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CRF01_AE subtype is associated with X4 tropism and fast HIV progression in Chinese patients infected through sexual transmission

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Background: The molecular epidemiology of the HIV-1 CRF01_AE subtype as a risk factor for fast HIV-1 progression remains poorly understood.

Methods: We analyzed HIV-1 tropism by utilizing samples from 201 treatment-naive patients in our multicenter cohort (12 research centers in different provinces of China). Tropism was determined by V3 loop sequencing. Data from 235 treatment-naive patients infected sexually (including aforementioned 201 patients) in this cohort with date of estimated seroconversion (EDS) were retrospectively evaluated. Median time from EDS to AIDS was analyzed by Kaplan–Meier curves. Hazard ratios were determined by Cox proportional model.

Results: CRF01_AE subtype was predominant (46.0%), especially in the MSM group. Further analysis revealed that the proportion of X4 tropism was higher in the CRF01_AE subtype (45.5%) than in others (C/CRF07_BC/CRF08_BC, 4.3%; B, 6.1%; $P<0.001$). CRF01_AE subtype was associated with faster progression from EDS to AIDS (4.8 vs. 6.4 years, $P=0.018$) compared with non-CRF01_AE subtypes. In a multivariate model, the adjusted hazard ratio (aHR) of CRF01_AE was 1.42 (95% confidence interval, CI 0.99–2.03, $P=0.057$), independent of HIV-1 viral load; it was also associated with fast progression to advanced immunodeficiency (aHR, 1.81, 95% CI 1.03–3.18, $P=0.038$).

Conclusion: CRF01_AE, a predominant HIV-1 subtype in Chinese HIV-1 sexually infected patients, tends to be associated with fast progression to AIDS and advanced immunodeficiency, which might be ascribed to high proportion of X4 tropism. Further investigation of these risk factors may have significant implications to clinical practice and policy-making.

AIDS 2014, **28**:521–530

Keywords: CRF01_AE, HIV, HIV progression, MSM, X4 tropism

Introduction

The progression of HIV-1 infection is determined by multiple factors including viral tropism and subtypes [1]. HIV requires coreceptors (either CCR5 and/or CXCR4) to enter cells, and thus the virus has R5 tropism, X4 tropism, or R5/X4 dual tropism. As the disease progresses, coreceptor usage shifts from CCR5 to

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CXCR4, which is in turn associated with increased rates of $CD4^+$ cell count decline and progression to advanced immunosuppression [2,3]. The HIV-1 subtype, another important viral factor, is also related to HIV-1 progression, but most studies focus on faster progression associated with subtype D in African countries [4,5] and subtype B in western countries [6]. The CRF01_AE strain is prevalent in southern Asia and some provinces in China, especially among sexually infected patients [7–10], but the clinical significance and coreceptor usage of the CRF01_AE subtype remains poorly understood. Previous studies suggested that CRF01_AE subtype was associated with higher viral load compared with subtype B only within 3 months of seroconversion [11]. Studies in Thailand also demonstrated that the CRF01_AE subtype was associated with shorter survival in comparison with Concerted Action on SeroConversion to AIDS and Death in Europe (CASCADE) study results in western countries [12,13]. Coreceptor usage in the CRF01_AE subtype remains unclear. A recent study in Singapore suggests that prevalence of X4 tropism is high in CRF01_AE among treatment-naive CRF01_AE-infected HIV-1 patients in comparison with B subtype-infected patients [14], which is in accordance with another study in Belgium [15]. In comparison, previous studies from Thailand and Cambodia, where CRF01_AE is quite prevalent, show that most CRF01_AE strains have R5 tropism [16,17]. Unfortunately, the current situation in China remains uncharacterized.

In China, sexual transmission has become the primary mode of transmission of HIV-1. It has been estimated that 780 000 patients were living with HIV-1 by the end of 2011, of which 46.5% were infected heterosexually and 17.4% homosexually. There were estimated 48 000 new HIV-1 infections in 2011, of which 52.2% were infected heterosexually and 29.4% homosexually [18]. In the meantime, the proportion of patients infected via intravenous drug use (IVDU) has been decreasing, which could be ascribed to clean syringe and needle programs [18]. Of note, in patents with history of IVDU, limited data do show that they tend to be engaged in high-risk sexual behavior [19]; however, the prevalence of IVDU in patients infected via sexual contact remains unclear. During our clinical practice, we discovered that in these sexually infected patients, time from seroconversion to AIDS was usually shorter than 6 years. Unfortunately, few studies have addressed this rapid progression or have identified risk factors for rapid progression to AIDS. Moreover, patients from this population do not initiate combination antiretroviral therapy (cART) until $CD4^+$ cell count drops to a low level [20], which could probably be ascribed to fear of adverse effects of cART. Therefore, identification of risk factors for fast progression is necessary to justify early initiation of cART and to reduce further morbidity, mortality, and transmission rates [21,22]. In this study, we wanted to characterize the natural history and molecular epidemiology of HIV-1 in sexually infected patients and to identify risk factors associated with faster HIV-1 progression in our nationwide, multicenter cohort.

