A9 Deep sequencing analysis to investigate the importance of within host genetic diversity and evolution of influenza A viruses for the development of resistance against neuraminidase inhibitors

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Currently approved neuraminidase inhibitors (NAI) for the treatment of influenza A virus infections are prone to induce viral drug resistance development due to the rapid evolutionary dynamics of the neuraminidase (NA) and hemagglutinin (HA) proteins. Both HA and NA proteins are subject to antigenic drift and the epistatic interactions within and between these proteins can lead to genetic diversity that enables the virus to easily develop resistant mutations under selective pressure in a host. To study NAI resistance and clinical outcome, the global observational Influenza Resistance Information Study (IRIS; NCT00884117) was conducted. Patients that were clinically diagnosed with influenza were enrolled in the study. Nasal and throat swabs taken at baseline and on days 3, 6, and 10 were assessed by semi-quantitative real-time reverse transcription polymerase chain reaction (RT-qPCR) to determine the influenza virus type and subtype. NAI resistance was initially determined by mutation specific RT-PCR, Sanger sequencing and phenotypic susceptibility analysis. Genetic resistance mutations to oseltamivir were detected in 61 patients (43 H1N1pdm [H275Y] and 18 H3N2 [R292K]) by mutation specific PCR. Subsequently, samples of 43 patients were subjected to deep sequencing analysis to characterize both the between- and the within-host diversity and the evolutionary process of the HA and NA proteins of infected patients. The NAI resistance mutations (H275Y and R292K) in the NA protein of the H1N1pdm and H3N2 viruses were either detected in day 3 samples or at later time point. Additionally, viruses in several individuals had mutations that were located across the whole HA and NA proteins. Some low frequency mutations such as D114N, S200P, and D239N, that were located in the antigenic sites or near the receptor binding site of the HA protein, became fixed in the later time point viral samples. Also, some mutations in HA may have occurred in concert with the resistance mutations in NA. Further genetic analyses and phylogeny should provide further insights in the emergence of mutations in individual hosts and the larger population.

A10 The evolution and molecular epidemiology of epidemic GII.17 noroviruses

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In past decades, the GII.4 genotype with a higher evolutionary rate predominated in norovirus epidemics globally. In the winter of 2014–5, a novel GII.17 variant emerged, causing large outbreaks in mainland China and sporadic infections globally. The origin, evolution and transmission patterns of this new emerged variant are largely unknown. We generated 103 full capsid and 8 whole genome sequences of GII.17 strains collected between August 2013 and November 2015 in Guangdong province. Phylogeny reconstruction was performed by including all public available GII.17 sequences. Our evolutionary analysis revealed variable evolutionary rates during GII.17 evolution history. The newly emerged lineage GII.17_Kawasaki_2014 most likely originated from Africa around 2001 and evolved at 5.6×10^{-3} substitutions/site/year. In this lineage, a novel variant with series of important amino acids changes emerged around August 2013 and caused epidemics in 2014–5. Through Bayesian skyline plot analysis, we found that the phylodynamics of GII.17_Kawasaki_2014 lineage were similar to the epidemic pattern observed during GII.4 evolution. Hong Kong was inferred as the epicenter of local GII.17 outbreaks, and frequent virus transitions were observed among Hong Kong and several coastal cities in Guangdong. In this study, we provide a novel insights into GII.17 noroviruses by inferring virus evolution and local transmission patterns. Our analysis highlights the possibility that a rarely detected genotype of norovirus could rapidly cause local epidemics by replacement with a new variant. As the persistence of GII.17 has been observed in the winter of 2015–6, close monitoring the evolution of GII.17 globally is critical for current norovirus disease control and vaccine development.

A11 Phylogenetic and phylogeographic analysis of viral surveillance data to inform rabies control programmes in Cambodia

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Canine-mediated rabies is a serious zoonosis, responsible for at least 60,000 human deaths per year. The disease is caused by Lyssavirus genotype I, which is endemic in domestic dog populations, particularly in Asia and Africa where dogs are free-roaming. There is increasing evidence that the majority of free-roaming domestic dogs are owned; with human-mediated movement of dogs (some of them infected) contributing to the spatial spread of rabies and local persistence through reintroductions. The spatial spread of rabies through the translocation of dogs by people has been demonstrated in South Africa and Thailand using molecular epidemiological techniques. These studies have improved our understanding of the role of human behaviour and interference in conspecific transmission of this important zoonosis. Understanding the transmission dynamics of the virus is essential for its control; however, the spatial dynamics of canine rabies is poorly quantified in Cambodia, where the burden of rabies is substantial. Therefore, we will undertake phylogenetic and phylogeographic analysis of viral surveillance data from Cambodia to inform control programmes. These analyses may be combined with local-level contact tracing and ecological data to generate a more complete picture of conspecific transmission dynamics involving human interference in the region.

A12 Predictors of treatment failure among Irish individuals infected with hepatitis C virus

