Characterization of HIV Type 1 Genotypes and Drug Resistance Mutations Among Drug-Naive HIV Type 1-Infected Patients in Northern Vietnam

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Abstract

To evaluate HIV-1 drug resistance-associated mutations among drug-naive HIV-1-infected patients in Northern Vietnam, we performed sequence analysis of HIV-1 pol-PR and pol-RT in samples collected from 206 (161 men and 45 women) consenting patients in 2008. From these 206 samples, we successfully sequenced 173 pol-PR and 155 pol-RT genes. Phylogenetic analysis revealed that all patients were infected with HIV-1 CRF01_AE. Major protease inhibitor resistance mutations, such as L33F, M46I, and M46L, were found in three patients (1.7%). Major reverse-transcriptase inhibitor (RTI) resistance mutations were found in seven patients (4.5%), four of whom had single mutations: A62V (nucleoside RTI resistance mutation) in two cases and K103N and Y181C (nonnucleoside RTI resistance mutation) in one case each. Three patients had multiple RTI resistance mutations: two, three, and seven, respectively. Thus, monitoring for drug-resistant HIV-1 and performing drug resistance testing before initiating antiretroviral therapy (ART) are recommended to facilitate selection of the appropriate ART and better clinical outcomes in Vietnam.

The first case of HIV-1 infection in Vietnam was detected in Ho Chi Minh City in 1990. By the end of 1998, the HIV epidemic had spread to all 61 provinces and cities. The number of people living with HIV-1 in Vietnam has increased steadily from $112 \times 10^3$ in the year 2000 to $293 \times 10^3$ in 2007.1,2 Previous studies showed that HIV-1 CRF01_AE is the predominant strain in Vietnam,3–7 although subtype B, subtype C, and intersubtype recombinants have also been identified.8–11 A number of governmental, civilian, and international programs have been implemented to reduce endemic HIV-1 infection in Vietnam, and the availability of treatment, care, and support programs for HIV-1-infected individuals has also increased. In 2003, antiretroviral treatment (ART) was introduced into Vietnam by the Vietnamese government, WHO, and international donors, resulting in an increase in ART coverage of HIV-1-infected individuals from 1% in 2003 to 28.4% in 2007.1–3 Because ART was introduced into Vietnam rapidly, it is anticipated that the appearance and spread of ART-resistant strains of HIV-1 will become problematic. ART-resistant HIV-1 among drug-naive HIV-1-infected patients was reported to be 6.5% in Ho Chi Minh City (Southern Vietnam) in 2003, less than 5% in Hanoi (Northern Vietnam) in 2006,6,7 and 2.9% in Hai Phong (Northern Vietnam) in 2007.3 The aim of this study was to characterize the HIV-1 pol gene, which encodes protease (PR) and reverse transcriptase (RT), and to monitor the prevalence of drug resistance mutations among ART-naive patients in the northern part of Vietnam.

Two hundred and six HIV-1-infected patients (men/women, 161/45; mean age, 32.0 years; age range, 17–54 years) were recruited from four HIV/AIDS centers in Northern Vietnam (two centers in Hanoi, one each in Ninh Binh and Nam Dinh provinces). The patients were admitted to or visited these centers regularly. No patients reported prior exposure to ART and/or single-dose nevirapine for the prevention of mother-to-child transmission of HIV-1. The study protocol was reviewed and approved by the ethical committees of the National Institute of Hygiene and Epidemiology in Vietnam and Kanazawa University in Japan. After obtaining informed consent, we collected blood samples from the participants in June and July of 2008. HIV-1 RNA was extracted from 100 µl of HIV-1-positive plasma using an SMITEST EX-R&D Nucleic Acid Extraction Kit (Genome Science Laboratories, Fukushima, Japan), according to the manufacturer’s instructions. The HIV-1 pol gene was amplified by both

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one-step reverse transcription-polymerase chain reaction (RT-PCR; SuperScript III One-step RT-PCR system with Platinum Taq High Fidelity; Invitrogen, Carlsbad, CA) and nested PCR using AmpliTaq Gold (Applied Biosystems, Japan) and/or KOD FX (Toyobo, Osaka, Japan), as described previously. The amplified products were directly sequenced and analyzed using an ABI Prism 310 Genetic Analyzer (Applied Biosystems) with BigDye Terminator v1.1 (Applied Biosystems). The sample nucleotide sequences were aligned with HIV-1 subtype/CRF reference sequences from the Los Alamos database and with previously reported sequences of HIV-1 strains isolated from Southeast Asia and Southern China using Clustal W (version 1.83), with minor manual adjustments. Phylogenetic trees were constructed and visualized as described previously. The presence of drug resistance-associated mutations in the PR and RT regions was analyzed using an ABI Prism 310 Genetic Analyzer (Applied Biosystems) with BigDye Terminator v1.1 (Applied Biosystems). The amplified products were directly sequenced and analyzed, seven (4.5%) had major reverse transcriptase inhibitor (RTI) resistance-associated mutations: five had nucleoside RTI (NRTI) resistance-associated mutations such as M41L, M46I, M46L, and I50V (Table 1). Of the 155 RT region sequences analyzed, seven (4.5%) had major reverse transcriptase inhibitor (RTI) resistance-associated mutations; five had nucleoside RTI (NRTI) resistance-associated mutations such as M41L, A62V, D67N, M184V, L210W, and T215Y, and five had non-nucleoside RTI (NNRTI) resistance-associated mutations such as K103N, V108I, Y181C, and G190A. Three had multiple mutations (two, three, and seven, respectively; Table 1). GenBank/EMBL/DBJ accession numbers of the sequences reported in this study are AB519452 to AB519595 for pol-PR/RT, AB519596 to AB519624 for pol-PR, and AB519625 to AB519635 for pol-RT.

