

## Molecular Epidemiology and Trends of HIV-1 Subtypes in Taiwan

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**Summary:** To understand the trends of distribution and risk factors associated with different HIV-1 subtypes in different populations in Taiwan, blood samples and questionnaires were collected from 267 male and 21 female HIV-1-infected people in a multicenter survey from 1993 to 1996. This group represented about one quarter of the total registered HIV-1 cases in Taiwan. The HIV-1 subtypes were determined using V3-based peptide-enzyme immunoassays complemented by heteroduplex mobility assay and phylogenetic tree analysis. The results showed that in Taiwan, men were primarily infected with HIV-1B (68.2%) and HIV-1E (27.3%), whereas women were mainly infected with non-B subtypes (4.8% A, 4.8% C, 71.4% E, and 9.5% G). In addition, 71.4% of men with HIV-1B were homosexual or bisexual, whereas 56.2% of men with HIV-1E were heterosexual ( $p < .001$ ). Although HIV-1E subtype came to Taiwan later than HIV-1B, it has become a major subtype in the heterosexual population. **Key Words:** HIV-1 subtypes—Molecular epidemiology—Heteroduplex mobility assay—HIV-1 genetic sequences—Taiwan.

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The HIV pandemic did not obviously manifest its arrival in Asia until the period from 1987 to 1988 when Thailand experienced an explosion of HIV-1 infection among intravenous drug users (IDUs), with rates rising from 1% to 40% in only 8 months (1). At present, at least 1% of the country's 55 million population carries the virus (2). The HIV epidemic in Thailand may affect, or repeat itself, in other countries in Asia. In Taiwan, the first indigenous AIDS case was not reported until 1986 (3). By December 12, 1997, a cumulative total of 1484 HIV-1-infected people, of whom 516 were AIDS cases, had been reported to the Department of Health. Most cases had been detected in the past 5 years. According to risk factor analysis, sexual contact was the main route of transmission and the male:female ratio of HIV-1 infection was 12:1 (4). Only 3% of the HIV-1/

AIDS cases in Taiwan had a history of intravenous drug use (4).

Deduced from the variation of env and gag sequences, it has been demonstrated that, besides the outlier (O) group (5), 10 HIV-1 subtypes (A–J) in the major group (M) of HIV-1 infection exist in the world (6,7). The global distribution of these genotypes shows that multiple subtypes may exist in different geographic regions (8). It has been reported that 3 main HIV-1 subtypes were present in Asia: B, C, and E (9–15). Previously, epidemiologic studies in Thailand and South Africa showed that HIV-1 subtypes segregate among people with different risk behaviors (9,16). It is important to determine whether HIV-1 subtype segregation occurs in different risk groups in other areas, such as Taiwan, and to determine the factors that contribute to any segregation. About one quarter of the total HIV-1/AIDS cases registered at the Department of Health of the Republic of China participated in the study. Their HIV-1 subtypes were determined and associated risk factors were analyzed. In addition, the trends of distribution of HIV-1

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subtypes in different Taiwanese populations were analyzed.

## SUBJECTS AND METHODS

### Patients

Between April 1993 and September 1996, 288 serum and/or heparinized whole blood samples were collected from HIV-1-infected patients attending AIDS treatment centers in different regions in Taiwan. The treatment centers included Taipei Venereal Disease Control Institute, Taipei Veterans' General Hospital, National Cheng-Kung University Hospital, and Kao-shiung Medical School Hospital. In addition, serum samples dating back to 1988, which had been sent to the centralized laboratory of the National Institute of Preventive Medicine for HIV-1 confirmatory tests, were also included in this study. A short history including date of the first diagnosis, possible risk factors, current symptoms, T4 cell counts, and medication taken was obtained from the patients. Informed consent was obtained from all patients who participated in this study. All serum samples were stored at  $-80^{\circ}\text{C}$  before serologic assays.

### Isolation of Viruses

Lymphocyte separation medium (Organon Teknika Corporation, Durham, NC, U.S.A.) was used to isolate peripheral blood mononuclear cells (PBMCs) from patients. A T-cell line (SUP-T1) (17) and PBMCs from healthy donors were used for virus isolation.

### Polymerase Chain Reaction and Heteroduplex Mobility Assay

For the polymerase chain reaction (PCR) assay, conditions were as recommended by the manufacturer (Perkin Elmer Cetus, Norwalk, CT, U.S.A.), except that the concentration of  $\text{MgCl}_2$  was 2 mM (18). 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 [HMA]) and ES7/ES8 (second-run primer pair for automated DNA sequencing) as described by Delwart et al. (19). For HMA, 5  $\mu\text{l}$  (containing 100–250 ng of DNA) of second-round product from each sample was mixed with 5  $\mu\text{l}$  of homologous product from a subtype reference and 1  $\mu\text{l}$  of 10 $\times$  reannealing buffer (1M NaCl, 100 mM Tris, pH 7.8, 20 mM ethylenediaminetetraacetic acid [EDTA]). The mixture was heated to  $94^{\circ}\text{C}$  for 2 minutes, cooled rapidly on ice, mixed with 1/5 volume of 5 $\times$  loading dye (25% Ficoll, 1% orange G), and loaded onto a 5% polyacrylamid gel. Electrophoresis was done at 70 mA (constant) for 1000 V-hr (19). Subtype references used in the HMA were as follows: A1 (RW20), A2 (IC144), A3 (SF170), B1 (TH14), B2 (SF162), C1 (ZM18), C2 (IN868), D1 (UG21), D2 (UG46), E1 (TH22), E2 (TH06), F1 (BZ162), F2 (BZ163), G1 (LBV217), G2 (VI525), H1 (CAR7), and H2 (VI557).

### DNA Sequencing

Ten microliters of PCR product from the first round of amplification was used as the template for the second-round PCR assay with primer pair ES7/ES8 (19). Then 10  $\mu\text{l}$  of the amplified DNA was used for automated DNA sequencing (Applied Biosystems, Model 373A, Version 1.0.2, Foster City, CA, U.S.A.). The procedures were as recommended by the manufacturer (20). For sequence analysis of each DNA

fragment, two different primers from both directions of the DNA double helix were employed to double check the results.

