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Title

Global and regional molecular epidemiology of HIV-1 in 1990-2015: a systematic review, global survey, and trend analysis.

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Summary

Background

Global HIV-1 genetic diversity is a major obstacle to HIV vaccine development. We aimed to estimate the regional and global distribution of HIV-1 subtypes and recombinants during 1990-2015.

Methods

We conducted a systematic literature review by searching PubMed, EMBASE, CINAHL, and Global Health databases for studies published from Jan 1, 1990, to Dec 31, 2015. Additionally, unpublished HIV-1 subtyping data was collected through a global survey. We included prevalence studies with HIV-1 subtyping data collected during 1990-2015. The primary outcome was the number of samples designated as subtypes A, B, C, D, F, G, H, J, K, circulating recombinant forms (CRFs), and unique recombinant forms (URFs). Countries were grouped into 14 regions and the analysis conducted for the periods 1990-1999, 2000-2004, 2005-2009 and 2010-2015. The distribution of HIV-1 subtypes, CRFs and URFs in individual countries was weighted according to the UNAIDS estimates of the number of people living with HIV (PLHIV) in each country to generate regional and global estimates of HIV-1 diversity in each time period. The systematic review is registered with PROSPERO, number CRD42017067164.

Findings

The systematic review and global survey yielded a total of 2,203 data sets with 383,519 samples from 130 countries over 1990-2015. Distinct distributions of HIV-1 subtypes and recombinants were seen in different countries and regions. Globally, subtype C accounted for nearly half

(46·6%; 16,280,897/34,921,639 of PLHIV) of all HIV-1 infections in 2010-2015. Subtypes B and A were responsible for 12·1% (4,235,299/34,921,639) and 10·3% (3,587,003/34,921,639) of infections, respectively, followed by CRF02_AG (7·7%; 2,705,110/34,921,639), CRF01_AE (5·3%; 1,840,982/34,921,639), subtype G (4·6%; 1,591,276/34,921,639) and D (2·7%; 926,255/34,921,639). Subtypes F, H, J, and K combined accounted for 0·9% (242,345/34,921,639) of infections. Other CRFs accounted for 3·7% (1,309,082/34,921,639), bringing the proportion of all CRFs to 16·7% (5,844,113/34,921,639). URFs constituted 6·1% (2,134,405/34,921,639), resulting in recombinants accounting for 22·8% (7,978,517/34,921,639) of all global HIV-1 infections. The distribution of HIV-1 subtypes and recombinants changed over time in countries, regions and globally. Over the most recent decade (2005-2015) subtype B increased, subtypes A and D were stable, and subtypes C and G and CRF02_AG decreased at a global level. CRF01_AE, other CRFs, and URFs increased, leading to a consistent increase in the global proportion of recombinants over time.

Interpretation

Global and regional HIV diversity is complex and evolving and forms a major challenge to HIV vaccine development. Surveillance of the global molecular epidemiology of HIV-1 remains crucial for the design, testing, and implementation of HIV vaccines.

Funding

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Human Immunodeficiency Virus, HIV, subtype, recombinant, circulating recombinant form (CRF), unique recombinant form (URF), clade, serotype, epidemiology, surveillance, systematic review.

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Introduction

The HIV pandemic remains a major global public health problem, with 36·9 million people living with HIV in 2017.[1] Despite the expansion of antiretroviral treatment programmes, 940,000 people died from AIDS-related illnesses and 1·8 million people became newly infected with HIV in 2017.[1] An HIV vaccine is likely to be necessary to end the HIV pandemic.[2]

One of the key characteristics of the HIV pandemic is its extraordinary global genetic diversity.[3] After zoonotic transmission from non-human primates to humans in the beginning of the 20th century, HIV-1 group M diversified into distinct subtypes, designated by the letters A, B, C, D, F, G, H J, and K. In addition, recombinants between subtypes formed, which are designated as Circulating Recombinant Forms (CRFs) or Unique Recombinant Forms (URFs).[4] CRFs are strains propagated in the population and named consecutively according to internationally agreed guidelines (96 distinct CRFs have been identified to date).[5] URFs refer to unique recombinant sequences without evidence of onward transmission.[4] The spread and evolution of HIV has resulted in the differential distributions of HIV-1 subtypes, CRFs and URFs across the world.[3]

Global HIV-1 genetic diversity forms a major obstacle to HIV vaccine development, as a globally effective HIV vaccine will need to protect against divergent HIV subtypes and recombinants.[6] Designing, testing and implementing HIV vaccines requires up-to-date and accurate knowledge of the distribution of HIV subtypes and recombinants in different parts of the world, as vaccine immunogen sequences need to match as closely as possible the viral sequences circulating in the target population.[7] Furthermore, HIV diversity also affects HIV diagnostic assays and viral load measurements,[8] and impacts the development of drug resistance and response to antiretroviral treatment.[9,10]

