Journal of Infection (2010) **61**, 164—172

www.elsevierhealth.com/journals/jinf

High genetic diversity of HIV-1 viruses in Macao, China

Yi Tan [a], Desmond Chan [a], Denise Chan [a], Peng Kei Ip [d], Chong Lam [e], Nga Yin Fong [c], Stephen Kwok-Wing Tsui [c], Shui Shan Lee [a,b,*]

[a] *Stanley Ho Centre for Emerging Infectious Diseases, School of Public Health, The Chinese University of Hong Kong, Shatin, N.T. Hong Kong, China*
[b] *Department of Microbiology, The Chinese University of Hong Kong, Shatin, N.T. Hong Kong, China*
[c] *School of Biomedical Sciences, The Chinese University of Hong Kong, Shatin, N.T. Hong Kong, China*
[d] *Public Health Laboratory, Macao, China*
[e] *Macao CDC, Macao, China*

Accepted 30 April 2010
Available online 20 May 2010

KEYWORDS
BF genotype;
Molecular epidemiology;
Injection drug users;
IDU;
Macao;
China

Summary *Objective:* To investigate the molecular epidemiology of recently diagnosed HIV-1 infection in Macao for better understanding the epidemiology in this Chinese city, in context of its relationship with other countries in Asia and the rest of the world.
Methods: Serum samples of HIV positive cases reported between 2005 and 2007 were collected from the Macao Public Health Laboratory. HIV genotype was determined by phylogenetic analysis of sequences from *gag*, RT, and *env* regions.
Results: A total of 30 HIV positive samples were genotyped. The HIV-1 viruses circulating in Macao were characterized by their relatively high genetic diversity. CRF01_AE was predominant (56%), followed by subtype B (13%), CRF12_BF (10%), G/CRF12_BF, A1/CRF10_AD and CRF07_BC, of which CRF12_BF and G/CRF12_BF were first reported in Southeast Asia. Phylogenetic analysis showed that there was no clear clustering of CRF01_AE strains but a distinct CRF12_BF cluster associated with injection drug use could be delineated.
Conclusion: The results suggested that there were multiple introductions of HIV strains in Macao that have been circulating for an extended period of time, superimposed by an outbreak in injection drug users.

* Corresponding author. 2/F Postgraduate Education Centre, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong, China. Tel.: +852 2252 8812; fax: +852 2635 4977.
E-mail address: sslee@cuhk.edu.hk (S.S. Lee).

0163-4453/$36
doi:10.1016/j.jinf.2010.04.012

Introduction

Globally, there were an estimated 33 million people living with human immunodeficiency virus (HIV) as of 2007. The HIV epidemic has stabilized, albeit with unacceptably high levels of new HIV infections and AIDS deaths.[1] The pandemic currently is contributed largely by HIV-1, while HIV-2 is restricted to Western and Central Africa.[2] The diversity of HIV-1 with its division into subtypes (A—D, F—H, J and K of Group M) and 43 circulating recombinant forms (CRFs) offers a unique opportunity for mapping the spread of the infection geographically.[3] In Asia, HIV transmission is gaining momentum, the molecular epidemiology of which would be useful for tracking its spread in the continent. CRF01_AE circulates in major parts of Asia, particularly Southeast Asia,[4–6] a pattern unlike that in Western Europe and Americas where Subtype B has always been the main epidemic component.[7,8] Globally, Subtype C is at the heart of the pandemic, accounting for 60% of HIV infections worldwide.[9,10]

There are disparate epidemic trends in Asia, home to 5 million people with HIV. Although the prevalences in Cambodia, Myanmar and Thailand have shown declines,[1] new infections are increasing steadily in China, especially in the southern provinces.[11,12] Macao, a small Chinese city of 28.6 km^2 in the Pearl River Delta (PRD), is one of the linkages between China and countries in and outside Asia. Macao has been a territory of Portugal and was both the first and last European colony in China. However, little information is available about the epidemiology of HIV-1 in this small city. According to the World Health Organization (WHO) and Joint United Nations Programme on HIV/AIDS (UNAIDS), the epidemic state of HIV in Macao is described as low, as HIV prevalence is generally less than 0.1%.[1] Macao is however facing the challenges of maintaining the low HIV infection level. Injection drug use and sexual activities in the region, for example, can lead to an increase of HIV transmission and the introduction of new genetic forms. There are no studies addressing the emergence of HIV genetic variants in Macao. The objective of this study is, therefore, to investigate the molecular epidemiology of HIV-1 infection in Macao, so as to contribute to effective surveillance as well as to improve prevention and treatment.

Materials and methods

Study participants

This is a cross-sectional study on the molecular epidemiology of HIV-1 infections in Macao, which forms an extended part of HIV surveillance under the disease notification mechanism. Left-over sera from reported HIV cases were collected from Macao Public Health Laboratory from June 2006 to November 2007. Serum samples with viral load at least 50 copies per ml and volume not less than 500 μl were archived. All laboratory tests were performed at Stanley Ho Centre for Emerging Infectious Diseases, the Chinese University of Hong Kong, as part of a collaborative arrangement.

HIV RNA extraction, reverse transcription, nested-PCR amplification, and sequencing

HIV RNA was extracted from 200 μL of serum by using QIAamp Viral RNA Mini Kit (Qiagen Inc., Hiden, Germany), followed by standard protocols. Complementary DNA (cDNA) was synthesized from 5 μL extracted RNA with First Strand Synthesis System for RT-PCR (Invitrogen Corporation, San Diego, CA). Primers for *gag* and *env* genes were obtained from the Centers for Disease Control and Prevention, United States (US CDC). The primers for the RT gene were designed in-house (Table 1). First round PCR using primer F1 and R1 was conducted with the following conditions: 95 °C for 5 min, then 39 cycles of 95 °C for 1 min, 45 °C for 1 min and 72 °C for 1 min 45 s. The PCR ended with a final extension of 72 °C for 10 min. Second round PCR using primer F2 and R2 was conducted with the same

Table 1 List of the PCR primers used in the study.

Region[a]	Name	Sequences (5′—3′)	Position[b]	Gene
gag	Rv-typing-gag-F1	GCG AGA GCG TCA RTA TTA AGI GG	796—818	P17 and P24
	Rv-typing-gag-F2	GGG AAA AAA TTC GGT TAA GGC C	836—857	
	Rv-typing-gag-R1	TCT GAT AAT GCT GWR AAC ATG GG	1319—1297R	
	Rv-typing-gag-R2	CTT CTA YTA CTT TYA CCC ATG C	1271—1249R	
env	Rv-typing-env-F1	ACA GTR CAR TGY ACA CAT GG	6954—6973	gp120
	Rv-typing-env-F2	CTG TTI AAT GGC AGI CTA GC	7002—7021	
	Rv-typing-env-R1	CAC TTC TCC AAT TGT CCI TCA	7668—7648R	
	Rv-typing-env-R2	RAT GGG AGG RGY ATA CAT	7541—7524R	
RT	BF_1740F1	GGA AAA TGG AAA CCA AAA ATG A	2370—2391	Protease and
	BF_1745F2	ATG GAA ACC AAA AAT GAT AGG G	2375—2396	P51RT
	BF_2718R1	TTT CCC ACT AAC TTC TGT ATG TC	3337—3315R	
	BF_2619R2	GTT CAT AAC CCA TCC AAA GGA A	3249—3228R	

[a] Genomic regions amplified.
[b] Nucleotide positions with reference to the numbering of HIV HXB2 strain (Genbank accession number K03455).