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Nigeria has been reported to have the highest number of AIDS-related deaths in the world. In this study, we aimed to use molecular epidemiology to investigate the HIV-1 diversity and phylodynamics in Nigeria. We analyzed 1,442 HIV-1 *pol* sequences collected from 1999 to 2014 from seven geopolitical zones in Nigeria. The main circulating strains, CRF02_AG (44.1% of the analyzed sequences), Subtype G (8.3%), and CRF43_02G (16.4%), were introduced to Nigeria in the 1960s, 1970s, and 1980s, respectively. The number of effective infections decreased in Nigeria after the introduction of free antiretroviral treatment in 2006. We also found a significant number of unique recombinant forms (22.7%), the majority of which were recombinants between the two or three of the main circulating strains described above. In addition, phylogeographic analysis indicates multiple occasions of HIV-1 transmission between Lagos and Abuja (two of the main cities in Nigeria). Our results may be relevant for HIV-1 intervention and contribute in making informed decisions in strategies aiming at reducing further spread of HIV-1 in Nigeria.

A4 An amplicon-based approach for universal amplification, sequencing, and assembly of full-length HIV-1 samples from the DRC

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Phylogenetic studies have contributed to our understanding of the early epidemic onset of HIV-1 in the Democratic Republic of Congo (DRC); however, the factors driving its early emergence and establishment in human populations still remain unresolved. In order to determine the key aspects of its successful epidemic spread, complete genome data are required from samples representative of the viral diversity in the DRC. In this study, we have established a universal PCR-assay that uses seven different panels of primers to produce overlapping amplicons covering the complete HIV genome. To circumvent the limitations of purifying these fragments and sequencing them with traditional approaches, we have developed a massive parallel sequencing method and a protocol for efficiently assembling HIV-1 genomes. A total of thirty-six samples, collected between 1997 and 2001 from different locations across the DRC, have been obtained, and, at this stage, we are focusing on complementing our dataset with more archival samples that can be used as HIV 'molecular fossils'. By generating complete genome phylogeographic data from the DRC, we aim to create a genomic window into the past evolutionary and epidemiological dynamics of HIV-1 in Central Africa and understand the natural history of this devastating pandemic.

A5 Near full-length HIV-1 genome sequencing in newly diagnosed individuals in Sweden

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The Swedish HIV-1 epidemic is characterized by a high diversity in HIV subtypes and recombinants as a result of migration. To study the time from infection through viral diversification, transmission patterns, and drug resistance in minor quasispecies, a robust protocol for pan-genotypic near full-length HIV-1 genome (HIV-NFLG) next-generation sequencing (NGS) is key. Our group has established two protocols for HIV-NFLG on the Illumina platform that we aim to compare and, if necessary, modify to find a method with optimized coverage, depth, and subtype inclusivity. Zanini et al. (https://doi.org/10.7554/eLife.11282.001) have developed a method with one-step RT-PCR with six overlapping primer sets, followed by NGS and quality filtering and assembly with in-house methods. Aralaguppe et al. (https://doi.org/10.1016/j.jviromet.2016.07.010) have designed amplification in two fragments, followed by multiplexed NGS and quality control and assembly with Iterative Virus Assembler and VICUNA. Both methods have high coverage per nucleotide and low error rates in amplification and sequencing and can reliably identify SNPs at 1 per cent of the viral population with linkage within the quasispecies. Subtype inclusivity remains a challenge even though both methods show success in amplifying and sequencing subtypes B, C, and the common recombinants 01_AE and 02_AG. Therefore, we aim to evaluate and optimize our NFLG NGS methods on a panel of patient samples that more completely reflects HIV-1 diversity in Sweden. Patient samples from fifty treatment-naïve viremic individuals representing the genotypic HIV-1 panorama in Sweden, including CRFs, are being amplified and sequenced by both protocols. Coverage of the genome, error rate, and possible depth of quasispecies analysis is being evaluated. We will compare number of reads, coverage across the HIV genome, and representation of minor single nucleotide variants as well as subtype inclusivity and impact of plasma RNA levels. To do this we will use an in-house bioinformatic pipeline. The NFLG sequences will also be analyzed with phylogenetic tools for determination of subtypes including CRFs and URFs.

A6 Does treatment cause virulence changes in HIV-1?

