

Sequence Note

The Molecular Epidemiology of HIV Type 1 among Vietnamese Australian Injecting Drug Users in Melbourne, Australia

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ABSTRACT

The proportion of human immunodeficiency virus type 1 (HIV-1) among Vietnamese injecting drug users (IDUs) in Melbourne, Australia exceeds that of the background population. To investigate the molecular epidemiology of HIV-1 among this group, the C2-V4 region of the HIV-1 envelope was directly sequenced from 11 Vietnamese Australians and 19 non-Vietnamese Australian controls. A significant difference in the distribution of the HIV-1 subtypes was demonstrated, with greater than 50% of Vietnamese Australian IDU shown to be infected with CRF01_AE—the predominant subtype in Southeast Asia, rather than subtype B, which dominates the Australian epidemic and which was found in 89.5% of the non-Vietnamese controls. The genetic diversity of the CRF01_AE epidemic in Vietnamese Australian IDUs was substantially lower than that of the background subtype B, consistent with a more recent introduction of a limited number of viral strains from Vietnam. These results support public health policy targeting Australian IDUs of Vietnamese ethnicity as a distinct vulnerable population.

ALTHOUGH AUSTRALIA HAS BEEN SUCCESSFUL in maintaining a low prevalence of human immunodeficiency virus type 1 (HIV-1) among its population of injecting drug users (IDUs), there is concern about specific subgroups of injectors being at greater risk of infection. The proportion of Vietnamese Australian IDUs infected with HIV in Victoria is high compared with other IDUs; since 1996 there have been 38 reports of IDU-associated HIV-1 infection in Victoria and 11 of these 38 (29%) reported their country of birth as Vietnam.¹ Previous studies have reported that Vietnamese Australian IDUs are a relatively marginalized group who are less likely to access harm reduction campaigns and are thus at heightened risk of HIV-1 infection.^{2–4} Furthermore, other reports have suggested that this

population has an additional, unique risk factor for HIV-1 acquisition—travel to Vietnam, a country where IDU carries a notable risk of HIV-1 infection.^{2,4–6}

The principal subtypes of HIV-1 in Australia and Vietnam differ. In Australia, subtype B predominates in all risk categories^{7,8} whereas the recombinant form CRF01_AE is accountable for the majority of infections among IDUs and commercial sex workers (CSWs) in the expanding Vietnamese epidemic.^{9–13} This investigation aimed to further characterize the HIV epidemic occurring among Vietnamese IDUs in Australia at a molecular level and to determine whether travel back to Vietnam could be considered a significant risk factor for HIV-1 acquisition.

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Thirty HIV-1-positive patients were recruited as part of a multicenter retrospective study of HIV-1 among Vietnamese Australian IDUs conducted in hospital and primary care HIV clinics in Melbourne, Australia. Ten milliliters of whole blood was collected into acid citrate dextrose anticoagulant from 11 ethnic Vietnamese Australian and 19 non-Vietnamese Australian patients between June 1997 and March 2003. RNA was extracted from the stored plasma and used in a reverse transcriptase-polymerase chain reaction (RT-PCR) to amplify the C2-V4 region of the HIV-1 *env* gene (nucleotide coordinates 6948–7541 on reference genome HXB2 accession number K03455). The reverse transcription and first 40 cycles of amplification were performed using the OneStep RT-PCR kit (Qiagen, Germany) and the outer primers CRAF-5'-TGGGCGYACACATGCCTGTGTACC-3' and CRAR-5'-TTGCTTTGCTGTTGCACTATACCAGAC-3'. Qiagen (Germany) HotStarTaq DNA polymerase and the nested primers CRCF-5'GTCAGCACAGTACAATGCACACA-3' and CRCR -5'-GATGGGAGGGGCATACATTGC-3' were used in the subsequent 40 rounds of amplification. The manufacturer's conditions were used in all cases. The amplified fragments were directly sequenced on both strands using an ABI automated DNA Sequencer. The resultant sequences were aligned in DNAMAN version 4.1 (Lynnon Biosoft), uploaded into the WebANGIS web interface at the Australian National Genomic Information Service (www.angis.org.au) and subject to phylogenetic analysis with reference strains of each HIV-1 group M subtype as defined by the HIV Sequence Database, Los Alamos National Laboratory. Phylogenetic analysis was performed using the EDNADIST, ENEIGHBOUR, ESEQBOOT, and ECONSENSE Embossed versions of the Phylip software suite. Phylogenetic trees were inferred on the basis of pairwise distances calculated using the two-parameter algorithm described by Kimura and drawn with TreeView (Win 32) (Rod Page, 1998). Distance matrices created using the Jukes-Cantor algorithm were used to calculate pairwise diversity as average genetic distance. A two-sample test of proportions was employed to statistically analyze the data. Sequences were submitted to the GenBank database under the accession numbers AY686502–AY686531.

