

High Genetic Diversity in Human Immunodeficiency Virus – Type 1 in Jamaica

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ABSTRACT

The subtypes of the human immunodeficiency virus – type 1 (HIV-1) strains from 54 HIV-1 – infected persons including 44 strains which were typed previously by heteroduplex mobility assay (HMA) were determined by DNA sequencing and phylogenetic analysis. Of 54 HIV- infected persons, 92.5% were infected with HIV-1 subtype B and 7.5% with other HIV-1 subtypes including subtypes D (3.7%), A (1.9%) and J (1.9%). In the phylogenetic analysis, the subtype A virus found in the sample clustered with subtype A reference strains and a circulating recombinant form (CRF) reference strain which originates in Central Africa and is circulating in Cuba indicating a close relationship between these viruses. There was 86% concordance between HMA and DNA sequencing in assigning subtype B viruses. For the non-B subtype viruses, there was less concordance between the two methods (67%). The results confirm the predominance of HIV-1 subtype B strains and the high genetic diversity of HIV-1 strains in circulation in Jamaica. The efficacies and some limitations of the HMA as a method of HIV-1 subtyping also were noted. It is important that the HIV/AIDS epidemic in Jamaica be monitored meticulously for possible expansions in non-B subtypes and the emergence of inter-subtype recombinant forms. We recommend that the more expensive DNA sequencing and phylogenetic analysis, including HIV-1 genotyping for antiretroviral drug resistance testing, be used as an adjunct to the more cost-effective HMA to track the HIV/AIDS epidemic in Jamaica.

Elevada Diversidad Genética del Virus de la Inmunodeficiencia Humana – Tipo 1 en Jamaica

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RESUMEN

Los subtipos de cepas de virus de la inmunodeficiencia humana-tipo-1 de 54 personas infectadas con el VIH-1, que incluyeron 44 cepas previamente clasificadas según su tipo mediante ensayo de movilidad de heterodúplex (HMA), fueron determinados mediante secuenciación de ADN y análisis filogenético. De 54 personas infectados con VIH, 92.5% estaban infectadas con VIH-1 subtipo B y 7.5% con otros subtipos de VIH-1 incluidos los subtipos D (3.7%), A (1.9%), J (1.9%). En el análisis filogenético, el virus de subtipo A hallado en la muestra, se agrupa con las cepas de referencias del subtipo A y una cepa de referencia de forma recombinante circulante (CRF), que tiene su origen en África Central y está circulando en Cuba, lo que indica una estrecha relación entre estos virus.

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Hubo un 86% de concordancia entre el HMA y la secuenciación del DNA en la asignación de virus de subtipo B. Para los virus de subtipo no B, hubo menos concordancia entre los dos métodos (67%). Los resultados confirman el predominio de las cepas del subtipo B del VIH-1, y la alta diversidad genética de las cepas del VIH-1 en circulación en Jamaica. También se señalaron las eficacias y algunas limitaciones del HMA como método de clasificación del VIH-1 en subtipos. Es importante monitorear meticulosamente la epidemia de VIH/SIDA en Jamaica, a fin de detectar posibles expansiones de subtipos no B y la aparición de formas recombinantes inter-subtipos.

Recomendamos que por ser ambos métodos más costosos, tanto la secuenciación de ADN como el análisis filogenético – incluyendo el genotipado del VIH-1 para probar la resistencia antiretroviral del medicamento – sean usados como complementos del HMA, el cual es más costo-efectivo, para seguir de cerca el rastro de la epidemia VIH/SIDA en Jamaica.

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INTRODUCTION

An estimated 50 million persons, worldwide, are infected with the human immunodeficiency virus- type 1 (HIV-1), the aetiologic agent of acquired immune deficiency syndrome [AIDS] (1, 2). The HIV-1 strains which infect humans belong to three distinct phylogenetic lineages which derive from simian immunodeficiency virus (SIVcpz) which infects apes and include groups M (main), O (outlier) and N (non-M/non-O). The viruses which belong to Group M, the largest group, show extensive genetic diversity and are responsible for the Global HIV/AIDS pandemic (3).