Surveillance of the global molecular epidemiology of HIV-1 is therefore essential, but recent data and information on trends are lacking.[11,12] HIV sequence databases contain sequences with known dates and countries of collection, but samples are not representative of populations.[5,13] Estimates based only on published data have limited coverage, especially for recent years, and are prone to publication bias.[14] We therefore endeavoured to estimate the regional and global distribution of HIV-1 subtypes and recombinants during 1990-2015, by combining country-specific HIV subtyping data obtained from a systematic review and a global survey with UNAIDS estimates of the number people living with HIV (PLHIV) in each country throughout 1990-2015.

Methods

Systematic literature review

We conducted a comprehensive electronic literature search to identify all published studies reporting HIV-1 subtyping data. We searched four electronic literature databases (Pubmed, EMBASE (Ovid), CINAHL (Ebscohost) and Global Health (Ovid)) to identify studies published between Jan 1, 1990, and Dec 31, 2015. Search terms included Mesh headings and Emtree terms, as well as free text words and synonyms, including "HIV", "subtype", "recombinant", and "epidemiology" (appendix, pp2-5). No methodological or language filters were applied. All references obtained by the searches were combined to form a central database of citations in Endnote reference manager (Endnote X7). Duplicate references were removed. Reviewers RE, JY, LD and JH screened titles and abstracts of references, retrieved full text articles of potentially eligible studies, and assessed articles against the eligibility criteria (figure 1).

Additional published data sources

Further published data was obtained from four sources (appendix, pp6-8): i) WHO HIV Drug Resistance Report 2012[15]; ii) References in published reports and reviews on HIV diversity; iii) Screening of tables of content of four specialist journals (*AIDS, Journal of AIDS, Journal of Virology, AIDS Research and Human Retroviruses*) of issues published between January 1990 and February 2016; and iv) Review of papers indexed on the Scopus citation database which referenced previous publications on global HIV-1 molecular epidemiology.[16]

Global survey

Unpublished original HIV-1 subtyping data was collected through a survey among experts in the field under the umbrella of the WHO-UNAIDS Network for HIV Isolation and Characterisation. Researchers who were known to be working on HIV-1 molecular epidemiology based on previous publications, conference abstracts or informal networking were contacted by email/fax and asked to contribute unpublished HIV-1 subtyping data collected as part of independent studies by completing a pre-formulated data collection template. Over time, data from each country may have been collected from different sites and populations, as is the case for the published data. A full list of contributors is found at the end of this article. The eligibility criteria for unpublished data sources were the same as those applied to published sources.

Eligibility criteria

We included prevalence studies of PLHIV with known country and year of sample collection (between 1990-2015) and with original HIV-1 subtyping data, with a minimum of 20 samples in each study. All study types providing prevalence HIV-1 subtyping data were eligible, including randomized controlled trials, cohort studies, and cross-sectional studies. Studies containing only incident infections or new diagnoses were excluded. No restrictions were applied in relation to age, gender, ethnicity, nationality, duration of infection, CD4 count, viral load, treatment with antiretrovirals or other medications, or co-infections. The country designation of a data set was determined by the country where the (blood) samples were taken and not by the country of origin of the participants.

Subtyping methods included sequencing (genotyping), heteroduplex mobility assay (HMA), and serotyping (appendix, p10). Any genome segment could be used for subtyping, including *gag, pol, env,* other genome segments, or the full-length genome (appendix, pp11-12). No

minimum sequence length was specified and all online subtyping tools were accepted. The subtyping data as provided in each manuscript/submitted data set was taken as correct. Untyped samples were excluded from the analysis.

Data extraction

Reviewers RE, JY, LD and JH extracted the following data items from both published and unpublished sources, as reported: country, city/region, year(s) when samples were collected, study type, population, subtyping method(s), genome segment(s) analysed, and the subtyping data (minimum of 20 samples per data set). The primary outcome was the number of samples designated as subtypes A, B, C, D, F, G, H, J, K, Circulating Recombinant Forms (CRFs), and Unique Recombinant Forms (URFs) in each data set. No formal study quality assessments were performed. No patient identifiable information was retrieved at any stage and consent was presumed to have been obtained by the researchers who submitted or published each data set. Any ambiguities and discrepancies were resolved by the senior reviewer (JH).