### Nucleotide Sequence Accession Numbers

The nucleotide sequences of HIV-1 isolates from this study have been deposited into the GenBank and their accession numbers are AF041108 to AF041124 for the following isolates: L6034, L6044, L6064, L6145, L6186, L6214, L6242, L6461, L6067, L6077, L6080, L6099, L6103, L6132, L6171-1, L6171-2, and L6195. For phylogenetic tree analysis, several well-characterized reference sequences were selected to represent subtypes A–H: A, RM-SF1703 (LNL accession number M66533), ZR-Z321 (M15896); B, US-MN (M17449), US-SF2 (K02007); C, ZA-NOF (L07426), ZM18 (L22954); D, ZR-JY1 (J03653), ZR-ELI (X04414); E, TH22 (09131), CM243 (L03703); F, BZ162 (L22084), BZ163 (L22085); G, LBV217 (U09664), VI525 (U09665); H, VI557 (U09666), CA13 (U09667). An HIV-2 isolate (HIV-2NIHZ [J03654]) was used as the outgroup to transform unrooted trees to rooted trees.

### Phylogenetic Tree Analysis

The nucleotide sequences obtained from the sequencing were edited using the MacDNASIS program (Version 3.0, Hitachi Software Engineering Co., San Bruno, CA, U.S.A.) and the resultant 318-bp fragment representing the C2-V3-C3-V4 domains (7062–7379 nucleotide residues of HXB2) were subjected to phylogenetic tree analysis. The "bootstrap" method was used in the SEQBOOT program (PHYLIP package version 3.52c, University of Washington, Seattle, WA, U.S.A.) to produce 1000 data sets that were randomly resampled. Phylogenetic trees were constructed using the neighbor-joining method. To run the program, distances from each of the 1000 replicated data sets were calculated using the DNADIST program (PHYLIP package) with Kimura's two-parameter model (21). From each of the tree files created previously, a consensus tree was constructed using the CONSENSUS program with the "majority rule" criteria.

### Peptide-Enzyme Immunoassay for Serotyping

The antibody reactivity to different V3 loop synthetic peptides was measured using indirect enzyme immunoassays (EIAs). The amino acid sequences (one letter code was used) of those synthetic peptides were as follows: A, RKSVMHIGPGQAFYATGDIIGD; B, RKSIMHIGPGR-AFYTTGDIIGD; B', RKRIHIGPGRAFYTTGDIIGD; C, RKSIRI-GPGQTFYATGGIIGD; D, NTRQRTFHGPGQALYTTRI; E, RTSI-TIGPGQVFYRTGDIIGD; E', RTSITIGPGHVFYKTGDIIGD; E'', YNTRTKITRGPRVFYRTGDM; and G, RKSIRIGPGQTLAT-GAIIGD. Each synthetic peptide was coated on a flat-bottomed EIA polystyrene plate (Beckman Instruments, Inc., Fullerton, CA, U.S.A.), 100  $\mu\text{l}$ /well at a concentration of 5  $\mu\text{g}/\text{ml}$  in 50 mM sodium carbonate buffer, at pH 9.6. The plates were incubated overnight at  $4^{\circ}\text{C}$ . and washed with phosphate-buffered saline (PBS) once before they were blocked with PBS with 3% bovine serum albumin (Sigma Co., St. Louis, MO, U.S.A.) at  $37^{\circ}\text{C}$  for 1 hour. After the wells were washed 5 times with PBS containing 0.05% Tween 20, each patient's serum at a 1/100 dilution in duplicate was added to each well. After incubation at  $37^{\circ}\text{C}$  for 1 hour, the plates were washed 5 times and 100  $\mu\text{l}$  1/500 diluted alkaline phosphatase conjugated goat anti-human immunoglobulin G (Sigma) was added to each well. The plates were incubated at  $37^{\circ}\text{C}$  for another 1 hour and washed 3 times and 100  $\mu\text{l}$  of substrate solution (1 M 2-amino-2-methyl-1-propanol [Sigma], pH 9.9, 25  $\mu\text{M}$

ZnCl<sub>2</sub> and 1.0 mM MgCl<sub>2</sub>) were added to each well. After incubation for 35 minutes, measurements were made using a Titertek Multiskan spectrophotometer (Flow Laboratories Inc., McLean, VA, U.S.A.) at an optical density (OD) of 405 nm.

HIV-1-negative samples ( $n = 8$ ) were included in each run of the EIA. The cutoff value of the assay was determined using the following formula:  $2 \times (\text{mean OD of the HIV-1-negative samples} + 3 \text{ standard deviations [SD]})$  (22). The serum binding ratio of each serum sample to the cutoff value (OD/control) tested in different EIAs was calculated. The serotype was determined according to the following principle: the highest ratio of antibody binding to a particular peptide with a ratio  $>1$ .

### Statistical Analysis

Fisher's exact and  $\chi^2$  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 subtypes B or E.

## RESULTS

### Demographic Characteristics of HIV-1-Infected People in Taiwan

In this study, blood samples and questionnaires were collected from 267 male and 21 female HIV-1-infected people, which constitute about one quarter of the cumulative HIV-1/AIDS cases in Taiwan. As shown in Table 1, the mean age of heterosexual men in this study was significantly older than that of homosexual men ( $t$ -test,  $p$

TABLE 1. Demographics and risk factors of different groups of HIV-1-infected people from Taiwan

