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Molecular Epidemiology of HIV-1 Subtypes and Drug Resistant Strains in Taiwan

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The Taiwanese government has provided free highly active antiretroviral therapy since April 1997. Previously, we have reported on the molecular epidemiology of HIV-1 in Taiwan from 1988 to 1998. In addition, an outbreak of circulating recombinant form (CRF) 07_BC among intravenous drug users was noted in 2004. Therefore, the purposes of this study were to elucidate the distribution of HIV-1 subtypes among different high-risk groups in Taiwan from 1999 to 2000 and to conduct surveillance on drug resistance among treatment naïve patients from 1997 to 2000. Blood samples from 239 HIV-1/AIDS patients and their subtypes were examined using DNA sequencing and phylogenetic analysis. The results showed that among 226 male patients, 213 (94.2%) had subtype B, 11 (4.9%) had CRF01_AE, 1 had unique recombinant strain related to both CRF07_BC and CRF08_BC (strain T12-99TW) and 1 had CRF08_BC (strain L9312-00TW). The patients infected with T12-99TW and L9312-00TW were intravenous drug users and had needle-sharing experiences in Yunnan Province, China. Of the 13 HIV-1-infected females, 7 (53.8%) had subtype B, 5 (38.5%) had CRF01_AE, and 1 (7.7%) had subtype C. Phylogenetic analysis demonstrated that the neither strain T12-99TW nor L9312-00TW clustered with CRF07_BC strains isolated from Taiwanese intravenous drug users in 2004. In addition, 126 treatment naïve patients were selected for genotypic DR analysis and the results showed that 4.3% (2/47) homosexual males had M184V mutations. This is the first report on the identification of CRF08_BC and a unique recombinant strain related to both CRF07_BC and CRF08_BC in Taiwan. ***J. Med. Virol. 80:183–191, 2008.*** © 2007 Wiley-Liss, Inc.

KEY WORDS: HIV drug resistance; molecular epidemiology; HIV subtype

INTRODUCTION

Phylogenetic analyses of human immunodeficiency virus type 1 (HIV-1) strains have identified three distinct groups—major (M), outlier (O), and new (N) groups. More than 99% of the HIV-1 strains in this pandemic belong to group M and can be classified into nine subtypes (A, B, C, D, F, G, H, J, and K), 16 circulating recombinant forms (CRFs) and at least 30 unique recombinant forms (URFs) [Los Alamos National Laboratory, 2004; Takebe et al., 2004]. If an intersubtype recombinant virus is transmitted from one patient to another, and becomes one of the circulating strains in the HIV epidemic, it can be classified as a CRFs [Robertson et al., 2000]; if there is no evidence of

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epidemic spread, then it is called a URF [McCutchan, 2000].

Molecular epidemiological investigations have been a powerful tool for analyzing the origin [Korber et al., 2000; McCutchan, 2000; Lemey et al., 2003; Travers et al., 2004] and tracking the dissemination [Ou et al., 1992] of different HIV-1 subtypes or CRFs in the world. The Taiwanese Molecular Epidemiology Study was initiated in 1993 to monitor and report on the molecular epidemiology of HIV-1 in Taiwan. Monitoring is accomplished through the collection and analysis of clinical samples and questionnaires from patients living with HIV/AIDS (PLWHAs) from different AIDS treatment centers throughout island [Chen et al., 1998].

Trends of the subtype distribution in Taiwan between 1988 and 1998 have been reported previously [Chen et al., 1998, 2001]. HIV-1 subtype B has been the major subtype of HIV-1 infection in Taiwan since 1988. However, according to the previous analyses, other subtypes of CRFs have emerged, especially CRF01_AE [Chen et al., 2001]. Therefore, the first purpose of this study was to continue monitoring the distribution of various subtypes and CRFs in different high-risk groups in Taiwan between 1999 and 2000.

Previously, a severe outbreak of CRF07_BC among intravenous drug users in Taiwan was reported in 2004 [Chen et al., 2006]. Furthermore, phylogenetic analysis demonstrated that the Taiwanese CRF_07BC strains belonged to two phylogenetic clusters, and the first cluster contained only CRF07_BC strains from the southern part of Taiwan [Lin et al., 2007]. Since the earliest case found in the study were diagnosed to have HIV-1 infection in 2002, it was concluded that the CRF07_BC was first introduced into the southern region in 2002 and then spread to other regions in Taiwan in 2004 [Lin et al., 2007]. Therefore, the second purpose of this study was to search for CRF07_BC strains earlier than 2002.

The Taiwanese government has started to provide all HIV-infected citizens with free access to highly active antiretroviral therapy (HAART) since April 1997 [Fang et al., 2004; Chen and Kuo, 2007]. The public health concern is whether the drug resistant (DR) strains will arise as a new epidemic in Taiwan. If so, which high-risk group will be its major target? Therefore, another purpose of this study was to conduct a surveillance of HIV-1 DR strains among different high-risk groups in Taiwan according to the protocol provided by the World Health Organization [HIV Drug Resistance Program, WHO and CDC, 2004].

