PREVALENCE OF HIV-1 DRUG RESISTANCE IN ANTIRETROVIRAL-NAIVE PREGNANT THAI WOMEN

Somsri Auswinporn[1], Ekachai Jenwitheesuk[1], Panyu Panburana[2], Sayomporn Sirinavin[3], Asda Vibhagool[4] and Wasun Chantratita[1]

[1]Virology and Molecular Microbiology Unit, Department of Pathology; [2]Department of Obstetrics and Gynecology; [3]Department of Pediatrics; [4]Department of Internal Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Abstract. HIV-1 drug resistance may limit the use of antiretrovirals when attempting to reduce the vertical transmission rate. Establishing the prevalence of the HIV-1 mutations associated with antiretroviral resistance in pregnant women will enable clinicians to maximize the chances of preventing vertical transmission. In order to determine the prevalence of HIV-1 resistant strains among antiretroviral-naive pregnant Thai women, the nucleotide sequences of the HIV-1 polymerase (*pol*) gene were evaluated. The plasma samples were collected from the women during the 34th week of pregnancy: numerous secondary mutations could be found in the reverse transcriptase (RT) and protease gene, while no primary mutations in the *pol* gene were found. The result also showed that by detecting the △32bp deletion within the CCR 5 locus, it was evident that none of HIV-1 infected individuals had homozygous or heterozygous △32bp deletions of the CCR5 gene; moreover, no CCR5 gene mutations were found in any individual.

INTRODUCTION

Despite the remarkable efficacy of antiretrovirals in preventing perinatal HIV-1 transmission, a small but significant proportion of mothers still transmit infection. Failure to prevent perinatal transmission may, in some cases, be linked to maternal antiretroviral resistance. Vertical transmission of antiretroviral-resistant HIV-1 has been reported following the incomplete suppression of maternal viremia and extensive antiretroviral exposure prior to delivery (Johnson *et al*, 1999). In this case, the mother had a resistant virus prior to delivery, which suggested that the antiretroviral was unlikely to prevent transmission.

Establishing the prevalence of resistant virus in the community, and espicially in pregnant women, will provide valuable information that could guide treatment and result in the modification of drug regimens, in turn maximizing the chances of preventing vertical transmission from pregnant women to their offspring.

In the present study, HIV-1 RT and protease gene sequence data were analysed; the subjects were 21 antiretroviral-naive pregnant Thai women. The aim of the study was to establish the prevalence and appearance of the mutations associated with antiretroviral resistance.

Correspondence: Dr Wasun Chantratita, Virology and Molecular Microbiology Unit, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand.
Tel: ++66 (0) 2201 1369; Fax: ++66 (0) 2201 1324
E-mail: rawct@mahidol.ac.th

MATERIALS AND METHODS

Sample preparation

This study was conducted at Ramathibodi Hospital, Bangkok, between October 2000 and October 2001. The project and informed consent have been reviewed and approved by the Ethics Committee of Ramathibodi Hospital. Twenty-one HIV-infected women enrolled con-

secutively, and all were during the 34th week of pregnancy naive for antiretrovirals at inclusion. Venous blood samples were collected from the 21 subjects. Viral RNA was extracted by using the QIAamp™ RNA extraction kit (Qiagen Inc, California, USA). Human DNA was purified from the peripheral blood mononuclear cells (PBMC) by using the High Pure PCR Template Preparation Kit (Boehringer Mannheim, Indianapolis, USA), which was used in accordance with the manufacturer's instructions.

HIV-1 RNA load

The HIV-1 RNA load was determined by the Amplicor HIV-1 MONITOR Test (Roche Diagnostics) according to the manufacturer's instructions. A new set of gag primers (SK145 and SK151), provided by the manufacturer, was added to the master mix reagent before amplification to ensure efficient quantification of RNA from the HIV-1 subtype CRF01_AE. With a starting volume of 200 ml, the assay had a threshold sensitivity of 200 HIV RNA copies/ml and a linear quantitation range of 400-750,000 copies/ml.

Gene amplification and sequencing

The entire protease and 75% of 5′ reverse transcriptase (codon 1-320) of HIV-1 were reverse transcribed and PCR-amplified by the Superscript One-step RT-PCR (Life technology/Invitrogen) with forward primers P1 (5′ TTG GAA ATG TGG AAA GGA AGG AC 3′) and reverse primer P2 (5′ AAT CTG ACT TGC CCA ATT CAA TTT 3′). The amplified DNA product was directly sequenced by the Dye Terminator Cycle Sequencing ready reaction kit (HIV Genotyping System, PE Biosystems). The products of cycle sequencing were analyzed by an ABI PRISM 310 automated DNA sequencer. All DNA sequences were then compared with the HIV-1 reference sequence in order to explore the amino acid changes associated with drug resistance. Interpretation of the genotype in terms of drug resistance was based on an algorithm established by the Los Alamos HIV-1 genotypic database. The sequences reported here have been submitted to Genbank under accession numbers AF187037-187039, AF191189-191195 and AF345955-345976.