HIV epidemiology data and country groupings

Country-specific estimates of the number of PLHIV in each year for the period 1990-2015 were obtained from UNAIDS.[17] Countries were grouped into regions according to the UNAIDS classification, with a few modifications based on subtype distributions in countries and regions (appendix, p9). India and Ethiopia were separated from their respective regions due to a highly distinct subtype distribution compared to the other countries in their regions.

Data analysis

The data were split into four time periods, chosen based on the spread of data sets and samples across the years: 1990-1999, 2000-2004, 2005-2009 and 2010-2015. The earliest (1990-1999)

and latest (2010-2015) time periods encompass more years to account for the relatively fewer data available in these years.

For each time period, the average number of PLHIV in each country was calculated. Coverage was calculated as the proportion of PLHIV in a region who lived in the countries for which HIV subtyping data was available in that region. Depth of sampling was calculated as the number of HIV samples subtyped in a region as a proportion of the average number of PLHIV in that region.

For each country, the HIV-1 subtyping data was split into the four time periods and the subtype distribution (i.e. proportions) in each time period was determined based on the number of samples for each subtype/CRF/URF. For country-specific data sets with sampling years that crossed multiple time periods (e.g. 2003-2006), the total number of samples for each subtype/CRF/URF was divided by the number of sampling years, giving the number of samples for each subtype/CRF/URF per year, which were then assigned to the relevant time periods.

In order to estimate regional HIV-1 subtype distributions in each time period, the proportion of each subtype/CRF/URF in each country was multiplied by the estimated average number of PLHIV in the country. The resulting (absolute) numbers of each subtype/CRF/URF in each country were added together per region and used to calculate the proportions of each subtype/CRF/URF in each region. Countries that lacked HIV-1 subtyping data were not included in these regional calculations.

To estimate global HIV-1 subtype distributions, we multiplied the regional proportions of each subtype/CRF/URF by the regional estimates of the number of PLHIV. The resulting regional (absolute) numbers of each subtype/CRF/URF were summed in order to generate a total number of each subtype/CRF/URF globally, which then resulted in the global proportions of each subtype/CRF/URF. Monte Carlo simulations were used to generate repeated random samples from the cumulative distribution defined by the observed set of subtypes from which

we assessed the level of uncertainty and derived 95% confidence intervals (CIs) on the proportions of subtypes for the different time periods (appendix, pp13-14).

All calculations were conducted in Excel. The systematic review is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines, as applicable.[18] This review is registered online with PROSPERO (https://www.crd.york.ac.uk/prospero/), number CRD42017067164.

Role of the funding source

The study received no funding. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Data collection

The systematic literature review for the period 1990-2015 yielded 894 data sets with 257,276 samples (figure 1). This was supplemented with 1173 data sets (112,404 samples) from the global survey of the WHO-UNAIDS Network for HIV Isolation and Characterisation and a further 136 data sets (13,839 samples) from references from reports, reviews and journals, yielding a total of 2,203 data sets and 383,519 samples from 130 countries over 1990-2015 (figure 1, table 1).

For the analysis, the data set was split into four time periods: 1990-1999, 2000-2004, 2005-2009 and 2010-2015 (table 1). There was excellent global coverage, as the countries for which HIV-1 subtype distribution data were available harboured >90% of global HIV-infected individuals in each time period, and >95% in 2000-2004 and 2005-2009 (table 1). Regional coverage was also good with 10 out of 14 regions having >90% coverage in the first three time periods, and seven regions in 2010-2015 (table 1). Low coverage was seen in some time periods for the Caribbean, Oceania and Middle East & North Africa (MENA). Sampling depth, defined as the number of samples out of the number of PLHIV, varied between regions and sampling depth was highest in Western and Central Europe and North America (WCENA) and Oceania and lowest in South Asia (India), Ethiopia and Southern Africa (table 1), though these latter three regions have large HIV-infected populations and very little subtype diversity (see below).

Global distribution of HIV-1 subtypes, CRFs and URFs

In the most recent time period, 2010-2015, subtype C accounted for nearly half (46·6%; 16,280,897/34,921,639 of PLHIV) of all HIV-1 infections worldwide (figure 2A, table 2A, appendix pp13-15). Subtypes B and A were responsible for 12·1% (4,235,299/34,921,639) and

10·3% (3,587,003/34,921,639) of infections, respectively, followed by CRF02_AG (7·7%; 2,705,110/34,921,639), CRF01_AE (5·3%; 1,840,982/34,921,639), subtype G (4·6%; 1,591,276/34,921,639) and D (2·7%; 926,255/34,921,639). Subtypes F, H, J, and K combined accounted for 0·9% (242,345/34,921,639) of infections globally. Other CRFs accounted for 3·7% (1,309,082/34,921,639), bringing the total proportion of CRFs to 16·7% (5,844,113/34,921,639). URFs constituted 6·1% (2,134,405/34,921,639), which brought the proportion of all global HIV-1 infections attributable to recombinants to 22·8% (7,978,517/34,921,639).

