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Case Report

An unusual cluster of HIV-1 B/F recombinants in an Asian population

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SUMMARY

We report the detection of multiple HIV strains in injection drug users (IDU) in Macao, which appear to be derived from subtypes F, G, and CRF12_BF. A total of 14 HIV-infected IDU samples were collected and examined. Direct sequencing was performed to obtain the *gag*, *pol*, and *env* fragments. The subtypes of individual viral sequences were determined using the REGA subtyping tool. The concatenated sequences were aligned with reference sequences retrieved from the Los Alamos National Laboratory HIV database. We found 11 unusual cases in Macao, which showed characteristics of CRF12_BF ($n = 2$) and CRF14_BG ($n = 8$), and one that could not be classified into an existing subtype/CRF, along with three cases of CRF01_AE. Interestingly, the sequences derived from subtypes BG and BF recombinants have not been previously reported in any other Asian cities. Another subtype, CRF14_BG, has also been introduced into Macao among the IDUs. In conclusion, human activity, including travel over long distances and injection drug usage have fueled the spread of HIV and have provided a platform for recombination, which may otherwise have taken years to happen.

1. Introduction

In recent years, human migration has accounted for the spread of pathogens, such as SARS-associated coronavirus, *Mycobacterium tuberculosis*, and HIV, on a worldwide scale. Molecular epidemiological study is an important tool to investigate such epidemics at the level of virus subtypes or genotypes. In many of the articles on HIV-1, the subtype BF recombinant is often found in South America and is occasionally seen in Europe, but has so far been unheard of in Asian countries.[1–4] We hereby report the detection of multiple HIV strains in injection drug users (IDUs) in Macao, which appear to be derived from subtypes F, G, and CRF12_BF.

Macao is a special administrative region of China with a total population of less than half a million. The HIV prevalence in Macao has remained low over the years with an average of 20 to 30 HIV/AIDS cases reported to the Health Bureau under the notifiable diseases ordinance each year. In those reported, sexual transmission has been the main route of HIV transmission. In 2002 and 2003, none of the reported cases came from the drug injecting community. In 2004, however, 18 of the 30 reported HIV infections (60%) were found in IDUs.

We examined the sequences of the HIV-1 strains from IDUs in order to determine whether there was a common source for the outbreak, and to assess the relationship among similar sequences in other parts of the world.

2. Methods

A total of 14 HIV-infected IDU samples (GenBank accession numbers listed in Figure 1) collected from 2002 through 2007 by the Macao Public Health Laboratory were examined. Nested PCR followed by sequencing was performed to obtain the *gag*, *pol*, and *env* fragments. We used sample sequences of polymerase of 634 bp (regions covered: protease, 256–297; reverse transcriptase (RT), 1–592), gag of 284–296 bp (p17, 144–396; p24, 1–43), and envelope of 242–249 bp (gp160, 908–1158; gp120, 908–1158) to align with reference sequences retrieved from the Los Alamos National Laboratory HIV database (http://www.hiv.lanl.gov); the resulting neighbor-joining phylogenetic trees are shown in Figure 1 a–c.

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3. Results

The results revealed 11 unusual cases, which showed characteristics of CRF12_BF ($n = 2$) and CRF14_BG ($n = 8$), and one that could not be classified into an existing subtype/CRF, along with three cases of CRF01_AE. Antiviral drug resistance analysis (http://www.hiv.lanl.gov/content/sequence/ADRA/adra2.html) showed no special drug resistance mutations in the *pol* sequences. In addition, since six samples were epidemiologically linked (MO007, MO021, MO106, MO108, MO112, MO113), one of the samples, MO108, was subtyped by REGA subtyping tool after whole genome sequencing

