Global and regional distribution of HIV-1 genetic subtypes and recombinants in 2004

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Objective: To estimate the global and regional distribution of HIV-1 subtypes and recombinants in 2004.

Design: A study was conducted in which molecular epidemiological data on HIV-1 subtype distribution in individual countries were combined with country-specific estimates of the number of people living with HIV.

Methods: HIV-1 subtype data were collected for 23 874 HIV-1 samples from 70 countries, which together accounted for 89% of all people living with HIV worldwide in 2004. The proportions of HIV-1 infections due to various subtypes detected in each country were combined with the number of HIV infected people in the respective countries to generate regional and global HIV-1 subtype distribution estimates.

Results: Subtype C accounted for 50% of all infections worldwide in 2004. Subtypes A, B, D and G accounted for 12%, 10%, 3% and 6%, respectively. The subtypes F, H, J and K together accounted for 0.94% of infections. The circulating recombinant forms CRF01_AE and CRF02_AG each were responsible for 5% of cases, and CRF03_AB for 0.1%. Other recombinants accounted for the remaining 8% of infections. All recombinant forms taken together were responsible for 18% of infections worldwide.

Conclusion: Combining data on HIV-1 subtype distribution in individual countries with country-specific estimates of the number of people living with HIV provided a good method to generate estimates of the global and regional HIV-1 genetic diversity in 2004. The results could serve as an important resource for HIV scientists, public health officials and HIV vaccine developers.

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Introduction

The World Health Report 2004 of the World Health Organization identified HIV as the world's most urgent public health challenge at present [1]. Over 25 million individuals are estimated to have died of AIDS since the start of the pandemic. Up to 38.6 million individuals are currently infected with HIV, the majority living in

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sub-Saharan Africa and Asia. An estimated 4.1 million individuals were newly infected with HIV in 2005, 95% of whom are living in developing countries [2].

The high genetic variability and rapid evolution of HIV-1 are important factors in its worldwide spread. HIV-1 genetic heterogeneity originates from the high mutation and recombination rates of the reverse transcriptase enzyme combined with a high turnover rate. This results in genetically diverse populations of viral species in each infected individual, termed 'quasispecies'. Viral species in a single individual can differ by up to 10%. Selection pressures applied by, for example, the immune system or antiviral drugs result in further viral evolution [3].

Phylogenetic analysis of HIV samples has led to the classification of HIV-1 into three genetic groups, M (major), O (outlier) and N (non-M, non-O). Most HIV-1 infections globally are caused by group M viruses, with group O and N causing a small minority of infections in central Africa. Within group M, nine subtypes are recognized, designated by the letters A–D, F–H, J and K [4]. Although this classification was initially based on *env* sequences, it applies to all regions of the genome. Genetic variation within a subtype can be of the order of 15–20%, whereas variation between subtypes is approximately 25–35%, depending on the subtypes and genome regions examined [5]. Analyses of multiple genome regions and in particular full-length genome sequencing have revealed inter-subtype recombinants, which are thought to originate from individuals with multiple infections, i.e. infected with viruses of two or more subtypes simultaneously. Recombinant viruses identified in at least three epidemiologically unlinked individuals and characterized by full-length genome sequencing are designated as circulating recombinant forms (CRFs) [4]. To date, 19 CRFs have been identified [6]. Some CRFs, e.g. CRF01_AE and CRF02_AG, play important roles in regional epidemics. With increased global availability of sequencing techniques, it is becoming apparent that recombinant forms are widespread and are contributing significantly to the pandemic. The different subtypes have distinct global distribution patterns [7].

The high level of genetic variability of HIV-1 may have important implications for HIV pathogenesis, transmission, diagnosis, treatment and vaccine development. The possibility that different subtypes may have different biological properties resulting in differences in transmissibility and pathogenicity is obvious, but has been difficult to establish. Most subtypes conform to the non-syncytium-inducing CCR5 receptor usage phenotype early in infection, followed by a shift to the syncytium-inducing CXCR4 receptor usage phenotype late in infection. However, at least some subtype C and D viruses do not seem to follow this pattern [8]. Several studies have suggested that the maternal HIV subtype could play a role in the likelihood of vertical transmission, although others discount this notion [9–11]. Consistent subtype-associated differences in efficiency of transmission via different routes remain to be proved. Some studies have reported a difference between subtypes in rates of progression to AIDS [12], whereas others have not found such differences [13]. Such variation in reported results could be caused by virus, host and environmental factors, as well as differences in study design, sample size, the use of surrogate markers of progression and duration of clinical follow up.

HIV-1 diversity has an impact on HIV diagnosis, as well as on the determination of viral load. HIV-1 immunoassays need to be capable of detecting all known group M subtypes [14]. Polymerase chain reaction-based assays for viral load measurements also need to reliably quantify HIV-1 RNA from all known genetic variants of HIV-1 [15].

Resistance to antiretroviral drugs is an important issue in the clinical management of HIV-related disease [16]. It is therefore important to determine whether HIV subtypes differ in their primary susceptibility to antiretroviral drugs or their capacity to develop antiretroviral resistance. Group O viruses are known to be naturally resistant to non-nucleoside reverse transcriptase inhibitors. It appears that different group M subtypes have similar susceptibilities to currently used antiretroviral drugs, at least *in vitro* [17]. Some studies have shown differences between B and non-B subtype viruses in the generation of drug resistance mutations after the commencement of treatment [18]. Others have shown no effect of viral subtype on outcomes of antiretroviral therapy [19].

