Human immunodeficiency virus type 1 intersubtype recombinants predominate in the AIDS epidemic in Cameroon

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SUMMARY

A broad and rapidly changing HIV Type 1 (HIV-1) diversity has been reported from different populations in Cameroon since the early epidemic. Our understanding of HIV-1 dynamics can be improved by a systematic surveillance in Cameroon as accessibility and use of antiretroviral drugs increase. To contribute to this, we genotyped 30 samples by sequencing the protease and reverse transcriptase (proRT) genes of HIV-1. Phylogenetic analysis of the HIV-1 proRT sequences using the MEGA3 software showed that 26 (86.7%) were recombinant forms which included 20 (66.7%) circulating recombinant forms: CRF02_AG, (50%), CRF06_cpx (3.3%), CRF11_cpx (10%) and CRF37_cpx (3.3%), and 6 unique recombinant forms (URF, 20%). Two of the six URFs were second generation recombinants and 4 contained unclassified segments. HIV-1 subtypes A1 (3.3%), C (3.3%) and D (6.7%) were also identified. Although partial sequences of HIV-1 genome were analysed, our results indicate that recombinant HIV-1 variants predominate in the AIDS epidemic in Cameroon. With the widespread use of antiretroviral drugs in Cameroon and the circulation of several HIV-1 variants within this population, the emergence of recombinants with unknown diagnostic and clinical consequences is a concern.

KEY WORDS: HIV, Diversity, Recombinant forms, Cameroon

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INTRODUCTION

The molecular epidemiology and genetic diversity of the human immunodeficiency virus type 1 (HIV-1) reflect an old infection in Cameroon, although the prevalence is low by African standards. Contemporary HIV-1 strains isolated from Cameroon in the early epidemic show a viral population of non recombinant forms compared to reports of recent studies. Complete genomes of HIV-1 subtype D (Kijak *et al.*, 2004), subtype F2 (Peeters *et al.*, 2000; Carr *et al.*, 2001; Kijak *et al.*, 2004) subtype K (Triques *et al.*, 1999; Peeters *et al.*, 2000), CRF02_AG (Kijak *et al.*, 2004), CRF25_cpx (Luk et *et al.*, 2008; Carr JK, unpublished), CRF36_cpx (Powell *et al.*, 2007), CRF37_cpx (Powell *et al.*, 2007) and partial gag, pol and/or env sequences of subtypes A, B, C, D, F2, G, J and K and CRF06_cpx, CRF09_cpx, CRF11_cpx, CRF13-cpx (Triques *et al.*, 1999; Peeters *et al.*, 2000; Carr JK, *et al.*, 2001, Fonjungo *et al.*, 2002; Konings *et al.*, 2004; Kijak *et al.*, 2004; Ndongmo *et al.*, 2006, Brennan *et al.*, 2008) have been reported from Cameroon. The rare HIV-1 Group O (Gurtler *et al.*, 1994; Ayouba *et al.*, 2000; Yamaguchi *et al.*, 2003; Bodelle *et al.*, 2004; Brennan *et al.*, 2008), HIV-1 Group N (Simon *et al.*, 1998; Ayouba *et al.*, 2000; Bodelle

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et al., 2004; Yamaguchi *et al.*, 2006; Brennan *et al.*, 2008), HIV Type 2 (HIV-2, Ndembi *et al.*, 2007; Brennan *et al.*, 2008), mixed infections A/J/U (Fonjungo *et al.*, 2000), A/C, A/D/Group O, B/A (Takehisha *et al.*, 1998), A/G/Group O (Peeters *et al.*, 1999), and HIV-2 intergroup recombinant (Yamaguchi *et al.*, 2008) have also been reported from Cameroon.

Genetic variation of HIV-1 was recognized early in the epidemic and the challenges it presents to the development of diagnostic tests, antiretroviral (ARV) drugs, and AIDS vaccines were well appreciated. The questions that are not fully addressed are related to the factors that modulate a rapidly changing epidemic in the central African region. We describe the genetic diversity of HIV-1 from a population recently sampled to provide more information and an update of the variants co-circulating and that could be transmitted.