The prevalence of mutations that induce antiretroviral drug (ARV) resistance among ART-naive HIV-1-infected patients sampled in 2008 in Northern Vietnam was 1.7% for PI and 4.5% for RTI. However, previous studies reported this prevalence at less than 5% for both in Hanoi in 2006 and 2.9% (0.3% for PI and 2.6% for RTI) in Hai Phong in 2007. These findings could indicate that the HIV-1 drug resistance rate among ART-naive HIV-1-infected individuals has been increasing in Northern Vietnam, although the area used for the present study differs from the areas used in previous studies.

In this study, ARV-resistant mutants were detected in 10 patients. Of them, three had a virus that harbored multiple drug resistance mutations; in particular, two had multiple NRTI resistance mutations. Because the NRTI resistance mutations have a high fitness cost for the virus and these mutations would not develop in the absence of drug pressure, the possibility that our patients had previous unreported contact with ARV cannot be excluded. Therefore, we could have overestimated the number of HIV-1 drug-resistant strains transmitted in our study population. Considering these and other limitations of a self-reporting system, performing drug resistance testing before initiating ART would likely result in better clinical outcomes, even in resource-limited countries.

HIV-1 CRF01_AE strains were predominant in Northern Vietnam. Phylogenetic analysis revealed that the majority of HIV-1 CRF01_AE strains analyzed in this study were closely related to strains prevalent in Hai Phong (Northern Vietnam) and Guangxi (Southern China), and were different from strains prevalent in Ho Chi Minh (Southern Vietnam), Cambodia, and Thailand. These findings are consistent with our previous findings in Hai Phong in 2007 and with those of previous studies, which reported that CRF01_AE strains in the northern provinces along the Vietnam–China border were introduced from Guangxi province and reached Hanoi through heroin-trafficking routes, or vice versa.

In conclusion, the most prevalent HIV-1 strains in Northern Vietnam were confirmed to be CRF01_AE, and the majority strains were similar to strains in Hai Phong (Northern Vietnam) and Southern China. The prevalence of ARV-resistant HIV-1 among drug-naive individuals was 6.2% (1.7% for PI and 4.5% for RTI), which is slightly higher than the prevalences reported in the threshold survey in Hanoi in 2006 and

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Age (years)/gender</th>
<th>Center</th>
<th>HIV-1 subtype</th>
<th>PI resistance mutations</th>
<th>RTI resistance mutations</th>
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<tr>
<td>NB56</td>
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<td>Ninh Binh</td>
<td>CRF01_AE</td>
<td>L33F</td>
<td>NRTI</td>
</tr>
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<td>Nam Dinh</td>
<td>CRF01_AE</td>
<td>M46I</td>
<td>NNRTI</td>
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<td>NB15</td>
<td>27/M</td>
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<td>CRF01_AE</td>
<td>M46L</td>
<td></td>
</tr>
<tr>
<td>NB14</td>
<td>29/M</td>
<td>Ninh Binh</td>
<td>CRF01_AE</td>
<td>A62V</td>
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</tr>
<tr>
<td>AH40</td>
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<td>A62V</td>
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<td>CRF01_AE</td>
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<tr>
<td>09.28</td>
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<td>CRF01_AE</td>
<td>M184V</td>
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<td>CRF01_AE</td>
<td>K65R, M184V</td>
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</tr>
<tr>
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<td>Hanoi</td>
<td>CRF01_AE</td>
<td>M41L, D67N, M184V, L210W, T215Y</td>
<td></td>
</tr>
</tbody>
</table>

*PI, protease inhibitor; RTI, reverse transcriptase inhibitor; NRTI, nucleoside RTI; NNRTI, nonnucleoside RTI.
the prevalences in Hai Phong in 2007. Thus, drug resistance monitoring is recommended to ensure selection of the appropriate ART for HIV-1-infected individuals in Vietnam.

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Author Disclosure Statement
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