

Temporal Trends and Molecular Epidemiology of HIV-1 Infection in Taiwan From 1988 to 1998

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Summary: Eight hundred and seventy-nine HIV-1-infected patients (comprising 46% of reported HIV-1/AIDS cases in Taiwan) were recruited for this study of the molecular epidemiology of HIV-1 in Taiwan from 1988 to 1998. HIV-1 subtypes were determined using a modified peptide-enzyme immunoassay complemented with DNA sequencing and phylogenetic analysis. Of the 807 HIV-1 infected men, 68.2% were infected with HIV-1B, 29.5% with HIV-1 circulating recombinant form (CRF)01_AE and 2.3% with other subtypes. Of the 72 HIV-1-infected women, 72.2% were infected with HIV-1 CRF01_AE, 13.9% with HIV-1B, and 13.9% with other subtypes. All of 8 foreign-born, Southeast Asian women and 6 of 7 (85.7%) Taiwan-native female commercial sex workers were infected with HIV-1 CRF01_AE. Fourteen of the 33 (42.4%) heterosexual married men with CRF01_AE had transmitted HIV-1 to their wives, whereas only 1 of 17 (5.9%) men with HIV-1 B had transmitted HIV-1 to their spouses ($p < .01$). Of 18 heterosexual male injecting drug users, 1 of 12 (8.5%) with HIV-1B and 5 of 6 (83.3%) with HIV-1 CRF01_AE had had sexual contact with female commercial sex workers ($p < .01$). Therefore, in this population, CRF01_AE was preferentially associated with heterosexual risk groups, a finding compatible with differences in transmission capability between B and non-B subtypes. **Key Words:** HIV-1 subtypes—Molecular epidemiology—Heterosexual transmission—Injecting drug users—Female sex workers.

HIV-1 is characterized by a high degree of genetic diversity. Most HIV-1 isolates in the global epidemic cluster in the M (main) group, whereas fewer belong to O (outlier) and N (Non-M, Non-O) groups (1–3). The group M HIV-1 viruses include subtypes A, B, C, D, F, G, H, and J and several important circulating recombinant forms (CRFs) (<http://hiv-web.lanl.gov/>). The HIV pandemic was not widely recognized in Asia until 1988, when Thailand experienced an explosion of HIV-1 infection among injecting drug-users (IDUs), with rates of

infection rising from 1% to 40% in only 8 months (4). Since then, the HIV epidemic in Thailand has affected other countries in Asia. The first AIDS case registered in Taiwan and Thailand was the same person, an American who was seeking medical care in both countries while he was traveling in Asia in 1985 (C.-Y. Chuang, M.D., oral communication, November, 1998). The first indigenous AIDS case in Taiwan was found in 1986 (5). By September 2000, a cumulative total of 2,755 native HIV-1-infected people, of whom 962 were AIDS patients, had been reported to Taiwan's Department of Health. According to risk factor analysis, 85.7% of the cases resulted from sexual contact (ratio for heterosexual versus homosexual contact was 41.6%:44.1%), and the male:fe-

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male ratio of reported HIV-1/AIDS cases was 12:1. In only 1.9% of the HIV-1/AIDS cases in Taiwan was there a history of injecting drug use. The remaining cases resulted from vertical transmission (0.3%), coagulation factor usage (1.9%), blood transfusion (0.4%), and unknown causes (9.8%).

Global distribution of HIV-1 subtypes shows that multiple subtypes may exist in different geographic regions (6). It has been reported that there were three main HIV-1 subtypes in Asia: B, C, and E (CRF01_AE) (7–13). Several epidemiologic studies have shown that HIV-1 subtypes are segregated among people with different risk behaviors (14,15). Previously, in a multicenter survey in Taiwan from 1993 to 1996, we found that among men who had subtype B infection, 71% were homosexual or bisexual, whereas among men who had CRF01_AE, 56% were heterosexuals ($p < .001$). In addition, most Taiwanese women were infected with subtype E (CRF01_AE) (13). To verify and monitor the trends of distribution of different HIV-1 subtypes in several high-risk populations, we invited more AIDS treatment centers from different regions in Taiwan to participate in this survey. Therefore, the data collected for this study represent 46% of all HIV-1/AIDS cases reported to the Department of Health of the Republic of China (Taiwan) by the end of 1998.

STUDY SUBJECTS AND METHODS

Patients

Five hundred and ninety-one serum samples were collected from HIV-1-infected patients seen at AIDS treatment centers in different regions in Taiwan between October 1996 and December 1998. The treatment centers included the Taipei Venereal Disease Control Institute, Taipei Veterans' General Hospital, Cheng-Kung University Hospital in Tainan, Kaohsiung Medical University Hospital, and Chinese Medical and Pharmacological School Hospital in Taichung. In addition, molecular epidemiologic data from 288 HIV-1/AIDS patients collected between 1993 and 1996 were also included for trend analysis (13). A short history including date of the first diagnosis, possible risk factors, sexually transmitted diseases, current symptoms, CD4 cell counts, and medication profile was obtained from the patients. Informed consent was obtained from all patients who participated in this study. All serum samples were stored at -80°C before serologic assays were undertaken.