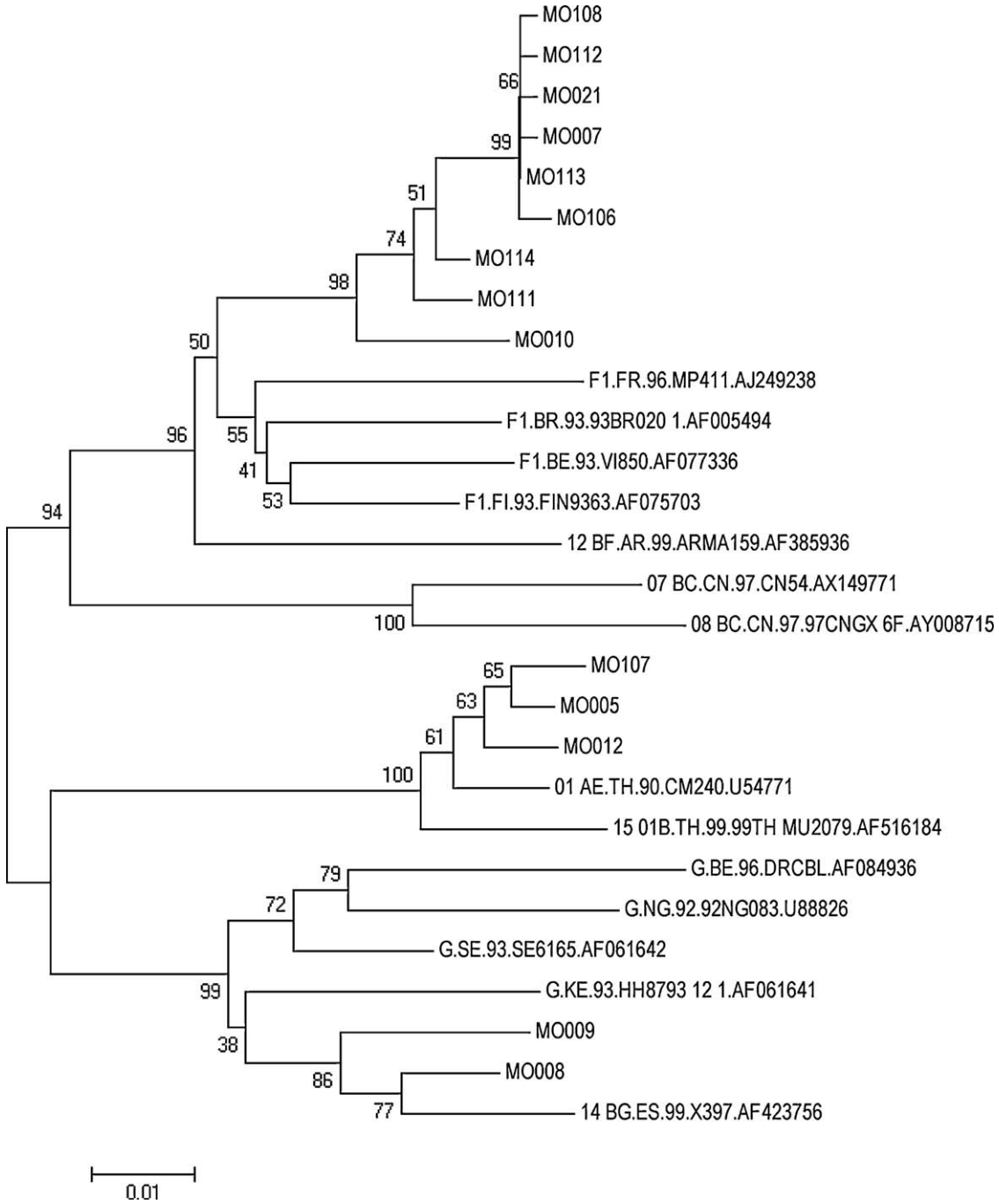

Figure 1. Phylogenetic tree analysis of (a) reverse transcriptase, (b) *gag*, (c) *env*. 'MO005, MO012 and MO107' and 'MO007, MO021, MO106, MO108, MO111–MO114' form two distinct monophyletic groups, showing characteristics of subtypes AE and F, respectively. Since MO108 is subtyped as BF recombinant (see Figure 2), the monophyletic group should be better classified as BF. While MO008 and MO009 usually cluster with G/BG characters, the incongruence of the positions (reverse transcriptase and *env* of BF character but *gag* of G character) of MO010 suggests there has been a recombination event. Accession numbers of sequences used are as follows: reverse transcriptase sequences (MO108: FJ012834, MO112: FJ012836, MO021: FJ012845, MO007: FJ012841, MO113: FJ012837, MO106: FJ012832, MO114: FJ012838, MO111: FJ012835, MO010: FJ012843, MO107: FJ012833, MO005: FJ012839, MO012: FJ012844, MO009: FJ012840, MO008: FJ012842); gag sequences (MO108: FJ012807, MO113: FJ012810, MO112: FJ012809, MO106: FJ012805, MO021: FJ021817, MO007: FJ012813, MO111: FJ012808, MO114: FJ012811, MO005: FJ012812, MO012: FJ012816, MO107: FJ012806, MO008: FJ012814, MO010: FJ012815); env sequences (MO112: FJ012822, MO113: FJ012823, MO108: FJ012820, MO106: FJ012818, MO021: FJ012831, MO007: FJ012827, MO010: FJ012829, MO111: FJ012821, MO114: FJ012824, MO009: FJ012826, MO008: FJ012828, MO107: FJ012819, MO005: FJ012825, MO012: FJ012830).