The best hope for controlling the HIV pandemic is a preventive vaccine that is safe, simple, highly effective and affordable [20]. It has, however, been recognized that the global genetic and antigenic variability of HIV-1 may pose a major challenge for the development of globally effective HIV vaccines [21]. Correlates of immune protection against HIV remain unknown, but it seems likely that, given the high genetic diversity of HIV, both humoral and cell-mediated immune responses are needed to confer broad and long-lasting protection against primary isolates [22]. However, during primary HIV infection both the antibody and the cell-mediated immune responses exert a selection pressure on the infecting virus, resulting in the rapid generation of escape mutants resistant to antibody neutralization and/or cytotoxic T-cell-(CTL) mediated killing [23,24]. Although antigenic variation between subtypes is likely to be important for antibody-mediated protection, neutralization serotypes do not appear to correlate with HIV-1 genetic subtypes [25]. Cytotoxic T-cell responses in HIV-1-infected individuals are more broadly crossreactive between different subtypes, although intrasubtype responses are often stronger and more frequent than intersubtype reactivities [26,27].

It is clear that HIV-1 diversity may have important implications for many aspects of the HIV pandemic and its control. It is therefore imperative to monitor the global and regional distribution of HIV-1 subtypes and recombinants. We have previously examined the global distribution of HIV-1 subtypes [7]. The aim of the current study was to improve further on the global surveillance of HIV-1 subtypes and epidemiological data collection to generate reliable and up-to-date estimates of the global and regional diversity of HIV-1. To this end, we estimated the global and regional distribution of HIV-1 genetic subtypes and recombinants by combining molecular epidemiological data on HIV-1 subtype distribution in individual countries with country-specific estimates of the number of individuals living with HIV in 2004.

Methods

Country-specific HIV-1 subtype distribution data

A questionnaire was sent out to research laboratories across the world specializing in the characterization of HIV samples from diverse geographical regions. Data were requested from studies in molecular epidemiology conducted on HIV blood samples taken in the years 2000–2004. In particular, researchers were asked to detail the subtypes of the analysed samples as A, B, C, D, CRF01_AE, CRF02_AG, CRF03_AB, other subtypes (F, G, H, J, K) and other CRFs and recombinants. The majority of subtyping data were obtained by nucleotide sequencing of one or more genome segments, whereas the remainder were typed by heteroduplex mobility assay. Researchers were asked to indicate the country of origin of the samples, the year in which the samples were taken, the likely route of transmission through which HIV was acquired, the detection method used, and the genome segment that was analysed (*gag*, *env*, *pol*, long-terminal repeat or full length). Only samples with complete data were included in our analyses. Data sets representing different routes of transmission (heterosexual including perinatal transmission and female sex workers, men who have sex with men and injecting drug users) within a country were combined into one country dataset, weighted according to the relative importance of each transmission route. When no information was received from countries, in particular from countries in regions with a high HIV-1 prevalence, the subtype profile was constructed by conducting literature searches to find publications containing the necessary data from samples collected in 2000–2004. In all cases, the resulting aggregates were used to determine the overall proportions of HIV-1 subtypes and recombinants in the country.

HIV-1 epidemiology data and geographical regions

The HIV epidemiology data used in this study were obtained from the UNAIDS/WHO estimates of the burden of HIV in 2004 [28]. The only exception was Ethiopia, for which 2003 estimates were used, because more recent data were not available at the time of our study.

Countries were grouped together in geographical regions according to the classification used by UNAIDS [28], with a few modifications. Sub-Saharan Africa was divided into four separate regions (west, east, central and southern), because the region has the largest number of HIV-1 infections and a high level of regional diversity in HIV-1 subtypes, warranting a more detailed analysis. The data analyses for India and Ethiopia were conducted separately from the other countries in their respective regions because they harbour a large number of infections caused by a single subtype, which would have skewed the results for their respective regions. These modifications resulted in the grouping of countries into 15 regions. The countries comprising each region are specified in the legend to Table 2.

Data processing

For the countries for which appropriate data were obtained, the proportions of HIV-1 subtypes and recombinants were calculated as described above. To determine the distribution of HIV-1 subtypes in the regions, the proportions of all HIV-1 subtypes present in each country in a region were first multiplied by the absolute number of individuals living with HIV in the same country. The resulting estimated numbers of individuals living with each subtype in the countries in each region were then added up. The total absolute numbers of the different subtypes in each region were finally used to derive the proportions of the different HIV-1 subtypes and recombinants in the regions (Table 2). Countries for which no HIV-1 subtype distribution data had been obtained were left out of this analysis.

Similarly, to calculate the global HIV-1 subtype distribution, the regional subtype proportions were multiplied by the number of individuals living with HIV in the regions (which included HIV-infected individuals in countries for which no HIV subtype distribution data had been obtained). The resulting absolute numbers of the different subtypes in the regions were added up by subtype across regions, and the global total numbers of each subtype were used to determine the global distribution of HIV-1 subtypes and recombinants (Table 2).

The regional distribution of infections caused by individual subtypes was determined using the absolute numbers of infections caused by each subtype in each region (see above). The number of infections caused by a subtype in a region was taken as a proportion of the global number of infections caused by that same subtype (Table 3).

Results

Collection of HIV-1 subtype distribution data

A total number of 23 874 samples were included in our analyses (Table 1). Data on the distribution of HIV-1 subtypes and recombinants were collected for a total of 70 countries across the globe as listed in the legend of Table 2. For our analysis, the world was divided into 15 regions, as specified in the Methods section and the legend of Table 2. For each region, special efforts were made to ensure that data were obtained for countries with the highest absolute numbers of infections in the region. As a result, in nine of the 15 regions the countries for which HIV subtype distribution data were collected represented more than 90% of individuals living with HIV (Table 1, coverage of region). In a further three regions, the countries with subtype distribution data covered between 80 and 90% of HIV-1 infections in the region. In the remaining three regions, a lack of data from some countries meant that less than 80% of individuals living with HIV in the region were represented. Overall, the 70 countries from which data were obtained accounted for 89% of individuals living with HIV worldwide (Table 1).