Peptide-Enzyme Immunoassay (PEIA) for Subtyping

An EIA plate containing 8 V3 loop synthetic peptides from 6 HIV-1 subtypes (A, B, C, D, E, and G) was developed for subtyping. Each serum sample was diluted at 1:100 and aliquoted into different wells on the same lane of the EIA plate for subtyping.

The amino acid sequences (one letter code was used) of the synthetic peptides were as follows: A, RKSVHIGPGQAFYATGDIIGD; B, RK-SIHIGPGRAFYTTGEIIGD; B', RKRIHIGPGRAFYTTGDIIGD; C,

RKSIRIGPGQTFYATGGIIGD; D, NTRQRTHFPGQALYTTRII; E, RTSITIGPGQVFYRTGDIIGD; E', YNTRTKITRGPRVFYRT-GDM; and G, RKSIRIGPGQTLATGAIIGD. Detailed procedures of the EIA have been described previously (13). The subtype was determined according to the following principles: the highest optic density (OD) of antibody binding to a particular peptide and differences in ODs greater than 1. If the subtypes could not be determined using these principles, the PEIA was repeated with lower or higher dilutions of the serum samples.

RNA Extraction

For those samples that could not be determined using PEIA, HIV-1 RNA was extracted for reverse transcriptase-polymerase chain reaction (RT-PCR). The HIV-1 pellet was obtained from 1-ml plasma by centrifugation at 14,000 rpm for 90 minutes (Eppendorf centrifuge model 5420, Hamburg, Germany). GTC solution (4.5 M guanidine isothiocyanate, 25 mM sodium citrate, pH7, 0.5% sarkosyl, 0.1 M 2-mercaptoethanol) was mixed well with the virus pellet. Then, the HIV-1 RNA was extracted with 50 μl 3M sodium acetate (pH5.2), 50 μl acid phenol (pH5.4) and 200 μl chloroform:isoamyl alcohol solution. The mixture was refrigerated for 15 minutes before centrifugation at 14,000 rpm for 15 minutes. The supernatant was extracted using the same procedure. HIV-1 RNA was alcohol precipitated from the final supernatant. The resultant RNA pellet was dissolved in 20 μl water treated with diethyl pyrocarbonate (DEPC).

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

For RT, 2 μl RNA was mixed with 2 μl primer ED14 (5 μM) (14) and placed at 65°C and room temperature for 5 and 10 minutes, consecutively. Next, 2 μl 10 \times RT buffer (Stratagene), 0.5 μl deoxyribonucleoside triphosphate (100 mM), 1 μl RNase inhibitor (40 U μl), 0.5 μl Moloney murine leukemia virus RT (50 U μl , Stratagene) and 12 μl DEPC-treated water were added into the mixture and incubated at 37°C for 90 min. After being heated at 90°C for 5 minutes, the reaction was refrigerated and readied for PCR assay. The PCR was 96°C for 15 seconds, 50°C for 45 seconds, and 72°C for 1 minute for 40 cycles. The condition was as specified by the manufacturer (Perkin Elmer Cetus, Norwalk, CT, U.S.A.), except that the concentration of MgCl_2 was 2 mM (15). The primer pairs used in this study were ED3/ED14 (for the first round PCR), ED5/ED12 (second round primers for the heteroduplex mobility assay) and ES7/ES8 (second run primer pair for automated DNA sequencing), as described by Delwart et al. (14).

DNA Sequencing and Phylogenetic Analysis

The sequencing method has been described previously (13). The nucleotide sequences from different HIV-1 isolates were edited using the MacDNASIS program (Version 3.0, Hitachi Software Engineering Co., San Bruno, CA, U.S.A.) and the resultant 318 base-pair fragment representing the C2-V3-C3-V4 domains (7062 to 7379 nucleotide residues of HXB2) were subjected to phylogenetic tree analysis according to the procedures reported previously (13).

Statistical Analysis

Fisher exact and χ^2 tests were performed in univariate analysis to find the statistical significance for all comparisons between patient

groups with different risk factors, or patients infected with HIV-1 subtype B or CRF01_AE.

RESULTS

In this study, blood samples and questionnaires were collected from 540 male and 51 female HIV-1-infected patients. Those risk factors associated with HIV-1 infection were analyzed in conjunction with 288 cases previously collected from 1993 to 1996 (13). In total, this cohort consisted of 807 men and 72 women, who represent, respectively, about 41% and 45% of the male and female HIV/AIDS cases reported to the Department of Health of the Republic of China (Taiwan). The sex ratio (male:female) of the cohort in this study was 11.2:1, which is similar to the Taiwanese national registration statistics. In addition, the distributions of different risk-groups in both cohorts mentioned above were compatible, except that the percentage of the unknown risk-factor group in this study was much lower than that of the Taiwanese national registration data (Table 1).

Demographic Data and Risk Factors of the Cohort

Over 70% of the HIV-1-infected people in this study were between the ages of 20 and 39 (Table 2). Both the mean ages of male heterosexuals and bisexuals were significantly older than that of male homosexuals (*t*-test, $p < .001$). Among men, the education level of homosexuals was significantly higher than that of either heterosexuals or bisexuals (χ^2 test, $p < .001$). The general education level of the men was significantly higher than that of the women (χ^2 test, $p < .001$). Among women, 36.5% (19 of 68) had only a primary school level education.