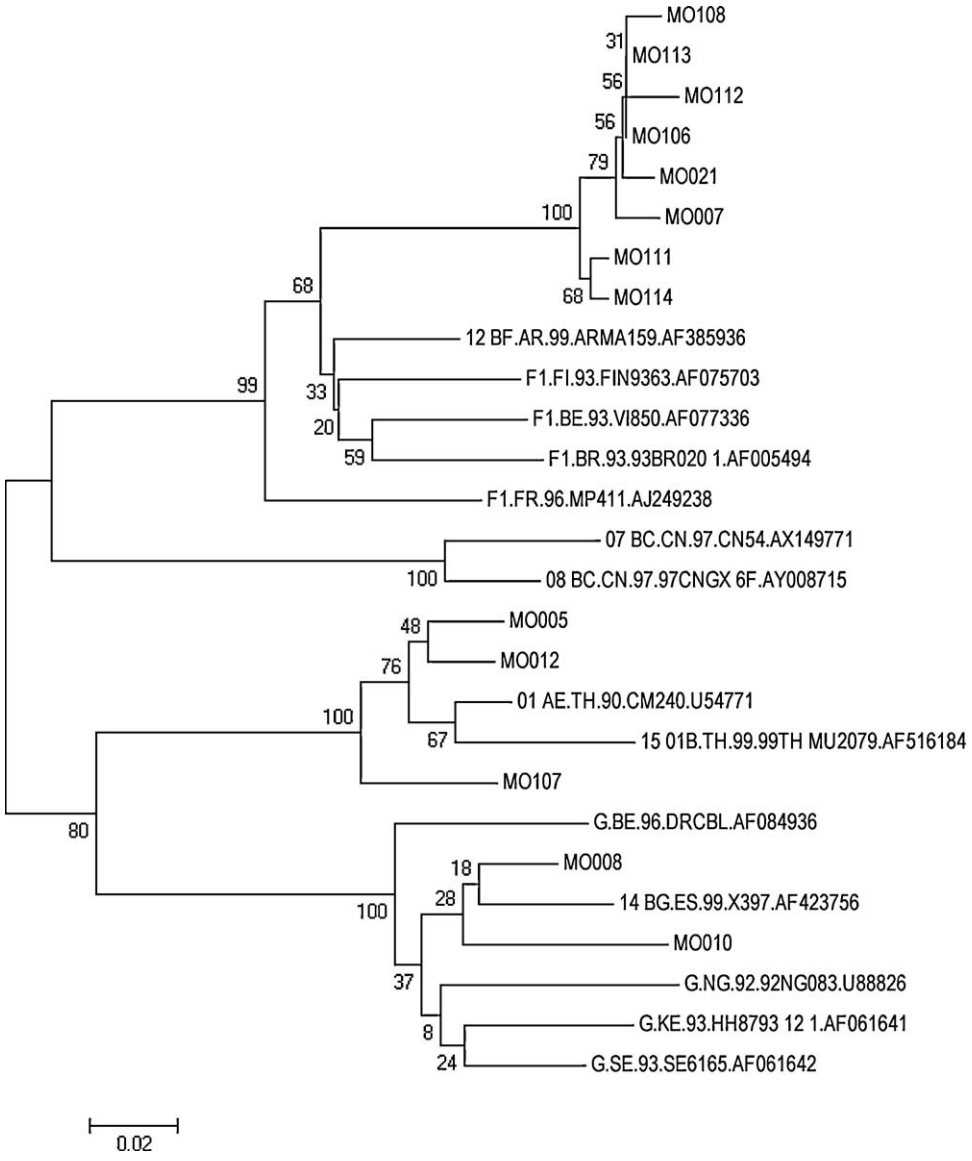

Fig. 1. (*Continued*).

(Figure 2). In the figure, it can be seen that it is a triple recombinant BFG.

Except for the three CRF01_AE sequences probably originated from the same Southeast Asian region, the sequences derived from subtypes BG and BF recombinants have not been previously reported in any other Asian cities, including the neighboring areas of Hong Kong and Mainland China.[5] To study the possible geographical origins of these sequences, a BLAST search was performed and the GenBank records and literature of the matches were studied. Samples MO008 and MO009, from two Portuguese manifesting characteristics of subtype G matched with sequences from Spain and Portugal; whereas the monophyletic group similar to CRF12_BF (MO007, MO021, MO106, MO108, MO111, MO112, MO113 and MO114) matched with sequences mainly from Brazil and a few from Europe (Supplementary Table). The high query coverage and high similarity in *gag* and *pol* regions of MO008 and MO009 suggested that the year of first HIV-positive diagnosis was around 2000, which is in concordance with our records. Sample MO010 shared characters of both BF and BG recombinants – its *gag* shared some similarity with that of MO008, while its *pol* and *env* shared some similarity with MO108, implying a recombination event.

4. Discussion

The key question is how the two uncommon strains emerged. CRF12_BF circulates in South America, and has been reported to be associated with sexual, IDU, and mother-to-child transmission.[4,6,7] Although a major human migration between Macao and South America seems unlikely, as a colony administered by Portugal before 1999, migration between Macao and Europe could reasonably have occurred. The most common HIV subtypes in Portugal are B, G and CRF14_BG.[8,9] Coincidentally a cluster of CRF12_BF was reported in IDUs in southern Portugal in 2003, shortly before the Macao outbreak.[10] Subtype F strain has also been reported in the European countries of Spain,[2] Portugal,[8] and Italy.[11] The temporal and spatial information alongside the BLAST results suggests that IDUs in Portugal could have acquired CRF12_BF from South America and in turn introduced the strain into Macao. Another subtype CRF14_BG has also been introduced into Macao among the IDUs.

In conclusion, human activity, including travel over long distances and injection drug usage have fueled the spread of HIV and have provided a platform for recombination, which may otherwise have taken years to happen.