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Antiretroviral treatment (ART) has provided substantial benefits for HIV-1-infected patients and has reduced incidence in areas with high uptake since its introduction in the late 1980s. As ART has led to shifts in the worldwide epidemiology of HIV-1, it may also have the potential to cause concomitant selective pressure on the virus population. Evidence for changes in HIV-1 virulence since the introduction of ART appears to be inconsistent. As well as reviewing both empirical and theoretical studies on the likely impact of ART on HIV-1 virulence, we developed a mathematical framework to evaluate the likely impact of ART on virulence selection under the widespread treatment programs and the future impact of recent test-and-treat recommendations. By quantifying both the relationship between virulence changes with the transmissibility through disease progression and the speed of diagnosis and treatment, we reconcile observational studies on virulence changes with the mathematical model predictions. On adoption of new test-and-treat programs—synonymous with early detection and immediate treatment—it is likely that increased virulence will be observed. Our findings highlight the potential public health consequences of mass treatment and the ensuing requirement for greater access and adherence to nullify the public health effect of these virulence changes.

A7 Co-receptor tropism determined by genotypic assay in HIV-1 non-B subtypes circulating in Cuba: Implications for pathogenesis and Maraviroc resistance

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The V3 loop of the HIV-1 envelope (*env*) gene is involved in binding to the chemokine receptors CCR5 and CXCR4, thus determining viral tropism. With the aim of genetically characterizing the C2V3 *env* region of HIV-1 samples from Cuban patients, naive to Maraviroc (MVC) therapy, 115 plasma samples were taken in the period of 2014–6 and analyzed by sequencing of the C2V3 region. HIV-1 subtyping was performed using COMET V.2 and Rega subtyping toolV.3 software. Subtypes were confirmed by phylogenetic analyses using Mega-6. Prediction of co-receptor tropism was performed using the geno2pheno algorithm. The viral

mutations associated to MVC resistance were analyzed, as well as the association of the subtype with clinical, epidemiological, virological, and immunological variables. The subtypes detected using the C2V3 region were CRF20, 23, 24_BG (35 patients, 30.4%); Subtype B (33 patients, 28.7%); CRF19_cpx (30 patients, 26.1%); CRF18_cpx (10 patients, 8.7%); and others (7 patients, 6.1%). Overall, 60 per cent of the viruses exhibited R5 phenotype, 14.8 per cent were R5X4 and 25.2 per cent were X4. Interestingly, CRF19_cpx virus was associated with having phenotype X4 [46.7%, $P = 0.0047$, odds ratio (OR): 3.96, 95% confidence interval (95% CI): 1.59–9.84], with infection in young individuals (39.1%, $P = 0.025$, OR: 3,548; 95% CI: 1,136–11,077) and with higher values of viral load ($P \leq 0.05$). The comparison of the amino acid sequences of the V3 loop showed differences between the B and non-B subtypes ($P = 0.0001$). Mutations reported to be associated with MVC resistance, were detected in 75.7 per cent of the samples, in positions 11 (6.1%), 13 (49.6%), 25 (6.1%), 316 (7.0%), 323 (11.3%), and 319 (3.5%) of Gp120, particularly in the recombinant forms CRF19_cpx and CRF_BGs. HIV variants that use the CXCR4 co-receptor were associated with more than 10 years of diagnosis, with older individuals, in the AIDS stage, with low CD4 counts and higher viral load levels ($P < 0.05$). The results support the hypothesis previously stated that CRF19_cpx viruses could be more pathogenic and would have limitations for the use of MVC. The high rate of mutations associated to MVC among non-B Cuban subtypes should be further studied.