The number of samples analysed as a proportion of the number of individuals living with HIV varied considerably between regions (Table 1, second last column). The representation was better for north America, western Europe and Oceania, whereas populations of individuals living with HIV in India, Ethiopia and southern Africa were less well represented.

Global distribution of HIV-1 subtypes and recombinants

The global proportions of HIV-1 subtypes and recombinants are shown in Fig. 1 and Table 2. Subtype C accounted for half (50%) of all infections worldwide. The subtypes A, B, D and G were responsible for 12, 10, 3 and 6% of infections, respectively. The subtypes F, H, J and K together caused 0.94% of infections. The two major CRFs, CRF01_AE and CRF02_AG, were each responsible for 5% of infections, whereas CRF03_AB was responsible for only 0.1% globally. Other recombinants accounted for the remaining 8% of infections. All recombinant forms taken together were responsible for 18% of infections worldwide.

Regional distribution of HIV-1 subtypes and recombinants: analysis by region

The distribution of HIV-1 subtypes and recombinants within each region is shown in Table 2 and depicted in Fig. 2. Of all HIV-1 infections worldwide, 64% are present in sub-Saharan Africa (Table 1). For the whole of sub-Saharan Africa, 56% of infections are caused by subtype C, with smaller proportions of infections caused by subtype A (14%), subtype G (10%), CRF02_AG (7%) and other recombinants (9%). However, there are marked differences between the five subregions of sub-Saharan Africa.

Table 1. Global and regional HIV-1 epidemiology and sample collection.

Region of the world	Numbers of individuals living with HIV in 2004 (*n*)[a]	HIV-1 infections as proportion of global total in 2004 (%)[a]	HIV-1 prevalence in 2004 (%)[b]	Number of samples collected (*n*)	Proportion of HIV-1 infected population represented (%)[c]	Coverage of region (%)[d]
North America	1 000 000	2.57	0.6	3098	0.3098	95.0
Caribbean	440 000	1.13	2.3	204	0.0464	26.9
Latin America	1 700 000	4.36	0.6	2476	0.1456	91.5
Western Europe	570 000	1.46	0.3	7442	1.3056	86.5
Eastern Europe and central Asia	1 500 000	3.85	0.7	1022	0.0681	91.7
India	5 100 000	13.08	0.9	426	0.0084	100.0
South and south-east Asia (excl. India)	1 800 000	4.62	0.3	1155	0.0642	73.7
East Asia	1 100 000	2.82	0.1	1173	0.1066	99.2
Oceania	35 000	0.09	0.2	684	1.9543	43.1
North Africa and middle east	540 000	1.39	0.3	291	0.0539	89.5
West Africa	6 300 000	16.16	4.5	2815	0.0447	91.0
East Africa (excl. Ethiopia)	3 900 000	10.00	5.7	1268	0.0325	90.9
Ethiopia	1 500 000	3.85	4.4	143	0.0095	100.0
Central Africa	2 000 000	5.13	4.8	771	0.0386	95.6
Southern Africa	11 500 000	29.50	17	906	0.0079	81.1
Global	38 985 000	100	1.1	23874	0.0612	88.6

[a]The numbers of individuals living with HIV were obtained from the UNAIDS/WHO estimates of the burden of HIV in 2004 (see Methods).
[b]The percentage of adults aged 15–49 years living with HIV. Data obtained from the UNAIDS/WHO estimates of the burden of HIV in 2004 (see Methods).
[c]The number of samples collected from a region as a proportion of the number of individuals living with HIV in the region (%).
[d]The combined number of individuals living with HIV in the countries for which HIV subtype distribution data were collected in a region as a proportion of the total number of individuals living with HIV in the region (for countries per region: see caption Table 2).

Table 2. Distribution of HIV-1 subtypes and recombinants within each region and globally in 2004 (%).

Region of the world	A	B	C	D	F	G	H	J	K	CRF01_AE	CRF02_AG	CRF03_AB	Other recombinants	CRFs & other recombinants (%)[a]
North America	0.26	98.42	0.45	0.06	0.03	0.00	0.00	0.00	0.00	0.58	0.10	0.00	0.10	0.77
Caribbean	0.00	94.07	0.97	0.03	0.03	0.13	0.03	0.03	0.00	0.00	0.00	0.00	4.72	4.72
Latin America	0.00	74.50	12.60	0.15	4.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.40	8.40
Western Europe	1.69	87.62	2.15	0.46	0.35	2.43	0.00	0.00	0.02	0.62	2.05	0.00	2.60	5.27
Eastern Europe and central Asia	78.88	14.54	1.86	0.00	1.18	0.01	0.00	0.00	0.00	0.60	0.27	2.66	0.00	3.53
India	1.17	0.23	96.95	0.00	0.00	0.00	0.00	0.00	0.00	0.47	0.00	0.00	1.17	1.64
South and south-East Asia (excl. India)	0.00	8.09	3.28	0.00	0.00	0.00	0.00	0.00	0.00	84.28	0.00	0.00	4.36	88.63
East Asia	1.28	38.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	15.53	0.31	0.00	44.85	60.69
Oceania	0.32	88.06	5.01	0.20	0.12	0.00	0.00	0.00	0.00	4.58	1.22	0.00	0.49	6.29
North Africa and middle east	6.21	7.46	28.66	47.03	0.00	0.00	0.00	0.14	0.00	0.00	1.05	0.00	9.45	10.50
West Africa	20.63	0.01	0.59	0.54	0.41	34.94	0.05	0.22	0.01	0.25	27.87	0.00	14.48	42.60
East Africa (excl. Ethiopia)	34.97	0.11	25.09	10.93	0.11	0.24	0.00	0.00	0.00	0.00	0.00	0.00	28.55	28.55
Ethiopia	1.40	0.00	98.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Central Africa	37.60	0.23	11.64	11.08	4.02	11.14	3.14	1.77	0.84	3.99	3.98	0.00	10.57	18.55
Southern Africa	0.47	0.21	98.30	0.39	0.21	0.13	0.00	0.03	0.00	0.00	0.00	0.00	0.25	0.25
Global	12.30	10.42	49.91	2.53	0.59	6.32	0.17	0.14	0.04	4.69	4.77	0.10	8.02	17.59