Methods

Study population

Our cohort consisted of 529 patients enrolled from 12 research centers in different parts of China and 48 patients from the outpatient clinic of Peking Union Medical College Hospital. These patients were recruited between November 2008 and January 2010. Inclusion criteria for this study were age 18–65 years and both genders, $CD4^+$ cell count lower than 350 cells/μl, antiretroviral-naive, willingness to sign informed consent, sexually infected, and with estimated date of seroconversion (EDS) available. The main exclusion criteria were acute phase of HIV-1 infection, pregnancy or breastfeeding, anticipated poor adherence, IVDU or IVDU and other risk factors. All the patients in our study were sexually infected with HIV-1. Among all the participants, 235 had EDS available, and these patients were included in this study (Supplementary Fig. S1, http://links.lww.com/QAD/A449). After recruitment, cART was initiated in all patients. A flowchart of this study is shown in Supplementary Fig. S1, http://links.lww.com/QAD/A449.

Definitions

Earliest date of blood test showing HIV-1 positivity and earliest date of unprotected sexual contact with someone known to have HIV-1 infection was used to calculate EDS as previously reported [23]. AIDS was defined as either $CD4^+$ cell count less than 200 cells/μl at enrollment or AIDS-defining diseases before enrollment according to CDC criteria [24]. Advanced immunodeficiency was defined as $CD4^+$ cell count less than 100 cells/μl [25].

Viral load and $CD4^+$ cell count

Methods for viral load and $CD4^+$ cell count measurement have been described elsewhere [26]. The HIV-1 viral load was determined with COBAS Ampliprep/TaqMan48 real-time RT-PCR (Roche Diagnostics, Indianapolis, Indiana, USA). $CD4^+$ cell count was measured by three-color flow-cytometry (Epics XL flow cytometer; Beckman-Coulter, Brea, California, USA).

Subtype analysis

In order to determine HIV-1 subtype, HIV-1 RNA was extracted from patient plasma by using the QIAamp RNA mini Kit (QIAGEN, Hilden, Germany). The Pol gene was amplified by using PrimeScript One Step RT-PCR Kit Ver.2. PCR primers used for Pol were 5'- TGGAAATGTGGRAARGARGGAC-3' (sense) and 5'- CCTGTATGCARMCCCCAATATGTT -3'

Table 1. General characteristics of the study population at recruitment classified by viral subtypes.

Characteristics	All ($n=235$)	CRF01_AE ($n=108$)	Non-CRF01_AE ($n=127$)	P value
Age at seroconversion (median years, IQR)	29 (24–36)	28 (23–36)	30 (24–38)	0.115
Age at conversion, category (n, %)				0.448
12–24 years	67 (28.5)	35 (32.4)	32 (25.2)	
25–34 years	95 (40.4)	43 (39.8)	52 (40.9)	
35–44 years	52 (22.1)	23 (21.3)	29 (22.8)	
45–54 years	15 (6.4)	6 (5.6)	9 (7.1)	
55–64 years	6 (2.6)	1 (0.9)	5 (4.0)	
Male sex (n, %)	176 (74.9)	88 (81.5)	88(69.3)	0.032
Ethnic category (n, %)				0.311
Han	220 (93.6)	103 (95.4)	117(92.1)	
Minority	15 (6.4)	5 (4.6)	10 (7.9)	
Transmission category (n, %)				0.038
MSM	122 (51.9)	64 (59.3)	58 (45.7)	
Heterosexual	113 (48.1)	44 (40.7)	69 (54.3)	
Tropism (n, %)				<0.001
R5	128 (54.5)	28 (25.9)	100 (78.8)	
Dual R5/X4	28 (11.9)	20 (18.5)	8 (6.3)	
X4	45 (19.1)	40 (37.1)	5 (3.9)	
Unknown	34 (14.5)	20 (18.5)	14 (11.0)	
Median viral load at enrollment (log copies/ml, IQR)	4.55 (3.98–4.95)	4.66 (4.14–5.04)	4.45 (3.78–4.92)	0.008
Median $CD4^+$ cell count at enrollment (cells/μl, IQR)	192 (109–258)	170 (68–266)	201 (130–254)	0.117
EDS (median year, IQR)	2006 (2004–2007)	2006 (2005–2007)	2006 (2003–2007)	0.052
Median time from seroconversion to treatment (years, 95% CI)	3.5 (3.2–3.8)	3.4 (3.1–3.8)	3.8(2.9–4.7)	0.040[a]

EDS, estimated date of seroconversion; IQR, interquartile range.
[a]P value for median time from seroconversion to enrollment was calculated using log-rank method.