Changes in the global distribution were seen over time (figure 2B, table 2A, appendix pp13-15). Subtype C increased its proportion over 1990-2009 followed by a decrease in 2010-2015. Subtype B showed a consistent increase over the last 15 years. In contrast, subtypes A, D and G all showed decreasing trends throughout 1990-2015, although subtypes A and D stabilised in 2010-2015. Subtypes F, H, J and K remained very small contributors, with a combined global total of around 1% throughout 1990-2015. Amongst the recombinants, CRF02_AG increased over 1990-2009, followed by a decrease in 2010-2015. CRF01_AE and other CRFs showed consistent increases in their proportions over 1990-2015, contributing to a consistent increase in the proportion of all CRFs over all time periods. URFs made a substantial contribution throughout 1990-2015. Overall, a consistent increase in the total proportion of recombinants was seen over 1990-2015 (figure 2B, table 2A, appendix pp13-15).

Regional distribution of HIV-1 subtypes, CRFs and URFs

The proportions of HIV-1 subtypes, CRFs and URFs are strikingly different in different regions across the globe and evolve over time (figure 3, table 2B, appendix pp13-14, 16-31).

The greatest diversity is found in Central Africa, where all HIV-1 subtypes and many CRFs and URFs are found, throughout all time periods (figure 4, table 2B, appendix pp13-14, p30).

Over time, decreases in the proportions of subtypes A, D, G, and H were accompanied by increases in proportions of subtype C, other CRFs, and URFs. Central Africa has the highest proportion of URFs (21·3%; 243,041/1,143,531) of any region, contributing to a total proportion of recombinants of 46·8% (534,997/1,143,531) in 2010-2015.

West Africa has the highest proportion of CRF02_AG in the world (46·2% (2,504,438/5,419,010) in 2010-2015), in addition to subtype G (26·8%; 1,454,444/5,419,010) as well as URFs (15·5%; 838,476/5,419,010), bringing the total proportion of recombinants to 68·4% (3,706,246/5,419,010) (figure 3, table 2B, appendix pp13-14, 16-31).

In East Africa, about half of all infections are caused by subtype A (53·4%; 2,510,665/4,704,986), which has remained stable over time, with further contributions by subtypes C (14·8%; 696,163/4,704,986) and D (16·8%; 791,501/4,704,986) and URFs (12·6%; 591,140/4,704,986) in 2010-2015.

Subtype C dominates in Southern Africa, Ethiopia and South Asia (India), where it contributed ≥95% of infections throughout 1990-2015 and hardly any other subtypes or recombinants are found.

Subtype B is the main contributor in WCENA, Caribbean, Latin America, and Oceania, where it accounts for ≥75% of infections in 2010-2015. Notably, however, there is a downward trend over time in the proportion of subtype B in WCENA and Latin America, with concomitant increases in CRFs and URFs.

In Eastern Europe & Central Asia (EECA), the majority of infections (>50%) are caused by subtype A in all time periods, with further significant contributions by subtype B and other CRFs.

In the Middle East & North Africa (MENA), infections are caused by a decreasing proportion of subtype B and an increasing proportion of other CRFs (mainly CRF35_AD), which

contributed 59·8% (102,853/171,944) of infections in the region in 2010-2015, the highest proportion of other CRFs in any region.

South-East Asia and East Asia are both dominated by CRF01_AE, contributing to an overall proportion of recombinants of 80% in each region. South-East Asia has consistently had the highest proportion of CRF01_AE infections of any region in the world in any time period, with 72·8% (1,378,602/1,893,512) of infections in 2010-2015. In East Asia, CRF01_AE has increased consistently over the periods to 47·2% (381,996/810,004) in 2010-2015, with further contributions by other CRFs (28·2% (228,720/810,004) in 2010-2015; mainly CRF07_BC and CRF08_BC), resulting in the highest proportion of total CRFs (75·5%; 611,520/810,004) and total recombinants (80·5%; 652,181/810,004) anywhere in the world (figure 3, table 2B, appendix pp13-14, 16-31).