	Men			Women ( $N = 21$ )	Total ( $N = 288$ )
	Heterosexual ( $N = 101$ )	Homosexual ( $N = 120$ )	Bisexual ( $N = 46$ )		
Age (y)					
≤19	0 (0%)	0 (0%)	1 (2.3%)	1 (5.3%)	2 (0.8%)
20-29	29 (32.6%)	43 (44.8%)	10 (22.7%)	9 (47.4%)	91 (36.7%)
30-39	36 (40.4%)	40 (41.7%)	25 (56.8%)	4 (21.1%)	105 (42.3%)
40-49	16 (18.0%)	11 (11.5%)	4 (9.1%)	3 (15.8%)	34 (13.7%)
≥50	8 (9.0%)	2 (2.1%)	4 (9.1%)	2 (10.5%)	16 (6.5%)
Total <sup>a</sup>	89 (100%)	96 (100%)	44 (100%)	19 (100%)	248 (100%)
Mean <sup>b</sup>	35.4 ± 10.9	31.4 ± 7.8	36.6 ± 10.3	32.8 ± 11.4	33.7 ± 9.9
Education					
<6th grade	9 (10.2%)	4 (4.3%)	5 (11.1%)	4 (25.0%)	22 (9.1%)
Junior high	13 (14.8%)	11 (12.0%)	6 (13.3%)	2 (12.5%)	32 (13.3%)
Senior high	35 (39.8%)	42 (45.7%)	15 (33.3%)	5 (31.3%)	97 (40.2%)
≥College	31 (35.2%)	35 (38.0%)	19 (42.2%)	5 (31.3%)	90 (37.3%)
Total	88 (100%)	92 (100%)	45 (100%)	16 (100%)	241 (100%)
IDU	3 (3.0%)	1 (1.7%)	0 (0%)	0 (0%)	4 (1.4%)
Marital status					
Single	62 (68.1%)	85 (90.4%)	32 (74.4%)	10 (47.6%)	189 (75.9%)
Married	26 (28.6%)	4 (4.3%)	5 (11.6%)	10 (47.6%)	45 (18.1%)
Divorced	3 (3.3%)	4 (4.3%)	6 (14.0%)	0 (0%)	13 (5.2%)
Widower/widow	0 (0%)	1 (1.1%)	0 (0%)	1 (4.8%)	1 (0.4%)
Total	91 (100%)	94 (100%)	43 (100%)	21 (100%)	249 (100%)
Travel					
Any foreign countries <sup>c</sup>	46 (45.5%)	45 (37.5%)	11 (23.9%)	6 (28.6%)	108 (37.5%)
In Southeast Asia	34 (33.7%)	26 (21.7%)	6 (13.0%)	4 (19.0%)	70 (24.3%)
Sexually transmitted diseases					
Gonorrhea	7 (6.9%)	3 (2.5%)	1 (2.2%)	0 (0%)	11 (3.8%)
Syphilis	10 (9.9%)	27 (22.5%)	12 (26.1%)	0 (0%)	49 (17.0%)
Any	21 (20.8%)	37 (30.8%)	16 (34.8%)	0 (0%)	74 (25.7%)
Sexual contact with prostitutes					
In Taiwan	24 (23.7%)	3 (2.5%)	11 (23.9%)	0 (0%)	38 (13.1%)
In Southeast Asia	15 (14.9%)	1 (0.8%)	0 (0%)	0 (0%)	16 (5.6%)
In other countries	0 (0%)	2 (1.7%)	0 (0%)	0 (0%)	2 (0.7%)
Total <sup>d</sup>	39 (38.6%)	6 (5.0%)	11 (23.9%)	0 (0%)	56 (19.4%)

IDU, intravenous drug user.

<sup>a</sup> There were missing age data in different populations.

<sup>b</sup> The mean age of HIV-1-infected male heterosexuals was significantly higher than that of HIV-1-infected male homosexuals ( $p < .05$ ).

<sup>c</sup> HIV-1-infected male heterosexuals had higher frequency of foreign travel than did HIV-1-infected male homosexuals/bisexuals ( $\chi^2$  test,  $p = .063$ ).

<sup>d</sup> HIV-1-infected male heterosexuals had significantly higher rate of having sexual contact with prostitutes than did HIV-1-infected male homosexuals/bisexuals ( $\chi^2$  test,  $p < .001$ ).

< .05). The women had significantly less education than the men ( $\chi^2$  test,  $p < .05$ ). Heterosexual men had a higher rate of foreign travel than the male homosexuals, bisexuals, or women, although it was not statistically significant. The heterosexual men had a significantly higher rate of having contact with commercial sex workers than the homosexual and bisexual men ( $\chi^2$  test,  $p < .001$ ).

### Establishment of Different Methods for HIV-1 Subtyping

To establish peptide-enzyme immunoassays (PEIAs) for subtyping, 9 synthetic peptides based on different HIV-1 subtypes' V3-loop sequences were used. Thirty-six serum samples from HIV-1-infected patients whose subtypes have been determined using phylogenetic tree analysis (PTA) were used to verify the sensitivity and specificity of the PEIA. The samples included 1 subtype A, 20 subtype B, 2 subtype C, 12 subtype E, and 1 subtype G. In total, 34 of 36 (94.4%) of the samples' serotypes can be determined and are consistent with their genotypes. A subtype B sample had low OD ratios in all PEIAs, so that its serotype could not be determined. Another sample was a subtype A case (L6171), which was serotyped as E because it had a higher OD ratio in the subtype E-PEIA than in the subtype A-PEIA (Table 2). Although the 1.2-kb PCR-amplified *env* DNA fragments from neither L6171-1 nor L6171-2 isolates (obtained in June and October 1996, respectively) formed heteroduplexes with the subtype A plasmid DNA in the HMA (Fig. 1A), they clustered with two HIV-1 subtype A strains, SF1703, UG37, and Z321, in PTA (Fig. 2). A 98% nucleotide sequence homology of the V3-V4 region occurred between L6171-1 and L6171-2, and their sequence homology with SF1703 and Z321 were 83% and 85%, respectively. However, as shown in Table 2, the amino acid sequences of the V3 loop of L6171-1 had a higher degree of homology with E-peptide than with A-peptide (17 of 21 versus 16 of 21) consensus sequence used in the PEIA.

Subsequently, PEIAs were used for the remaining cases in this study and 236 of 252 (93.7%) cases' subtypes could be determined. Among 16 cases whose serotypes could not be determined, 8 PBMC samples were available for genotypic analysis using HMA or PTA and all were subtype B. In total, 97.2% (280 of 288) of HIV-1/AIDS patients' subtypes were determined, and multiple HIV-1 subtypes, including A, B, C, E, and G, were present in different risk groups in Taiwan (Table 3).

### Distribution and Trends of HIV-1 Subtypes in Different Groups

Most infections among Taiwanese men were of subtype B (68.2% B; 27.3% E; 1.5% C; 0.8% G), whereas infections among Taiwanese women were mainly non-B subtypes, including subtypes A, E, and G (Table 3). Among men who had HIV-1B, 71.4% (130 of 182) were homosexual or bisexual, whereas among men who had HIV-1E, 56.2% (41 of 73) were heterosexual ( $\chi^2$  test,  $p < .001$ ). Findings showed that 66 of 182 (36.3%) men with subtype B and 33 of 74 (44.6%) men with subtype E had traveled to foreign countries, especially Southeast Asia (62.1% and 66.7%, respectively). Similarly, 2 of 3 men with subtype C and 1 of 2 men with subtype G also had traveled in Southeast Asia. Men with HIV-1E infection had a significantly higher rate of sexual contact with prostitutes than men with HIV-1B infection (28.8% versus 17.0%;  $\chi^2$  test,  $p < .05$ ). Among 4 male IDUs in this study, 3 were heterosexuals (2 HIV-1B and 1 HIV-1E) and 1 was homosexual (HIV-1E).