(antisense) for the first-round reaction, and 5'-ACTGAGAGACAGGCTAATTTTTTAGGGA-3' (sense) and 5'- CTCCTAGTGGGATRTGTACTTCTGARCTTA -3' (antisense) for the second-round reaction. Amplified viral DNA was purified with the QIAquick Gel Extraction Kit (QIAGEN). Sequencing was performed with ABI PRISM BigDyeTM Terminator CyCle SequenCIng Ready Reaction Kit (Applied Biosystems, Foster City, California, USA). Subtypes were determined by using Recombinant Identification Program (http://www.hiv.lanl.gov/content/sequence/RIP/RIP.html). In addition, we confirmed viral subtypes by using neighbor-joining phylogenetic analysis of these sequences along with reference sequences from the Los Alamos National Laboratory (http://www.hiv.lanl.gov/content/index). Sequencing of V3 loop was carried out in a subgroup of patients ($n=201$) to confirm subtypes, with 93.0% concordance with subtypes determined by Pol sequence. The length of pol amplicon is 2160 bp and the length of V3 loop amplicon is 360 bp. As there were no samples available for V3 region sequencing ($n=33$) or failure to replicate V3 region by PCR ($n=1$), 34 strains could not be sequenced in V3 region.

Tropism analysis

Sequencing of V3 loop was carried out in a subgroup of patients ($n=201$). We determined viral tropism by using Geno2pheno, with false positive rate (FPR) cut-off values 5% for X4 tropism following German guideline; R5 usage was defined as FPR over 15% and R5/X4 dual tropism was defined as FPR between 5 and 15%. (http://coreceptor.geno2pheno.org/index.php and http://www.daignet.de/site-content/hiv-therapie/leitlinien-1/Leitlinien%20zur%20Topismus_Testung%20Stand%20Juni%202009.pdf) [27]. According to recent studies, when the FPR cut-off value is set at 5%, the concordance of genotype and phenotype in CRF01_AE subtype range from 77.0 to 79.5% [28,29].

Statistical analysis

Baseline characteristics were analyzed by using the Mann–Whitney U-test (noncategorical variables) and χ^2 test (categorical variables). Kaplan–Meier curves were used to evaluate median time from EDS to different endpoints, and log-rank test was used to compare different subtype groups (determined by Pol sequence in the 235 patients). Cox proportional hazard model was utilized to estimate the hazard ratios of different risk factors. In a multivariate model, age and sex were forced to enter, and all other factors were subjected to backward-stepwise likelihood ratio (LR) regression with $P<0.20$ as exclusion criterion. For all tests, $P<0.05$ was considered to be statistically significant. SPSS 20.0 statistical package was used for all analyses. Bootscanning was conducted by using SimPlot [30].

Protocol approval

After obtaining informed consent from each patient, this research was approved by Institutional Review Board of Peking Union Medical College Hospital. It was in

accordance with relevant local laws and the ethical requirements of the Declaration of Helsinki.

Results

Population characteristics

The population characteristics are summarized in Table 1. Most patients in our cohort were Han Chinese. Median EDS was January 2006 (IQR, January 2004 to August 2007). Median time from EDS to treatment ($CD4^+$ cell count <350 cells/μl) was 3.5 years (95% confidence interval, CI 3.2–3.8), and to AIDS was 5.7 years (95% CI 5.0–6.4). The median age of seroconversion in MSM group (27 years) was younger than that in heterosexual group (31 years, $P=0.032$), but $CD4^+$ cell count and viral load were comparable in these two transmission groups.

We classified our patients as CRF01_AE and non-CRF01_AE groups according to the viral subtypes (determined by Pol sequence). As is shown in Table 1, CRF01_AE group was associated with higher viral load ($P=0.008$) and faster time to the initiation of cART ($P=0.040$).

We also confirmed subtypes by using V3 loop sequence in a subgroup ($n=201$), of which 187 subtypes were in accordance with those derived from Pol sequence. This subgroup ($n=187$) had similar population characteristics in comparison with those of the 235 patients (Supplementary Table S2, http://links.lww.com/QAD/A447). We compared the differences in age, sex, routes of transmission, $CD4^+$ cell count at recruitment, and viral load at recruitment between different tropism and discovered no statistical significance (Fig. 1a and b, all comparisons were not statistically significant) in this subgroup. However, we did discover that HIV-1 virus tended to be X4-tropic when $CD4^+$ cell count was less than 50 cells/μl (Fig. 1c).