Based on DNA sequencing and phylogenetic tree analysis group M viruses have been classified into 9 distinct clades or subtypes: A-D, F-H, J, K; sub-subtypes designated A1, A2, A3, A4, F1, F2 and 43 described circulating recombinant forms (CRFs) [LANL HIV Sequence database], (<http://hiv-web.lanl.gov>). The CRFs are inter-subtype recombinants resulting from recombination between subtypes within a dually infected person from whom the recombinant forms are then passed on to other persons (3–5). The recombinant progeny are classified as CRFs if they are identified in 3 or more persons with no direct epidemiologic linkage; otherwise, they are called unique recombinant forms (URFs) (5).

The HIV-1 subtypes vary in geographical distribution. The complex and constantly evolving molecular epidemiology of the virus globally has been described in several recent publications (1–6). There are conflicting data on whether or not subtype variation might affect disease transmission, progression, viral response factors and HIV-1 viral loads (5, 6). However, HIV-1 subtype diversity may influence susceptibility to antiretroviral drugs, resistance pathways and the development of an effective preventive vaccine (5–9).

A recent report showed that, in Jamaica, over 90% of HIV-1 infections were subtype B, the predominant subtype in North America, Western Europe, Australia and South America (4, 5, 10, 11). In that study, the heteroduplex mobility assay (HMA) was used to assign the HIV-1 subtypes in circulation in Jamaica (12).

Genetic sequencing of the envelope (*env*), group antigen (*gag*) or polymerase (*pol*) gene is the definitive method of subtyping (10–13). In addition to subtype determination, DNA sequencing of different *pol* gene regions is used in antiretroviral drug resistance testing (7, 10, 11). The HMA correlates well with genetic sequencing and characterization by phylogenetic analysis. This method was introduced in developing countries due to its simplicity to perform and low economic cost. The combination of results from HMA performed on the HIV-1 *env* and *gag* genes allows for the recognition of inter-subtype recombinant strains (12–15). However HMA does not allow for identification, evolutionary analysis of the intra-subtype nucleotide polymorphisms and other variations which may be important in antiretroviral therapy (7, 8).

The results of a study in which DNA sequencing and phylogenetic analysis were used to determine HIV-1 subtypes and the efficacy of HMA, in the Jamaican setting, are reported herein.

SUBJECTS AND METHODS

The residual EDTA blood samples, from HIV-1 infected adult patients, submitted to the laboratory for immune monitoring, were studied following ethical approval from the ethics committee of the University of the West Indies/University Hospital of the West Indies. The HIV-1 strains (n = 63) studied were isolated by nested *env*- and *gag*- polymerase chain reaction (PCR) assays performed on peripheral blood mononuclear cell (PBMC) lysates using previously described primers and procedures (12, 13). The DNA sequencing was performed using the ABI 3100 analyser (Applied Biosystems, Foster City, CA), directly on the purified PCR products (Qiagen DNA Purification Kit; QIAGEN Inc, Valencia, CA) and/or after cloning using the pGEM-T Easy Vector System (Promega Corp, Madison, WI). The sequencing reactions were prepared using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and the second round *env* and *gag* gene PCR primers as the sequencing primers (12, 13). For the commercially prepared reagents, the manufacturers' instructions were followed. The

chromatograms generated were edited, assembled and saved in the FASTA format using Sequencher software (Gene Codes Inc, Ann Arbor, MI). The sequences were aligned with those of HIV-1 subtypes reference viruses using ClustalX software (available at <http://inn-prot.weizmann.ac.il/software/clustalX.html>) (16). Phylogenetic analysis and molecular evolutionary relationships were determined using the PAUP 4.0, Beta version 8 software (Sinauer Associates Inc, MA). Phylogenetic trees for each data set were constructed using the neighbour-joining method. Of the 63 sequences included in the study, 48 were also characterized by *env* and *gag* heteroduplex mobility assay (HMA) as previously described (12, 15).