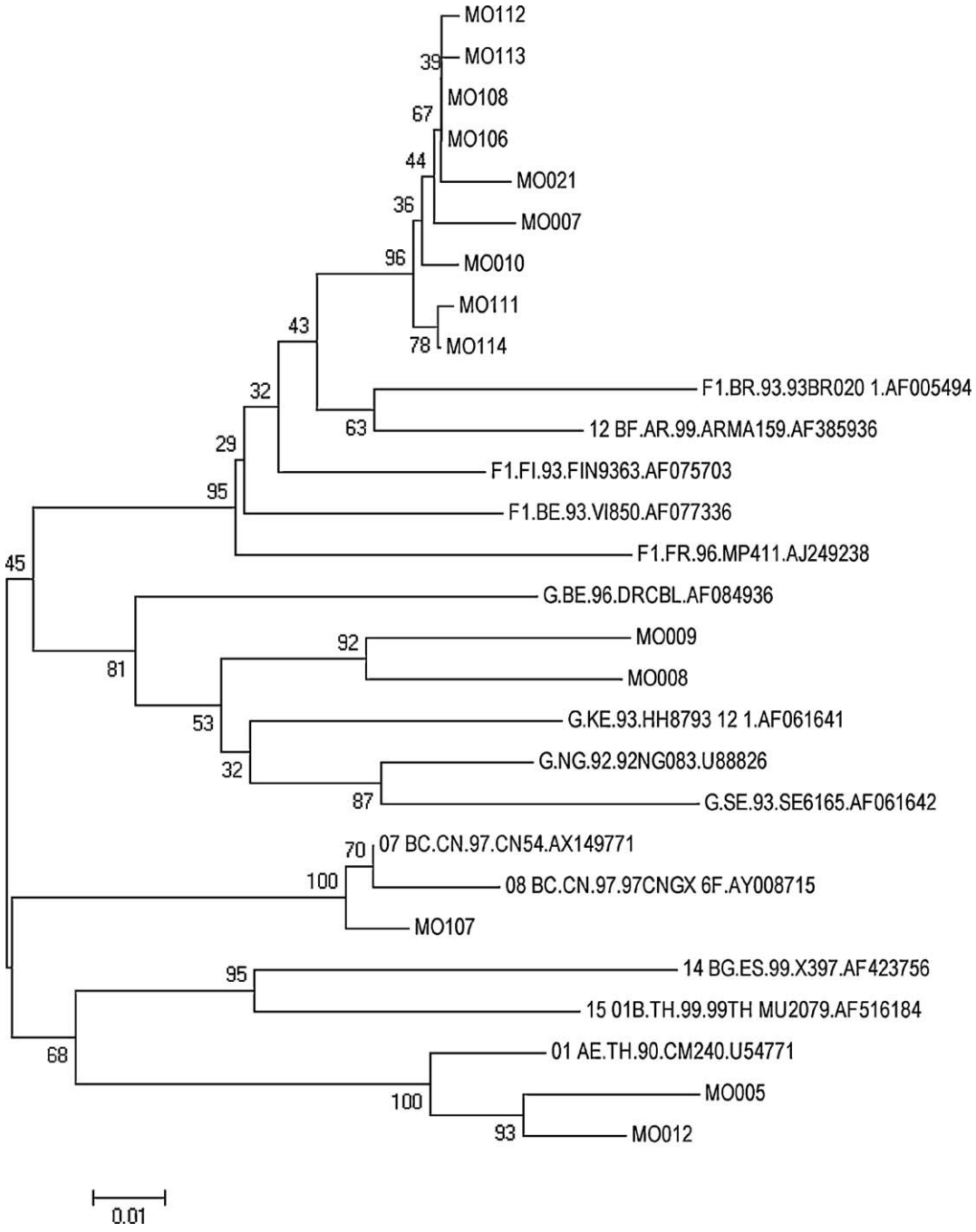

Fig. 1. (*Continued*).

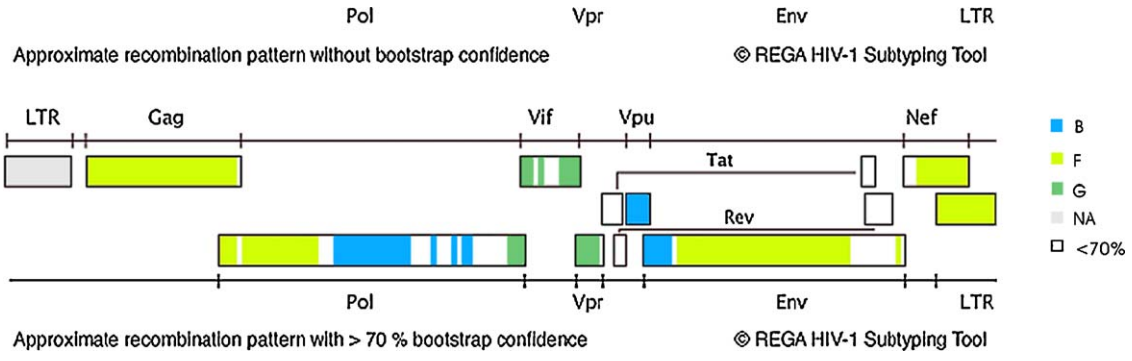

Figure 2. HIV-1 subtyping results of MO108 (approximate recombination pattern with >70% bootstrap confidence).

Conflict of interest

No conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2010.01.003.

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