The proportions of HIV-1 subtypes and recombinants within each region and the world. The countries comprising each region are specified below. Underlined are the countries for which HIV-1 subtype distribution data were obtained.

*: The combined proportions of CRF01_AE, CRF02_AG, CRF03_AB and other recombinants.

1. Americas.

North America: Canada, USA;

Caribbean: Bahamas, Barbados, Cuba, Dominican Republic, Haiti, Jamaica, Trinidad and Tobago;

Latin America: Argentina, Belize, Bolivia, Brazil, Chile, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, Suriname, Uruguay, Venezuela.

2. Europe.

Western Europe: Albania, Austria, Belgium, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Serbia and Montenegro, Slovenia, Spain, Sweden, Switzerland, The Former Yugoslav Republic Macedonia, the United Kingdom;

Eastern Europe & Central Asia: Armenia, Azerbaijan, Belarus, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Estonia, Georgia, Hungary, Kazakhstan, Kyrgyzstan, Latvia, Lithuania, Poland, Republic of Moldova, Romania, Russian Federation, Slovakia, Tajikistan, Turkmenistan, Ukraine, Uzbekistan.

3. Asia.

India;

South & South-East Asia (excl. India): Afghanistan, Bangladesh, Bhutan, Brunei Darussalam, Cambodia, Indonesia, Iran, Lao People's Democratic Republic, Malaysia, Maldives, Myanmar, Nepal, Pakistan, Philippines, Singapore, Sri Lanka, Thailand, Viet Nam.

East Asia: China, Democratic People's Republic of Korea, Hong Kong Special Administrative Region, Japan, Mongolia, Republic of Korea;

Oceania: Australia, Fiji, New Zealand, Papua New Guinea.

4. North Africa & Middle East.

Algeria, Bahrain, Cyprus, Egypt, Iraq, Israel, Jordan, Kuwait, Lebanon, Libyan Arab Jamahiriya, Morocco, Oman, Qatar, Saudi Arabia, Sudan, Syrian Arab Republic, Tunisia, Turkey, United Arab Emirates, West Bank and Gaza Strip, Yemen.

5. Sub-Saharan Africa.

West Africa: Benin, Burkina Faso, Cameroon, Côte d'Ivoire, Equatorial Guinea, Gambia, Ghana, Guinea, Guinea-Bissau, Liberia, Mali, Mauritania, Niger, Nigeria, Senegal, Sierra Leone, Togo;

East Africa (excl. Ethiopia): Burundi, Djibouti, Eritrea, Kenya, Mauritius, Rwanda, Somalia, Uganda, United Republic of Tanzania;

Ethiopia;

Central Africa: Angola, Central African Republic, Chad, Democratic Republic of the Congo, Gabon, Republic of the Congo;

Southern Africa: Botswana, Comoros, Lesotho, Madagascar, Malawi, Mozambique, Namibia, South Africa, Zambia, Zimbabwe.

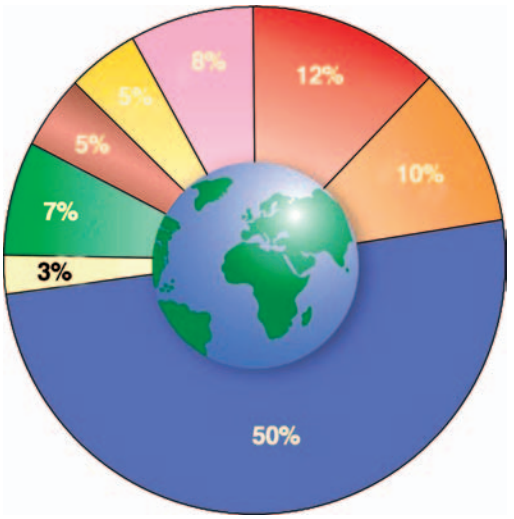

Fig. 1. Global distribution of HIV-1 subtypes and recombinants in 2004. The number of infections caused by HIV-1 subtypes and recombinants are represented as a proportion of the global total number of individuals living with HIV-1. The colours representing the different HIV-1 subtypes are indicated in the key below. Subtypes F, G, H, J and K were combined (for details see Table 2). A; B; C; D; F, G, H, J, K; CRF01_AE; CRF02_AG; CRF03_AB; other recombinants.

In southern Africa, which accounts for 30% of all global HIV-1 infections (Table 1), and Ethiopia (4% of global infections), the HIV-1 infections are caused nearly exclusively by subtype C, 98 and 99%, respectively (Table 2), with only minor differences between countries in southern Africa (93–100% subtype C).