RESULTS

A total of 54 (54/63, 85.7%) HIV-1 isolates comprising 26 *gag* and 28 *env* sequences were sequenced. Representative neighbour-joining phylogenetic trees constructed from the

gag and *env* gene sequences are shown in Figures 1–2. Fifty (50/54, 92.5%) genetic sequences were assigned subtype B, two (2/54, 3.7%) subtype D and one each (1/54, 1.9%) assigned subtypes A and J, respectively. In the *gag* gene 84.6% (22/26) clustered with HIV-1 B subtype reference strains (BUS98, BFR, BTH90), 7.6% (2/26) with subtype D reference strain (DUG94), 3.8% (1/26) each with subtype A and CRF reference strains (A1 UG92, 18CPXCM) and subtype J reference strain (JSE93) supported by bootstrap values $\geq 70\%$ in 96.0% (25/26) of isolates. The 28 (100%) *env* gene sequences clustered with B subtype reference strains (BUS98, BFR, BTH90), the majority (89.0%, 25/28) supported by bootstrap values $> 90\%$.

For the subtype B viruses which were characterized by both methods, there was an overall 86.0% (38/44) concordance between phylogenetic analysis and HMA. Of the six discordant subtype B isolates, five were indeterminate and one identified as subtype D in the HMA. With respect to

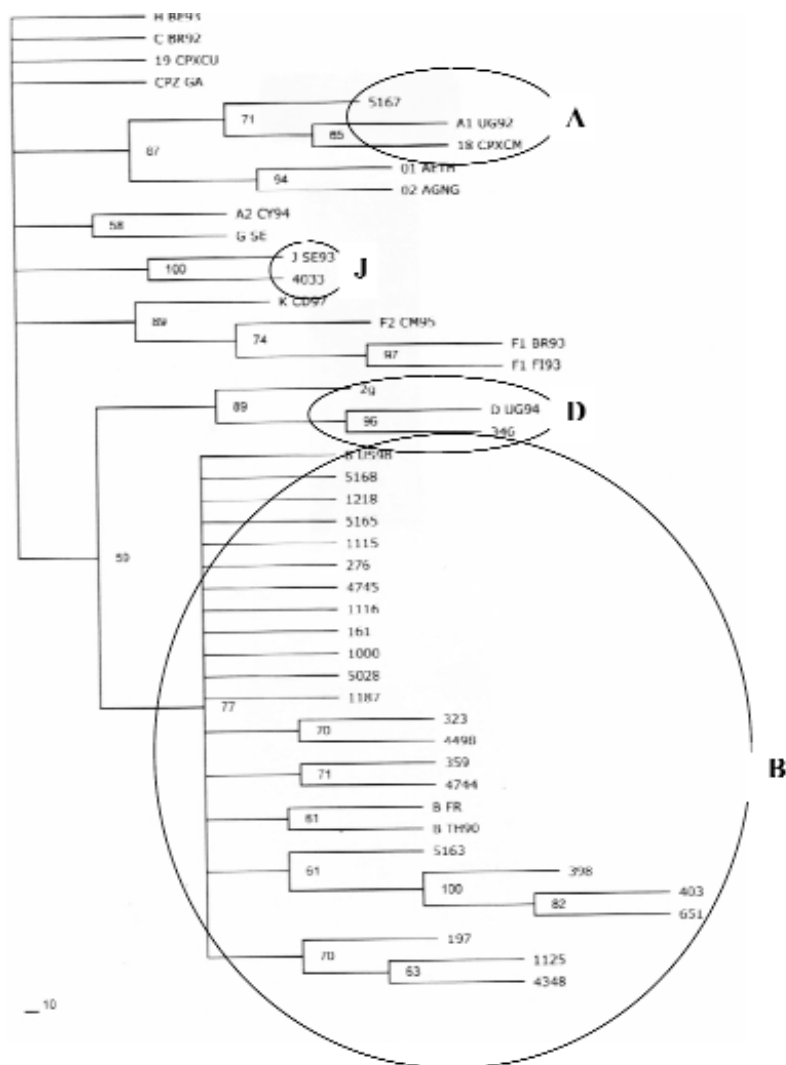


Fig. 1: Neighbour-joining phylogenetic tree, constructed from 26 HIV-1 *gag* sequences, and reference subtypes (A1UG92, bus98, BFR, bth90, DUG94, 18 c[xcm, JSE93). Numbers at the nodes of the tree indicate bootstrap values expressed as the percentage of 1000 replicates supporting each subtype.