TABLE 1. Comparisons of the distributions of different genders and risk factors between two HIV/AIDS-patient cohorts

	National registration ^a	This study
Gender		
Men	1,980 (92.6%)	807 (91.8%)
Women	159 (7.4%)	72 (8.2%)
Total	2,139 (100%)	879 (100%)
Risk factors		
Heterosexuals	868 (40.6%)	373 (42.4%)
Male homosexuals	592 (27.7%)	324 (36.9%)
Male bisexuals	365 (17.1%)	136 (15.5%)
Injecting drug users	53 (2.5%)	22 (2.5%)
Patients with hemophilia	51 (2.4%)	12 (1.4%)
Blood transfusion	9 (0.4%)	1 (0.1%)
Vertical transmission	3 (0.1%)	1 (0.1%)
Unknown	198 (9.3%)	10 (1.1%)

^a Statistics provided by the Taiwan Department of Health at the end of 1998.

In this cohort, both male heterosexuals and bisexuals had significantly higher rates of sexual contact with commercial sex workers than male homosexuals (χ^2 test, $p < .001$). Nevertheless, both male homosexuals and bisexuals had significantly higher rates of sexually transmitted infections (STIs) than male heterosexuals (χ^2 test, $p = .0027$ and $.0026$, respectively). As indicated in Table 2, 44% of the homosexuals and 52% of the bisexuals in this cohort had histories of syphilis infection. In terms of the status of the disease when they were found to be HIV-1 seropositive, 38.7% of the male heterosexuals had CD4 cell counts $<200/\text{ml}$. In contrast, only 23.6% and 25.1% of the male bisexuals and homosexuals had CD4 cell counts $<200/\text{ml}$.

HIV-1 Subtyping

An EIA plate containing eight synthetic peptides from six HIV-1 subtypes was developed for rapid serotyping. Thirty serum samples from HIV-1-infected patients whose genotypes have been determined previously (13) were used in a single-blind test to verify the sensitivity and specificity of the modified PEIA. The samples included 1 HIV-1A, 14 HIV-1B, 2 HIV-1C, 12 CRF01_AE, and 1 HIV-1G. Results showed that the subtypes that were determined using PEIA were consistent with that defined by phylogenetic tree analysis.

Subsequently, the subtypes of 494 cases were determined using PEIA at a dilution of 1:100. Eighty-seven of the remaining 97 patients were repeatedly tested with lower dilutions of their serum samples and their subtypes were determined. Therefore, 98.3% (581 of 591) of the HIV/AIDS patients' subtypes could be decided using PEIA in this study. Among 10 patients whose subtypes could not be determined by PEIA, 4 were determined using RT-PCR and found to be HIV-1B.

Distribution of HIV-1 Subtypes in Different Risk Groups

Data of HIV-1 subtypes from 591 HIV/AIDS patients were pooled with that of 288 cases from the previous study (13) and analyzed. The percentages of different subtypes in this cohort were as follows: HIV-1A, 0.5%; HIV-1B, 63.7%; HIV-1C, 1.6%; HIV-1D, 0.1%; CRF01_AE, 33.0% and HIV-1G, 0.5% (Fig. 1A). As shown in Figure 1B and C, the ratio of the B versus non-B subtypes in male study subjects was 2.4:1 (68%:28%), whereas this ratio was reversed among female study subjects (0.37:1 or 26%:71%). In addition, there were variations in the ratio of B versus non-B sub-

TABLE 2. Demographics and risk factors of different groups of HIV-infected patients from Taiwan