In west Africa, home to 16% of the world's HIV-1 cases (Table 1), the dominant HIV-1 subtypes are A (accounting for 21% of all HIV-1 infections in the region), G (35%), CRF02_AG (28%) and other recombinants (14%; mostly CRF06_cpx; Table 2). All other subtypes and major CRFs were also detected, albeit at less than 1% at the regional level. The country with by far the largest number of HIV-1 infections in the region is Nigeria, where the epidemic is dominated by subtypes A (29%) and G (54%). Other countries in the region, such as Cameroon, Ghana and Côte d'Ivoire, have a distinct subtype distribution pattern in which CRF02_AG dominates (between 39 and 83%), with smaller contributions by subtype A and recombinant strains.

In east Africa, which accounts for 10% of the world's individuals living with HIV-1 (Table 1), the most prevalent subtypes are: A (35%), C (25%), D (11%) and a large proportion (29%) of mostly unique recombinant forms (Table 2). The same four subtypes are found in all countries in the region, although the distribution varies considerably between the individual countries. In Kenya and Rwanda, 57 and 79% of infections are caused by subtype A, whereas in the United Republic of Tanzania subtype C (44%) and various recombinants (37%) dominate. In Uganda, subtype D causes almost half of all infections (46%).

In central Africa, with 5% of the world's HIV-1-infected individuals (Table 1), the greatest diversity of subtypes and recombinants is found. Subtype A is the most prevalent, accounting for 38% of all infections. Subtypes C, D, G and other recombinants (CRF11_cpx and others) are each responsible for 11% of the total number of infections in the region. Subtypes F, H, CRF01_AE and CRF02_AG have each been the cause of 3–4% of infections. Subtypes B, J and K each accounted for less than 2% of infections (Table 2). Overall, the countries in the region all harbour a great diversity of subtypes and recombinants, but differ in the subtype that dominates. Subtype A dominates the epidemic in the Democratic Republic of the Congo and in the Central African Republic, causing 38 and 83% of infections, respectively. CRF02_AG was frequently found in Gabon (39%), whereas in Angola and Chad the epidemics are driven by other recombinant forms, found in 39 and 40% of cases, respectively.

India on its own is home to 13% of the world's individuals living with HIV (Table 1). The epidemic there is dominated by subtype C strains, which account for 97% of infections (Table 2).

In the rest of south and south-east Asia, which accounts for a further 5% of the global number of HIV-1-infected individuals (Table 1), the epidemic is dominated by CRF01_AE, which is responsible for 84% of all infections (Table 2). Other recombinants account for 4%, bringing the combined proportion of CRF and other recombinants to 89%, the highest in the world (Table 2). In Cambodia, Thailand and Viet Nam, CRF01_AE is responsible for more than 95% of infections, with the remaining infections caused by subtype B and other recombinants. In Myanmar, CRF01_AE accounts for 52% of infections, along with subtypes B (24%), C (12%) and other recombinants (12%).

In east Asia, with 3% of the global number of HIV infections (Table 1), China harbours the vast majority of HIV-1-infected individuals, and the distribution of subtypes in east Asia is therefore nearly identical to the distribution found in China. In China, subtype B strains are found in 38% of cases, whereas CRF01_AE (15%) and other recombinants (45%; mostly CRF07_BC and CRF08_BC) are also major players, resulting in a total proportion of recombinants of 61% (Table 2). In Hong Kong Special Administrative Region, subtype B (50%) and CRF01_AE (45%) are most prevalent. In Japan, subtype B strains dominate the epidemic at 81%, with remaining infections being caused by subtypes A, C and CRF01_AE.