The △32 bp deletion of the human CCR5 gene was detected by PCR-amplification using the CCR5 gene specific primers CCR5/1 (5′CCAGATCTCAAAAAGGTCT 3′) and CCR5/2 (5′GGTCTTCTCTTTTATTTGTTAGT 3′). The PCR product was then run on agarose gel electrophoresis and directly sequenced using specific primers, N5/1 (5′CTGCATCCTAGGT GCAATGT3′) and N5/2 (5′GGTCTTCTCTT TTATTTGTTAGT 3′).

Phylogenetic analysis

In order to rule out laboratory contamination or multiple sampling of the same patients at different testing sites, and determine the subtype of HIV, phylogenetic analysis was performed using the Phylogeny Inference Package (version 3.6); PHYLIP (Felsenstein, 1989) programs DNADIST, SEQBOOT, NEIGHBOR, CONSENSE and DRAWTREE. The tree was outgroup-rooted by using the Simian Immunodeficiency Virus (SIV) in the alignment. Distance matrices were generated by DNADIST using a Kimura 2-parameter model. Statistical significance was tested using SEQBOOT to produce bootstrapped datasets (n = 100). Trees were constructed from the distance matrices using the neighbor-joining (NEIGHBOR) method and a consensus tree was calculated by CONSENSE. Trees were visualized using DRAWTREE or TreeView.

RESULTS

Twenty-one blood samples were collected for analysis of the *pol* gene: positive PCR results were obtained in all cases. No primary mutation associated with high-level antiretroviral resistance was found. However, secondary mutations, which contributed to resistance when accompanied by primary mutations, were found at positions 10, 36, and 93 of the protease gene and position 43 of the RT gene (Table 1).

Table 1
HIV genotypic resistant mutations in the protease and reverse transcriptase genes.

Patient number	Age (year)	Viral load (copies/ml)	Protease	Reverse transcriptase	Subtype
P1	24	38,000	M36I	K43E	CRF01_AE
P2	24	20,000	M36I	K43E	CRF01_AE
P11	22	4,700	M36I	K43E	CRF01_AE
P12	23	<400	M36I	K43E	CRF01_AE
P19	36	470	L10I, M36I, I93L	K43E	CRF01_AE
P26	18	590	M36I, I93L	K43E	CRF01_AE
P31	27	34,000	M36I	K43E	CRF01_AE
P35	30	<400	M36I, I93L	K43E	CRF01_AE
P45	23	16,000	M36I	K43E	CRF01_AE
P60	27	25,000	M36I	K43E	CRF01_AE
P61	28	37,000	M36I	K43E	CRF01_AE
P79	31	1,300	M36I	K43E	CRF01_AE
P86	29	6,500	M36I, I93L	K43E	CRF01_AE
P96	23	3,400	M36I	None	CRF01_AE
P102	26	8,200	M36I	K43E	CRF01_AE
P120	27	8,700	None	None	B
P123	32	50,000	M36I	None	CRF01_AE
P140	21	18,300	M36I	None	CRF01_AE
P141	24	14,000	M36I	None	CRF01_AE
P146	27	76,000	M36I	None	CRF01_AE
Px	32	<400	M36I	None	CRF01_AE