	Men			Women (N = 68)	IVDU (N = 22)	Hemophiliacs (N = 12)	Total ^a (No./%) (N = 879)
	Heterosexual (N = 305)	Homosexual (N = 324)	Bisexual (N = 136)				
Age:							
≤19	5 (1.7)	9 (3.0)	4 (3.0)	3 (4.7)	0 (0)	1 (9.1)	24 (2.9)
20–29	80 (27.9)	160 (53.9)	41 (30.8)	25 (39.1)	5 (26.3)	4 (36.4)	319 (38.9)
30–39	106 (36.9)	101 (34.0)	59 (44.4)	21 (32.8)	10 (52.6)	3 (27.3)	302 (36.8)
40–49	49 (17.1)	22 (7.4)	15 (11.3)	9 (14.1)	2 (10.5)	2 (18.2)	101 (12.3)
50–59	26 (9.1)	4 (1.3)	6 (4.5)	3 (4.7)	2 (10.5)	1 (9.1)	42 (5.1)
≥60	21 (7.3)	1 (0.3)	8 (6.0)	3 (4.7)	0 (0)	0 (0)	33 (4.0)
Total ^a	287 (100)	297 (100)	133 (100)	64 (100)	19 (100)	11 (100)	821 (100)
Mean ^b	37.4 ± 12.9	29.3 ± 7.4	34.8 ± 11.8	32.8 ± 11.4	33.6 ± 9.1	32.7 ± 11.8	33.4 ± 11.4
Education (yr) ^{c,d}							
≤6	30 (11.2)	8 (2.7)	16 (12.3)	19 (36.5)	5 (25.0)	0 (0)	82 (10.5)
7–9	53 (19.9)	24 (8.2)	17 (13.1)	7 (13.5)	5 (25.0)	5 (50)	113 (14.5)
10–12	152 (56.9)	195 (66.3)	75 (57.7)	23 (44.4)	9 (45.0)	5 (50)	461 (58.8)
≥13	32 (12.0)	67 (22.8)	22 (16.9)	3 (5.8)	1 (5.0)	0 (0)	126 (16.1)
Total	267 (100)	294 (100)	130 (100)	52 (100)	20 (100)	10 (100)	782 (100)
Marital status							
Single	155 (54.0)	281 (93.6)	90 (68.2)	21 (31.8)	16 (76.2)	8 (80)	580 (70.2)
Married	111 (38.7)	12 (4.0)	29 (22.0)	33 (50.0)	3 (14.3)	2 (20)	191 (23.1)
Divorced	15 (5.2)	6 (2.0)	9 (6.8)	4 (6.1)	1 (4.8)	0 (0)	35 (4.2)
Widow(er)	4 (1.4)	1 (0.3)	2 (1.5)	7 (10.6)	0 (0)	0 (0)	14 (1.7)
Separation	2 (0.7)	0 (0)	2 (1.5)	1 (1.5)	1 (4.8)	0 (0)	6 (0.7)
Total	287 (100)	300 (100)	132 (100)	66 (100)	21 (100)	10 (100)	826 (100)
Travel							
To any country outside							
Taiwan	167 (57.4)	131 (41.7)	50 (39.4)	28 (49.1)	11 (52.4)	1 (8.3)	389 (50.6)
To Southeast Asia	123 (42.3)	60 (19.1)	33 (26.0)	21 (36.8)	10 (47.6)	0 (0)	241 (31.3)
Sexual contact with							
commercial sex workers ^e	145 (49.8)	52 (16.6)	39 (30.7)	0 (0)	5 (23.8)	0 (0)	241 (31.3)
Sexually transmitted							
diseases ^f	(N = 122)	(N = 106)	(N = 56)	(N = 20)	(N = 8)	—	(N = 312)
Gonorrhea	8 (6.5)	3 (2.3)	1 (1.8)	0 (0)	1 (12.5)	—	13 (4.2)
Syphilis	31 (25.2)	47 (44.3)	29 (51.8)	1 (5.0)	1 (12.5)	—	109 (34.9)
Others	4 (3.3)	8 (7.5)	3 (5.4)	0 (0)	0 (0)	—	15 (4.8)
Total	43 (35.0)	58 (54.7)	33 (58.9)	1 (5.0)	2 (25)	—	137 (43.9)
CD4 cell counts ^g							
<200	92 (38.7)	62 (25.1)	25 (23.6)	14 (31.1)	5 (22.7)	4 (36.4)	202 (30.4)
200–499	108 (45.4)	138 (55.9)	52 (49.1)	28 (62.2)	10 (45.5)	6 (54.6)	337 (50.7)
≥500	38 (16.0)	47 (19.0)	29 (27.4)	3 (6.7)	7 (31.8)	1 (9.1)	126 (18.9)
Total	238 (100)	247 (100)	106 (100)	45 (100)	22 (100)	11 (100)	665 (100)

^a Age data for some populations were missing.^b Mean ages of both HIV-1-infected male heterosexuals and bisexuals were significantly above those of HIV-1-infected male homosexuals, $p < .001$.^c The education level of HIV-1-infected men was significantly higher than that of the HIV-1-infected women, $p < .001$.^d The education level of HIV-1-infected male homosexuals was significantly higher than that of either male heterosexuals or bisexuals, $p < .001$.^e Both the HIV-1-infected male heterosexuals and bisexuals had significantly higher rates of having sexual contact with female commercial sex workers than HIV-1-infected male homosexuals, $p < .001$.^f Both the HIV-1-infected male homosexuals and bisexuals had significantly higher rates of having sexually transmitted diseases than HIV-1-infected male heterosexuals, $p = .0027$ and $.0026$, respectively.^g The mean of T4 cell counts of HIV-1-infected male bisexuals was significantly higher than that of HIV-1-infected male heterosexuals, $p = .0165$. IVDU, (illicit) intravenous drug user.

types in the different male subgroups. The ratio of B versus non-B subtypes varied from 1.1:1 (52%: 48%) in the heterosexual men to 3.8:1 (79%: 21%) in the homosexual/bisexual men (Table 3).

Furthermore, 134 of 238 (56.3%) men infected with CRF01_AE and 157 of 550 (28.5%) men infected with HIV-1B were heterosexual ($p < .001$). Conversely,

66.0% (363 of 550) men infected with HIV-1B were homosexual/bisexual, whereas only 39.1% (93 of 238) men infected with CRF01_AE were homosexual/bisexual ($p < .001$).

