regimen. We enrolled a prospective cohort of children newly initiating ART at one of two main pediatric hospitals in Ho Chi Minh City from December 2011 to March 2014. Demographic and clinical data were collected at baseline and supplemented by genotyping and VL at start and after 12 months or when first-line ART ends if it comes first. Of 136 patients enrolled, the mean age was 4.7 years; 17 (12%) exposed to ARV to prevent maternal to child HIV transmission; seven (5.15%) carried at least one strain of HIV with mutations related to ARV resistance, two (1.47%) against Nucleotide Reverse Transcriptase Inhibitors (NRTIs) (AZT, D4T), one (0.74%) against Non-Nucleotide Reverse Transcriptase Inhibitors (NNRTIs) (NVP), and four (2.94%) against Nelfinavir of Protease Inhibitors (PIs). At 12 months, 121 (89%) children were still receiving the first line ART, 7 (5%) died and 8 (6%) were lost to follow-up. Among 121 children on ART, 107(88%) achieved VL suppression (<1,000 copies/mL); 9 (7%) had acquired HIVDR mutations, three against NRTIs only, and six (4.96%) against both NRTIs and NNRTIs. The most prevalent mutation was the M184V (4.96%, $n = 6$) causing high-level resistance to 3TC, FTC and low-level resistance to ddI and ABC. Some TAMs were also found (D67N, K70R, T215F, K219Q). No major resistance mutations to PIs were detected. Viral load at initiation is associated with HIVDR at 12 months. Low levels of virologic failure and HIVDR were observed in pediatric patients. However, since some multidrug-resistant or cross-resistant mutations were recorded, continued monitoring of HIV drug-resistance in pediatric patients is needed.

### A38 Diversity analyses of HIV-1 envelope glycoproteins in HIV-infected individuals with and without broadly neutralizing antibodies

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High levels of HIV envelope glycoprotein (Env) diversity have been associated with the development of broadly neutralizing antibodies (bNAbs). Here, we compare chronically HIV-1 infected subjects who develop bNAbs with those who did not, to assess whether lack of breadth can be attributed to low levels of viral diversity. Env nucleotide sequences were generated using Single Genome Amplification from four CAPRISA 002 cohort participants. Two participants developed neutralization breadth (CAP256 and CAP257) whereas the other two did not (CAP88 and CAP228) despite equivalently long duration of infection. Longitudinal diversity analyses were performed using Sequence Demarcation Tool (SDT). Phylogenetic analyses were performed using Bayesian Evolutionary Analysis by Sampling Trees (BEAST) software. Overall diversity increased with time in all subjects, as expected. Highest diversity was observed in CAP256 and CAP228, followed by CAP257 and least diversity in CAP88. The highest nucleotide substitution rates were observed in CAP257 (2.63 substitutions/100 nucleotides/year), CAP256 (2.28 subs/100n/yr.) and CAP228 (2.07 subs/100n/yr.), and the lowest in CAP088 (0.99 subs/100n/yr.). The time to the most recent common ancestor (tMRCA) inferred from BEAST was longer than the actual time of infection for CAP256 and CAP228, suggesting the possibility of super-infection or multivariant infection. We conclude that the absence of viral diversity may limit bNAb development, as in CAP88. However, increased diversity through high mutation rates and/or recombination, while likely necessary, is not sufficient for driving the development of bNAbs.

### A39 Human exome sequencing to evaluate the impact of rare coding variation on HIV-1 control

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Common variants (>5% frequency) in the MHC and CCR5 regions are known to influence set point HIV-1 viral load (spVL) yet explain only a portion of the total trait variance. The impact of rare coding variation on HIV-1 disease progression has not been as thoroughly investigated. Here we utilize exome sequencing in 392 HIV-1 infected individuals with stable spVL to look for rare and functional variants that mediate control of HIV-1 infection. Set point HIV-1 viral load was calculated as the average of at least 3 measurements obtained during the chronic phase of infection. We captured and sequenced all coding exons in 392 HIV-1 infected individuals of the Swiss HIV Cohort Study using the Illumina Truseq 65Mb enrichment kit and the Illumina HiSeq2000. Quality control and variant calling were performed using the GATK and variant functional annotation was performed using snpEff version 3.3. Individual variants were tested for association using linear regression. Testing of the combined effects of multiple low frequency variants across each of >18,000 genes was performed using SCORE-Seq and SKAT. Consistent with previous results, single marker variant tests showed a strong signal of association in the MHC. The top association was observed between spVL and rs1131446 ($P = 2.3 \times 10^{-11}$) in exon 3 of HLA-B. Conditioning on this SNP, residual association was observed at rs2308622 (conditional $P = 2.2 \times 10^{-6}$) in HLA-C. Accounting for these two SNPs, no other variants showed evidence for association. Analyses aimed at detecting the combined effect of multiple low-frequency variants within a gene showed no significant associations. Restricting this analysis to only those variants that result in a change in protein sequence did not reveal further signals. Outside of the MHC, no significant impact of rare variation on spVL was detected by exome sequencing in 392 individuals.

Larger samples are likely required to fully explore the role of rare coding variation on this phenotype. Additional classes of variation not detected by GWAS or current sequencing technologies may also contribute to host HIV-1 control.