MATERIALS AND METHODS

Reverse transcription - Polymerase chain reaction and DNA Sequencing

Blood specimens were collected from HIV-positive individuals attending the "Chantal Biya" International Reference Centre (CIRCB) clinical services for routine follow-up from 2007 to 2008. Plasma RNA samples (extracted using the Qiamp RNA minikit, Qiagen) were genotyped using the ViroSeq HIV-1 Genotyping System v2.0 (Abbott Diagnostics). The RT-PCR steps amplify HIV-1 protease gene (from codons 1 to 99) and the reverse transcriptase gene (from codons 1 to 335) to generate a 1.8kb amplicon.

Positive and negative controls were included in each RT-PCR reaction run. 1% agarose gel electrophoresis was used to confirm the amplification of the appropriately sized PCR product using a 100bp DNA ladder. This amplicon was sequenced using seven primers to generate continuous overlapping bidirectional sequences of about 434 codons of the HIV-1 protease and reverse transcriptase (RT) genes using the ABI Prism 3130 Genetic Analyzer (Applied Biosystem). The ViroSeq® HIV-1 Genotyping System software was used to assemble the sequence and compare it with the reference sequence, HXB2.

Analysis of sequence data sets

The DNA Sequence Analysis software version 5.2 (Applied Biosystems) was used for editing and the DNAMAN sequence analysis and alignment software to trim and resolve any ambiguities in the consensus sequences. Alignment was obtained with HIV-1 reference sequences followed by neigbour-joining performed with the Kimura two-parameter method of distance calculation and bootstrap analysis with 100 replicas. A Phylogenetic tree was constructed using MEGA 3 software (http://www.megasoftware.net) for the sequences that were not unique recombinant forms (URF).

RESULTS

From 30 individuals (15 sampled in 2007 and the remaining in 2008), HIV-1 proRT sequences were genotyped. Of these, 13 (43%) were female and 9 (30%) were children below the age of ten years (Table 1).

TABLE 1 - *Sex, Age and Year of Collection of Blood Specimens.*

Code	*Sex*	*Age (years)*	*Year of specimen collection*
795circb	Female	9	2007
881circb	Female	30	2007
931circb	Male	not available	2007
940circb	Male	43	2007
941circb	Female	9	2007
961circb	Female	49	2007
966circb	Male	40	2007
992circb	Male	5	2007
994circb	Female	45	2007
1016circb	Female	39	2007
1017circb	Male	53	2007
1049circb	Female	39	2007
1054circb	Male	29	2007
1331circb	Female	34	2007
1645circb	Male	2	2007

2870circb	Male	not available	2008
2894circb	Female	10	2008
2924circb	Male	49	2008
3071circb	Female	10	2008
3072circb	Male	2	2008
3346circb	Male	4	2008
3375circb	Male	42	2008
3559circb	Female	37	2008
4862circb	Male	35	2008
5150circb	Male	4	2008
5272circb	Female	4	2008
5504circb	Male	4	2008
5611circb	Female	36	2008
5841circb	Male	13	2008
6366circb	Male	34	2008

Analysis of these 30 sequences showed that 26 (86.7%) were recombinant strains. Bootscan and phylogenetic analysis of 23 sequences that were not URFs were performed with reference sequences representing different HIV-1 clades (Fig. 1).

1) HIV-1 Circulating Recombinant Forms (CRF)
Of the 30 samples analysed, 20 (66.7%) were circulating recombinant forms (CRF) (Figure 1). Phylogenetic analysis of the 30 samples showed that 26 (86.7%) were recombinants of which 20 (66.7%) were Circulating Recombinant Forms (CRF). Of the 20 CRFs identified in this group of patients, 15 were CRF02_AG (50%), 1 of CRF06_cpx (3.3%), 3 of CRF11_cpx (10%) and 1 of CRF37_ cpx (3.3%).