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With the increasing number of licensed direct-acting antivirals (DAA) for the treatment of chronic HCV infection, choosing the right treatment regimen for the right patient has become paramount. We believe baseline sequencing for the presence of

resistance-associated substitutions (RAS) is an important contributor to this decision-making process. In the present study in an Irish cohort, we performed a retrospective analysis on all HCV samples received for drug resistance testing at the Irish National Virus Reference Laboratory between September 2014 and May 2016. Particular attention was paid to patients who experienced virological failure in an attempt to identify predictors of failure. Sanger sequence data covering the HCV NS3 protease coding region were obtained for 682 samples received during the study period. These were analysed using PAUP phylogenetic software. Sequence data for the NS5A and NS5B regions in some samples were also obtained. The rs12989860 single nucleotide polymorphism site was examined by allelic discrimination real-time PCR. Analysis of the NS3 viral sequences demonstrated that 85.5% (583/682) were HCV subtype 1a, 14.2% (97/682) subtype 1b and 0.3% (2/682) subtype 1c infections, subtype 1a was further differentiated into 76% clade 1 (443/583) and 24% clade 2 (140/583). RAS proven to reduce susceptibility to NS3 inhibitor treatment were detected in 45.9% of cases (313/682). Although the vast majority of all RAS detected were found in subtype 1a viruses, 7.2% (7/97) subtype 1b samples also contained one or more RAS. The Q80K polymorphism was found in 313/583 (57.3%) of HCV subtype 1a, and almost exclusively in clade 1 (242/443; 54.6%) versus clade 2 viruses (2/140; 1.4%). This distribution is reflected in the neighbour joining tree. Among the cohort of patients who experienced virological failure whilst on treatment, RAS could be detected in 11/17 (64.7%) patients for whom sequence could be generated. These included V36M/L (6/11; 54.5%), Q80K (5/11; 45.5%), R155K/T (3/11; 27.3%) and T54S (1/11; 9.1%). The majority of these patients were found to possess the deleterious "T" single nucleotide polymorphism (SNP) at the rs12989860 site within the Interferon lambda 4 (INFλ 4) gene locus. Nine of eleven patients with detected RAS were found to also be either CT or TT at rs12989860, one patient was CC at this SNP. Preliminary data from patients experiencing treatment failure on NS5A/B inhibitors also indicate the presence of RAS in 4 of 7 individuals. The high incidence of RAS within HCV NS3 protease sequences, the detection of RAS in NS5A sequences, and the apparent risk of treatment failure, albeit in a small number of patients, when the RAS are present, highlights the importance of sequencing these viruses prior to commencing treatment with protease inhibitors, and the need to identify additional predictors of failure.

A13 HIV drug resistance over a decade of antiretroviral therapy scale-up for HIV/AIDS patients in Vietnam

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Since 2005, Vietnam has remarkably scaled-up Antiretroviral therapy (ART) for HIV-infected people. The number of people receiving ART has increased from 2,670 in 2005 to 78,438 adults and 4,204 children at the end of 2013. ART coverage increased to 67% (60.0% in adults and 78.1% in children), against current eligibility criteria per National Guideline (CD4 cells <350 cells/ml). Standardized ART was delivered at 364 outpatient clinics at the end of 2013. Since 2010, the Ministry of Health has recommended the first-line prioritized ART regimens with two NRTIs (d4T + 3TC or ZDV + 3TC) plus one NNRTI (nevirapine [NVP]). In the context of rapid ART scale-up, the extent of HIV drug resistance (HIVDR) in Vietnam has been concerned, studies on transmission and emergence of HIV drug resistance were carried out in 2013–4. HIVDR study protocols were adapted from WHO guidelines for transmitted drug resistance (TDR) (2012) and acquired drug resistance (ADR) (2014). In brief, the TDR survey was implemented in a total of 15 voluntary counseling and testing (VCT) sites located in the old Hanoi. A total of 74 eligible VCT clients, aged 18–24, detected HIV positive, had no history of ART exposure, had no previous pregnancy if female, and were sequentially sampled. HIV genotyping was done in order of enrollment date until DR prevalence could be classified. For the ADR survey, 8 ART outpatient clinics were sampled from a total of 114 clinics that had ART available for more than 3 years up to the end of 2010, in the North, using probability proportional to proxy size (PPPS) sampling method. From each selected VCT, 23 patients who had received ART for more than 36 months were consecutively recruited into the study. All patients were taken blood for evaluating viral suppression and HIV drug resistance if viral load above 1,000 copies/ml. The prevalence of transmitted HIV drug resistance was classified as moderate between 5 and 15%, mainly to NRTIs/NNRTIs, no protease inhibitor (PI) resistance. In 181 patients on ART for more than 36 months, 93.9% (95% CI: 90.4–97.4%) had viral load suppression and 5.5% (95% CI: 2.2–8.9%) had drug resistance. Notably, 100% of individuals with viral suppression failure are resistant to all drugs in both their initial and current ART regimes receiving. Against 7 NRTIs and 4 NNRTs recommended for the first-line ART as per the national guideline, resistance rates ranged between 75 and 100%. No resistance to PIs was found. The most common mutations are M184V (90%), D67G (60%), K70RES (60%), K103N (50%), Y184C (50%), T215FYN (50%), and K219QE (50%). The scaled-up ART program in Vietnam was proven to be effective with high rate of viral suppression at 36 months on the first-line prioritized ART regimens. Transmitted HIV DR to NRTIs/NNRTIs was increased, requiring the national program on HIV DR surveillance and prevention be strengthened to maximize long-term effectiveness of first-line ART regimens.

A14 Comprehensive characterisation and evolutionary analysis of endogenous retroviruses in the mouse genome

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It is well established that the genome of the mouse (Mus musculus musculus) contains large numbers of transposable elements, including many endogenous retroviruses (ERVs). Murine ERV lineages have been characterized piecemeal, but a comprehensive analysis has yet to be implemented. In this study, we address this by combining high-throughput *in silico* screening of the mouse genome with in-depth phylogenetic analysis of murine ERVs. Based on phylogenetic analysis of ERV polymerases, we establish the presence of at least 22 major ERV lineages in the murine genome, of which only 14 have been previously described. The majority of the previously unreported lineages are relatively low copy number (<100). Using a combination of automated and manual approaches we were able to recover representative internal regions and long terminal repeats (LTRs) for four of the eight novel lineages. LTR sequences were used to infer calibrated timelines of ERV invasion and intragenomic expansion within the mouse genome. These data were transposed against a timeline of murine evolution and phylogeography, providing new insights into the coevolutionary