

Acknowledgments

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HIV-1 CRF07_BC Infections, Injecting Drug Users, Taiwan

To the Editor: To date, Taiwan's human immunodeficiency virus type 1 (HIV-1) epidemic has primarily spread via sexual contact. The subtype B and circulating recombinant form (CRF) 01_AE account for >95% of all infections (1). However, since

2003 Taiwan has experienced a major outbreak of CRF07_BC among injecting drug users (IDUs).

The first wave of HIV-1 infections in Taiwan can be traced to the early 1980s, when a group of hemophilia patients received imported HIV-1-contaminated antihemophilia medications. By the time these medications had been replaced by heat-treated factor VIII concentrates, at least 53 patients had contracted HIV-1 infections (2). According to Taiwan's Center for Disease Control (CDC), HIV infections have been diagnosed in 9,229 persons (including 523 foreigners) as of July 31, 2005 (3). The number of persons living with HIV-1/AIDS has increased rapidly in the past few years, with a 77% increase in 2004, compared to 11% in 2003 (online Table, available at <http://www.cdc.gov/ncidod/EID/vol12no04/05-0762.htm#table>). According to the results of a risk factor analysis of people living with HIV-1/AIDS reported to the Taiwan CDC, the proportion of IDUs increased from 1.7% (13/773) in 2002 to 8.1% (70/861) in 2003 to 30.3% (462/1,521) in 2004 (online Table). The Taiwan CDC received reports of 1,241 IDUs diagnosed with HIV-1 infections from January 1 to July 31, 2005; these account for >75% of all reported HIV-1 infections in 2005 (3). The evidence points to an explosive epidemic of HIV-1 infections among IDUs in Taiwan since 2003, with no indication of a slowdown.

Taiwan has ≈60,000 IDUs (1). According to the Republic of China Ministry of Justice, the number of incarcerated drug offenders increased from 5,988 in 2003 to 9,303 in 2004; the rate of HIV-1 seropositive inmates increased from 13.3/100,000 in 2002 to 56.8/100,000 in 2004 (Y-M. Wu, Ministry of Justice, pers. comm.). Since all inmates are routinely tested for HIV-1 in detention centers, and all infected inmates are separated from HIV-1-seronegative inmates, the

potential of HIV-1 transmission in prisons is remote. We therefore suggest that the Taiwanese IDU population and its HIV-1 seropositive rate have both increased rapidly in the past few years.

To identify the primary HIV-1 strains in the current epidemic, we collected blood specimens from HIV-1-infected inmates in 3 detention centers (1 each located in the northern, central and southern regions of Taiwan). HIV-1 subtypes were determined by polymerase chain reaction, DNA sequencing, and phylogenetic analyses of *pol* or *env* genes. Our results indicate that 145 (96%) of 151 IDUs were infected with CRF07_BC and 6 (4%) were infected with subtype B; 97% of the CRF07_BC cases were diagnosed in 2003 or 2004. According to our phylogenetic analysis of the *env* gene, the Taiwanese CRF07_BC strains clustered with CRF07_BC strains drawn from IDUs in China (Figure).

CRF07_BC is a recombinant of the B' and C subtypes. Several studies have suggested that CRF07_BC originated in China's Yunnan Province, with subtype B' from Thailand mixing with subtype C from India before moving northwestward to Xinjiang Province along a major Chinese heroin trafficking route (4–6). To our knowledge, this is the first report of a large group of IDUs in northeastern Asia having CRF07_BC infections. It may have followed another drug trafficking route from Yunnan Province to southeast China, moving through Guangxi Province and Hong Kong to Taiwan (7–9). In a bid to combat skyrocketing HIV/AIDS infection rates among IDUs, the Taiwan CDC has proposed a 5-year harm reduction program to the Republic of China Executive Yuan.

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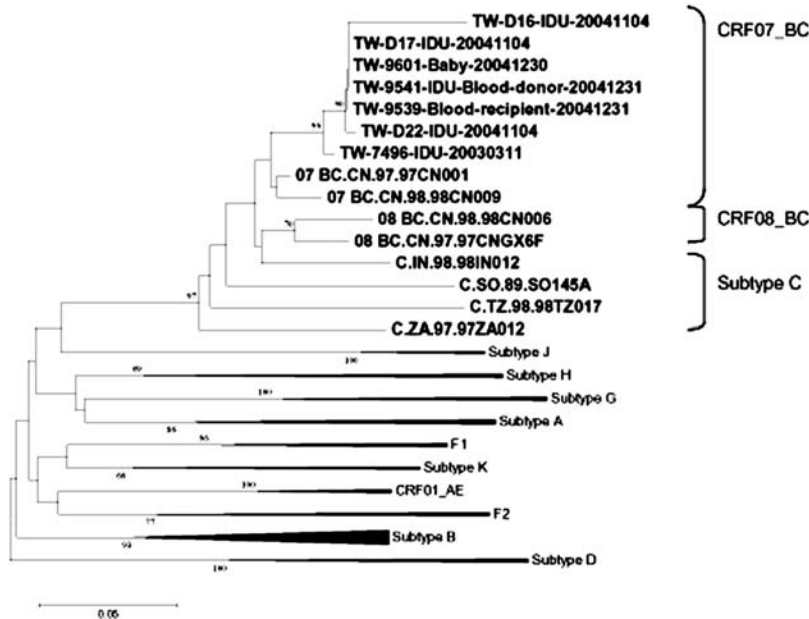