Two hundred and thirty-eight persons admitted that they had had sexual contact with commercial sex workers before contracting HIV infection, and the country in

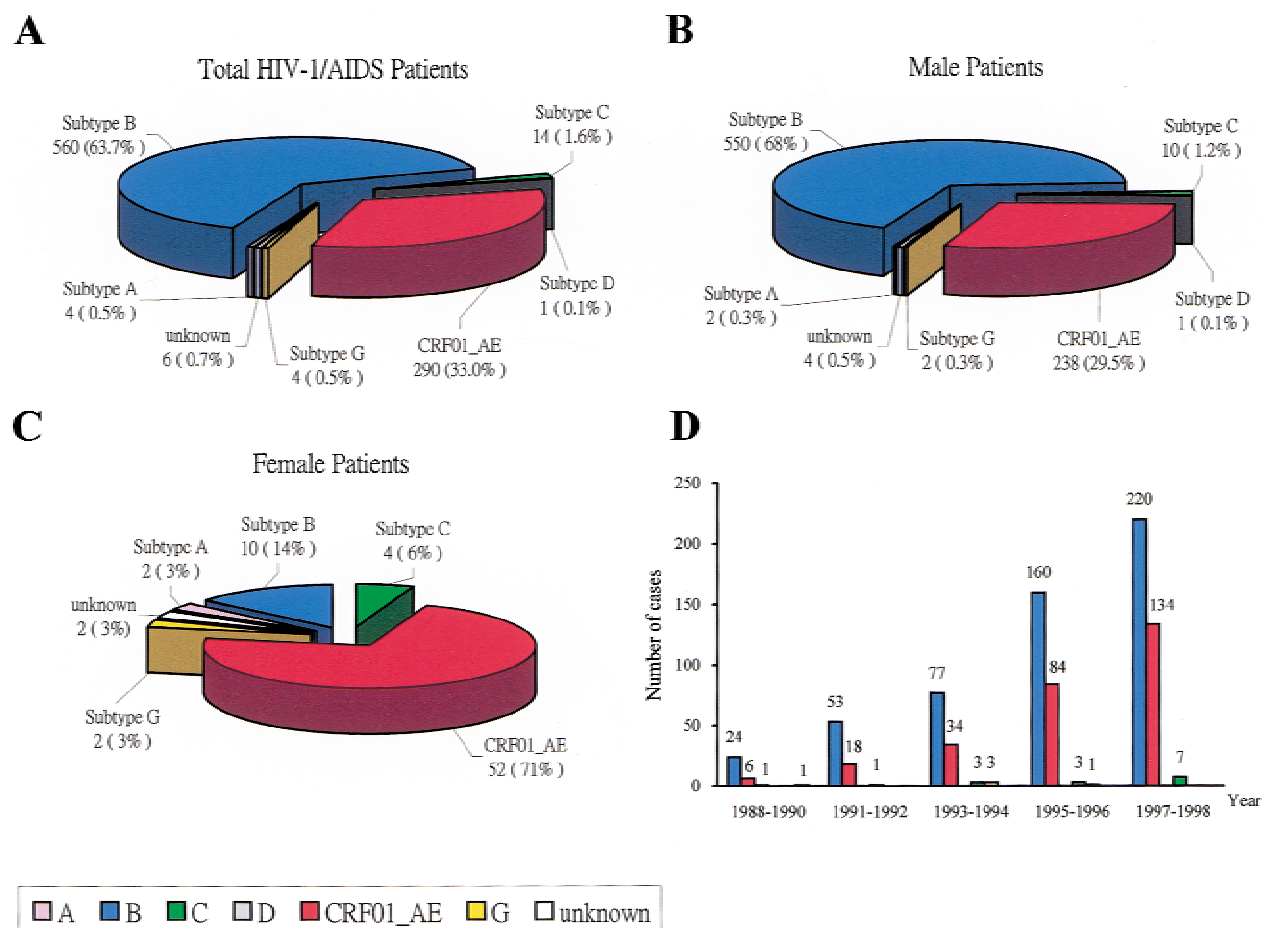


FIG. 1. Proportions of HIV-1 subtypes in different groups of patients from 1988 to 1998 in Taiwan. (A) Total patients. (B) Male patients. (C) Female patients. (D) Numbers of different HIV-1 subtypes found from 1988 to 1998.

which this contact took place was also noted. As shown in Table 4. In contrast, men with HIV-1B infection had significantly higher rate of contact with Taiwanese commercial sex workers than men with CRF01_AE infection (88.3% vs. 30.8%).

The 12 patients with hemophilia who were included in this study were all infected with subtype B. In terms of IDUs, there were 12 heterosexuals and 2 homosexuals. According to risk factor analysis, 83.3% (5 of 6) of men infected with CRF01_AE and only 8.3% (1 of 12) men

TABLE 3. Distribution of HIV-1 subtypes in different male groups in Taiwan

HIV-1 subtype/CRF	Male HIV-AIDS patients (No./%)							Total
	Heterosexual	Homosexual	Bisexual	Hemophiliacs	Heterosexual IDU	Homosexual IDU	Others ^a	
A	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (7.1)	1 (10.0)	2 (0.2)
B	157 (51.5)	252 (77.8)	111 (81.6)	12 (100)	12 (66.7)	1 (50.0)	4 (40.0)	550 (68.2)
C	9 (3.0)	1 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	10 (1.2)
D	1 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.1)
CRF01_AE	134 (43.9)	68 (21.0)	25 (18.4)	0 (0)	6 (33.3)	1 (50.0)	5 (50.0)	238 (29.5)
G	2 (0.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (0.2)
Untypable	1 (0.3)	3 (0.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (0.5)
Total	305 (100)	324 (100)	136 (100)	12 (100)	18 (100)	2 (100)	10 (100)	807 (100)

^a Includes 1 HIV-1 CRF01_AE who was infected through vertical transmission. IDU, injecting drug user; CRF, circulating recombinant form.