Among 21 HIV-1-infected women, 7 (6 HIV-1E and 1 HIV-1G) were commercial sex workers, 7 (6 HIV-1E and 1 HIV-1A) were infected by their husbands, 4 (3 HIV-1E and 1 HIV-1G) were infected by their boyfriends, 1 (HIV-1E) contracted the infection through blood transfusion, and 2 were untypable (Table 3).

The subtype distribution among different risk groups were further analyzed chronologically according to the

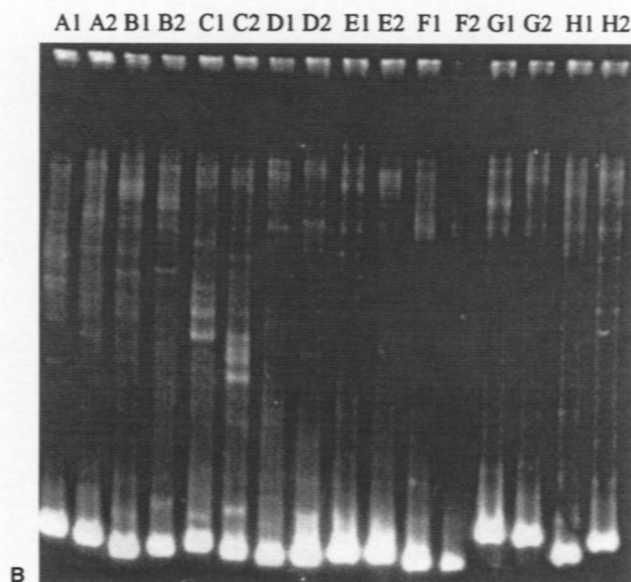
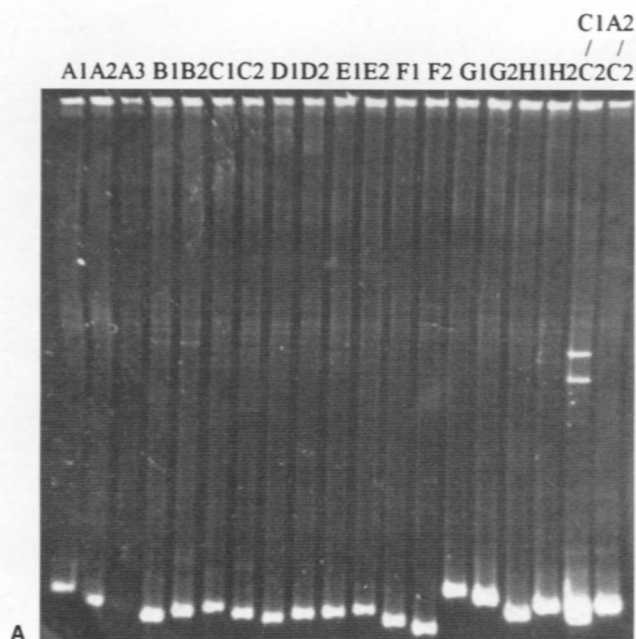
**TABLE 2.** The HIV-1 V3 loop amino acid sequences of two isolates from patient L6171 and its homology with subtype E and B peptides used in the peptide-enzyme immunoassays

	V3 loop																				PEIA (OD ratio) <sup>a</sup>	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	A	E
L6171-1	C	T	R	P	H	N	N	T	R	Q	S	T	R	I	G	P	G	Q	V	F	0.907	1.641
L6171-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	-	0.813	1.101
Peptide A	-	-	-	-	-	-	-	-	-	K	-	V	H	-	-	-	-	-	A	-	-	-
Peptide E	-	-	-	-	-	-	-	-	-	T	-	I	T	-	-	-	-	-	-	R	-	-

OD, optical density.

<sup>a</sup> The OD ratio of the serum samples obtained from patient L6171 at two occasions and tested by peptide-enzyme immunoassay with subtype A or E peptide.





**FIG. 1.** Heteroduplex mobility assay (HMA) of a subtype A (1a: patient L6171) and a subtype C (1b: patient L6034) HIV-1 strains from Taiwan. The last two lanes on the right of **A** showed the positive (heteroduplex DNA formed between C1 and C2) and negative (no heteroduplex DNA formed between A2 and C2) controls of the assay. Reference subtypes shown in different lanes of the HMA are mentioned in the subjects and methods.

year when the patients were diagnosed as seropositive. As shown in Figure 3, HIV-1B was present in the male population as early as 1988, while HIV-1E did not enter any of the three male groups until 1989. In addition,

several HIV-1C and HIV-1G infections have been found among male heterosexuals starting as early as 1993. Among heterosexual men, HIV-1E started to increase from 1993. Until 1996, a significant alteration was found in total B vs E infections in male heterosexual cases. A similar but less obvious trend was also observed in male homosexuals, bisexuals, and women during 1995 and 1996.