MATERIALS AND METHODS

Patients

Two hundred thirty-nine blood samples were collected from HIV-1 infected patients seen at AIDS treatment centers in different regions in Taiwan between 1999 and 2000. The treatment centers included the Taipei Venereal Disease Control Institute, Tao-Yuan General Hospital, Lin-Kou Chang-Gung Memorial Hospital, and China Medical University Hospital in Taichung. These 239 study subjects for subtype analysis included 226 males (41 heterosexuals, 118 homosexuals, 49 bisexuals, 5 Intravenous Drug Users, and 13 others who could not be classified into any of the above groups) and 13 females (10 heterosexuals, 3 commercial sex workers). In addition, molecular epidemiologic data from 879 HIV/AIDS patients collected from 1988 to 1998 were included in order to examine a trend analysis [Chen et al., 1998, 2001]. A short history including date of the first diagnosis, possible risk factors, sexually transmitted diseases, CD4+ counts, and medication profile was obtained from the patients. Informed consent was obtained from all patients who participated in this study.

For the genotypic drug resistance assay, the guidelines provided by WHO were used for the surveillance of HIV drug resistance [HIV Drug Resistance Program, WHO and CDC, 2004]. One hundred twenty-six treatment naïve patients which included 34 heterosexual males, 47 homosexual males, 34 bisexual males, 7 females, and 4 intravenous drug users were randomly selected from our cohort for analysis. All were diagnosed from 1997 to 2000. In addition, plasma samples from four intravenous drug users patients who have been treated under HAART and had clinical parameters which suggested that they had developed drug resistance were also included in the study.

RNA Extraction and RT-PCR

RNA was extracted from all 239 patient plasma samples using QIAamp viral RNA mini kits (Qiagen, Valencia, CA). The primer pairs used in the RT-PCR are listed as follows: for the *env* region, ed5: 5′-ATGGGATCAAAGCCTAAAGCCATGTG-3′ and ed12: 5′-AGTGCTTCCTGCTGCTCCCAAGAACCCAAG-3′; for the *gag* region, gag1: 5′-CATGCGAGAGCGTCAGTATTAAGCGG-3′ and gag4: 5′-CATTCTGATAATGCTGAAAACATGGG-3′; for the *pol* region, F1849: 5′-GATGACAGCATGTCAGGGAG-3′ and R-3500: 5′-CTATTAAGTCTTTTGATGGGTCATAA-3′. RT-PCR was performed in a single-tube reaction (Promega, Madison, WI) using primers for each region mentioned above. First strand cDNA synthesis consisted of a reverse transcription at 48°C for 45 min followed by 1 cycle at 94°C for 2 min for denaturation. Second strand synthesis and PCR amplification followed by 40 cycles of denaturing at 94°C for 30 sec, annealing at 55°C in the *env*, *gag* regions and at 50°C in the *pol* region for 1 min, with extension at 68°C for 2 min and a final extension at 68°C for 7 min.

Nested PCR and Sequencing

The products of the RT-PCR process continued with nested PCR by TaKaRa Ex Taq™ (TaKaRa Bio Inc., Otsu, Shiga, Japan). PCR primer pairs are listed as follows: for the *env* region, es7: 5′-tgtaaaacgacggccagtCTGTTAAATGGCAGTCTAGC-3′ and es8: 5′-caggaaacagctatgaccCACTTCTCCAATTGTCCCTCA-3′;

for the *gag* region, gag2: 5′-CATAAGCTTGGGAAAAAATTCGGTTAAGGCC-3′ and gag3: 5′-CATGAATTCCTTCTACTACTTTTACCCATGC-3′; for the *pol* region, POL1: 5′-GCTAATTTTTTAGGGAA-3′ and POL2: 5′-TTCTCTTCTGTGTCAATGGCCATTGTTT-3′. The PCR thermocycler program consisted of predenaturing at 94°C for 5 min followed by 35 cycles of denaturing at 94°C for 15 sec, annealing at 55°C in the *env*, *gag* regions and 50°C in the *pol* region for 45 sec, initial extension at 72°C for 1.5 min and final extension at 72°C for 10 min. PCR products were checked using 1.5% agarose gel prior to purification. The resulting product was gel-purified for DNA sequencing, which was completed using a BigDyeTM Terminator Cycle Sequencing Ready Reaction Kit with an ABI PRISMTM 3700 DNA Analyzer (Applied Biosystems, Foster City, CA). The PCR product of env region was used for phylogenetic analysis. But, in the case which cannot amplify the PCR product of env region, the gag and pol region were use for subtyping.

Phylogenetic Analysis

The HIV-1 subtypes were determined based on the phylogenetic analysis of their *env*, *gag* or *pol* (protease) sequence. The sequences were aligned with reference sequences from different subtypes using the BioEdit program (North Carolina State University, Raleigh, NC: http://www.mbio.ncsu.edu/bioedit/bioedit.html) and the dataset was used to construct a phylogenetic tree using the MEGA program (Molecular Evolutionary Genetic Analysis Software, Version 2.0, http://www.megasoftware.net/). Kimura-2-parameter model was used employing the Neighbor-Joining method (unrooted tree). Phylogenetic groups were evaluated by using the bootstrap test (1,000 bootstrap replicates).