In 14 patients with discrepant subtypes, we did not find recombination in Pol except for patient 6, 116, and 396, from whom we discovered CRF01_AE and B recombination in Pol area (Supplementary Fig. S2, http://links.lww.com/QAD/A448). We assigned all these subtypes as non-CRF01_AE subtypes. Details in subtype assignment

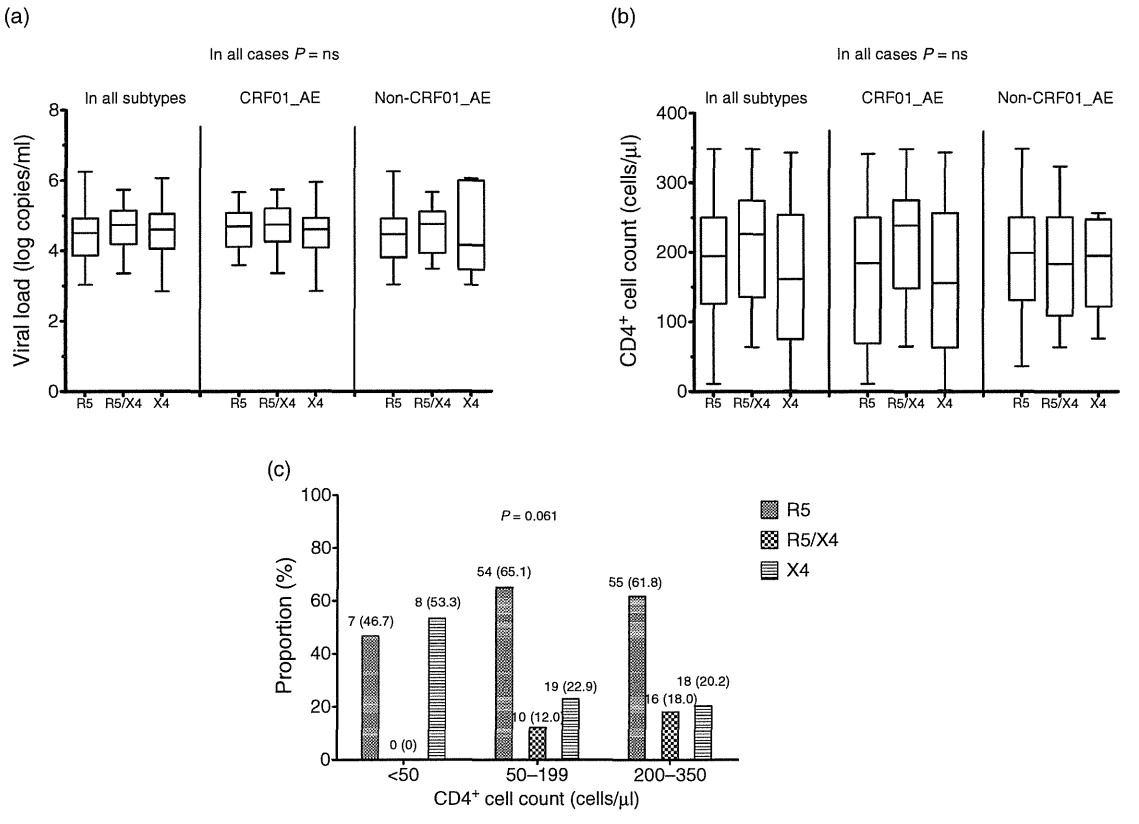

Fig. 1. Viral load and $CD4^+$ cell counts in different tropisms. (a) Viral load distribution. (b) $CD4^+$ cell count distribution. Medians with interquartile range (boxes) and maximal and minimal values (whiskers). All comparisons between tropisms in different HIV subtypes are nonsignificant. (c) Tropism distribution in different $CD4^+$ cell count intervals. First numbers indicate case numbers in each group and numbers in parentheses indicate proportion of certain tropism in each $CD4^+$ cell count interval. As there were over 20% of cells having estimated number less than 5, we used Fisher's exact test to calculate the *P* value.

in these 14 strains are shown in Supplementary Table S1, http://links.lww.com/QAD/A447.

CRF01_AE subtype is predominant in Chinese HIV-1 sexually infected patients

We identified the following subtypes in our cohort: CRF01_AE (46.0%), B (17.0%), CRF07_BC/CRF08_BC/C (32.3%), and other recombination subtypes (4.7%). CRF01_AE was more prevalent in the MSM group (52.5%) than in the heterosexual group (38.9%, $P<0.001$; Fig. 2a).