### Characteristics of the V3 Loop Sequences in Different Subtypes

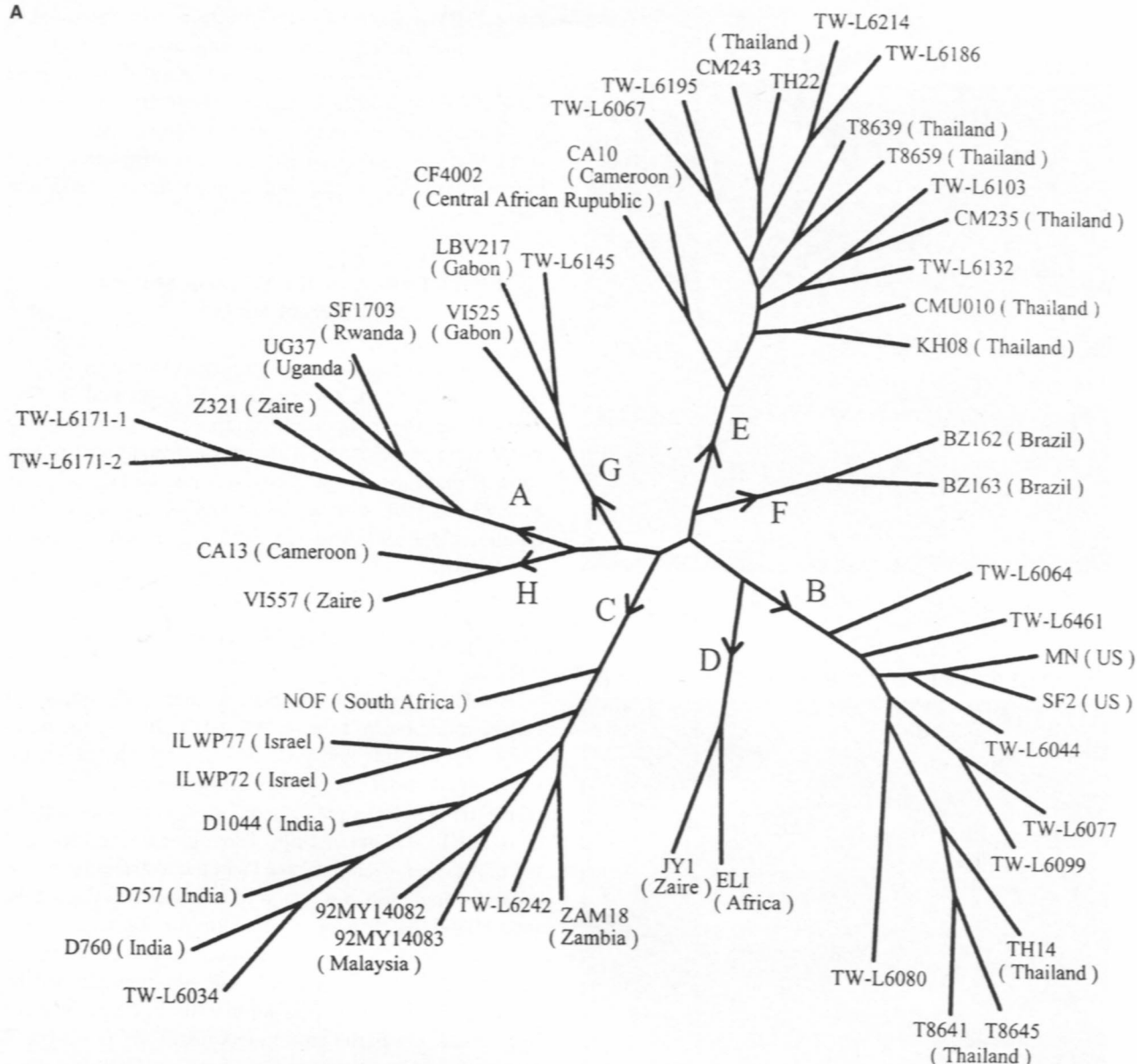
The predicted amino acid sequences of the C2-V3-C3 region for some of the isolates are presented in Figure 4. When the tetrapeptide motifs of the V loop among different subtypes from Taiwan were analyzed, results showed that most of the subtype B had GPGR, whereas 4 of 33 (12.1%) subtype B had GPGK; among 12 subtype E isolates, 9 (75%) had GPGQ, 2 had GPGH, and 1 had GPGR.

### DISCUSSION

This is the first analysis of molecular epidemiology of HIV-1 infection in Taiwan. As stated already, the number of HIV-1-infected people who participated in this study equal about one quarter of the cumulative number of the HIV-1/AIDS patients reported to the Department of Health by the end of 1996. The regions of residency of the participants were analyzed and the distribution of our sample population was found to be similar to that of the total HIV-1/AIDS patient population in Taiwan.

In this study, a good correlation existed between the serotype determined by PEIA and genotype defined by HMA and PTA, which is similar to findings in other reports (22,23). In the preliminary test, 1 of 20 subtype B cases was not serotyped due to a low OD ratio (low antibody titer). Another case (L6171) had discordant results in serotyping and genotyping assays. The discrepancy between the serotyping and genotyping results was due to the V3 consensus peptide sequences used for serotyping. As shown in Table 2, both L6171-1 and L6171-2 had valine (V) at amino acid position 19, which is identical to the consensus peptide E and different from the peptide A used in the PEIAs. The most important epitopes for antibody binding are located at the crown and the downstream of the V3 loop with a general sequence motif XGPGXXXX, which may explain the preferential serum antibody reactivity of L6171 to the E peptide (24). It has been proposed that the subtype E isolates identified in most regions were the recombination prod-

A



**FIG. 2.** Phylogenetic tree analyses of 16 Taiwanese (TW-) HIV-1 isolates. The consensus neighbor-joining trees were obtained from 1000 bootstrap samples of aligned *env* sequences corresponding to the nucleotide residues 7062-7379 of HXB2 from different HIV-1 isolates. Sequences from 34 HIV-1 isolates representing subtypes A-H from different regions in the world were also included for analysis. An HIV-2 isolate (HIV-2NIH2) was used as the outgroup to transform an unrooted tree (A) to a rooted tree (B). The numbers shown at the forks are consensus bootstrap values out of 1000 replications.

ucts of subtypes A and E (6,25) Another less favored hypothesis is that subtype E viruses are members of subtype A, but their *env* gene evolved faster than their *gag* gene, and a distinct group in the *env*-derived phylogenetic trees developed. The 22nd amino acid residue of the V3 loop sequence of L6171-1 has evolved from Y to H (L6171-2) within 5 months and has also exhibited higher amino acid homology with some of the subtype E isolates (26). Further genetic studies on the *gag* and

*gp41-env* regions of both L6171-1 and L6171-2 isolates are needed to determine the significance of this observation.

Patient L6171 was a 55-year-old housewife when she was diagnosed as seropositive in 1992. She contracted the HIV-1 infection from her husband, who on multiple occasions had sex with female commercial sex workers in Thailand. The peptide used in the PEIAs to detect genotype A in Thailand was identical to the one that we