Genotypic Drug Resistance

Genotypic resistance to HIV-1 was determined from the randomly selected 126 treatment naïve and 4 treatment experienced patient plasma samples using the ViroSeq HIV-1 Genotyping System, Version 2 (Celera Diagnostics, Alameda, CA). Details of the procedures have been described previously [Elbeik et al., 2002]. Sequences were determined using the high throughput ABI Prism model 3100 Capillary Sequencer (Applied Biosystems). Derived sequences were aligned and the resulting contiguous Pro-RT sequence analyzed for resistant mutations using the ViroSeq HIV-1 Genotyping System (HGS). The sequence correlating with nucleotide residues 2262–3503 of HXB2 was used for this analysis.

RESULTS

In this study, blood samples and questionnaires were collected from 226 male and 13 female patients attending different AIDS treatment centers in Taiwan. The risk factors of these 239 subjects and 879 cases collected previously from 1988 to 1998 [Chen et al., 2001] were combined and analyzed. As shown in Table I, the HIV-1/AIDS patient cohort of our center consisted of 1,118 cases which represented 37.7% of the total cases reported to the Centers for Disease Control of Taiwan [Centers of Disease Control Taiwan, 2002]. In addition, the sex ratio (male/female = 12:1) and the proportions of different high-risk groups in both cohorts were similar.

HIV-1 Subtyping

Of the 239 samples processed successfully through the PCR (Table II), sequencing and phylogenetic analysis, 217 were infected with subtype B (90.8%). In addition, 19 (8.0%) were infected with CRF01_AE, 1 was infected with subtype C (0.4%), 1 with CRF07_BC related strain, and 1 with CRF08_BC. As shown in Figure 1A, the phylogenetic analysis of the *pol* region (HIV-1 HXB2 reference sequence nucleotides 2257–2521) demonstrated that an HIV-1 strain-L9312-00TW clustered with several CRF08_BC strains with a bootstrap value of 91%. In addition, another HIV-1 strain-T12-99TW clustered with both CRF07_BC and CRF08_BC strains (data not shown). To elucidate whether T12-99TW is CRF07_BC or CRF08_BC, the *gag* region (HIV-1 HXB2 reference sequence nucleotides 896–1199) was used for phylogenetic analysis and showed that T12-99TW was clustered with both CRF07_BC and CRF08_BC (Fig. 1B). Signature nucleotide sequence analysis of

TABLE I. Relative Representation of the Taiwanese Molecular Epidemiology Study as Compared to the National Surveillance Program for Study Subjects Enrolled Between 1988 and 2000

	National Registration Data[a], N = 2,969	Taiwanese Molecular Epidemiology Study, N = 1,118
Risk factors		
Heterosexual	1,300 (43.79%)	426 (38.1%)
Male homosexual	1,013 (34.12%)	443 (39.6%)
Male bisexual	434 (14.62%)	187 (16.7%)
Intravenous drug users	57 (1.92%)	28 (2.5%)
Patients with hemophilia	53 (1.79%)	12 (1.1%)
Blood transfusion	12 (0.40%)	1 (0.1%)
Vertical transmission	7 (0.24%)	1 (0.1%)
Infection by injury	1 (0.03%)	1 (0.1%)
Others	92 (3.10%)	20 (1.7%)

[a]By the end of 2000, from the Centers for Disease Control, Taiwan, Republic of China.

TABLE II. Summary of HIV-1 Subtype Determinations of 239 Study Subjects by Gender Enrolled in the Taiwanese Molecular Epidemiology Study Between 1999 and 2000

	HIV-AIDS patients		
HIV-1 subtype	Total, N = 239	Male, N = 226	Female, N = 13
Subtype B	217 (90.8%)	213 (94.2%)	7 (53.8%)
Subtype C	1 (0.4%)	0 (0 %)	1 (7.7%)
07URF	1 (0.4%)	1 (0.4%)	0 (0%)
CRF08_BC	1 (0.4%)	1 (0.4%)	0 (0%)
CRF01_AE	19 (8.0%)	11 (4.9%)	5 (38.5%)

the pol gene demonstrated that its 5′ region is similar with CRF07_BC, while its 3′ region shares greater similarity with CRF08_BC. The nucleotide sequence variations of the gag gene (303 nucleotides) between T12-99TW and CRF07_BC or CRF08_BC were 0.036 and 0.046, respectively. Therefore, T12-99TW is a unique recombinant strain related to both CRF07_BC and CRF08_BC.

Distribution of HIV-1 Subtypes in Different Risk Groups

Data of HIV-1 subtypes from 239 HIV/AIDS patients were pooled with that of 880 cases from a previous study [Chen et al., 2001] and analyzed. Subtype distribution of the total 1,119 patients from 1988 to 2000 is shown in Figure 2A. Among male patients, subtype B was most prevalent and after separating these male patients into different risk factors, the prevalence of subtype B was 100% (12/12) in hemophiliacs, 85.4% (158/185) in bisexuals, 83.5% (369/442) in homosexuals, 68.0% (17/26) in Intravenous Drug Users and 54.5% (188/345) in heterosexuals. CRF01_AE was detected in all groups except hemophiliacs and ranged from 41.3% (143/345) in heterosexuals, 28.0% (7/26) in intravenous drug users, 15.6% (69/42) in homosexuals and 14.6% (27/185) in bisexuals. Subtype C was detected in 2.6% (9/345) heterosexuals and 0.2% (1/442) homosexuals. Only one subtype A isolate was detected in the heterosexual and other risk group while two isolates of subtype G were only detected in heterosexual patients (Table III).