Figure. Phylogenetic analyses of 7 HIV-1 isolates identified in Taiwan. TW-7496, TW-D16, TW-D17, and TW-D22 were collected from detention center inmates; TW-9541 and TW-9539 were collected from a blood donor and 1 of his donation recipients. This neighboring tree was created from 100 bootstrap samples of aligned *env* sequences corresponding to the 7077–7340 nucleotide residues of HIV-1-HXB2 from different isolates. Bootstrap values are shown on branch nodes. Reference isolates from the GenBank HIV database are indicated by subtype.

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Chlamydialike Organisms and Atherosclerosis

To the Editor: *Chlamydomphila pneumoniae* causes pneumonia, but its role in the pathogenesis of atherosclerosis is controversial (1–4). The role of *C. pneumoniae* in atherosclerosis is supported by seroepidemiologic studies and detection in atherosclerotic lesions by polymerase chain reaction (PCR), immunohistologic analysis, culture, and electron microscopy (2,3). However, these results were not confirmed by other serologic or PCR-based studies (4). Meijer et al. evaluated abdominal aortic aneurysm biopsy specimens and detected *C. pneumoniae* membrane antigen more frequently than lipopolysaccharide antigens but did not detect heat shock protein 60 (1). In addition, they could not amplify or detect specific *C. pneumoniae* DNA by PCR and fluorescence in situ hybridization (1). They

hypothesized that this discrepancy may result from a chlamydialike organism present in aortic samples that has surface antigens similar to those of *C. pneumoniae*.

Parachlamydia acanthamoebae and *Neochlamydia hartmanellae* are chlamydialike organisms that share ≈86% 16S rRNA sequence similarity with *C. pneumoniae* (5). Like *C. pneumoniae*, they have elementary and reticulate bodies visible by electron microscopy (6). *Neochlamydia*-related DNA (GenBank accession no. AF097191) has been amplified from 5 different arterial samples, including 1 aortic aneurysm (7), and a relationship ($p = 0.009$) between cerebral hemorrhage and serologic evidence of *Parachlamydia* infection has been reported (8). Therefore, we investigated the role of *Parachlamydia* in pathogenesis of atherosclerosis by using a molecular approach.

We analyzed 78 surgical samples from 27 patients undergoing aortic or carotid surgery for atherosclerotic disease at Hôpital Nord in Marseille from June 1, 2003, to December 31, 2003. The study was approved by the local ethics committee, and written informed consent was obtained from all participants. Demographic and clinical data were prospectively recorded.

DNA was extracted from aortic or carotid samples with atherosclerotic lesions by using the QIAamp DNA tissue kit (Qiagen, Courtaboeuf, France), according to the manufacturer's instructions. A nested PCR was performed by using external primers 16SIGF (5'-CGGCGTGGATGAGGCAT-3') and 16SIGR (5'-TCAGTCCAGTGTGGC-3') (9) and internal primers CHL16SFOR2 (5'-CGTGATGAGGCATGCAAGTCTCGA-3') and CHL16SREV2 (5'-CAATCTCTCAATCCGCCTAGACGTCTTAG-3') (7). PCR included negative controls from the DNA extraction step. DNA extractions and PCR amplifications were conducted in a laboratory

in which parachlamydial DNA had not been extracted or amplified. PCR products were purified by using the QIAquick PCR purification kit (Qiagen) and sequenced by using the d-rhodamine terminator cycle sequencing reaction kit (Perkin-Elmer Biosystems, Warrington, UK) and a 3100 ABI Prism automated sequencer (Applied Biosystems, Courtaboeuf, France).

Sequences were analyzed with BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>) using gap existence and extension penalties of 5 and 2, respectively. Results were considered positive only when the sequence of the amplified product exhibited a best BLAST hit with a chlamydialike organism. Statistical analyses were performed with STATA software (Stata Corporation, College Station, TX, USA).

A positive PCR result was obtained with samples from 5 (18.5%) of 27 patients (Table). Three sequences had a best BLAST hit with the sequence of *Parachlamydia* sp. UV7 (GenBank accession no. AJ715410), with a sequence similarity ranging from 99% to 100%. The other 2 sequences had 98% sequence similarity with *Neochlamydia*-related symbiont TUME-1 (GenBank accession no. AF098330). PCR positivity was not associated with age, sex, or location of the atherosclerotic lesion.

All patients with positive PCR results were ≥68 years of age. Patients without cardiovascular risk factors were more likely than those with ≥1 risk factor to have positive PCR results ($p = 0.023$). Despite the small number of patients in this study, this association was also confirmed in a multivariate logistic regression model adjusted for sex and previous cardiovascular disease (odds ratio 0.035, 95% confidence interval 0.001–0.94).

These findings suggest that *Parachlamydia* and *Neochlamydia* are associated with atherosclerosis. In addition, these obligate intracellular