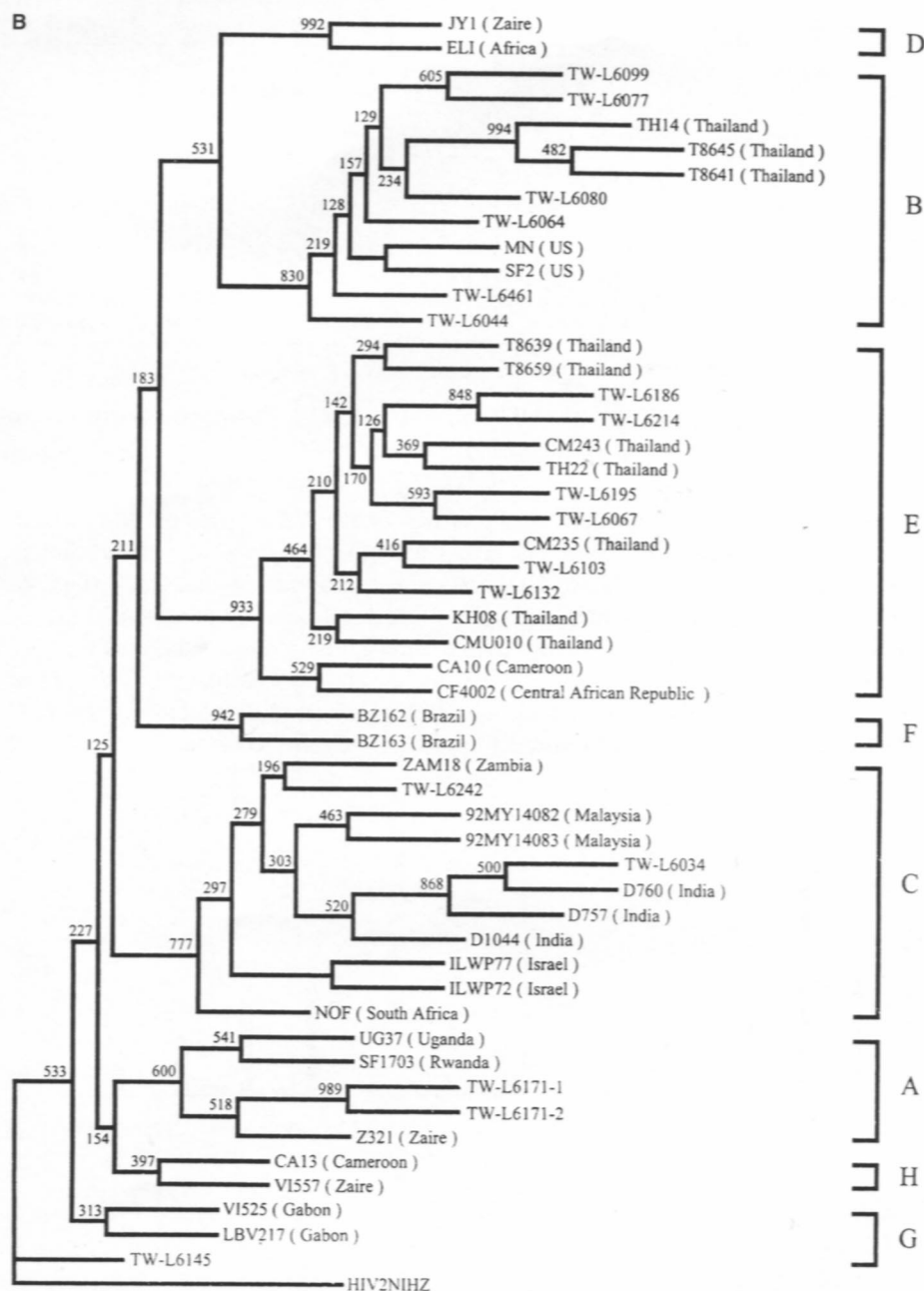


FIG. 2. Continued.

used in this study (22). Therefore, further studies with modified peptide A sequences are needed to elucidate the prevalence of this subtype A variant in both Thailand and Taiwan.

In total, the serotypes of 17 of 288 (5.9%) HIV-1-infected people could not be determined using the PEIA. This demonstrates the efficiency of the PEIA as an initial screening method for determining subtypes B and E in a large population. Due to the limited numbers tested, the usefulness of PEIA for the differentiation of other sub-

types besides B and E remains to be studied. In addition, considering the numbers of peptides used for the distinction between B and E (3 for subtype E and 2 for subtype B) and the mutation rate of HIV, constant monitoring of the evolution of different subtypes' env sequences is warranted.

In this study, we identified 5 Taiwanese (4 heterosexual men and 1 woman) infected with HIV-1C. Phylogenetic tree analysis of 2 HIV-1C isolates showed that L6034 clustered with an isolate from India, D760, and

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

HIV-1 subtype	Male HIV/AIDS patients No. (%)				Female HIV/AIDS patients No. (%)			Total no. (%)
	Heterosexual <sup>a</sup>	Homosexual <sup>b</sup>	Bisexual	Subtotal	Sex worker	Heterosexual <sup>c</sup>	Subtotal	
A	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (7.1)	1 (4.8)	1 (0.3)
B	52 (51.5)	91 (75.8)	39 (84.8)	182 (68.2)	0 (0)	0 (0)	0 (0)	182 (63.2)
C	4 (4.0)	0 (0)	0 (0)	4 (1.5)	0 (0)	1 (7.1)	1 (4.8)	5 (1.7)
E	41 (40.6)	25 (20.8)	7 (15.2)	73 (27.3)	6 (85.7)	9 (64.3)	15 (71.4)	88 (30.6)
G	2 (2.0)	0 (0)	0 (0)	2 (0.8)	1 (14.3)	1 (7.1)	2 (9.5)	4 (1.4)
Untypable	2 (2.0)	4 (3.3)	0 (0)	6 (2.3)	0 (0)	2 (14.3)	2 (9.5)	8 (2.8)
Total	101 (100)	120 (100)	46 (100)	267 (100)	7 (100)	14 (100)	21 (100)	288 (100)

<sup>a</sup> Including 3 patients (2 HIV-1 B and 1 HIV-1 E), who were intravenous drug users.

<sup>b</sup> Including 1 HIV-1 E patient who was an intravenous drug user.

<sup>c</sup> 1 HIV-1 A, 1 HIV-1 C, and 5 HIV-1 E patients were infected by their husbands; 3 HIV-1 E, 1 HIV-1 G and 2 untypable patients were infected by their boyfriends; 1 HIV-1 E patient was infected through transfusion.

L6242 clustered with an isolate from Zambia, Zam18 (Fig. 2). HIV-1C infections in Yunnan province of mainland China were mainly due to intravenous drug use (12,27). Unfortunately, the HIV-1C sequences from China were not available at the GenBank for PTA.

The intrasubtype variation of the C2-V3-C3 region (192 nucleotides) of 33 HIV-1B isolates in Taiwan was  $13.6\% \pm 3.9\%$ . The Asian subtype B variant-B' (GPGQ

motif at the crown of the V3 loop) seen in Thailand, Malaysia, and China (1,11,26) was not found among those HIV-1B isolates analyzed in this study (Fig. 4). Judging by the degree of sequence variation and the divergence of the tetrapeptide motifs at the crown of V3 loop, HIV-1B in Taiwan was less likely due to a local bloom phenomenon, rather, it might result from multiple exposures to people from other countries.