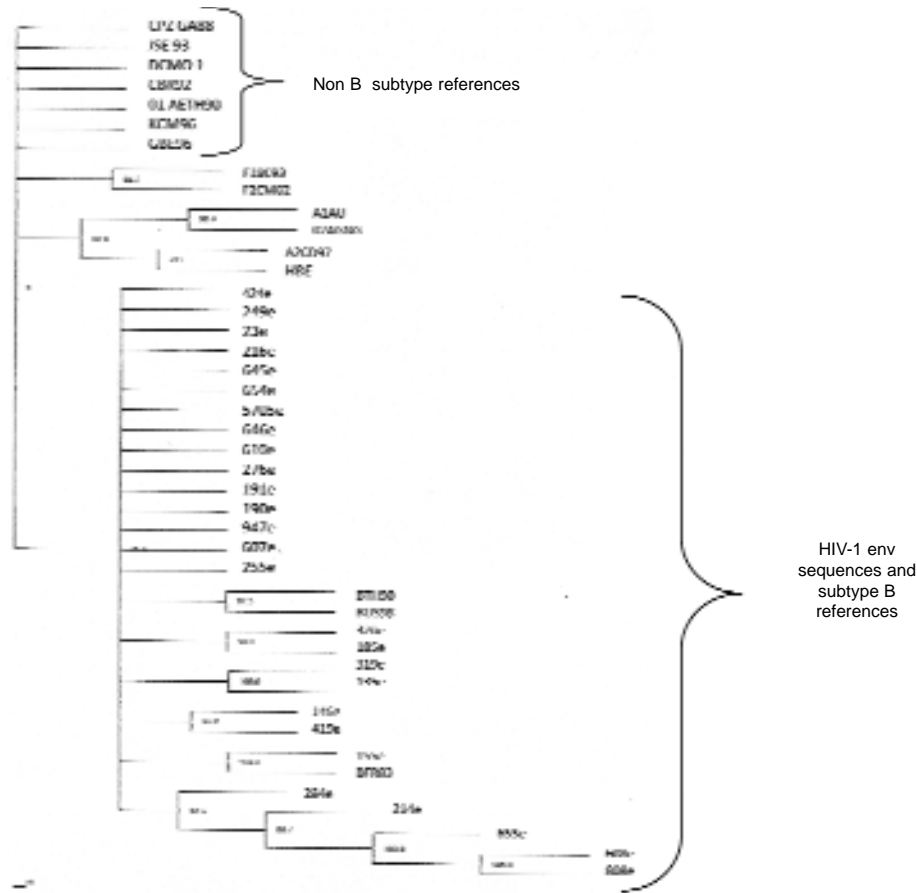


Fig. 2: Phylogenetic tree showing Jamaican HIV-1 *env* sequences and subtype reference strains. The 28 *env* sequences clustered with subtype B reference strains (BUS98, BTH90, BFR83). Bootstrap values, indicated by numbers at internal nodes, are expressed as the percentage of 1000 replicates.

non-B subtypes, the rate of concordance was lower (67.0%, 2/3) as the two subtype D viruses were identified, by HMA, as subtype E and subtype B, respectively. The HMA was concordant with phylogenetic analysis in discriminating the only subtype A virus in the sample.

DISCUSSION

The study revealed the presence of four HIV-1 subtypes in Jamaica including subtypes A, B, D and J. To date, five HIV-1 subtypes have been found in Jamaica as the presence of subtype C was reported previously (12). Outside of Central Africa, a limited number of HIV-1 variants are usually circulating in each country, rarely more than 2 or 3 representing multiple introductions, or in some cases locally generated CRFs (19).

Subtype B was confirmed as the predominant genetic form contributing to the HIV/AIDS epidemic, accounting for over 90% of infections in Jamaica (12). This is in keeping with other reports, from English-speaking Caribbean islands, which did not include Jamaican samples (10, 11).