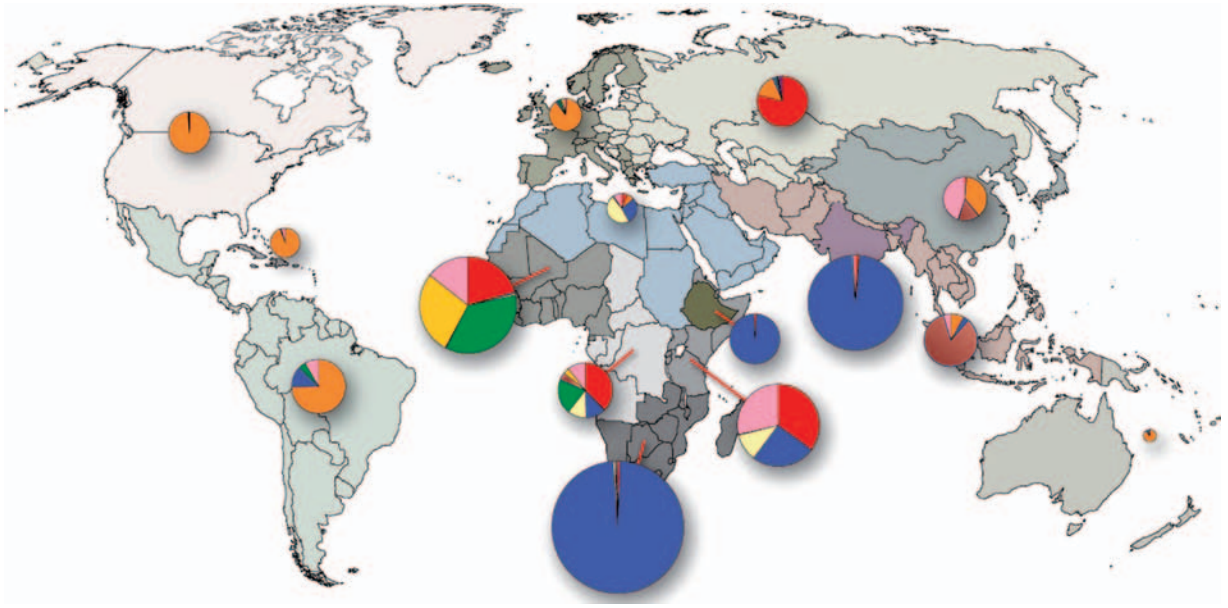

Fig. 2. Regional distribution of HIV-1 subtypes and recombinants in 2004. The world was subdivided into regions consisting of groups of countries as specified in the methods section and the legend of Table 2. Countries forming a region are shaded in the same colour. Different regions are shaded in different colours. Pie-charts representing the distribution of HIV-1 subtypes and recombinants in each region are superimposed on the regions or connected to the relevant regions by a line. The pie-charts were prepared using the data presented in Table 2. The colours representing the different HIV-1 subtypes are indicated in the key below. The proportions of the subtypes F, G, H, J and K were taken together (for details on these individual subtypes see Table 2). The relative surface areas of the pie-charts correspond to the relative numbers of individuals living with HIV in the regions (see column 2 of Table 1). A; B; C; D; F, G, H, J, K; CRF01_AE; CRF02_AG; CRF03_AB; other recombinants. Disclaimer: The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization or the Joint United Nations Programme on HIV/AIDS (UNAIDS) concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

Oceania is home to a very small absolute number of HIV-1-infected individuals, and the distribution is very similar in Australia and New Zealand, with the majority being caused by subtype B (88%) strains and a further 5% caused by subtype C and CRF01_AE (Table 2). Unfortunately, no data were collected that would allow an estimation of the subtype distribution in Papua New Guinea, which has the largest number of individuals living with HIV in that region.

North Africa and the middle east account for 1% of global HIV infections (Table 1). In that region, subtype D (47% of regional HIV-1 infections) and C (29%) are the major subtypes, with additional contributions by subtypes A (6%) and B (7%), as well as 9% of recombinant forms (Table 2). A similar distribution is found in Sudan, which harbours the vast majority of infected individuals in the region. In Algeria, Israel and Yemen the most prevalent subtype is B, which accounts for over half of all infections in those countries. However, CRF02_AG strains have also been reported in 25% of cases in Algeria, whereas subtype C strains are found in Israel (45%) and Yemen (22%). Subtype D strains have been found in 17% of cases in Yemen and 6% in Algeria.

Three per cent of all HIV-1-infected individuals live in north America (Table 1), with the vast majority of cases reported in the United States of America. In the United States the most prevalent subtype is B (98%), with only minor contributions by other subtypes and recombinants (Table 2).

The Caribbean region accounts for 1% of the global HIV-1 burden (Table 1), and the HIV-1 subtype distribution is very similar to the United States, with 94% of infections being caused by subtype B (Table 2). Unfortunately, no data were available from Haiti, which is one of the most affected countries in the region. In the Dominican Republic and Trinidad and Tobago more than 94% of HIV infections were caused by subtype B. In Cuba, on the other hand, a more diverse distribution was found, with subtype B being responsible for 48% of infections, 41% of cases being caused by various recombinant forms, and the remainder caused by subtypes C, D, F, G, H and J.

In Latin America, with 4% of the world's HIV-1 infections (Table 1), subtype B strains are found in 74% of cases. The subtypes C (13%), F (4%) and other recombinant forms (8%; mostly CRF12_BF and other B/F recombinants) have also been found to play a role in the region (Table 2).

Subtype B causes more than 94% of HIV-1 infections in Chile, Colombia, Ecuador, Honduras, Mexico and Venezuela. The other countries are characterized by more diverse patterns of subtype distribution. Subtype F strains play a visible role in the regional epidemic, and cause a significant proportion of infections in Brazil (8%), Paraguay (11%) and especially Uruguay (38%). Various recombinants feature strongly in the epidemics in Argentina (49%), Bolivia (16%), Paraguay (22%) and Uruguay (19%), along with subtype B strains, which account for 49% of infections in Argentina, and subtype C, which accounts for 28% of infections in Brazil.

The subtype distributions in western Europe and eastern Europe and central Asia are quite distinct from each other. In western Europe, which accounts for up to 1% of global HIV-infected individuals (Table 1), 88% of infections are caused by subtype B. The remainder is mainly caused by subtypes A, C, G and CRF02_AG, all of which account for approximately 2% each (Table 2). In countries with the highest absolute numbers of infection, France, Italy and Spain, the proportion of subtype B is equal to or exceeds 92%. In the other countries in the region, the proportion of subtype B infections was 82% or lower, with the lowest proportion of subtype B strains found in Portugal (39%). At the same time, in these other countries various non-B subtypes often constitute significant proportions of reported cases, such as subtype A in Austria (14%), Denmark (8%), the United Kingdom (8%) and Greece (6%); subtype C in Denmark (18%), Sweden (30%) and the United Kingdom (17%); subtype G in Portugal (26%); and other recombinant forms in Portugal (31%) and Greece (12%).

In eastern Europe and central Asia, where up to 4% of individuals infected with HIV live (Table 1), 79% of infections were caused by subtype A and a further 15% by subtype B. The remainder is attributed to subtypes C (2%), F (1%) and CRF03_AB (3%) (Table 2). Eastern Europe and central Asia is the only region in the world where CRF03_AB was found. Most individuals with HIV-1 infection in the region are living in the Russian Federation and the Ukraine. Subtype A strains account for more than 74% of infections in all countries, except the Czech Republic. In most countries the remainder of infections is largely represented by subtype B strains, except in Uzbekistan, where CRF02_AG accounts for 15% of infections. In the Czech Republic the subtype distribution is completely different from the other countries in the region and is much more similar to western Europe, with subtype B found in 73% of cases and the remainder caused by subtypes A (8%), C (9%) and CRF01_AE (5%), as well as a small proportion of other subtypes.