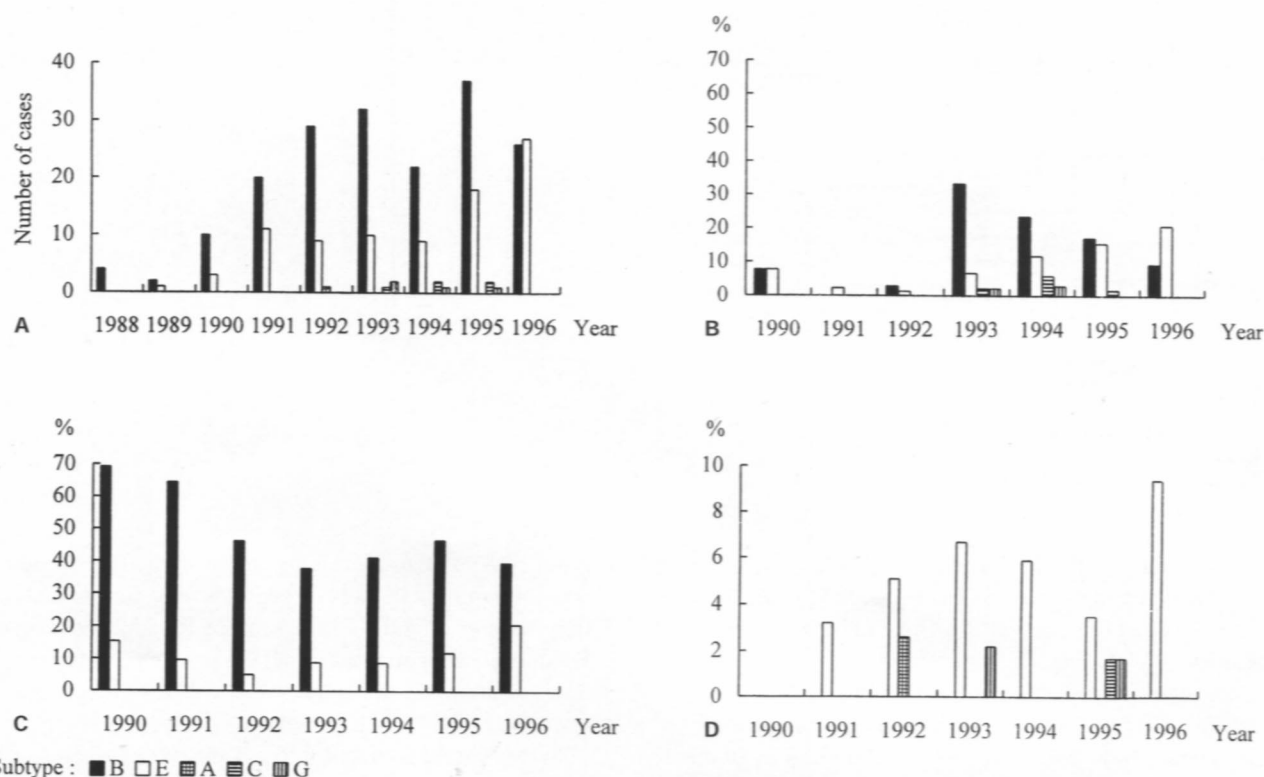


FIG. 3. Numbers and distribution of various HIV-1 subtypes in different populations from 1990 to 1996. (A) Total population. (B) Heterosexual men. (C) Homosexual and bisexual men. (D) Women. The percentages in parts B, C, and D are based on the total number of HIV-1 cases for each year.



Subtype B	V3		
Consensus	NAKTIIVQLNESVEIN	CTRPNNNTRKSIHIGPGRAFYTITGEIIGDIRQAHC	NISRAKWNNT
TW-L6002	- - - - - T - - - - -	- - - - - S - - - - - A - D - - - - N - - - -	- - - V E - - - R -
TW-L6033	- - - - - V - - - - -	- - - - - N - - - - - I - A - - - - - - - -	- - - L - - - N - - -
TW-L6035	- T - I - - - - K T - T -	- - - - - P - - - - - A - D - V - - - - K - -	- - - E T E - - - -
TW-L6044	- T - - - - - V - - - - -	- - - - - P - - - - - - - - P - - - - - -	- - - L - - - Q E K -
TW-L6058	- E V - - - - T V - - -	- - - - - G - - - - - - - - - - - - -	- - - L - - - A - E K -
TW-L6061	- - - V - - - - K - T - -	- - - - - P - - - - K - - - - - - - - K - Y -	- - - G - D - - - -
TW-L6064	- - - - - K K A - K - D -	- I - T - - - R - - L - Q - - L - A - - V - - - -	- - - S T N - T T -
TW-L6069	- T - N - - - H - K - A - -	- - - - - V - R - - - - H - - H V K - - - -	- - - - - D - D - -
TW-L6071	- - - - - K - P - K - A -	- A - - - - I - L N L - R - - - - A - - G - N - - P - R -	H L - I T G - T A
TW-L6073	- - - N - - - - T - - - -	- - - - - R - P - - - - - S - - - - - K - - -	- - - H L - K T A - H -
TW-L6077	- - - V - - - - T - - - -	- - - - - R - P - - - - - N A - - M - Y - - - -	- - - - - T H - - - -
TW-L6080	- P - N M V - - K - P - K -	- - - - - P - - - - K - L - - - - - N - - - -	- - - L - T T A - - - -
TW-L6082	- - - - - V - - - - - V - -	- - - - - P - - - - V M - A - Q - - - - - -	- - - L - T Q - K -
TW-L6086	- - - V - - - - T - H -	- - - G K I I - R R - - - - V - D - - G D K - E L T R -	- - - T E T N - - -
TW-L6088	- - - N - - - - K - P - K -	- - - - - R - - - - - - - - - - - N - - -	- - - L - Q T A - R -
TW-L6099	- - - D - - - - T - - - -	- - - - - R G - - - - - V - A - K - - - - K - -	- - - L - T D - - - -
TW-L6101	- - - V - - - - K - T - -	- - - - - R - P - - - - - - - - - - - K - -	- - - - - D - H -
TW-L6111	- - - - - - - - - A - - -	- - - - - - - - - A - - - - - - - - -	- - - K G Q - Q K -
TW-L6124	- - - V - - - - K - A - -	- - - - - G - - - - T - F - - - - - - K - -	- - - - - G D - R -
TW-L6156	- - - N - - - - T - - - -	- - - - - - - - - G - - - - - - K - -	- - - E T A - R -
TW-L6180	- - - V - - - - S - T - -	- - - - - G - M - - - V - A - D N - V - - K - Y -	- - - - - T D - R -
TW-L6183	- - - V - - - - K - T - -	- - - - - P - - - - K - - - - - - K - Y -	- - - - - D - H -
TW-L6189	- - - - - - - - - A - - -	- - - - - - - - - A - D V - - - - - -	- - - - - E - K -
TW-L6237	- - - - - K D P - Q - -	- - - R - L S - - - - - A - - - - - Y -	- - - T - T A - P -
TW-L6243	- - - - - K - P - K - -	- - - - - N - - - - - Y - D - - - - N - -	- - - L - K T E - - -
TW-L6249	- T - - - - - K - I - -	- - - - - G - N - - - - - - - - - - -	- - - L - - - D - - A
TW-L6254	- T - - - - - H K - - - -	- - - S S - - - P L - - - V - - D - T - - -	- - - L - K T D - - -
TW-L6277	- - - - - K - P - K - -	- - - - - N - - - - - Y - - - - - N - -	- - - L - K T E - - -
TW-L6321	- - - - - K D P - Q - T -	- - - R - - - - - - - A - D - - - - N - E -	- - - L T G T N - D -
TW-L6346	- - - V - - - - K - T - -	- - - - - - - - - K - - - - - - K - -	- - - - - D - D -
TW-L6348	- - - G - - - - - V - - -	- - - - - N - - - - L - A - - - - - -	- - - - - - - K -
TW-L6365	- - - I - - - - - T - - -	- - - - - P - - - - A - - S - - - - - K -	- - - - - K T A - H -
TW-L6461	- V D - - - - - - - - -	- - - - - - - - - W - A - - - - - -	- - - - - G - - - -