### A40 Persistent circulation of highly divergent HIV-1M lineages in the Congo Basin Region

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The evolutionary processes that within a century yielded the nine major HIV-1 group M (HIV-1M) lineages and over 72 circulating recombinant forms (CRFs) remain the most important obstacles to the development of both a cure and an effective vaccine. It is still unknown how global HIV populations will respond over the long-term when confronted with efficiently protective vaccines and drug therapies. It is entirely possible that divergent HIV variants that are presently circulating in diversity hotspots such as Cameroon and the DRC might be the source of future global multi-drug resistant or vaccine evasion re-emergence events. In an effort to discover and characterise more of these highly divergent lineages, we recently performed in-depth characterisation of HIV-1 diversity in Cameroon. We found that 10% of gag sequences and 5% of nef sequences were not easily classifiable into any of the known HIV-1M clades. In addition, the full length characterisation of 24 unique recombinant forms (URFs) from Cameroon showed that these divergent sequences contained large tracts of sequence that could not be credibly classified as having been derived from parental viruses in the known subtypes. Furthermore, we have found that many of the sequence fragments occurring within CRF04_cpx, CRF06_cpx, CRF11_cpx, CRF18_cpx, CRF25_cpx, CRF27_cpx and CRF49_cpx are in fact likely derived from divergent unclassified parental lineages that may pre-date the current subtypes, even though they are presently identified as derived from currently defined HIV-1M subtypes. The highly divergent sequence tracts evident within these various HIV-1M genomes might be the extant descendants of pre-epidemic HIV-1 group M lineages (i.e. they may, in a sense, be evolutionary relics). This suggests that large pools of undiscovered HIV-1M genetic diversity likely exist throughout equatorial West Africa. We hypothesise that viruses belonging to these lineages may have gone largely undetected because of their low pathogenicity and/or transmissibility: characteristics that would be expected to result in the long-term survival of individuals that they manage to infect. This should therefore be manifested by individuals infected with these viruses displaying substantially higher degree of within-individual diversity than is usually displayed by individuals infected by viruses in the main pandemic lineages.

### A41 Diversity and evolution of avian influenza (AI) viruses in poultry and wild birds

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Over the past decade, surveillance programs for Avian Influenza (AI) virus infections in the Netherlands have provided extensive information on the spatiotemporal distribution of AI virus infections in poultry and wild aquatic birds. Wild birds are the natural reservoir of AI viruses and suspected to be the source of AI virus infections in poultry. Surveillance focuses mainly on the early detection of AI virus infections of subtypes H5 and H7, which have the potential to mutate from a low pathogenic AI (LPAI) variant into a highly pathogenic AI (HPAI) variant. However, the introduction of AI viruses of other subtypes is also monitored. Here, we provide an overview of all AI virus subtypes that have been detected by virus specific antibody detection or virus isolation in poultry and wild birds in the Netherlands from 2006 to 2015. Results show that poultry as well as wild birds are frequently infected with LPAI viruses. However, the subtype distribution differs between the two groups, indicating that LPAI virus transmission from wild birds to poultry is not random and likely depends on viral factors that determine host range restriction. In multiple cases, LPAI virus infections of the same subtype have been detected on several poultry farms at approximately the same time, suggesting that these viruses have acquired the capacity to be efficiently transmitted from wild birds into poultry and/or between farms. In this study, the whole genome sequences of more than 300 AI viruses isolated from poultry and wild birds have been determined by next-generation sequencing (NGS). Phylogenetic analyses will be performed to understand the evolution of LPAI viruses in the Netherlands. Furthermore, we aim to identify specific mutations in the AI virus genome that correlate with an increased chance of LPAI virus introduction in poultry, within-farm spread of LPAI viruses, and transmission of LPAI viruses to other poultry farms. Increased knowledge of LPAI virus transmission is important to control virus spread and reduce the probability of mutation of LPAI viruses into HPAI viruses.

### A42 Evolution and spatial dissemination of the highly pathogenic Asian H5 avian influenza viruses

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After emerging in 1996, the Asian highly pathogenic avian H5Nx influenza viruses had spread to more than sixty countries across Asia, Europe, and Africa through three major transmission waves by 2006. Phylogenetic analysis of all H5 influenza virus sequence data in our long-term surveillance in southern China revealed that the virus was widespread and enzootic in China, continuously developing into different clades and reassortant variants, some of which disseminated to other regions and became enzootic. This indicates that continuous circulation of H5 viruses in China is not only a local risk factor, but also poses a broader threat to birds and humans in other regions. In late 2014, clade 2.3.4.4 of H5Nx viruses emerged and caused sporadic human infections in China and outbreaks in poultry in Eurasia and spread to North America, the first-time Asian highly pathogenic H5 viruses had been detected there. To ascertain how the Asian H5Nx influenza viruses evolved into the wide-spread clade 2.3.4.4 viruses, over 3,000 H5 avian viruses isolated from 2009 to 2015 have been sequenced. I wish to use more sophisticated phylodynamics analysis on our large genomic sequence datasets of H5Nx viruses to examine the emergence of new wide-spreading 2.3.4.4 clade of global concern.