Regional distribution of HIV-1 subtypes and recombinants: analysis by subtype

The spread of each major HIV-1 subtype and recombinant form across the globe is shown in Table 3. The

Table 3. Distribution of HIV-1 subtypes and recombinants by region in 2004 (%).

Region of the world	A	B	C	D	F	G	H	J	K	CRF01_AE	CRF02_AG	CRF03_AB	Other recombinants
North America	0.05	24.23	0.02	0.07	0.14	0.00	0.00	0.00	0.00	0.32	0.05	0.00	0.03
Caribbean	0.00	10.19	0.02	0.01	0.05	0.02	0.18	0.22	0.00	0.00	0.00	0.00	0.66
Latin America	0.00	31.18	1.10	0.25	32.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.57
Western Europe	0.20	12.29	0.06	0.27	0.88	0.56	0.03	0.00	0.55	0.19	0.63	0.00	0.47
Eastern Europe and central Asia	24.68	5.37	0.14	0.00	7.75	0.00	0.00	0.00	0.00	0.49	0.21	100.00	0.00
India	1.25	0.29	25.41	0.00	0.00	0.00	0.00	0.00	0.00	1.31	0.00	0.00	1.91
South and south-east Asia (excl. India)	0.00	3.58	0.30	0.00	0.00	0.00	0.00	0.00	0.00	83.00	0.00	0.00	2.51
East Asia	0.29	10.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.35	0.18	0.00	15.77
Oceania	0.00	0.76	0.01	0.01	0.02	0.00	0.00	0.00	0.00	0.09	0.02	0.00	0.01
North Africa and middle east	0.70	0.99	0.80	25.74	0.00	0.00	0.00	1.38	0.00	0.00	0.30	0.00	1.63
West Africa	27.11	0.01	0.19	3.47	11.16	89.38	4.42	25.76	2.55	0.88	94.32	0.00	29.16
East Africa (excl. Ethiopia)	28.45	0.11	5.03	43.21	1.88	0.37	0.00	0.00	0.00	0.00	0.00	0.00	35.60
Ethiopia	0.44	0.00	7.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Central Africa	15.69	0.11	1.20	22.45	35.13	9.04	95.37	65.70	96.90	4.37	4.28	0.00	6.76
Southern Africa	1.13	0.60	58.11	4.52	10.69	0.61	0.00	6.95	0.00	0.00	0.00	0.00	0.91

The number of infections caused by a subtype in a region is presented as a proportion of the global number of infections caused by that subtype.

majority of subtype C infections are present in southern Africa (58% of global subtype C infections) and India (25%), with the remainder in Ethiopia (8%) and east Africa (5%; Table 3). Subtype A is evenly spread between eastern Europe and central Asia (25% of global total of subtype A), west Africa (27%), east Africa (28%) and central Africa (16%). Subtype B is present in the Americas (together accounting for 66% of total subtype B infections), western Europe (12%) and east Asia (10%). Subtype D causes significant numbers of infections in north Africa and the middle east (26% of global subtype D infections), east Africa (43%) and central Africa (22%). Eighty-nine per cent of subtype G infections occur in west Africa and 9% in central Africa (Table 3).

Subtypes F, H, J and K cause a relatively small number of infections globally (0.94%). Overall, 53% of subtypes F, H, J, and K together are found in central Africa, where they account for 10% of infections, with a further 12% in west Africa, where they account for 1% of infections. Subtypes H, J and K are found nearly exclusively in sub-Saharan Africa. The largest numbers of subtype F infections occur in Latin America (32% of the global total of subtype F) and central Africa (35%). West and southern Africa each contain 11% of the global total of subtype F (Table 3). However, even in Latin America and central Africa subtype F constitutes only 4% of the total number of infections with HIV-1 (Table 2).

The majority of CRF01_AE (83%) is found in south and south-east Asia, whereas an additional 9% of the global total is found in east Asia. CRF02_AG is mainly found in west Africa (94% of global CRF02_AG), whereas CRF03_AB is exclusively found in eastern Europe and central Asia. The majority of other recombinant forms is found in east Asia (16% of global total of other recombinants; Table 3), west Africa (29%) and east Africa (36%). Taken together, all HIV-1 recombinants (that is CRF01_AE, CRF02_AG, CRF03_AB and other recombinants combined) account for 18% of HIV-1 infections worldwide (Table 2). High proportions of recombinants are found in south and south-east Asia (89%), east Asia (61%), west Africa (43%), east Africa (29%) and central Africa (19%; Table 2).

Discussion

We conducted a large study in which molecular epidemiological data on HIV-1 subtype distribution in individual countries were collected and combined with WHO/UNAIDS country-specific estimates of the number of HIV-infected individuals in 2004 [28]. Our analysis indicates that subtype C is responsible for up to 50% of all infections worldwide (Fig. 1, Table 2). Subtypes A, B, D and G accounted for 12, 10, 3 and 6%, respectively. The CRFs CRF01_AE and CRF02_AG were each responsible for 5% of cases. The subtypes F, H, J and K together caused 0.94% of infections. CRF03_AB plays only a small role in eastern Europe and central Asia (0.1% of global HIV-1 infections). Other recombinants accounted for the remaining 8% of infections. All recombinant forms taken together were therefore responsible for a large proportion, 18%, of infections worldwide (Table 2). This information, as well as the more detailed analysis presented in this paper, should be an important landmark and resource for all HIV scientists, public health officials and HIV vaccine developers.