**A**

Subtype E	V3		
Consensus	NAKTIIVHLNKSVEIN	CTRPSNNNTRTSITIGPGQVFYRTGDIIGDIRKAYC	EINGTKWNEA
CM235	- - - - - - - - - - -	- - - - - P - - - - A - - - - - - - -	- - - - - - - - V
TW-L2027	- - - - - - - - - - -	- - - Y - - - K - R - - R - - - M - - - -	- - - - - - - K V
TW-L6036	- - - - - - - - - - -	- - - I - - - M R - - - - - K - G - R - - - -	- - - - - - - S - V
TW-L6037	- - - - - - - - - - -	- - - A - L R - - - - T - G - S - - - - R - -	- - - - - - - V
TW-L6067	- - - - - E - Q - - - -	- - - F - - R M - M - - H - - - E - - - -	- - - - - - - K V
TW-L6070	- - - - - - - - - - -	- - - - - - - - - P - - - - - - - -	- - - - - - - K V
TW-L6103	- - - - - - - - - - -	- - - - - H - - - A - - - - T - - - -	- - - - - N - K V
TW-L6143	- - - - - - - - - - -	- - - - - N - - - - - - - - - - - - -	- - - - - - - T
TW-L6132	- - - - - - - - - - -	- - - D - V - - - R - - - - K - - - -	- - - - - - - K V
TW-L6164	- - - - - - - - - - -	- - - - - L - - - - - - - - - - - - -	- - - - - Q - Y K V
TW-L6186	- - - - - - - - M - -	- - - - - M - - - - - - - - - R - - -	- - - - - E - - - T
TW-L6195	- - - - - - - - - - -	- - - D - I - - R M - M - - H - - K - E - T - - R -	- - - - - - - K V
TW-L6214	- - - I - - - - E - - - -	- - - - - - - - - - - - - - - - -	- - - - - - - K V

**B**

Subtype A	V3		
Consensus	NAKTIIVQLVKPVKIN	CTRPNNNTRKSVHIGPGQAFYATGDIIGDIRQAHC	NVSRTEWNKT
TW-L6171-1	T Q - - - - - - - - -	- - - H - - - Q - T R - - - - V - T - - - - K - -	E - N - - - - T -
TW-L6171-2	T Q - - - - - - - - -	- - - H - - - Q - T R - - - - V - H T - - - - K - -	E - N - - - - T -

**C**

Subtype C	V3		
Consensus	NVKT-IIVHLNESVEIV	CTRPNNNTRKSIIRIGPGQTTFYATGDIIGDIRQAHC	NISKDKWNKT
TW-L6034	- - - V - - - - Q - - - -	- - - G - - - - - - - - - Q - - - - - -	- - - Q G R - D E -
TW-L6242	- - - V - - - - I G - T	- - - - - - - - - A - - - - N - - - -	- - - E G - D -

**D**

Subtype G	V3		
Consensus	NAKNIIIVQFNKSIDIV	CTRPNNNTRKSIHIGPGQALYATGAIIGDIRQAHC	NVSETDWRDM
LBV217	- - - I V - - - L - E T - E - N	- - - - - - - R - R F - - - T F - - - D - - - - -	- - - R - K - N - -

**FIG. 4.** Amino acid sequence alignment of the C2-V3-C3 Env domain from different strains of Taiwanese (TW-) HIV-1 subtypes. Consensus sequences derived from previously published subtypes are shown at the top of each subtype section. Dashes indicate amino acids identical to those at the top of each block, and periods indicate deletions.

As shown in Figure 2, all the Taiwanese E isolates clustered with Thailand isolates. Notably, in this study, 3 PEIAs with divergent subtype E V3 peptides were used for serotyping. Although most HIV-1E sequenced had the GPGQ motif seen in isolates throughout Thailand, 2

isolates had GPGH and 1 had GPGR, which resemble specific patterns found among AIDS patients in northern Thailand (26). The intrasubtype variation of the C2-V3-C3 region among 12 Taiwanese HIV-1E isolates was  $7.4\% \pm 2.1\%$ .

In this study, the B/E subtype distributions in male heterosexual and homosexual/bisexual groups were significantly different. Some 71.4% of men with HIV-1B were homosexual or bisexual, whereas 56.2% of men with HIV-1E were heterosexual ( $p < .001$ ). However, when the data are organized chronologically, although HIV-1 non-B subtypes, which include A, C, E, and G, came to Taiwan later than HIV-1B, subtype E has become a major subtype in the male and female heterosexual populations (Fig. 3B and D, respectively). A similar but less obvious trend was also observed in the homosexual/bisexual population (Fig. 3C). These observations should be confirmed through prospective cohort studies on the molecular epidemiology of HIV-1 infection in different high-risk groups (28). However, the findings in this study may indicate future trends of HIV-1 epidemics in other countries sharing the same geographic area of affinity with Taiwan (29).

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