2) Unique Recombinant Forms (URF)
Six (20%) URFs were identified from this cohort of patients and their composition is shown in schematic diagrams below. The Recombinant HIV-1 Drawing tool was used to map the recombinant breakpoints for HIV-1 onto a map of the

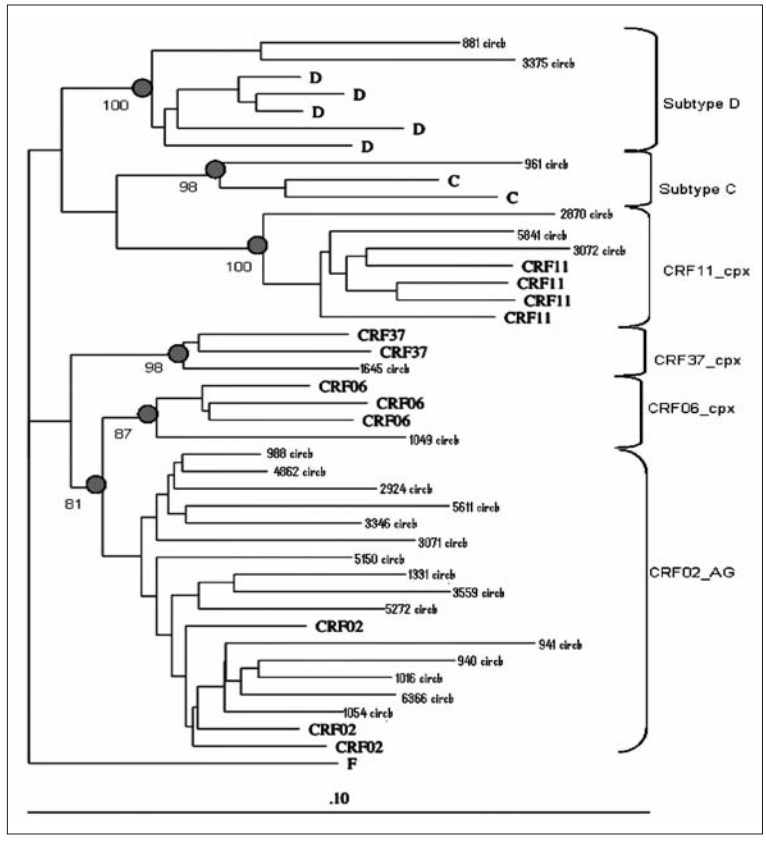

FIGURE 1 - *Phylogenetic Analysis of 23 HIV-1 protease-Reverse Transcriptase genes. Bootstrap values greater than 70% are indicated. Reference strains from the pandemic representing the different genetic forms are indicated by the name of the genetic form. Scale bar reflects 10% genetic difference. The query sequences are indicated by "circb".*