TABLE 4. Rates of sexual contact with female commercial sex workers (FCSWs) in patients infected with different HIV-1 subtypes or circulating recombinant forms (CRFs)

Geographic region in which sexual contact occurred	Patients with different HIV-1 subtypes/CRFs (No./%) ^a					
	B	C	D	CRF01_AE	G	Total
	(N = 154)	(N = 4)	(N = 1)	(N = 78)	(N = 1)	(N = 238)
Taiwan	136 (88.3)	1 (25.0)	0 (0)	42 (30.8)	1 (100)	180 (75.6)
Southeast Asia	40 (26.0)	1 (25.0)	1 (100)	39 (50.0)	0 (0)	81 (34.0) ^b
Other regions	27 (17.5)	2 (50.0)	0 (0)	12 (15.4)	0 (0)	41 (17.2)

^a The patients might have sexual contact with FCSWs from more than one geographic region.

^b Men who had contact with FCSWs in Southeast Asia exhibited a much higher rate of HIV-1 CRF01_AE infection than HIV-1B infection ($p < .001$).

infected with HIV-1B had histories of sexual contact with female commercial sex workers ($p < .01$). Therefore, sexual transmission is a major risk factor for male IDUs infected with CRF01_AE in this cohort.

Distribution of HIV-1 Subtypes in Different Female Groups

As shown in Table 5, among 72 HIV-1-infected females, 2 (2.8%) were infected with HIV-1A, 10 (13.9%) with HIV-1B, 4 (5.6%) with HIV-1C, 52 (72.2%) with CRF01_AE, and 2 (2.8%) with HIV-1G. The rates of CRF01_AE infection among heterosexual females, commercial sex workers, and Southeast Asian immigrants were 70.4% (38/54), 85.7% (6/7), and 100% (8/8), respectively. Two female IDUs were infected with HIV-1B.

Subtype Analysis Among HIV-1 Antibody Seroconcordant and Serodiscordant Married Couples

Among 152 married men in this cohort, 111 (73.0%) were heterosexual. The statuses of HIV-1 infection of those heterosexual men's wives were traced. The results

showed that 17 of 52 (32.7%) wives were concordant. All HIV-1-infected wives of study subjects were further interviewed and all denied having other risk factors of contracting HIV-1 infection. When the subtypes of both the husbands and wives were analyzed, as shown in Table 6, 14 of 33 (42.4%) husbands infected with CRF01_AE had transmitted HIV-1 to their spouses, whereas only 1 of 17 (5.9%) men with subtype B had transmitted HIV-1 to their wives ($p < .01$). Both of the 2 husbands infected with subtype C had transmitted HIV-1 to their wives.

Trends of HIV-1 Subtypes' Distributions in Different Groups

The subtype distribution among different risk groups was further analyzed chronologically according to the year when the patients were diagnosed as seropositive. As shown in Figure 1D, HIV-1B has been the major subtype of HIV-1 infection in Taiwan since 1988. However, other subtypes have emerged, especially CRF01_AE, since 1989. HIV-1C has been identified in several heterosexual men since 1993. In addition, the rate of CRF01_AE infection has surpassed that of HIV-1B infection in the male heterosexual population since 1997

TABLE 5. Distribution of HIV-1 subtypes in different female populations in Taiwan

HIV-1 subtype/CRF	Female HIV/AIDS patients (No./%)					
	Heterosexual	Commercial sex worker	Foreign brides ^a	IDUs	Other ^b	Total
A	2 (3.7)	0 (0)	0 (0)	0 (0)	0 (0)	2 (2.8)
B	8 (14.8)	0 (0)	0 (0)	2 (100)	0 (0)	10 (13.9)
C	4 (7.4)	0 (0)	0 (0)	0 (0)	0 (0)	4 (5.6)
CRF01_AE	38 (70.4)	6 (85.7)	8 (100)	0 (0)	1 (50.0)	52 (72.2)
G	1 (1.9)	1 (14.3)	0 (0)	0 (0)	0 (0)	2 (2.8)
Untypable	1 (1.9)	0 (0)	0 (0)	0 (0)	1 (50.0)	2 (2.8)
Total	54 (100)	7 (100)	8 (100)	2 (100)	2 (100)	72 (100)

^a Including 1 patient from Cambodia, 3 from Myanmar, 3 from Thailand, and 1 from Indonesia.

^b Including 1 patient with HIV-1 CRF01_AE who had been infected through blood transfusion. CRF, circulating recombinant form; IDU, injecting drug user.