Discussion

This is the largest study to date reporting on the global and regional distribution and trends of HIV-1 subtypes and recombinants, with a sample size of 383,519, and spanning the years 1990-2015, covering the whole period for which reliable estimates of national HIV prevalence are available for most countries. With our approach we achieved a very high coverage both globally and regionally throughout the time periods (table 1).

Distinct distributions of HIV-1 subtypes and recombinants and dynamic changes were seen in different countries and regions. Over the most recent decade (2005-2015) the proportion of subtype B increased, subtypes A and D were stable, and subtypes C and G and CRF02_AG decreased at a global level. CRF01_AE, other CRFs, and URFs increased, leading to a consistent increase in the global proportion of recombinants over time (figure 2B, table 2A). The global and regional trends observed are the product of changes in HIV-1 subtype distributions in countries and changes in the number of PLHIV in countries over time. Overall, trends reflect the relative differences in the numbers of new infections and deaths associated with each subtype/recombinant. The factors that lead to these patterns are complex and multifactorial, and include possible biological differences between subtypes leading to differences in transmission and disease progression.[19,20] However, many geographic and socioeconomic factors are also at play, including transportation links, founder effects, migration, urbanisation, transmission networks, and population growth.[21,22] Furthermore, the wider availability of anti-retroviral treatment globally has led to reductions in both HIV-related deaths and new infections, leading to a slower renewal of PLHIV. However, disparities in treatment and prevention coverage and effectiveness between different geographical regions and different risk groups may lead to differential control of the HIV epidemic in different

regions/risk groups with different HIV variants, thereby shaping the global and regional HIV subtype distributions.

A consistent increase in the proportion of CRFs, and recombinants overall, throughout the time periods was seen globally and in most regions (table 2, appendix pp13-16). Over time the number of different CRFs identified has increased, with 96 identified thus far,[5] and formation of new recombinants is ongoing.[3] In the past, recognition of CRF02_AG led to a re-classification of some strains which would previously have been assigned as 'pure' subtype A based on characterisation of *env* only.[23] This led to a decrease in subtype A and an increase in CRF02_AG between the periods 1990-1999 and 2000-2004 reported for West Africa and, to a lesser extent, Central Africa (table 2B), which translated into global changes (table 2A,figure 2B), which do not represent genuine epidemiological changes and should be interpreted with caution. However, the increase over time in the reported regional and global proportions of CRFs are unlikely to be due to better characterisation and assignment of CRFs only. Sequencing has been the mainstay of subtyping since 2000 (>97% of samples throughout 2000-2015; 99·8% in 2010-2015)(appendix, p10), the vast majority in *pol* (>77% of samples throughout 2000-2015; 94·2% in 2010-2015)(appendix, p11). In addition, the proportion of samples assessed in only one genome segment actually increased from 77·0%-83·4% in the first two time periods (1990-2004) to 87·2%-90·1% in the latter two time periods (2005-2015) and the proportion of full length sequences decreased from 1·4-1·9% to 0·3-0·4% in the same time (appendix, p12). Therefore, while the proportion of recombinants reported has increased over time, the methods and number and type of genome segments used to subtype samples have actually narrowed and are now dominated by *pol* sequencing, often as part of resistance testing. Indeed, it is likely that the prevalence of recombinants is actually underestimated in

our study because of the limited proportion of samples which are subtyped in more than one genome segment.

Our study had some further limitations. Although coverage was generally very high, some sample sizes were small and few data were from nationally representative surveys. Low coverage in some time periods for the Caribbean, Oceania and Middle East & North Africa (MENA), with different countries and populations sampled in different time periods, led to regional variations in subtype distribution over time, which do not represent genuine epidemiological change and should be interpreted with caution (appendix, pp17, 25-26). However, these three regions contain the smallest absolute numbers of PLHIV of all regions and hence these changes have very little impact on global proportions (table 1). Furthermore, dividing data sets which straddled different analytic time periods assumed that subtype data was collected evenly over the sampling years and/or that subtype distribution was stable during the sampling years, which may not have been the case. Publication bias may have occurred. Finally, heterogeneity among data sets in study types, populations sampled, subtyping methods used, and genome segments analysed may have introduced biases. In particular, different inclusion criteria for different studies and different risk groups sampled would lead to characterisation of subtype distributions in specific population/risk groups which may not be fully representative of the subtype distribution in a country. Use of certain subtyping methods (e.g. HMA) would only detect a limited number of prespecified subtypes/CRFs and examination of different genome segments in different studies could lead to differential classification of recombinants which correspond to different subtypes in different genome segments. These biases may affect comparative results between countries or regions, but also within countries and regions over time.