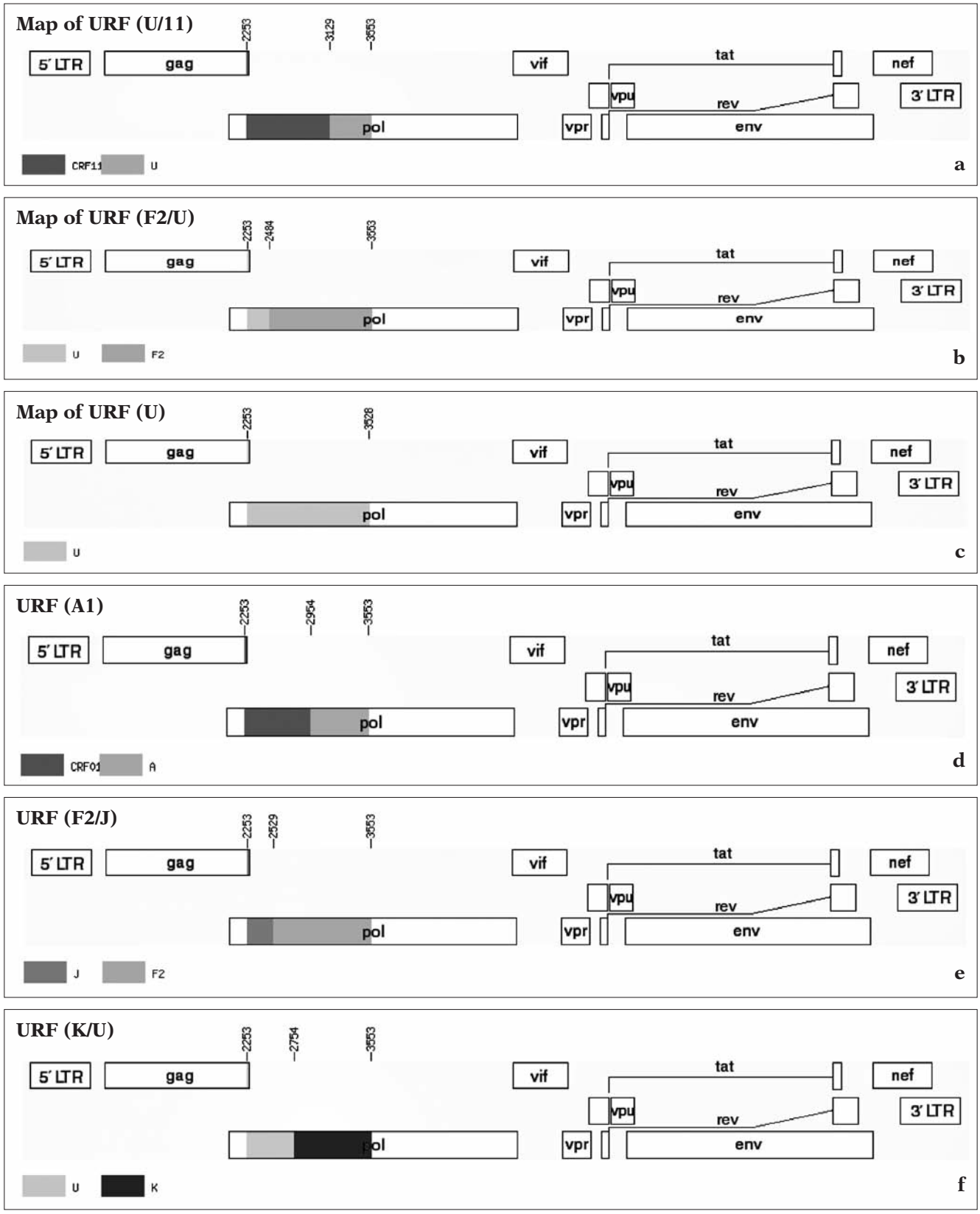

FIGURE 2 - *Maps of six Unique Recombinant Forms.*

HXB2 genome. The different subtypes that compose the genome appear as differently-coloured regions in the map (Figure 2) (http://www.hiv.lanl.gov/content/sequence/HIV/refer.html).
The mosaic composition of the 6 URFs consisted of segments derived from HIV-1 subtype A1, F2, J and K, CRF01_AE, CRF11_cpx and unclassified regions. Of these 6 URFs, 4(66.7%) contained an unclassified region and 2 (33.3%) were second generation recombinants: URF(A1) and URF (U/11). Analysis of the recombination pattern and breakpoint distribution among these URFs, reveal that breakpoints occurred at nucleotide positions 2484 and 2529 (protease gene) in 2 of the URFs (F2/U - Figure 2b and F2/J - Figure 2e), and also at 3129, 2954 and 2754 (reverse transcriptase gene) in 3 (U/211 - Figure 2a, A1 - Figure 2d and K/U - Figure 2f).

3) HIV-1 subtypes
The phylogenetic analysis of the 23 proRT sequences showed that 4 (13.3%) were non recombinant variants of subtype A1 (n=1, 3.3%), C (n=1, 3.3%) and subtype D (n=2, 6.7%) (Figure 1). The HIV-1 subtype A1 is not shown in the phylogenetic tree because it showed 77% of A1 and the remaining sequence was hyper mutated.