TABLE 6. Sexual transmission of HIV-1 infection among married couples in which the husbands were infected with different HIV-1 subtypes or circulating recombinant forms (CRFs)

Husbands infected with different HIV-1 subtypes	Transmitted to their wives ^a	
	Yes	No
B (N = 17)	1 (5.9%)	16 (96.1%)
CRF01_AE (N = 33)	14 (42.4%)	19 (57.6%)
C (N = 2)	2 (100%)	0 (0%)
Total (N = 52)	17 (32.7%)	35 (67.3%)

^a Husbands with HIV-1 CRF01_AE infection, compared with husbands with HIV-1B infection, had a significantly higher rate of sexual transmission to their wives, $p < .01$.

to 1998 (Fig. 2A). Although subtype B was still the dominant subtype in bisexual and homosexual men, the increase of CRF01_AE in the bisexual men in recent years has been noted (Fig. 2C).

In the female population, CRF01_AE was detected in 1991 and has since become the dominant subtype (Fig.

2D). HIV-1C infection has been found in this group since 1995, 2 years after such infections were found in the male population.

DISCUSSION

There are several important characteristics of the AIDS epidemic in Taiwan: more than 80% of infection was due to sexual transmission, less than 2% of infection was due to needle sharing, and mother-infant transmission was rare.

As reported previously by us and other groups, there was a good correlation between the serotype determined by PEIA and the genotype defined by phylogenetic analysis (13,16,17). In this study, because each serum sample was reactive to eight synthetic peptides representing different subtypes in one EIA plate, the modified PEIA did not require normal human serum controls to calculate the cut-off values for each plate.

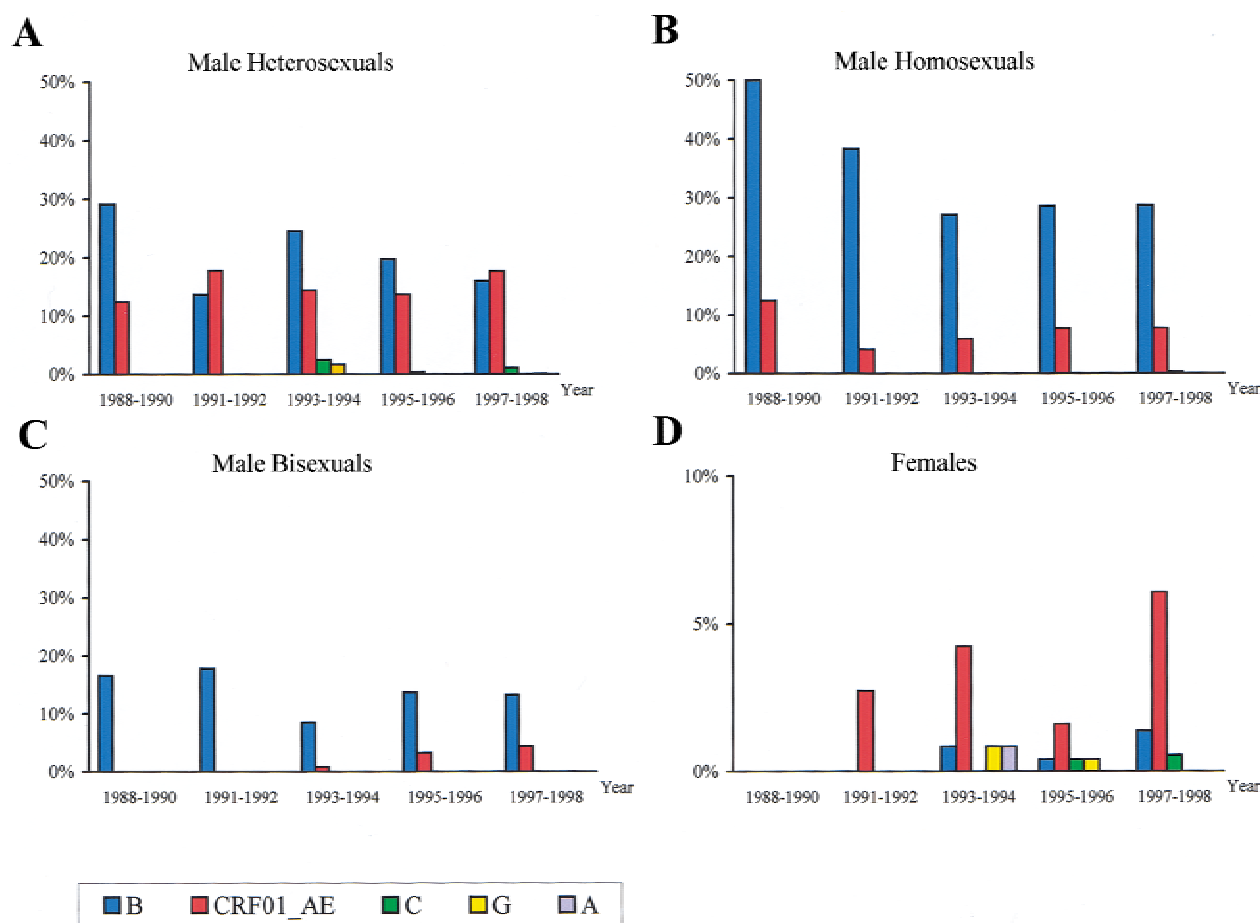


FIG. 2. Temporal trends of proportions of various HIV-1 subtypes detected in different groups of patients from 1988 to 1998 in Taiwan. (A) Male heterosexuals. (B) Male homosexuals. (C) Male bisexuals. (D) Women. Percentages are based on the total number of HIV-1 cases for each year.