Phylogenetic analysis revealed that there were two subtypes, B and CRF01_AE, present among the study population (Table 1). The majority (89.5%) of the non-Vietnamese Australians were infected with subtype B. Seventy-two percent of these individuals self-identified as gay or bisexual and half also reported IDU, however, none had any history of IDU outside Australia. In contrast, greater than half (55%) of the Vietnamese Australians were found to be infected with CRF01_AE. Of the eight individuals from whom CRF01_AE was isolated, six (75%) were Vietnamese Australian IDUs and two (25%) were non-Vietnamese, non-IDUs Australians. All individuals infected with CRF01_AE reported only heterosexual activity.

In the Australian IDU epidemic, as sampled in this Melbourne population, the genetic pairwise diversity over C2-V4 is similar for both subtype B (19.3%, SD = 0.03, $n = 22$) and CRF01_AE (20.6%, SD = 0.03, $n = 8$). Both these figures are high in comparison with regional IDU epidemics,^{14–16} reflecting the indolent progression of the Australian epidemic in comparison with the explosive spread seen elsewhere. These results

TABLE 1. SUBTYPE DISTRIBUTION OF HIV-1 AMONG THE STUDY POPULATION^a

<i>Non-Vietnamese Australian</i>	<i>Subtype^b</i>	<i>Vietnamese Australian</i>	<i>Subtype^c</i>
AU03C01	B	AU03CA2	CRF01_AE
AU03C05	B	AU03CA3	CRF01_AE
AU03C07	B	AU03CA4	B
AU03C010	B	AU03CA6	CRF01_AE
AU03C011	B	AU03CA8	B
AU03C013	B	AU03CA9	B
AU03C015	B	AU03CA14	CRF01_AE
AU03C016	B	AU03CA23	B
AU03C017	B	AU03CA24	CRF01_AE
AU03C018	B	AU03CA25	B
AU03C019	B	AU03CA26	CRF01_AE
AU03C020	B		
AU03C021	B		
AU03C022	B		
AU03C027	CRF01_AE		
AU03C028	B		
AU03C029	B		
AU03C030	CRF01_AE		
AU03C031	B		

^aSubtypes were determined based on the C2-V4 region of the HIV-1 envelope gene.

^bSubtype B, 89.5%; subtype CRF01_AE, 10.5%.

^cSubtype B, 45%; subtype CRF01_AE, 55%.

support previous reports that rapid transmission of HIV-1 is not occurring between Australian IDUs.¹⁷

Among IDUs of Vietnamese ethnicity, however, pairwise diversity of CRF01_AE strains is much lower (10.9%, SD = 0.04, $n = 6$), reflecting the limited number of related circulating strains as also demonstrated in the phylogenetic analysis (Fig. 1). The pairwise diversity of subtype B strains in this group, however, is close to that of the epidemic as a whole (19.2%, SD = 0.03, $n = 5$).

To further assess viral diversity within the ethnic Vietnamese population, the amplified sequences of the CRF01_AE strains were compared to 125 Vietnamese CRF01_AE sequences obtained from the Los Alamos National Library (LANL) (Fig. 2). Accession numbers of sequences used in this analysis are available from the author. The lack of a single, distinct cluster suggests that the epidemic has resulted from a number of introductions of this subtype into Australia, although the high level of relatedness between AU03CA2 and AU03CA24 (Fig. 2) also provides evidence that transmission of HIV-1 may have occurred between Vietnamese Australian IDUs.