DISCUSSION

Since the first cases of HIV infection were identified in Cameroon (Zekeng *et al.*, 1990) several questions still linger about its evolution in the Central African region. In the early epidemic, a few subtypes were reported from Cameroon. As more samples from Cameroon were genotyped, a more statistically acceptable report on both the seroprevalence and molecular epidemiology of HIV was presented. Other pointers to the impact of the dynamics or rapidly changing epidemiology of HIV in Cameroon and the rest of the central African region were discordant results obtained from commonly used serological assays over the years. In the meantime, individuals became infected with two or more variants of HIV and/or SIV-like viruses (Peeters *et al.*, 1994, Peeters *et al.*, 2002, Van Heuverswyn *et al.*, 2007) which may have gone undetected due in part to inappropriate screening algorithms.
During the era when the use of highly active antiretroviral therapy in Cameroon was very low, the AIDS epidemic was driven by non recombinant subtypes A1, C, D, F, G, K and J and a few less complex recombinant variants. This epidemic changed to one predominated by CRF02_AG of up to 58.2% (Carr *et al.*, 2001; Nyambi *et al.*, 2002, Montavon *et al.*, 2002, Tebit *et al.*, 2002, Njai *et al.*, 2006, Brennan *et al.*, 2008). From samples collected in the late 1990s, new CRFs were identified: CRF06_cpx, CRF11_cpx, CRF13_cpx (Nyambi *et al.*, 2004; Kijak *et al.*, 2004; Ndongmo *et al.*, 2006). In 1998, Simon and colleagues described the HIV-1 Group N viruses from a cameroonian woman which possesses segments from HIV-1 Group M and simian immunodeficiency virus (SIVcpz) from chimpanzee *Pan troglodytes troglodytes* isolated in Gabon (Simon *et al.*, 1998).
Wolfe and colleagues have described zoonotic infections of simian foamy virus (SFV, Wolfe *et al.*, 2004) and primate T lymphotropic virus (PTLV, Wolfe *et al.*, 2005), from hunters in Cameroon. These facts also support Peeters and colleagues reports of the possible transmission of SIV from chimpanzees to humans in the central African region. Therefore, whether within humans or another reservoir, HIV-1, HIV-2 and SIV-like viruses show the propensity to recombine between groups, subtypes or circulating recombinant forms, as an immune escape mechanism. In this evolution process, new recombinant variants or forms of HIV-1 evolve suggesting that dual infection occurs frequently in this population.
Brennan *et al.* reported a mature AIDS epidemic in Cameroon after studying blood donor samples collected from 1994 to 2004 (Brennan *et al.*, 2008). From rural hunting populations of low HIV prevalence (Torimiro *et al.*, 2007), several recombinants have been reported (Nyambi *et al.*, 2002) and recently, Yamaguchi (Yamaguchi *et al.*, 2008) and Ndembi (Ndembi *et al.*, 2008) also described HIV-2, HIV-1 and HIV-1 & HIV-2 intergroup recombinants in Cameroon. We identified one patient infected with CRF37_cpx, an old strain of genetically diverse lineages of HIV-1 subtypes, which others have also reported (Powell *et al.*, 2007; Brennan *et al.*, 2008). These reports indicate the broad diversity of HIV-1 in Cameroon and therefore the need for regular and systematic surveillance in this region.
From thirty patients attending the CIRCB clinical services, 86.7% were infected with a CRF or an

URF, a report similar to Brennan and colleagues' of 2008. These reports raise concerns over the biologic properties of these recombinants in terms of diagnosis and virologic response to antiretroviral drugs. Our report also provides more clues to the choice of diagnostic tests and antiretroviral treatment and a caution on the interpretation of discordant serologic results. These challenges could be addressed through systematic surveillance of HIV variants circulating in Cameroon. These protease and reverse transcriptase sequences of HIV-1 can also be useful for determining the mutations associated to antiretroviral drug resistance to protease inhibitors, non nucleoside reverse transcriptase inhibitors and nucleoside reverse transcriptase inhibitors. Our report also supports the fact that recombinant HIV-1 variants could be transmitted which will evolve to new variants. From this report and those of other groups, we conclude that the current AIDS epidemic in Cameroon is mature, genetically diverse and driven by HIV-1 recombinant viruses of unknown biological properties.

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