A8 Epidemiological study of transmission clusters in a local HIV-1 cohort

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Integration of molecular, clinical, and demographic data represents a powerful tool to understand the dynamics of local HIV-1 transmission chains (TCs). The aim of our study was the phylogenetic analysis of the TCs within a HIV-1 cohort and the description of the relevant patient data within a TC. We performed a phylogenetic analysis of 757 sequences from newly HIV-1 diagnosed patients in Málaga (Southern Spain) during the period 2004–15. We used partial *pol* gene sequences in a preliminary phylogenetic reconstruction using the Neighbour Joining method (MEGA v6.06 program). After eliminating branches with bootstrap values <80 per cent, we constructed a new phylogeny by Maximum likelihood method (FastTree program). We considered as TC any cluster with bootstrap values ≥ 90 per cent. Patient sequences within and outside TCs were compared. Resistance mutations in the protease (PR) and reverse transcriptase (RT) sequences were analyzed using the Stanford algorithm. Four hundred and fifty-one out of 757 patients (59.6%) were grouped into fifty-three TCs, seventeen of them with five or more subjects. The largest number of patients associated within a TC was ninety. Patients younger than 40 years [odds ratio (OR) 1.75, 95% confidence interval (95% CI) 1.2–2.4, $P = 0.002$], men who have sex with men (MSM) (OR 2.14, 95% CI 1.3–3.2, $P < 0.0001$), non-Spanish (OR 1.48, 95% CI 1.0–2.1, $P = 0.038$), with a non-B subtype HIV-1 (OR 3.12, 95% CI 2.0–4.8, $P < 0.0001$), and presenting primary resistance mutations (OR 14.1, 95% CI 3.1–62.6, $P = 0.001$) were more likely to be associated within a cluster. Ninety-four out of 118 patients (79.6%) with transmission resistance mutations were included in some TC. The most frequent mutations associated with clusters were T69D/N, L210W, and K219E/Q, for NRTIs, K103N, and G190A/S for NNRTIs, and the I54L/M and L90M mutations for PIs. The prevalence for resistance to NNRTIs in TCs was 13.7 per cent. There were two TCs of rarer non-B subtypes: CRF19_cpx, with twenty-one individuals, sixteen of them (76.2%) with mutation G190A; and CRF51_01B with thirty-nine patients, twenty of them with the K103N mutation. Approximately 60 per cent of newly HIV-1 diagnosed patients were included in a TC. Younger patients, MSM, non-Spanish, with non-B subtype HIV-1 and primary resistance mutations were more likely to belong to a cluster. NNRTI mutations were the most frequent ones among patients in TCs. We observed two TCs represented by infrequent non-B subtypes in our area—CRF19_cpx and CRF51_01B—both of which were associated to the transmission of primary resistance.

A9 A method to obtain full-length HIV proviral sequences and their sites of integration

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Accurate definition of the HIV-1 reservoir on antiretroviral therapy (ART) is of paramount importance to the development of curative strategies. Much of this reservoir is derived from clonal expansion of latently infected CD4+ T cells. Methods used to characterize the reservoir include near full-length single-genome sequencing (NFL-SGS) and integration site analysis (ISA). However, current technologies do not link the intact proviruses detected by NFL-SGS to their sites of integration. Therefore, we developed a method to obtain both near full-length single-proviral sequences and their sites of integration. We call our method full-length integrated proviral single-genome sequencing (FLIP-SGS). Genomic DNA from ACH2 and CEM cells mixed at 1:1,000, or patient samples were diluted to a single proviral endpoint. An in-house, optimized whole genome amplification (WGA) method was performed on wells at the endpoint, generating multiple copies of all DNA molecules within each well. The number of proviral copies after WGA was determined by droplet digital PCR targeting the long terminal region (LTR). Forty per cent of each WGA reaction was used to obtain the provirus–host integration sites with ISA (linker ligation, nested PCR, and Illumina sequencing). The remaining fraction was used to amplify the full-length proviruses in four overlapping fragments (LTR-pol, gag-int, int-env, and env-LTR) for Sanger sequencing. WGA performed on the endpoint-diluted ACH2:CEM DNA amplified single-copy HIV-1 proviral templates greater than 500-fold, making it possible to obtain unique integration sites from single proviruses in ACH2 cells, including one that was previously reported (in the NT5C3A gene on chromosome 7) and two that were not previously reported (in the EIF4ENIF1 gene of chromosome 22 and an unknown region of chromosome 6). Near full-length PCR amplification and Sanger sequencing was performed on proviruses integrated in the NT5C3A gene. FLIP-SGS was applied to peripheral blood mononuclear cells from one HIV-1 infected donor with viremia suppressed on ART and yielded integration sites of four genomes that appear to contain large internal deletions. We report a method for near full-length HIV-1 single-genome sequencing combined with host integration site detection that we call FLIP-SGS. This assay will further define clonal expansion of infected CD4+ T cells as a mechanism that maintains the HIV-1 reservoir and as the source of identical sequences observed during therapy and rebound, rather than from ongoing replication.

A10 Presence and frequency of M184V mutation in the MOBIDIP trial

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The MOBIDIP trial evaluated the simplification by protease (PI/r) monotherapy for HIV infection versus dual therapy and boosted protease inhibitor plus lamivudine (PI/r + 3TC) in controlled patients under second-line regimens. MOBIDIP was interrupted because of a significant number of patients with virological failure (VF) at week 48 (W48) in PI/r (33/133, ~25%) versus in PI/r + 3TC (4/132, ~3%). At the time of first-line VF, 96 per cent of patients harbored the M184V mutation. The presence of the M184V mutation was related to a protective effect against VF in the