The choice of vaccine immunogen sequence is crucial to the efficacy of an HIV vaccine. Of the candidate HIV vaccines in development, the two most clinically advanced concepts very recently reported on phase I/IIa trials in humans, demonstrating adequate safety and immunogenicity to justify progression to human phase IIb/III efficacy trials.[24,25] One of these human HIV vaccine efficacy trials (HVTN702; NCT02968849), currently under way in South Africa, investigates a vaccine based on the vaccine used in the successful RV144 trial in Thailand,[26] with the important adaptation that subtype B/CRF01_AE immunogens have been replaced with subtype C isolate immunogens, to match the HIV subtype that dominates in South Africa.[24] Should this subtype C vaccine prove effective, one of the next steps would be to test whether the vaccine offers cross-protection against other subtypes and recombinants, by conducting trials in regions with other circulating subtypes/recombinants, such as East Africa, where subtypes A, C and D co-circulate and hence relative protection against diverse subtypes may be evaluated. The other vaccine which entered into an efficacy trial (HVTN705; NCT03060629) in southern Africa is a polyvalent mosaic vaccine designed to cover all global HIV-1 group M viruses.[25]

Matching immunogen sequences to circulating strains is challenging. Primary isolates belonging to the same HIV subtype typically differ in the order of 8-17% (maximum 30%) at the amino acid level, whereas this is 17-35% (maximum 42%) between isolates from different subtypes, depending on which subtypes and genome segments are examined.[27] Recombination further adds to this complexity. However, the amino acid difference between immunogen sequence and circulating viral strains of the same subtype may be halved by using artificial centralised sequences, such as consensus, ancestral, or centre-of-tree sequences.[7,28] These approaches can be further expanded to include all global subtypes, e.g. centralised sequences of HIV-1 group M.[7] Other approaches to address HIV diversity include mosaic and polyvalent

vaccines and focusing on evolutionarily conserved regions of the HIV genome.[6] All these strategies are not mutually exclusive and may be used in combination. Of course, other vaccine approaches involving different vectors and immunogens may prove more effective when tested in human trials.[6]

In addition to aiding vaccine design and development as described above, knowledge of the regional and global prevalence of HIV subtypes and recombinants is also instrumental in estimating the global "need" and market size for both therapeutic and prophylactic HIV vaccines based on different subtypes in order to prioritise vaccines based on subtypes with the greatest potential global benefit.[29] Our analysis shows that subtype C causes the greatest number of global infections and that subtype C is the near-exclusive strain in southern sub-Saharan Africa. Together with the fact that the highest HIV prevalence is found in Southern Africa, it is logical to focus on a subtype C specific HIV vaccine and test it in South Africa, the country with the largest number of PLHIV.[1,24] In many countries and regions, however, the HIV epidemic is not solely composed of 1-3 'pure' subtypes, but instead a significant and increasing proportion of infections is made up of a variety of minority subtypes and recombinants. It remains to be seen whether a subtype specific vaccine will be able to protect against all these diverse subtypes and recombinants or whether complex cocktails of subtype-specific vaccines or a global group M-specific vaccine will be required.[6,25] Moreover, since the distribution of HIV subtypes is evolving and the number and proportion of recombinants increasing, a future HIV vaccine may need to be changed periodically, like influenza vaccines.[27]

HIV genetic diversity also impacts the efficiency of HIV diagnostic assays and viral load assays, which in turn challenges blood donor screening, surveillance, clinical diagnosis and

management.[3] A recent study compared a range of commercially available fourth generation antigen/antibody, p24 antigen and viral load assays against a standardised panel of HIV-1 subtypes and recombinants.[8] Differences in the efficiency of detection of different subtypes/recombinants by diagnostic assays were seen but, interestingly, the different assays tested had the same rank order of detection of diverse isolates, with similar levels of detection across subtypes. Viral load assays performed well across a range of genotypes and viral loads.[8] However, ongoing recombination is expected to lead to the generation of new URFs and CRFs for which the assays have not been designed and vigilance is therefore required. Most major resistance mutations found in subtype B are also found in non-B subtypes, although the prevalence of some mutations is higher in certain subtypes (e.g. K65R/N mutation in RT with tenofovir treatment).[9] In addition, several novel mutations occur in non-B subtypes.[10,30] However, drug resistance is not well-studied in the less common subtypes, CRFs and URFs. Continued surveillance for transmitted drug resistance and treatment failure is therefore essential in all HIV subtypes and recombinants.