The CRF07_BC-related strain (T12-99TW) was isolated from drug using male heterosexual prisoner who was born in Myanmar and had needle-sharing experience in Yunnan Province, Mainland China before he came to Taiwan. Another male heterosexual patient infected with CRF08_BC strain was also born in Myanmar. This patient's first HIV-1 positive test was documented in

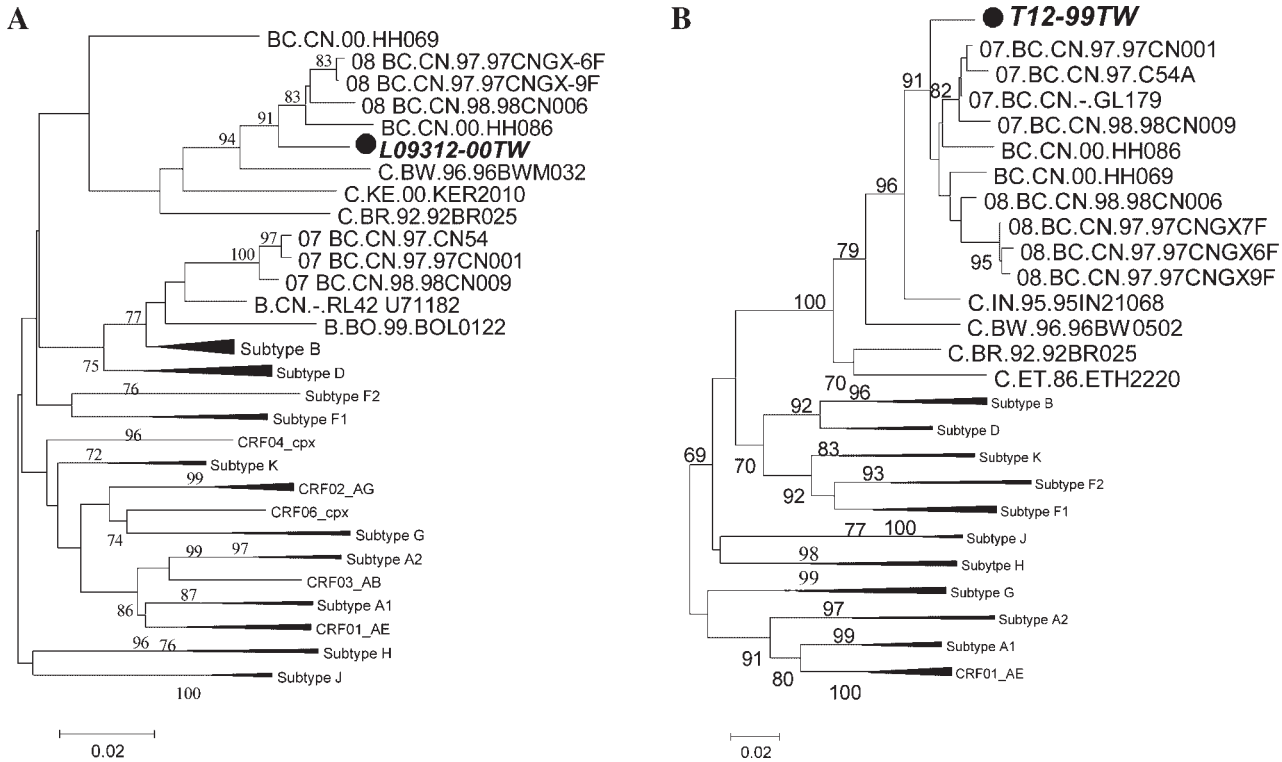

Fig. 1. **A**: Phylogenetic analysis of HIV-1 the *pol* region for defining the HIV-1 subtype (CRF07_BC or CRF08_BC) of isolate L9312-00TW. **B**: Phylogenetic analysis of the HIV-1 *gag* region for defining the HIV-1 subtype (CRF07_BC or CRF08_BC) of isolate T12-99TW.

TABLE III. Summary of HIV-1 Subtype Determinations of All Male Study Subjects by Risk Group Enrolled in the Taiwanese Molecular Epidemiology Study Between 1988 and 2000

	Male HIV-AIDS patients						
HIV-1 subtype	Total, N = 1,033	Heterosexual, N = 345	Homosexual, N = 442	Bisexual, N = 185	Hemophiliac, N = 12	IDUs[a], N = 26	Other, N = 23
Subtype A	2 (0.2%)	1 (0.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4.3%)
Subtype B	760 (73.6%)	188 (54.3%)	369 (83.5%)	158 (85.4%)	12 (100%)	17 (68.0%)	16 (69.6%)
Subtype C	10 (1.0%)	9 (2.6%)	1 (0.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
07URF	1 (0.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (4.0%)	0 (0%)
CRF08_BC	1 (0.1%)	0 (0.3%)	0 (0%)	0 (0%)	0 (0%)	1 (0%)	0 (0%)
Subtype D	1 (0.1%)	1 (0.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
CRF01_AE	252 (24.4%)	143 (41.3%)	69 (15.6%)	27 (14.6%)	0 (0%)	7 (28.0%)	6 (26.1%)
Subtype G	2 (0.2%)	2 (0.6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Indeterminate	4 (0.4%)	1 (0.3%)	3 (0.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

[a]Intravenous drug users.