The current study did not allow for the identification of inter-subtype recombinants as the subtype of the HIV-1 isolates were assigned on the DNA sequence of a single gene

region only (22, 23). The earlier study in which subtypes were assigned by HMA analysis of *gag* and *env* gene sequences of each isolate also failed to identify any recombinant strain in Jamaica except for the subtype E strain which was re-assigned subtype D in the present study (12). Also the subtype A virus identified in this Jamaican sample appears to be related, in the *gag* gene, to CRF18-cpx which originated in Central Africa and, currently, is circulating in Cuba (20). Reportedly, the genome of CRF18-cpx contains multiple segments clustering with subtypes A1, F, G, H and K as well as segments failing to cluster with any subtypes (20). The multiple subtypes of HIV-1 in circulation in Jamaica should be monitored for the likely emergence of inter-subtype recombinant strains as the epidemic progresses. Recombinant strains have been observed mostly in areas where multiple subtypes co-circulate (13). The emergence of recombinant strains in other Caribbean and Latin American countries, including Cuba and Brazil, following sudden shifts in HIV-1 subtype distribution from a predominance of subtype B is well documented (11, 19–21).

The limited divergence of the Jamaican HIV-1 strains, from the subtype reference sequences, in the *gag* and *env* gene sequences is another important observation. This was

evident from the high bootstrap values supporting the subtype classification of this sample especially subtype B viruses. This is not entirely surprising as most of this Jamaican cohort had predated the relatively recent and phased introduction of antiretroviral drugs in HIV-1 infected patients in Jamaica. The cohort was therefore largely antiretroviral therapy naïve. Secondly, failed PCR, HMA and DNA sequencing reactions occur in a substantial proportion of Jamaican HIV-1 isolates when the UNAIDS/WHO primer sets are used. These might include the more divergent strains which have not been genotyped (12). Gittens *et al* (10) reported a broad genetic diversity of *env* gene sequences in Barbados suggesting multiple introductions of subtype B viruses to the island (10). In contrast to the present and earlier Jamaican study, the HIV-1 *gag* gene was not genotyped in the studies conducted in the other English-speaking Caribbean countries (10–12).

The discordance between HMA and DNA sequencing/phylogenetic analysis in the assignment of subtypes is not unique to the present study (14, 16, 24, 25). The HMA has been shown to give excellent results for the detection of the subtypes B and F, the prevalent subtypes in Caucasian patients originating from western countries and Romania, respectively. Conversely, extensive viral variation might create problems in countries like Africa where different HIV subtypes have been circulating longer (25).

For example, in previous studies of international cohorts, the HIV-1 strains which were deemed untypable/indeterminate by HMA and were subsequently assigned by DNA sequencing turned out to be highly divergent subtypes A-D or G related strains (14, 16, 24, 25). In one study, almost two-thirds of the subtype D isolates were incorrectly genotyped by HMA (25). In the Jamaican sample, the two subtype D viruses were incorrectly genotyped, by HMA, as subtypes B and E, respectively (12). In a recent change of HIV-1 group M nomenclature former subtypes E and I have been relabelled as circulating recombinant forms CRFO1 and CRFO4 (3). The viruses belonging to subtypes B and D have been shown to be closely related with respect to *gag*, *env* and *pol* gene sequences and probably diverged relatively recently. Therefore, separation of subtype B and subtype D is not as well defined as between other subtypes (11, 26). Nonetheless, the HMA remains the recommended genotyping method second only to DNA sequencing. Consequently, it has been recommended that the plasmid selection in the HMA kit be constantly revised to cover viral diversification (16, 25).

Limitations of the study include the fact that this analysis includes only samples which were amplifiable by the primer sets which were used. The failure to sequence both the *env* and *gag* genes of each isolate to identify inter-subtype recombinants is another limitation. A more comprehensive study of the genetic diversity of the Jamaican HIV-1 isolates should include customized or more conservative primers such as those recently described by Angwale *et al* (27) and sequencing *pol* gene regions (10, 11, 27).

In concluding, HIV-1 subtype B remains the dominant subtype amidst high genetic diversity of the virus in Jamaica. The HMA is well established as a genotyping method in the Jamaican setting. The results in this study reiterate the value of this cost-effective and reliable method in tracking the HIV/AIDS epidemic. This does not abrogate the need for DNA sequencing and phylogenetic analysis which should be used as an adjunct methodology to resolve indeterminate HMA results and ensure the accurate assignment of non-B subtypes. Future plans to implement HIV-genotyping for antiretroviral drug resistance testing would provide a cost-effective and more comprehensive approach to genotyping to supplement the HMA in tracking the HIV/AIDS epidemic in Jamaica.

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