Molecular Characterization of HIV Type 1 Isolates from Untreated Patients of Mumbai (Bombay), India, and Detection of Rare Resistance Mutations

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ABSTRACT

The molecular characterization of HIV-1 isolates in drug-naive cases in the early stages of HIV disease was studied in 128 cases from Mumbai (Bombay), India. Subtype C was largely predominant followed by A–C intersubtype recombinants, one subtype A and one CRF01_AE. Compared to subtype B, subtype C exhibited an important polymorphism; the percentages of substitutions could reach more than 90%. Two isolates showed M184V substitution the reverse transcriptase indicating resistance to 3TC.

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Blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes and plasma was separated and stored at ~80°C.
HIV-1 MUTATIONS FROM MUMBAI, INDIA

Table 1. Sequences of the ANRS Consensus Primers Used for Amplification of RT, PR, and env C2/V3 Regions

<table>
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<tr>
<th>Zone primers</th>
<th>Inner primers</th>
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<tbody>
<tr>
<td>RT</td>
<td>5'-ATGAGAACCACTACATGTCACAC-3' bases 2480 to 2501</td>
</tr>
<tr>
<td>5'-GGCAACATGTGTTGATTGATTTC-3' bases 2082 to 2108</td>
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</tr>
<tr>
<td>5'-AATACATAGCTTCTGTATGTCATTGACAG-3' bases 6955 to 6973</td>
<td></td>
</tr>
<tr>
<td>Protease</td>
<td>5'-TCAGG-3' bases 2703 to 2734</td>
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<tr>
<td>5'-CAGTCAATGTACATCAGG-3' bases 2136 to 2163</td>
<td></td>
</tr>
<tr>
<td>5'-ATGGCAAGTCTAGCAGAAG-3' bases 7522 to 7540</td>
<td></td>
</tr>
<tr>
<td>C2/V3</td>
<td>5'-TTACAGTAGAAAAATTCCCCTC-3' bases 3300 to 3334</td>
</tr>
<tr>
<td>5'-TTATTTTATTTTTTTCTTCTGTCAATG-3' bases 2530 to 2564</td>
<td></td>
</tr>
<tr>
<td>5'-TGTTTCACTTTAAATTTTCCCATTAG-3' bases 2621 to 2650</td>
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The mean age of the studied population was 30.4 years and the median TCD4 T cell count was 594/µl (range 403–1586).

In the study population, subtype C is largely predominant (Fig. 1) followed by A–C intersubtype recombinants, one subtype A, and one CRFH1_AE. The predominance of subtype C in India has already been reported by different authors,5–6 the presence of A–C intersubtype recombinants is in agreement with previous work.7 Although the introduction of the B subtype in Bombay has been reported,5 this study did not identify the B subtype in any case. Subtype A and CRFH1_AE are rarely observed in Bombay as has been pointed out in India.5

The amino acid substitutions in the RT are presented in Fig. 2. Compared to subtype B, subtype C exhibits an important polymorphism; the percentages of substitutions can reach more than 90% at the following positions: 33, 39, 48, 60, 173, 177, 200, 207, 214, and 245. Resistance mutations at position 184 (M184V) could be detected in two isolates of subtype C; these mutations are known to be associated with resistance to 3TC, a molecule belonging to the NRTI group of drugs.

Concerning the protease gene (Fig. 3), the most frequently (above 50%) observed positions of amino acid substitutions for subtype C are 12, 15, 19, 36, 69, 89, and 93. Substitutions at positions of secondary mutations to PIs are rare (10, 20, 46, 77, 82) or exhibit a high polymorphism (36, 63). No major resistance mutation to PIs could be noted.

With the confirmed predominance of subtype C in Bombay, the main result of this study is the presence of resistance mutations to an NRTI in isolates from patients who denied having received treatment with ARV. It is difficult to find an explanation for these mutations: did the patients, in fact, receive ARV at improper doses or as a monotherapy with 3TC or as a bitherapy including 3TC? We must take into account the high level of resistance mutation to PIs could be noted.

The de novo transmission of HIV-1 isolates from patients who denied having received treatment with ARV. It is difficult to find an explanation for these mutations: did the patients, in fact, receive ARV at improper doses or as a monotherapy with 3TC or as a bitherapy including 3TC? We must take into account the high level of resistance mutation to PIs could be noted.
FIG. 2. Amino acid substitutions in the HIV-1 reverse transcriptase of isolates from 128 Indian patients. The gray columns concern significant polymorphism positions and the boxed columns indicate the positions of resistance mutations.
ACKNOWLEDGMENTS

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REFERENCES


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FIG. 3. Amino acid substitutions in the HIV-1 protease of isolates from 128 Indian patients. The gray columns concern significant polymorphism positions and the boxed columns indicate the positions of major and minor resistance mutations.