In conclusion, the current study, based on 383,519 subtyped samples, shows great global diversity, as well as dynamic trends over time. Continued effective surveillance of the global and regional molecular epidemiology of HIV-1 is therefore crucial for the design, testing and implementation of HIV vaccines.[31]

Research in Context panel

Evidence before this study

Global HIV diversity presents a major challenge to HIV vaccine development. Designing, testing and implementing HIV vaccines requires up-to-date and accurate knowledge of the distribution of HIV subtypes and recombinants in countries, regions and worldwide. Surveillance of the global molecular epidemiology of HIV-1 is therefore essential, but recent data and information on trends are lacking. HIV sequence databases contain sequences with known dates and countries of sample collection, but samples are not representative of populations. Estimates based only on published data have limited coverage, especially for recent years, and are prone to publication bias. Estimates based on both published and unpublished data and adjusted for the number of people living with HIV (PLHIV) in each country are only available for the period 2000-2007. We conducted a comprehensive electronic literature search to identify all published studies reporting HIV-1 subtyping data. We searched four electronic literature databases (Pubmed, EMBASE (Ovid), CINAHL (Ebscohost) and Global Health (Ovid)) to identify studies published between Jan 1, 1990, and Dec 31, 2015. Search terms included Mesh headings and Emtree terms, as well as free text words and synonyms, including "HIV", "subtype", "recombinant", and "epidemiology". Unpublished original HIV-1 subtyping data was collected through a survey among experts in molecular epidemiology of HIV-1 under the umbrella of the WHO-UNAIDS Network for HIV Isolation and Characterisation.

Added value of this study

This is the largest study to date reporting on the regional and global distribution of HIV-1 subtypes and recombinants, by combining data from a systematic literature review and a global

survey. The time period extended from 1990 to 2015, covering the whole period for which reliable estimates of national HIV prevalences are available. With our approach we achieved a very high coverage both globally and regionally. In total, we collected 2,203 data sets with 383,519 samples from 130 countries over 1990-2015.

Distinct distributions of HIV-1 subtypes and recombinants were seen in different countries and regions. Globally, subtype C accounted for nearly half (46·6%; 16,280,897/34,921,639 of PLHIV) of all HIV-1 infections worldwide in 2010-2015. Subtypes B and A were responsible for 12·1% (4,235,299/34,921,639) and 10·3% (3,587,003/34,921,639) of infections, respectively, followed by CRF02_AG (7·7%; 2,705,110/34,921,639), CRF01_AE (5·3%; 1,840,982/34,921,639), subtype G (4·6%; 1,591,276/34,921,639) and D (2·7%; 926,255/34,921,639). Subtypes F, H, J, and K combined accounted for 0·9% (242,345/34,921,639) of infections globally. Other CRFs accounted for 3·7% (1,309,082/34,921,639), bringing the proportion of all CRFs to 16·7% (5,844,113/34,921,639). URFs constituted 6·1% (2,134,405/34,921,639), resulting in recombinants accounting for 22·8% (7,978,517/34,921,639) of all global HIV-1 infections.

Changes in the distribution of HIV-1 subtypes and recombinants were seen over time in countries, regions and globally. Over the most recent decade (2005-2015) subtype B increased, subtypes A and D were stable, and subtypes C and G and CRF02_AG decreased at a global level. CRF01_AE, other CRFs, and URFs increased, leading to a consistent increase in the global proportion of recombinants over time.

Implications of all the available evidence

Global and regional HIV diversity is complex and evolving and forms a major challenge to HIV vaccine development. Surveillance of the global molecular epidemiology of HIV-1 therefore remains crucial for the design, testing, and implementation of HIV vaccines.

Author contributions

JH conceived, designed and coordinated the study, wrote the systematic review protocol, assisted with the literature search, assessed eligibility of manuscripts, collected additional published data, conducted the global survey, performed data extraction, designed and performed the analysis, designed figures and tables, interpreted the data and wrote the manuscript.

RE, JY, and LD screened the electronic literature search results for relevant manuscripts, assessed their eligibility, and extracted data. RE, JY, and LD collected additional published data. JY and LD performed analysis, made figures, and interpreted the data.

IF performed data analysis.

SK designed and conducted the electronic literature search.

BW assisted with the statistical analysis.

EG provided data on the number of people living with HIV in each country, assisted with the statistical analysis, and interpreted the data.

PG assisted with the analysis, and interpreted the data

JH had full access to all the data in the study and had final responsibility for the decision to submit the manuscript for publication.

All authors read and approved the final version of the manuscript.

Conflicts of interest

We declare that we have no conflicts of interest.

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Legends

Figure 1. Study flow diagram.

Sources of HIV subtyping data. See Methods for details of the electronic literature search, the global survey of the WHO-UNAIDS Network for HIV Isolation and Characterisation, and other published data sources.