This results of this investigation reveal that heterosexual Vietnamese Australians whose principal risk factor for HIV-1 acquisition is injecting drug use are more likely to be infected with CRF01_AE than non-Vietnamese Australians ($p = 0.0086$). The location of HIV-1 acquisition is uncertain, but the results suggest that Vietnamese Australian IDU may be getting infected in Vietnam, either prior to traveling to Australia or during a subsequent return visit to Vietnam. It is possible that some Vietnamese Australian IDUs have acquired their infection in

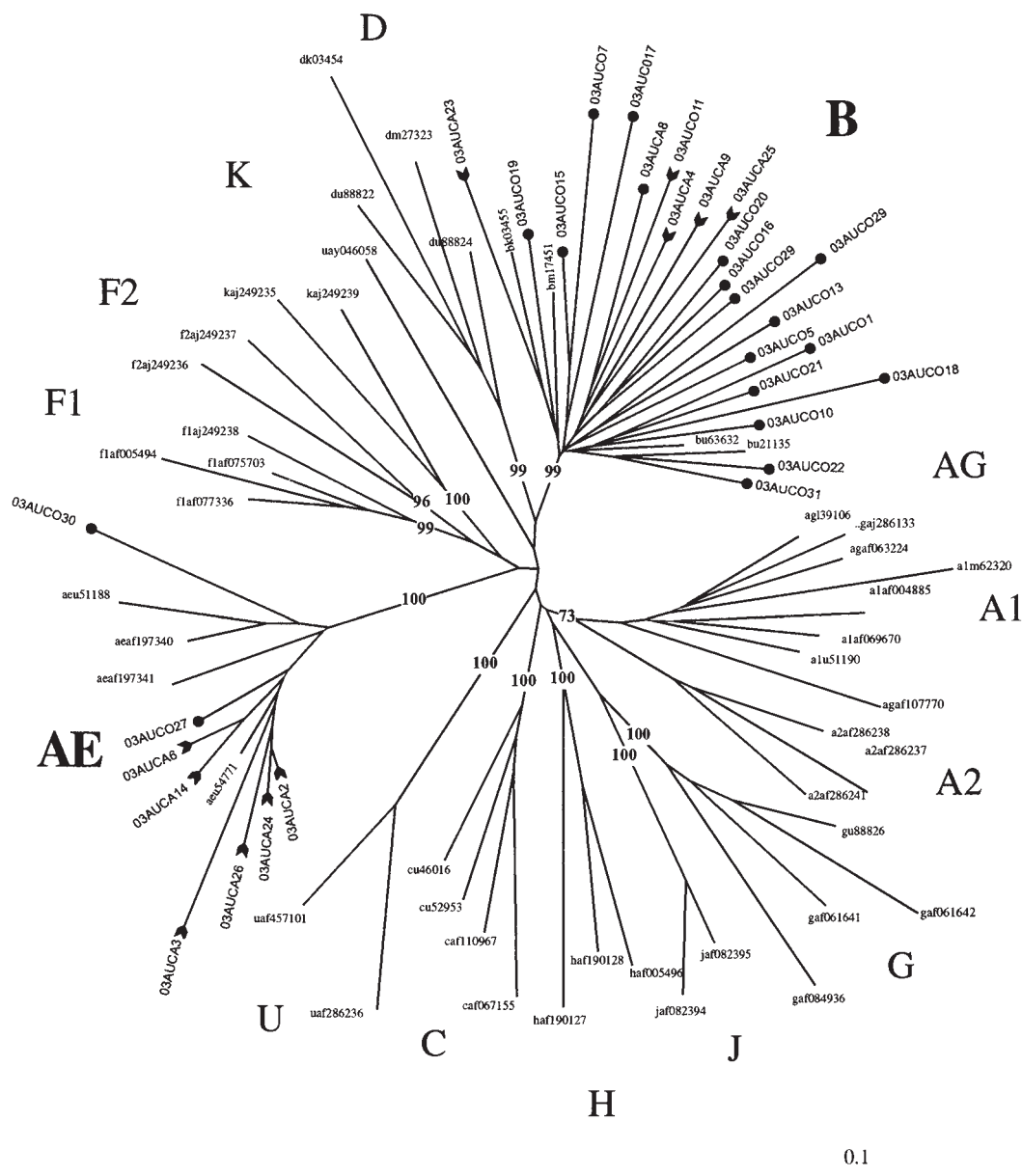


FIG. 1. Neighbor-joining tree illustrating the relationship between HIV-1 isolates from 11 Vietnamese Australians (arrowhead), 19 non-Vietnamese Australians (dot), and available reference strains of all subtypes and the first two CRFs. Sequences were analyzed over the C2-V4 region (corresponding to nucleotide position 6988–7508 on HXB2 reference genome—accession number K03455). Accession numbers of reference strains are indicated. All samples analyzed in this study cluster with either subtype B or CRF01_AE. The robustness of the phylogenetic tree was tested by bootstrapping (100 iterations) and the subsequent value is indicated at the relevant node.