CRF01_AE subtype was associated with higher proportion of X4 tropism

We then wanted to determine tropisms in different subtypes by utilizing 201 samples available from this study. To our surprise, we discovered that in CRF01_AE subtype, the proportions of X4 tropism (45.5%) and R5/X4 dual tropism (22.7%) were significantly higher than that in non-CRF01_AE subtypes ($P<0.001$), as is shown in Fig. 2b and Table 1. In addition, median time to AIDS was 6.4 years (95% CI 5.0–7.7) in R5 tropism and 4.6 years (95% CI 3.9–5.4) in X4 tropism. Similar results were seen in Supplementary Table S2, http://links.lww.com/QAD/A447 in subgroup analyses in 187 patients with concordant subtypes.

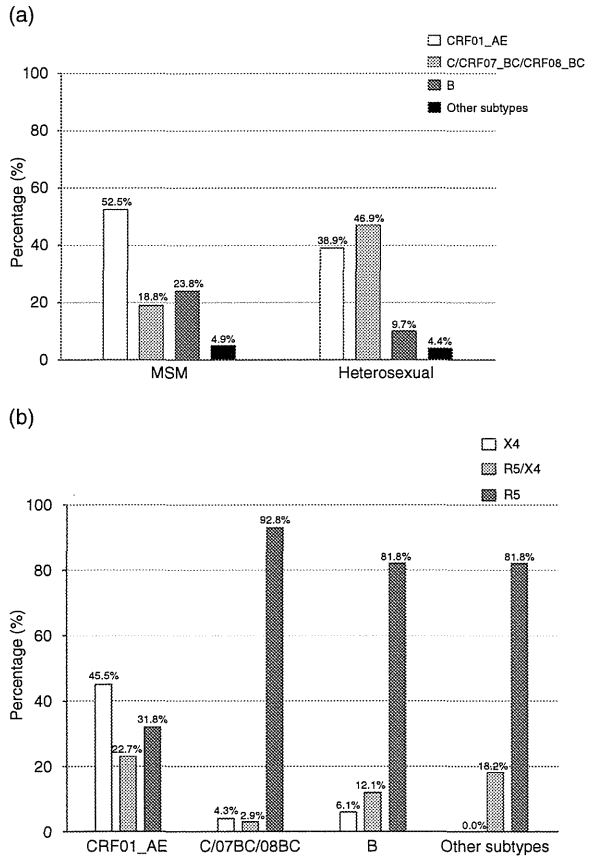

Fig. 2. Distribution of HIV-1 subtypes and tropism. (a) Distribution of HIV-1 subtypes among different risk groups. (b) Proportions of R5, R5/X4, and X4 tropism in different HIV-1 subtypes.

CRF01_AE was associated with fast progression to AIDS and advanced immunodeficiency

As X4 tropism is reported to be associated with fast HIV progression [1], and the CRF01_AE subtype is associated with a high proportion of X4 tropism, we hypothesized that CRF01_AE was associated with fast HIV progression. Median time from EDS to AIDS was 4.8 years (95% CI 3.8–5.9) in CRF01_AE group and 6.4 years (95% CI 5.4–7.4, $P=0.018$) in non-CRF01_AE group (Fig. 3a). The unadjusted hazard ratio of CRF01_AE was 1.53 (95% CI 1.07–2.18, $P=0.019$). In the multivariate regression model, the hazard ratio of CRF01_AE was 1.42 (95% CI 0.99–2.03, $P=0.057$; Table 2). Next, we wanted to determine whether these risk factors were associated with advanced immunodeficiency ($CD4^+$ cell count <100 cells/μl). Median time from EDS to $CD4^+$ cell count less than 100 cells/μl was 7.5 (95% CI 4.5–10.5) years in CRF01_AE group and 9.5 (95% CI, 7.0–12.0, $P=0.005$) years in non-CRF01_AE group (Fig. 3b). In multivariate analysis, hazard ratio of CRF01_AE was 1.81 (95% CI 1.03–3.18, $P=0.038$) and that of the viral load was 1.65 (per log copies/ml, 95% CI 1.08–2.52, $P=0.020$); route of transmission was excluded from backward-stepwise LR regression.

In order to determine whether the high proportion of X4 tropism could explain fast progression in CRF01_AE group, we also did a subgroup analysis in patients with concordant subtypes and tropism data. In CRF01_AE group, median years from EDS to AIDS were 5.57, 9.56, and 4.63 in R5, R5/X4 dual, and X4 tropism, respectively ($P=0.428$ calculated by log-rank test). In univariate and multivariate model, we failed to discover an association between tropism and disease progression in CRF01_AE subtype (Supplementary Table S3, http://links.lww.com/QAD/A447).