Most attempts to estimate the global distribution of HIV-1 subtypes are confounded by limitations regarding the representation of geographical regions, information regarding transmission routes, selection bias, small sample sizes and the accuracy of data on HIV prevalence [3,21,29,30].

In our study, an adequate representation of all geographical regions was pursued and HIV-1 subtype distribution data were collected from a total of 70 countries across the globe, ensuring that data were obtained for countries with the highest absolute numbers of infections in the region (see caption Table 2). Overall, the countries from which data were obtained accounted for 89% of individuals living with HIV worldwide (Table 2). Unfortunately, for two regions, the Caribbean and Oceania, no data were available for the countries with the largest number of individuals living with HIV.

Only datasets representative of the HIV-1-infected population groups in each country were included in our study. Datasets representative of different transmission routes were weighted according to the relative importance of each transmission route in that country. When multiple datasets for individual countries were received, the data were combined to increase representativity (see Methods). A total number of 23 874 samples were included in our analyses (Table 1). Despite the large database, the number of samples analysed as a proportion of the number of individuals living with HIV varied considerably between regions. Worldwide a sampling ratio of 0.0612% was achieved (Table 1).

In comparison with our previous study of this type [7], we collected three times more samples, giving a better representation of the global HIV-infected population. Data were collected from more countries and weighted according to the number of HIV-infected individuals in each country. In the current survey a higher proportion of subtyping data were generated by nucleotide sequencing of genome segments. A significant proportion of data were based on sequencing more than one genome segment, giving valuable information regarding recombination and unique and novel sequences. These improvements make the current regional and global

estimates more representative and accurate than in the previous survey. On the other hand, the above-mentioned changes also invalidate a direct comparison between the previous and current surveys. It is therefore not possible, based on our data, to make statements regarding recent changes in HIV-1 subtype distribution, nor to derive trends and make predictions for the future.

In view of the importance of accurate and up-to-date HIV-1 subtype distribution data, HIV-1 subtype surveillance needs to be continued and improved. The above-mentioned limitations with regard to geographical representation, combined with those related to the population groups represented in the studies, the lack of information regarding possible selection bias and the small sample sizes in most of the available studies, emphasize the need to obtain more representative information on the distribution of HIV-1 (as well as HIV-2) subtypes in countries and regions [29]. Future studies on the distribution of subtypes should be conducted in sufficiently large samples of the population that are representative of the national epidemic [31]. In generalized epidemics, pregnant women attending antenatal clinics constitute a population group that is easily accessible, is broadly representative of the general population, and is already included in the surveillance system to track changes in HIV prevalence [32]. National surveys with HIV testing are increasingly being conducted in countries with generalized epidemics, and have the advantage of including a nationally representative sample, but the disadvantage of being too expensive to conduct on a regular basis. In countries with concentrated HIV epidemics, it may be advisable to conduct subtype surveillance among those population groups in which HIV prevalence is concentrated and whose behaviour puts them at a higher risk of HIV infection, such as injecting drug users, men who have sex with men, and sex workers. For both types of epidemic, it is important to sample population groups that are representative of all individuals living with HIV in the country rather than of individuals with HIV infection who are in treatment programmes, to avoid selection bias. A reasonably representative sample can be obtained through the random or systematic sampling of individuals in the above population groups from sites included in the surveillance system. Sample sizes should be sufficiently large to detect changes in the proportion of the principal subtypes over time [31].

The building of a global subtype surveillance system can benefit from efforts in the area of antiretroviral drug resistance surveillance [33], although current efforts may only generate limited subtype information based on characterization of the *pol* sequence. To generate more comprehensive surveillance data, it is advisable that future molecular epidemiology studies include additional genome segments, which would allow for tracking the spread and distribution of HIV-1 subtypes and recombinants, in addition to generating drug resistance data. National programmes in all countries should explore the opportunities offered by existing and emerging HIV surveillance efforts to generate important information for their treatment and prevention programmes. It will also be important to promote studies that generate more comprehensive global datasets representative of all regions of the world.

In addition to improving the system of global HIV-1 subtype surveillance, further research is also required to elucidate the importance of HIV-1 sequence variation and evolution for pathogenesis, transmission, diagnosis, drug resistance and immune control. With the constant generation of new variants and recombinants, it remains necessary to review and update HIV diagnostic and viral load methodologies [15]. More research is needed to establish the association between natural resistance and subtypes, the selection of mutations under antiretroviral treatment and the frequency of transmission of drug-resistant non-B viruses. The best hope for controlling the HIV pandemic remains a preventive HIV vaccine [20]. However, the importance of HIV genetic subtypes for the efficacy of HIV vaccines remains unknown and will need to be evaluated as part of properly designed HIV vaccine efficacy trials. HIV vaccine strategies should include, at least initially, immunogens representative of the HIV subtypes prevalent in potential trial populations in the region where the candidate vaccine is meant to be tested and employed. To overcome potential problems as a result of HIV genetic diversity, candidate vaccines should aim to induce immune responses that are broad and against conserved regions of the virus so as to increase the chances of cross-protection and decrease the chances of the emergence of viral immune escape mutants [34]. Protection against infection by homologous and heterologous viruses should be compared in field trials, and the efficacy of multivalent vaccines that could protect against the many variants of HIV-1 should be explored.

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Appendix

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