1996. He admitted that he had needle sharing experiences and many instances of contact with female sex workers in Yunnan Province, Mainland China.

One teenager whose risk factor was "infection by injury" carried the CRF01_AE strain. The epidemiology investigation discovered that the only hint of contact was a traffic accident that occurred with a heterosexual HIV-1 positive male patient who also carried the CRF01_AE strain.

Distribution of HIV-1 Subtypes in Different Female Groups

For the female patients, CRF01_AE was most prevalent as it was detected in 67.4% (58/86) samples (Table IV). To differentiate by risk groups, all but the Intravenous Drug User patients contained CRF01_AE. Subtype B was detected in three risk groups including 100% of the intravenous drug users (2/2), 21.9% in heterosexuals (14/64) and 10% in commercial sex workers (1/10). One subtype G isolate was equally detected in the heterosexual and commercial sex worker patients.

Trends of HIV-1 Subtypes' Distributions

The subtype distribution among different risk groups was analyzed further chronologically according to the year when the patients were diagnosed as seropositive (Fig. 2D). While subtype B represented a decrease from 80% to 61.8% during previous years

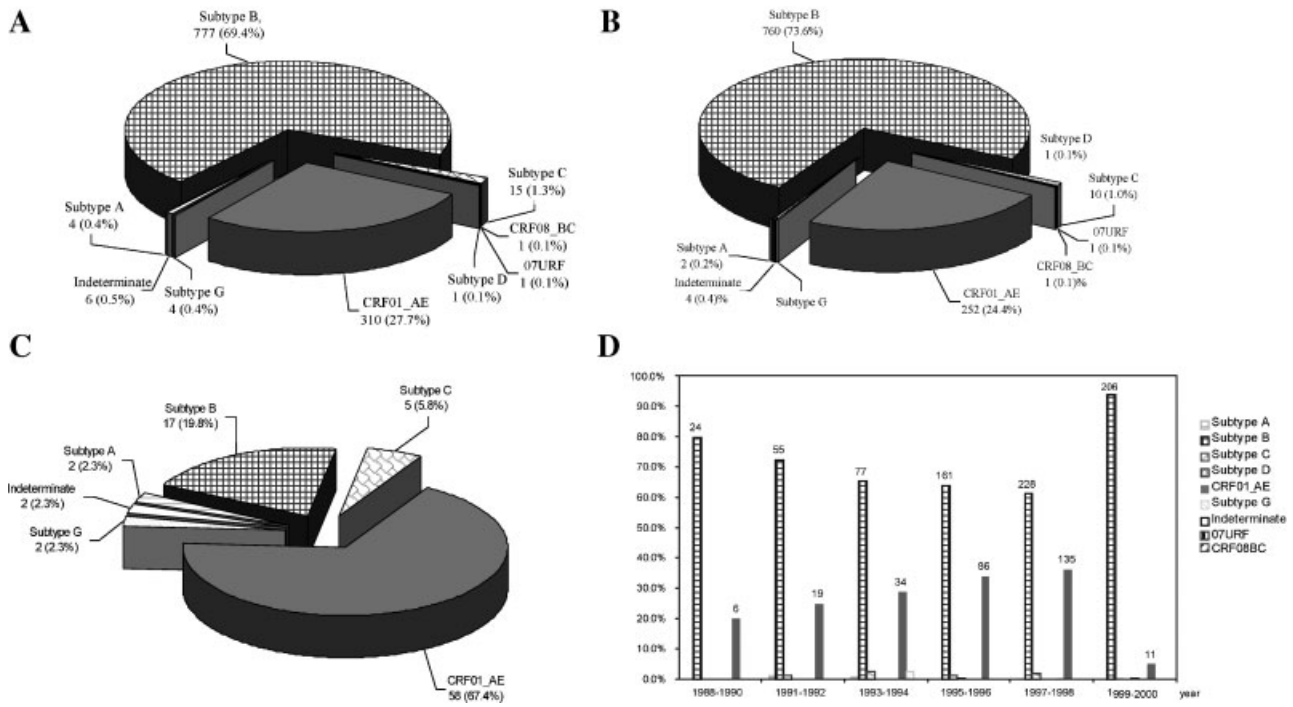

Fig. 2. **A**: Subtype distribution from all study participants enrolled between 1988 and 2000. **B**: Subtype distribution from all male study participants enrolled between 1988 and 2000. **C**: Subtype distribution from all female participants enrolled between 1988 and 2000. **D**: Relative proportion and numbers of HIV-1 subtypes determined from study participants enrolled from 1988 to 2000.