Discussion

Our study demonstrated that CRF01_AE, the dominant subtype in Chinese HIV-1 sexually infected patients, was associated with fast HIV-1 progression to AIDS; this could be due to a high proportion of X4 tropism in CRF01_AE subtype.

Notably, median time from EDS to AIDS was 5.7 years (95% CI 5.0–6.4) in our cohort, which was shorter compared with that in the CASCADE study [9.8 years (95% CI 9.5–10.1) for those aged 25–34 years at seroconversion, predominantly subtype B], a meta-analysis of 38 studies on the natural history of HIV-1 before the introduction of cART [23]. A Thai study

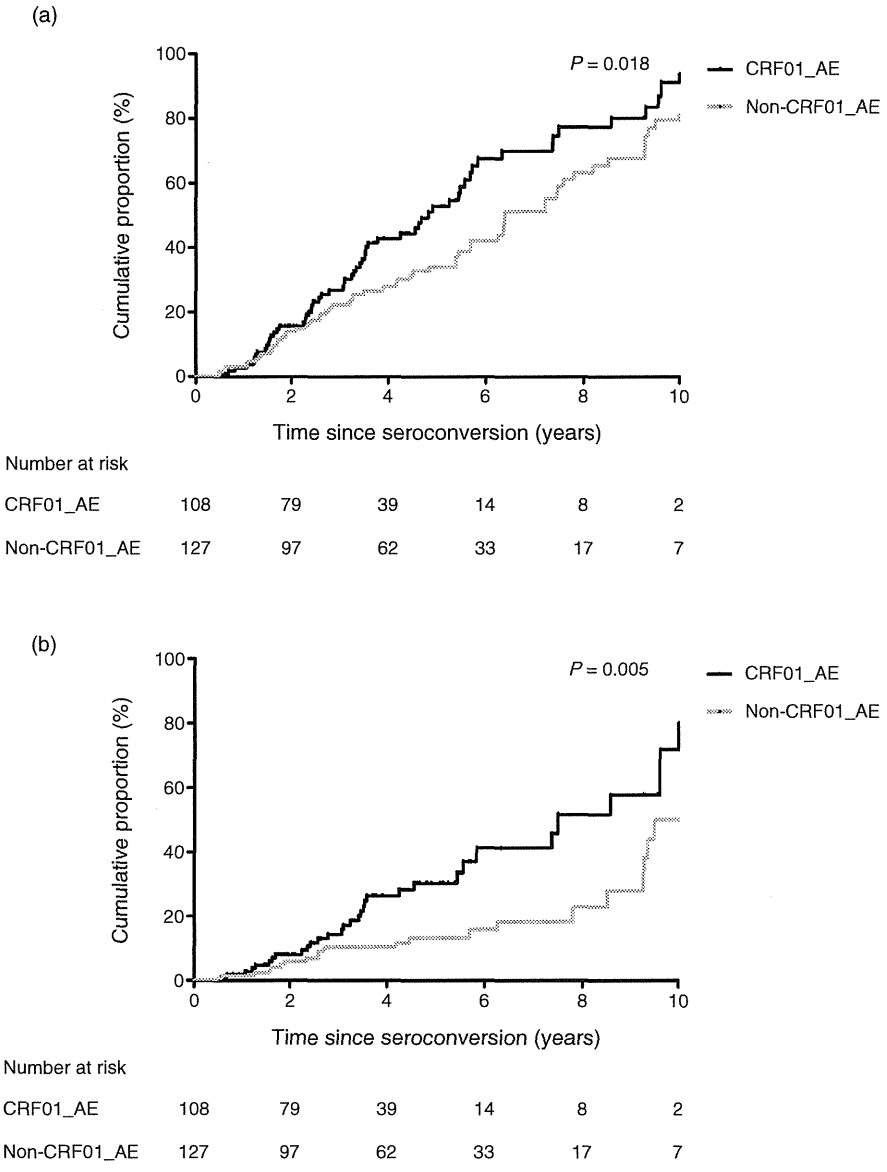

Fig. 3. Cumulative proportions of patients developing AIDS or advanced immunodeficiency classified by HIV subtypes. (a) Progression to AIDS. (b) Progression to advanced immunodeficiency. Estimated seroconversion (EDS) was defined as time point zero. As only nine participants experienced seroconversion or were censored 10 years after EDS, they were omitted in this figure. *P* values were calculated by log-rank test.

involving 228 HIV-1-infected men (predominantly subtype CRF01_AE) with seroconversion between 1991 and 1995 also reported longer time to AIDS (median time from seroconversion to clinical AIDS, 7.2 years, 95% CI 6.6–8.0) than ours (4.8 years, 95% CI 3.8–5.9 in CRF01_AE group) [12]. This difference can be partially explained by the high proportion of X4 tropism in the CRF01_AE subtype in our cohort (discussed later), although population and geographic factors may also play a role. Furthermore, most patients in our cohort experienced seroconversion after year 2000, and recent studies have suggested that HIV-1 could become more virulent since 1996 [31,32]. Further analyses are warranted to illustrate this issue.