* For example, HIV-positive immigrants only.

† For example, data given for 'B' and 'non-B' samples only.

‡ For example, 'subtypes' referred to disease states, not HIV subtypes.

Figure 2. Global distribution of HIV-1 subtypes, CRFs and URFs in 2010-2015, and trends during 1990-2015.

A. Pie-chart displaying the distribution of HIV-1 subtypes, CRFs and URFs as a proportion (percentage) of global HIV infections in the period 2010-2015. Colours representing the different HIV-1 subtypes, CRFs and URFs in the pie-chart are indicated in the legend on the left. Pie-charts for the periods 1990-1999, 2000-2004, and 2005-2009 can be found in the appendix, p15.

B. Stacked bar graphs displaying the distribution of HIV-1 subtypes, CRFs and URFs as a proportion of global HIV infections in the periods 1990-1999, 2000-2004, 2005-2009, and 2010-2015. Values for proportions and 95% confidence intervals can be found in table 2 and appendix, p13-14.

CRF = Circulating Recombinant Form, Other CRFs = CRFs other than CRF01_AE and CRF02_AG, Total CRFs = CRF01_AE + CRF02_AG + other CRFs, URF = Unique Recombinant Form, Total recombinants = Total CRFs + URFs.

Figure 3. Regional distributions of HIV-1 subtypes, CRFs and URFs in 2010-2015.

Countries were grouped into 14 regions as specified in appendix, p9. Countries forming a region are shaded in the same colour on the world map. Pie-charts displaying the distribution of HIV-1 subtypes, CRFs and URFs in each region in 2010-2015 are superimposed on the world map. Pie-charts were prepared using the data displayed in table 2B. The relative surface area of each pie-chart corresponds to the relative number of PLHIV in each region (table 1). Colours representing the different HIV-1 subtypes, CRFs and URFs in the pie-charts are indicated in the legend on the left. Equivalent maps for the periods 1990-1999, 2000-2004, and 2005-2009 can be found in appendix, p16.

CRF = Circulating Recombinant Form, Other CRFs = CRFs other than CRF01_AE and CRF02_AG, URF = Unique Recombinant Form.

Figure 4. Country and regional distribution of HIV-1 subtypes, CRFs and URFs in Central Africa in 1990-2015.

Pie-charts displaying the country distributions of HIV-1 subtypes, CRFs and URFs in Central Africa in 2010-2015 (A), 2005-2009 (B), 2000-2004 (C), and 1990-1999 (D) are superimposed on the regional maps. The relative surface area of each pie-chart corresponds to the relative number of people living with HIV in each country per time period. Colours representing the different HIV-1 subtypes, CRFs and URFs in the pie-charts are indicated in the legend on the left. The distribution of HIV-1 subtypes, CRFs and URFs in the whole region in each time period is given in the top-right corner of each panel. CRF = Circulating Recombinant Form, Other CRFs = CRFs other than CRF01_AE and CRF02_AG, URFs = Unique Recombinant Forms.

Table 1. Data collection.

HIV subtyping sample collection for 1990-2015. Regional and global data sets, samples collected, coverage, depth of sampling, and numbers of people living with HIV in the time periods 1990-1999, 2000-2004, 2005-2009, and 2010-2015.

* Number of countries for which HIV subtyping data was available (for countries with data see appendix, pp17-31).

† Coverage refers to the proportion (percentage) of people with HIV in a region which lived in the countries for which HIV subtyping data was available in that region. If any subtyping data was available for a country (independent of the number of samples collected), the whole HIV-infected population in that country was deemed to be represented in this analysis.

‡ Depth of sampling refers to the number of HIV samples subtyped in a region as a proportion (percentage) of the number of people living with HIV in that region.

§ The average number of people living with HIV in each region and the world in each time period.

WCENA = Western and Central Europe & North America, EECA = Eastern Europe & Central Asia, MENA = Middle East & North Africa.

Table 2. Global and regional distribution of HIV-1 subtypes, CRFs and URFs in 1990-2015.

Distribution of HIV-1 subtypes, CRFs and URFs as a proportion (percentage) of global (**A.**) and regional (**B.**) HIV infections in the periods 1990-1999, 2000-2004, 2005-2009, and 2010-2015. 95% confidence intervals of proportions can be found in appendix pp13-14.

CRF = Circulating Recombinant Form, Other CRFs = CRFs other than CRF01_AE and CRF02_AG, URFs = Unique Recombinant Forms, Total CRFs = CRF01_AE + CRF02_AG + other CRFs, Total recombinants = Total CRFs + URFs.

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