TABLE IV. Summary of HIV-1 Subtype Determinations of All Female Study Subjects by Risk Group Enrolled in the Taiwanese Molecular Epidemiology Study Between 1988 and 2000

HIV-1 subtype	Total, N = 86	Heterosexual, N = 64	Commercial sex worker, N = 10	Foreign brides, N = 8	IDUs[a], N = 2	Other, N = 2
Subtype A	2 (2.3%)	2 (3.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Subtype B	17 (19.8%)	14 (21.9%)	1 (10.0%)	0 (0.0%)	2 (100%)	0 (0.0%)
Subtype C	5 (5.8%)	5 (7.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
CRF01_AE	58 (67.4%)	41 (64.1%)	8 (80.0%)	8 (100.0%)	0 (0.0%)	1 (50.0%)
Subtype G	2 (2.3%)	1 (1.6%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Indeterminate	2 (2.3%)	1 (1.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)

[a]Intravenous drug users.

(1988–1998), the second predominant genotype CRF01_AE presented an increase from 20% to 36.6% during the same time period, and the B subtype went back up to 94.3% during the period of 1999–2000. After separating the different risk groups in Figure 3, we see the similar patterns presenting in male homosexuals and bisexuals. Male heterosexuals and female group had no such distinct increase or decrease between subtypes. The patient infected with 07URF strain was seropositive at 1999 and the CRF08_BC one was seropositive in 1996.

HIV-1 Genotypic Drug Resistance Analysis

Among the 126 drug naïve patients, subtype B was the most common. It was detected in 92.1% (116/126) of the isolates, followed by 7.1% (9/126) who had subtype CRF01_AE, and 0.8% (1/126) with subtype C. Subtype B isolates were detected in all male patients, and were detected exclusively in the 100% homosexual (47/47) and bisexual (34/34) groups. In addition to subtype B, subtype CRF01_AE was also detected in 11.8% (4/34) of the heterosexual males and 25% (1/4) of the Intravenous Drug User patients. Subtype C was not detected in the male patients. CRF01_AE was most common in female commercial sex workers and was detected in 57.1% (4/7) of the samples. This was followed by 28.6% (2/7) with subtype B and 14.3% (1/7) with subtype C. Subtype B was detected in all four drug experienced patients whose clinical parameters suggested that they had developed drug resistance, and all of them were intravenous drug users.

HIV-1 Primary Genotypic Drug Resistant Mutations

One primary resistant mutation, M184V in RT, was detected in 1.6% (2/126) of the HIV-1 isolates from a total of 126 drug naïve patients and was confined to 4.3% (2/47)