In terms of molecular epidemiology, our study suggests that CRF01_AE is dominant in patients infected via sexual transmission, especially in the MSM group. This finding is in accordance with other epidemiologic findings in different studies that were limited to certain provinces of China [8–10,33,34]. Of note, there were 14 patients with discrepant results for subtyping between Pol and V3 loop regions, which could potentially be due to recombination. Although V3 or Env sequence is typically used to determine HIV subtype, some studies also use other parts of HIV genome to determine subtype. We consider that this could potentially be due to recombination, as is shown in Supplementary Fig. S2, http://links.lww.com/QAD/A448 in details. Thus, we also did a

Table 2. Risk factors for faster progression to AIDS in patients infected via sexual transmission ($n = 235$).

Risk factors	Univariate model		Multivariate model	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age at seroconversion (years)		0.868		0.766
12–24	Reference		Reference	
25–34	1.02 (0.65–1.59)	0.943	1.10 (0.70–1.73)	0.687
35–44	1.27 (0.76–2.10)	0.361	1.25 (0.74–2.13)	0.407
45–54	1.12 (0.51–2.44)	0.777	1.37 (0.61–3.07)	0.443
55–64	1.33 (0.41–4.37)	0.637	1.99 (0.58–6.80)	0.273
Sex				
Female	Reference		Reference	
Male	2.00 (1.29–3.09)	0.002	1.18 (0.67–2.07)	0.561
Ethnic category				
Han	Reference			
Minority	0.53 (0.24–1.21)	0.134		
Transmission category				
Heterosexual	Reference		Reference	
MSM	2.07 (1.44–2.96)	<0.0001	1.97 (1.24–3.15)	0.004
Median viral load at enrollment (per log copies/ml)	1.34 (1.05–1.71)	0.019	1.28 (0.98–1.68)	0.068
Viral subtype				
Non-CRF01_AE	Reference		Reference	
CRF01_AE	1.53 (1.07–2.18)	0.019	1.42 (0.99–2.03)	0.057
Viral tropism		0.156		
R5	Reference			
R5/X4 dual	0.94 (0.49–1.79)	0.849		
X4	1.60 (1.02–2.52)	0.043		
Unknown	1.39 (0.83–2.33)	0.208		

CI, confidential interval; HR, hazard ratio.

subgroup analysis ($n = 187$) in which subtypes determined by pol and V3 were in concordance (Supplementary Table S2, http://links.lww.com/QAD/A447), which showed similar results to those in the analysis of the 235 patients' data.

In terms of viral tropism, almost a half of CRF01_AE strains were X4-tropic, and the proportion of X4 tropism was much higher than that in non-CRF01_AE groups. Zhang *et al.* [35] have reported that in all available China HIV-1 strains on the Los Alamos HIV Sequence Database, only 38.7% of CRF01_AE strains are R5-tropic, which is similar to our result; unfortunately, the clinical significance of this was not addressed. It has been established that X4 tropism is associated with faster $CD4^+$ cell count decline and HIV-1 progression in comparison with R5 tropism [1,36], and X4 tropism was indeed associated with faster progression in our subgroup analysis. Therefore, we suggest that Chinese CRF01_AE strains, with high proportion of X4-tropic virus, are associated with fast HIV-1 progression (discussed later). In fact, we did find the marginally significant association between tropism and late stage of HIV-1 infection. This result also suggests that in future, CCR5 inhibitors will need to be used with caution in Chinese patients infected with CRF01_AE strains, given that almost half of them are infected with X4-tropic viruses. Nevertheless, reports on tropism in CRF01_AE subtype are still lacking to date, and more studies are needed to shed light upon this issue.

In our study, CRF01_AE subtype tended to be an independent risk factor for faster HIV-1 progression to AIDS and to advanced immunodeficiency, which is probably associated with high proportion of X4 tropism. Of note, after adding tropism and subtype to our multivariate model, tropism became insignificant. We also failed to discover the association between tropism and disease progression in the CRF01_AE subtype. This is probably due to limited statistical power, given that tropism profiles were unavailable in 34 patients. In addition, the association between CRF01_AE subtype and fast progression can only be partially explained by tropism, as there were still 31.8% patients infected with R5-tropic virus in the CRF01_AE group. Another possibility is that CRF01_AE subtype could possibly lead to fast progression independent of X4 tropism, although we lack evidence to prove this. Further prospective studies with larger sample size are warranted to elucidate this question.