In Taiwan, the HIV-1-infected homosexual men were much younger than the HIV-1-infected heterosexual men (Table 2). In addition, the homosexual/bisexual patient group had both a larger number and higher percentage of persons younger than 20 than the heterosexual group, 3.0% (13 of 430) versus 1.7% (5 of 287), suggesting that homosexual/bisexual men might be engaging in high-risk behaviors at a younger age than heterosexual men in Taiwan. Among different high-risk groups in Taiwan, men who have sex with men (MSM) have the highest rate of HIV-1 infection. Recently, in a survey conducted in Kaohsiung city of Taiwan, we found that 8 of 108 (7.4%) MSM from bars frequented by homosexuals and 22 of 139 (15.8%) MSM from saunas frequented by homosexuals had HIV-1 infection (18). In this study, both the male homosexual and bisexual patients had significantly higher rates of syphilis infection than the heterosexual men. The synergistic relationship between HIV infection and certain STIs, especially genital ulcer diseases, has been reported previously (19). Therefore, it is important to develop an effective AIDS and STI prevention program for MSM in Taiwan.

Changes over time in the heterosexual population are very striking. When looking at the entire period of study, more than half of heterosexual males had HIV-1 B infection, but CRF01_AE surpassed HIV-1 B and became the major subtype in 1997 to 1998 (Figure 2A). According to the risk factor analysis, nearly half the heterosexual males admitted that they had sexual contact with female commercial sex workers when they went to Southeast Asia (Table 2). This is consistent with the results of the phylogenetic analysis reported previously, which showed that most of the Taiwanese CRF01_AE isolates clustered with CRF01_AE isolates from Thailand (13).

In the female population, over 85% were infected with non-B subtypes, with CRF01_AE as the predominant subtype (Fig. 1C). Because of this, we decided to analyze the rates of sexual transmission of different HIV-1 subtypes among married couples. To avoid other confounding factors, we chose only couples in which the husband was heterosexual. Men with CRF01_AE infection had a significantly higher rate of sexual transmission to their wives than those with HIV-1B infection (Table 6). Because the conclusion depends on the self-reported heterosexuality of the men in this study, a special effort has been made to ask physicians and social workers to confirm the sexual orientation of their patients. In addition, all wives who appeared to have been infected by their husbands were infected with the same subtype as their husband. Due to the study design, information regarding frequencies of sexual intercourse, condom use,

and other risk factors was unavailable for analysis. Because HIV-1B was present in the male population as early as 1988 and CRF01_AE did not appear until 1989, the prevalence of spousal infection with CRF01_AE cannot be explained as a temporal trend. Therefore, in this study, CRF01_AE was preferentially associated with heterosexual risk groups, a finding compatible with differences in transmission capability between B and non-B subtypes.

Previously, in a case-control study of heterosexual couples in Thailand, it has been shown that regular sex partners of men infected with CRF01_AE exhibited a significantly higher rate of seroconversion than women whose sexual partners were infected with HIV-1B (21). One aspect looked at in the study mentioned was the mode of acquisition of HIV-1 infection in the male index case: the cohort of men with CRF01_AE were mainly infected through sexual intercourse, whereas the cohort of men infected with HIV-1B were mainly drug users. In addition, some of the female patients had other risk factors such as needle-sharing (20). Such features were not present in this report.

In this study, among 7 female sex workers (FSW), 6 were infected with CRF01_AE and 1 with HIV-1 G. This suggests that there is great risk of increasing spread of CRF01_AE in the heterosexual population in Taiwan. Previously, in a survey of HIV-1 and STIs in FSWs in the Taipei metropolitan area from 1993 to 1996, 0.2% (2/1,036) of FSWs from karaoke bars were found to have HIV-1 infection (21). More comprehensive targeted interventions for female commercial sex workers need to be carried out immediately to counteract the spread of HIV successfully.

HIV prevalence among Taiwanese IDUs has never been systematically studied. It has been estimated that there are 60,000 IDUs in Taiwan. There is relatively little control of the medical supplies' market in Taiwan, and syringes are easy to obtain. Because of this, there appears to be a much lower rate of needle-sharing in Taiwan (with the possible exception of the prison inmate population). Among 18 heterosexual male IDUs who participated in this study, 12 were infected with HIV-1B and 6 were infected with CRF01_AE. In addition, 5 of 6 IDUs with CRF01_AE had histories of sexual contact with female commercial sex workers compared with only 1 of 12 IDUs. Therefore, sexual transmission is an important risk factor for male IDUs infected with CRF01_AE in Taiwan. In a study of the IDU population in Thailand, 65% were infected with HIV-1B (Thai B) and 35% with CRF01_AE. Younger age was a risk factor for IDUs infected with CRF01_AE; sexual risk behavior was not implicated (22). Further phylogenetic analysis may be

useful in elucidating the relationship between the CRF01_AE isolates from the heterosexual population and from the IDU group in this study.

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