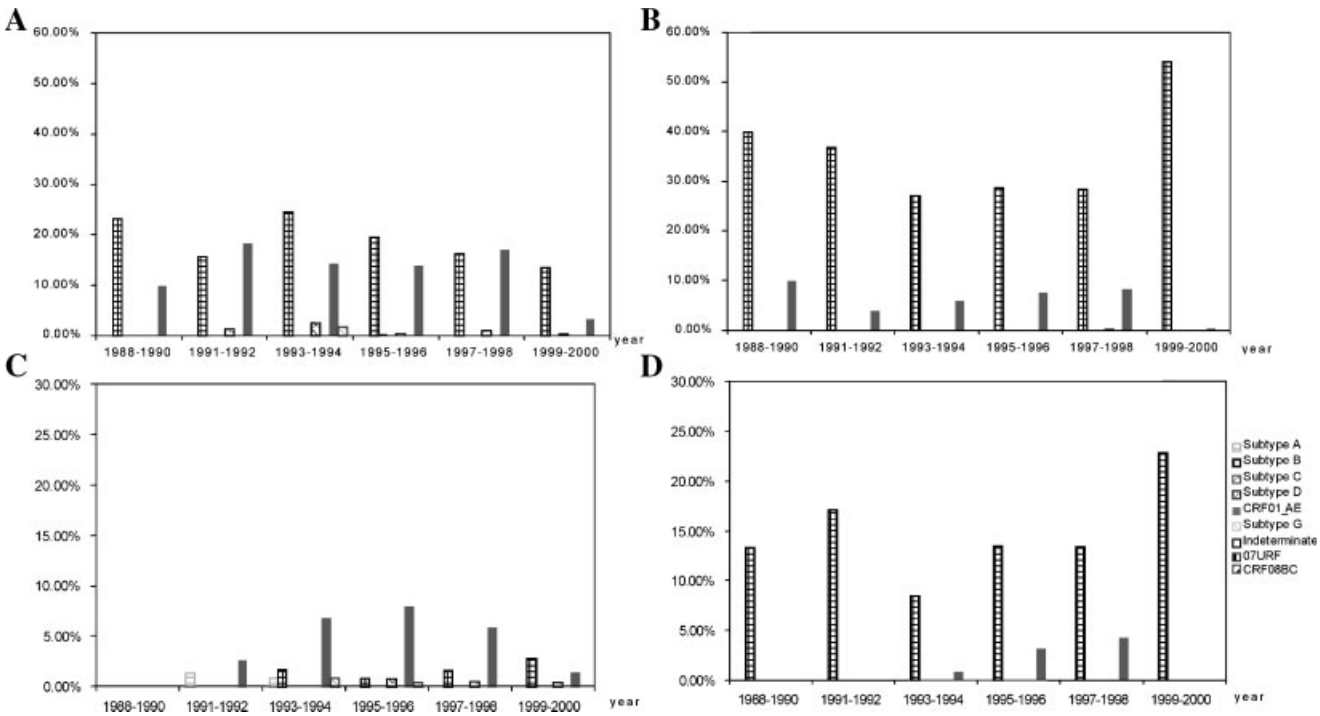

Fig. 3. Temporal trends of proportions of various HIV-1 subtypes detected in different groups of patients from 1988 to 2000 in Taiwan. **A**: Male heterosexuals. **B**: Male homosexuals. **C**: Male bisexuals. **D**: Women. Percentages are based on the total number of HIV-1 cases for each year.

TABLE V. Determination of HIV-1 Primary Drug Resistant Mutations From 126 Drug Naïve Study Subjects

		Male					Female
Primary mutation	Region	Total, N = 126	Heterosexual, N = 34	Homosexual, N = 47	Bisexual, N = 34	IDUs[a], N = 4	Commercial sex worker, N = 7
M184V	RT	2 (1.6%)	0 (0.0%)	2 (4.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

[a]Intravenous drug users.

of the homosexual group (Table V). All other patients lacked detectable primary resistance mutations. Five primary resistant mutations were detected in HIV-1 isolates from all four intravenous drug user patients, who were being treated under HAART and had developed drug resistance clinical pattern, and included one patient isolate containing T69D, two patient isolates containing M184V in RT and one patient isolate with three mutations including L90M in Pro, V75T and Y181C in RT.

HIV-1 Genotypic Drug Resistant Mutations in Different Subtype

All the primary mutation showed subtype B strains. The frequency (number of patient samples with the same mutation/total number of patients) of the most common secondary mutations was 69.8% (81/116) for L63P followed by 49.1% (57/116) for I93L, 45.7% (53/116) for V77I, and 20.7% (24/116) for M36I among the subtype B patients. In nine CRF01_AE strains, M36I was presented in all virus sequences and I93L was presented in 22.2% (2/9). Only one subtype C case exhibited M36I, L63P, and I93L mutations.

DISCUSSION

Former studies showed that a unique subtype B (B') spread primarily through Intravenous Drug Users in Southeast Asia. This B' strain was recombinant with subtype C which also was prevalent in the Intravenous Drug Users population. Two closely related CRFs, CRF07_BC and CRF08_BC, are disseminating rapidly among intravenous drug users networks in the Northwestern Xinjiang Province and the Southeastern (Guangxi Province) of China, respectively [Piyasirisilp et al., 2000; Su et al., 2000; McClutchan et al., 2002; Yang et al., 2003]. URFs that showed structural similarity to CRF07_BC were detected in Eastern Yunan [Yang et al., 2002, 2003]. This specific URF form was an example of CRFs recombining with each other (CRF07_BC and CRF08_BC). These specific subtypes were prevalent through the independent transmission networks among intravenous drug users in China [Yang et al., 2002].

A close historical and geographical relationship exists between Taiwan and China. Travelers intermix frequently in Hong Kong as well as other Southeastern parts of Asia thereby increase the potential spread of infectious disease. A recent example was the rapid spread, by air travel, of the infectious disease SARS [Ruan et al., 2003] around Southeast Asia and elsewhere. Although the molecular findings did not suggest an epidemiological link between HIV infection in Hong Kong and Mainland China between 1999 and 2001, the B' recombinant form was identified in a heterosexual men in 2001 in Hong Kong [Lim et al., 2004]. In a previous study in Taiwan, Sun et al. [2004] also reported that between 1994 and 2003, one CRF07_BC isolate which was collected from a primary HIV-1 infection patient in a Taiwan hospital during the same period in this study. The studies also show the CRF07_BC and B/C recombination virus circulated around intravenous drug user patient since 2003 [Chang et al., 2006; Chen et al., 2006; Lin et al., 2007]. In this study, we further presented the earliest time point of B/C recombination virus transport from China to Taiwan. The URFs 07URF and CRF08_BC isolated from two Taiwan intravenous drug user patients who were born in Myanmar, although the transmission between these two intravenous drug user patients could have occurred either in their hometown or from sharing needles after arrival in Taiwan. The epidemiological data still suggest that the 07URF came from China since one patient had visited Yunnan previously. The virus isolate which detected as 07URF will be further sequenced into its full-length genome in order to discover the genome pattern of this isolates.