A previous study in Thailand demonstrated that Thai patients with CRF01_AE subtype had shorter median survival time in comparison with western patients in CASCADE cohort (dominantly B subtype) [12], although this study could not exclude confounding factors such as geographic differences, and nutritional conditions. Recently, a Singaporean study has also suggested that CRF01_AE subtype is associated with 1.8-year earlier initiation of cART compared with non-CRF01_AE group [37], although they did not address the issue of whether CRF01_AE subtype is associated with progression to AIDS or advanced immunosuppression. In comparison, we only observed a 0.4-year earlier initiation in CRF01_AE group compared with non-CRF01_AE

group (Table 1). This discrepancy could be due to differences in monitoring, considering that these Singaporean patients received better monitoring than our patients did, and initiated cART earlier than ours did. Nevertheless, we did find that median time to AIDS was 1.6-year faster in CRF01_AE group than in non-CRF01_AE group. Although we could not determine whether viral subtype was associated with death in our cohort, we observed that CRF01_AE was associated with 2.0-year faster progression to advanced immunodeficiency, a condition closely related to even higher incidence of opportunistic infections and mortality [38].

This study has significant implications to clinical practice and policy-making. It justifies early treatment and encourages closer monitoring in patients infected by HIV through sexual contact, especially in the MSM population. For clinicians, the $CD4^+$ cell count of these patients should be monitored more closely, as these patients may progress more rapidly to AIDS than patients without these risk factors. In addition, early treatment may also be beneficial in these patients, as their disease progression is faster than patients without these risk factors. As is mentioned, patients infected via sexual transmission would not initiate cART until late stage of HIV infection [20]; this study, therefore, provides evidence justifying close monitoring of $CD4^+$ cell count and early initiation of cART. This is not only crucial in reducing transmission to their sexual partners, but also important in reducing HIV-associated morbidity and mortality [20,21]. Our study also has some implications on policy-making. In some Asian countries like China, where CRF01_AE is quite prevalent in patients sexually infected with HIV-1, the guideline indicates that patients are eligible for treatment after $CD4^+$ cell count becomes less than 350 cells/μl [39]. We suggest that more active monitoring and treatment strategies should be adopted in new guidelines, especially in those patients with higher risks, otherwise treatment would be postponed and mortality would remain higher in these groups in which HIV infection progresses quickly to AIDS [20]. Furthermore, this study may also have great implications for clinical practice in other Asian countries, as the CRF01_AE subtype is quite prevalent in Asia.

The limitations of this study are as follows. First, given that this study was a retrospective study, confounding factors like baseline $CD4^+$ cell count before seroconversion could not be controlled. In terms of EDS, this study may also have recall bias; however, given we have confirmed date of first seropositivity and earliest date of unprotected sexual contact with someone known to have HIV-1 infection as previously reported [23], this bias would be minor to affect the result. Nevertheless, prospective studies are warranted to give better estimation of HIV-1 natural history. Second, we only observed that CRF01_AE was associated with faster progression of HIV-1 in our cohort, but we were unable to elucidate the biological mechanisms. Right now we can only identify that viral tropism is an important factor for fast HIV-1 progression among Chinese patients; considering that HIV-1 progression is multifactorial, further studies on viral virulence and host genetics are necessary. Third, we can only make preliminary analyses on viral tropism via genotype methods; further endeavors to determine viral tropism by phenotypic assays are warranted. Fourth, patients who only met the criteria of China's National Free Antiretroviral Treatment Program (i.e., only with $CD4^+$ cell count less than 350 cells/μl) were recruited. In the future, patients with $CD4^+$ cell count over 350 cells/μl may be enrolled.

Noticeably, we also excluded intravenous drug users and patients with IVDU and other risk factors. As this cohort was primarily established for evaluating Chinese generic antiretroviral therapy, we wanted to ensure the adherence. Patients with IVDU usually have poor adherence [40,41], and therefore we exclude this group, making this study only powerful enough to evaluate patients infected via sexual transmission. A recent study suggests that IVDU is associated with high mortality in HIV-infected patients, and this is attributed to suboptimal management of HIV disease (including late diagnosis and late initiation of cART) [42]. Nevertheless, further studies are warranted to evaluate the role of HIV subtypes in HIV progression in IVDU group.

Taken together, this is the first cohort study to report that CRF01_AE subtype is associated with higher proportion of X4-tropic virus and faster HIV-1 progression among HIV-1-infected patients in China. Further studies focusing on these risk factors will help us improve our clinical practice and policy-making.

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