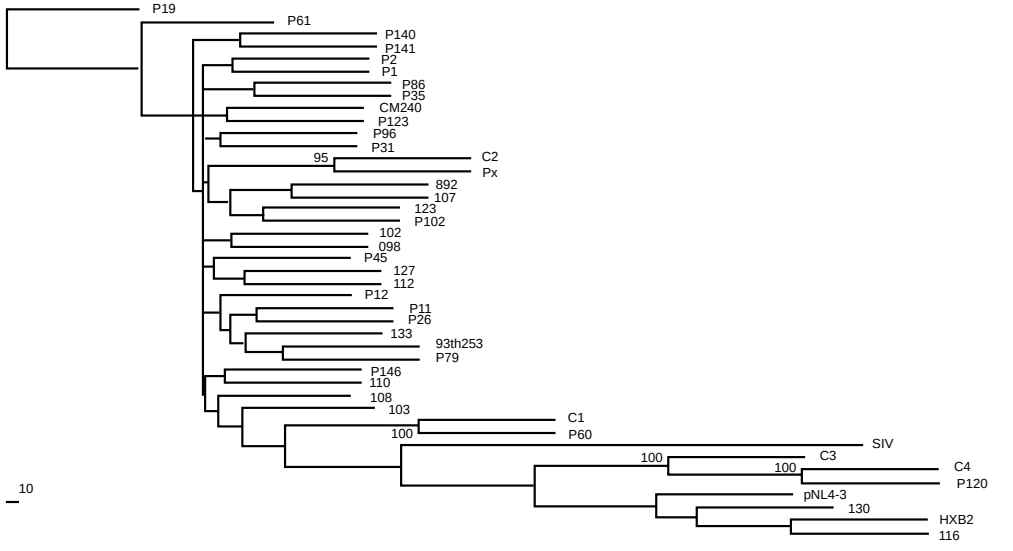

Fig 1–Phylogenetic tree of HIV-1 *pol* genes isolated from antiretroviral-naive pregnant Thai women. The tree was constructed by PHYLIP (version 3.6) with the HIV-1 subtype CRF01_AE (CM240 and 93th 253) and B (pNL4-3 and HXB2) reference sequences.

Two subtypes of HIV-1 were found among the 21 patients; phylogenetic analysis identified CRF01_AE in 20 of the women (95.5%); one patient (4.5%) had subtype B (Fig 1).

Defection of the △32 bp deletion within the CCR 5 locus made it clear that none of the subjects had homozygous or heterozygous △32-bp deletion of the CCR5 gene; moreover, no CCR5 gene mutations were found in any individual.

DISCUSSION

The data from this study show that their is a low frequency of any type of high-level drug resistant HIV strain among treatment-naive pregnant women in Thailand. The sequence of the HIV-1 *pol* gene from the subjects in this study suggests that a wild type was involved, which remains sensitive to all antiretrovirals.

In addition to antiretroviral therapy, cesarean sections, a low viral load, a high CD4 count, good nutrition, and healthy immunity all serve to reduce the vertical transmission of HIV. A genetic host factor and a 32 bp deletion in the CCR5 gene have been known to influence a low infection rate of HIV-1. A defective CCR5 allele or mutation of the CCR5 gene (32 bp deletion) results in non-functional chemokine receptors. This major factor can reduce the vertical transmission of HIV and impede the progress of the disease (Alexandra *et al*, 1997; Nathaniel, 1997). However, several studies have reported that the CCR5 32 bp deletion allele rarely occurs outside the Caucasian group. Gongalez *et al*, 1998; Yuanan *et al*, 1999. This study showed no evidence of a 32 bp deletion of the CCR5 gene in all 21 mother-child pairs, which included both homozygosity and heterozygosity.

The result of this study showed that none of the HIV-1 mutations that conferred a high level of antiretroviral drug resistance and 32 bp deletion in the CCR5 gene were found in antiretroviral-naive Thai pregnant women.

REFERENCES

Alexandra T, Tatjana D, Jame A, *et al*. CD4-dependent, antibody-sensitive interactions between HIV-1 and its co-receptor CCR-5. Fourth Conference on Retroviruses and Opportunistic Infections. January, 1997: S9.

Felsenstein J. PHYLIP - Phylogeny Inference Package (Version 3.2). *Cladistic* 1993; 5: 164-6.

Gonzalez S, Tirado G, Revuelta G, *et al*. CCR5 chemokine receptor genotype frequencies among Puerto Rican HIV-1-seropositive individuals. *Bol Asoc Med P R* 1998; 90: 12-5.

Johnson VA, Woods C, Hamilton CD, *et al*. Vertical transmission of an HIV-1 variant resistant to multiple reverse transcriptase and protease inhibitors. [Abstract]. Chicago: Sixth Conference on Retroviruses and Opportunistic Infections. January 31-Febuary 4, 1999: 266.

Lu Y, Nerurkar VR, Dashwood W-M, *et al.* Genotype and allele frequency of a 32-base pair deletion mutation in the CCR5 gene in various ethnic groups: Absence of mutation among Asians and Pacific Islanders. *Int J Infect Dis* 1999; 3: 186-91.

Nathaniel L. The role of CCR-5 in HIV entry and susceptibility of individuals to infection. Fourth Conference on Retroviruses and Opportunistic Infections. January, 1997: S11.