As shown in Figure 2B,C, the ratio of the subtype B versus non-B in the male population under study was 2.8:1 (760/269). However, this ratio was reversed in the female population at 0.25:1 (17/67). A change in trends and proportions of the subtypes is revealed from the temporal distribution between 1988 and 2000 (Fig. 2D). Subtype B and CRF01_AE represent the predominant forms, with subtype B derived chiefly from the male population, and CRF01_AE derived chiefly from the female population. These are correlated inversely; from 1988 to 1998 the proportion of subtype B gradually declined in relation to a gradual increase in CRF01_AE. Nevertheless, from 1999 onward the trend reversed itself. Other subtypes including A, C, D, and G, were detected since 1988 but their appearance is sporadic with no apparent trend. In addition, this study analyzed the subtype distribution in different risk-groups (Fig. 3). Similar patterns presented themselves in male homosexuals and bisexuals group. The distribution of these two risk groups increased between in 1999 and 2000. This finding suggested the virus rebound occurred among men who had sex with men. In addition, we should take note of the subtype profile for heterosexuals and intravenous drug users, which was heterogeneous as shown in Figure 3. The multiple subtypes present in

these groups might be caused by the multiple sources of virus isolate entry. In previous experience among Chinese intravenous drug users, multiple viral genotypes had been detected in Yunnan. Moreover, these viruses also spread rapidly among the intravenous drug user group [Zhang et al., 2004]. Further more, if we want to trace the origin of the subtype it would need to monitor the viruses in the intravenous drug users and heterosexual groups.

Furthermore, the previous observation gave us a lesson of the BC recombinant viruses spread and change very fast among intravenous drug users since they were be found in China during 1997. According to the date of HIV seropositive in this study, these BC recombinant viruses almost across the Taiwan Straits from China to Taiwan island immediately. As the SARS epidemic event, there should be concerned about the international public health issue of which these BC recombinant viruses would spread through the drug traffic routes over the sea quickly, especially Taiwan already suffered in a CRF07_BC epidemic since 2004 [Chen et al., 2006]. The cases in jail did not include in this study might cause the lower heterosexual group distribution than National data.

Although HIV-1 drug resistance is usually acquired during anti-HIV drug therapy, drug resistance can also be transmitted between individuals. The previous study showed that there was no major mutation among 11 isolates from patients in the primary HIV-1 infection stage during 1994–2003 in Taiwan [Sun et al., 2004]. But, primary genotypic DR mutations were detected in 1.6% (2/126) drug naïve patients in this study. The isolates from drug naïve patients contained mutations only in RT, while isolates from treatment experienced patients contained mutations in both Pro and RT. The nucleoside reverse transcriptase inhibitors (NRTI) such as zidovudine was introduced to Taiwan in 1987, followed by didanosine in 1992, deoxycysine in 1992, and deoxycytidine in 1995 [Fang et al., 2004]. On April 7, 1997, the government decided to provide free access to HAART to all HIV-infected citizens, and included NRTI and protease inhibitors (PI). The predominance of primary mutations in RT reflects the relatively longer use of RT targeted drugs, as compared with Pro targeted drugs which initiated more recently. The relatively low prevalence of primary genotypic DR mutations detected in Taiwanese samples collected between 1997 and 2000 is similar to earlier prevalence studies that demonstrate a temporal increase in primary DR isolates in newly infected individuals [Boden et al., 1999; Perez-Olmeda et al., 2001; UK Collaborative Group on Monitoring the Transmission of HIV Drug Resistance, 2001; Little et al., 2002].

For all drug naïve patient samples submitted to HIV-1 genotypic resistance testing, 92.1% (116/126) were subtype B, and 7.1% (9/126) were CRF01_AE. One had C. Subtype CRF01_AE, which is associated strongly with heterosexual transmission [Chen et al., 2001], and was highest in female commercial sex workers as well as detected in heterosexual male patients and intravenous drug users. Homosexual and bisexual groups contained all subtype B. The single subtype C isolate from female commercial sex workers, rarely detected in Taiwan, is more common in Southern China and Southeast Asia [Chen et al., 2001]. After comparing our findings with the study in China [Zhong et al., 2003], we found that secondary mutations were found in all non-B subtypes but in just 31% of the treatment naïve patient in China. This might because the free HARRT treatment was supported by the Taiwan government since 1997. Primary mutations were only associated with Subtype B in the treatment of naïve patients. However, the correlation of known DR mutations in non-B subtype as compared with B subtypes is not fully understood. The comparison of non-subtype B sequences in Pol and RT to corresponding Subtype B reference sequences may therefore not be adequate to determine drug resistance. Additional analyses comparing genotypic and phenotypic drug resistance assays on non-B subtypes are therefore necessary.

In 2000, the World Health Organization (WHO) Global HIV Drug Resistance Surveillance Programme, in collaboration with the International AIDS Society (IAS), concluded the need for a global drug resistance monitoring and surveillance program. In 2003, a WHO/IAS convened meeting in Bangkok established administrative guidelines, action items and recommendations to implement HIV-1 drug resistance surveillance in Asia [HIV Drug Resistance Program, WHO and CDC, 2004]. Our study which followed these guidelines, showed that the HIV-1 drug resistance for different risk-groups of drug naïve patient in Taiwan was less than 5% in the male heterosexual group and male bisexual group. In addition, the HIV drug resistance among male homosexual naïve patient was 5%. Accordingly, the Taiwanese Molecular Epidemiologic Study, which is a regionally developed working model defining cohort development, sample and patient survey collection, as well as methods used for HIV-1 genotypic resistance and subtype analysis, should potentially facilitate the rapid implementation of the WHOI/IAS HIV-1 drug resistance surveillance in Asia.

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