Australia from people infected in Vietnam. The results highlight the need to develop harm reduction programs that effectively target this subgroup of IDUs in Australia and the need to educate IDUs and their families about the risks of returning to Vietnam and injecting unsafely during that time.

SEQUENCE DATA

Nucleotide sequences were submitted to GenBank under accession numbers AY686502 to AY686531.

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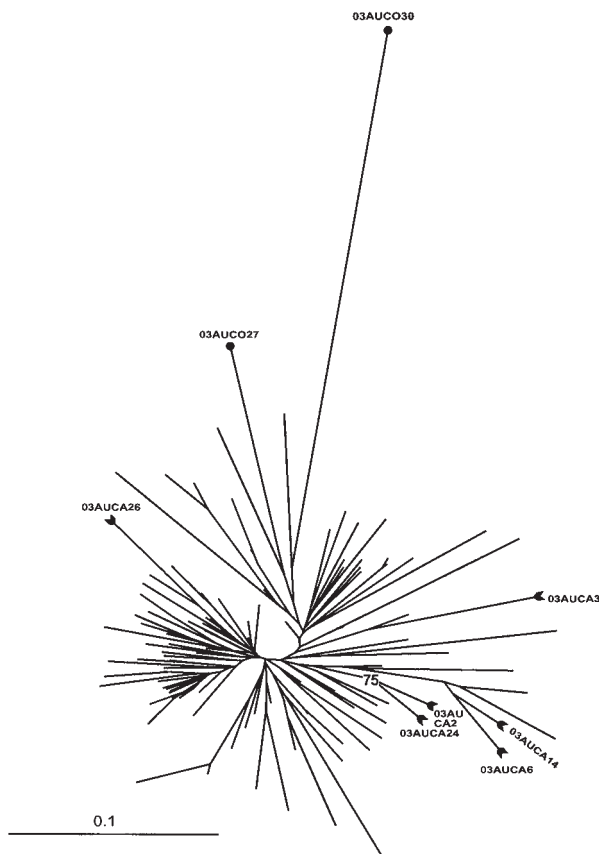


FIG. 2. Neighbor-joining tree illustrating the relationship between 125 strains of CRF01_AE from Vietnam with the eight CRF01_AE strains identified in this study over the envelope region (nucleotide positions 7049–7282 in the HXB2 reference genome—accession number K03455). Vietnamese Australian IDU cases are indicated by the arrowhead and non-Vietnamese Australian controls by the dot. The scale bar represents 10% genetic distance, significant bootstrap values (>70% of 100 iterations) are indicated on the nodes. The two Australian isolates, dispersed among the Vietnamese strains, show no evidence of a founder effect. The high level of relatedness between 03AUCA2 and 03AUCA24 is supported by a significant bootstrap value.

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