HIV-1 subtype C in Ethiopia: genotypic and phenotypic variation
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CHAPTER II

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HIV Type 1 Subtype C in Addis Ababa, Ethiopia

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HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 variants in different geographic regions have been phylogenetically classified into several distinct genetic subtypes (A–I and O) on the basis of sequence differences in the V3 regions of their gp120 envelope genes.1-3 In Africa the existence of all genetic subtypes except subtype I has been confirmed. The presence of this multitude of subtypes indicates the extensive divergence HIV-1 has accumulated in the African continent.3-5 This study describes the distribution of HIV-1 subtypes in Ethiopia, East Africa. The first Ethiopian AIDS case was reported in 1986 and the AIDS epidemic is a rapidly growing problem in Addis Ababa, the capital city with more than 2 million inhabitants.6 In Addis Ababa the HIV-1 seroprevalence is estimated to be 10–27% in pregnant women (PW), 47–59% in commercial sex workers (CSWs) (ENARP sentinel surveys 1995 and 1996) and 7% among blood donors (BDs) (Ethiopian Red Cross Society, National Blood Transfusion Service [ERCS-NBTS], unpublished data, 1994). In the countries neighboring Ethiopia subtypes A (Djibouti, Kenya), C (Kenya, Djibouti, Somalia), and D (Kenya) are present.1,3 Preliminary sequence data on a limited number of samples have indicated the presence of subtype C in Addis Ababa in 1988.8 To assess the distribution of HIV-1 subtypes in Addis Ababa in more detail, 94 sera were analyzed, collected from 3 different risk groups over 1989–1995 (Table 1).

HIV-1 RNA was isolated from the collected sera according to a standard procedure.9 Viral RNA was converted to cDNA and then subjected to a nested polymerase chain reaction (PCR) amplifying a 284-bp fragment covering the V3 region of the gp120 gene (positions 785–1069, HIV-1 subtype B consensus database, Los Alamos, 1993). First and second PCR conditions were as follows: 35 and 25 cycles of, respectively, 1 min at 95°C, 1 min at 55°C, and 2 min at 72°C, followed by a final incubation for 10 min at 72°C. The amplified products were sequenced directly by automated cycle sequencing with dye terminators, using an ABI (Foster City, CA) 370 A system. Sequence alignments and comparisons were performed by using CLUSTAL and the neighbor-joining algorithm.10,11 Figure 1 demonstrates the results of neighbor-joining sequence comparisons, clearly indicating that the vast majority of sequenced samples (93 of 94) are of the C subtype. The prevalences of C and A subtypes among the 57 samples analyzed from 1995 were, respectively, 56 of 57 (98.2%) and 1 of 57 (1.8%). In addition, our data show that the C subtype is highly abundant in samples collected before 1995.

The Ethiopian subtype C sequences tend to differ slightly from the consensus C sequence (HIV-1 subtype C consensus database, Los Alamos, 1995). Within the Ethiopian sequences, a subcluster could be identified that is fairly homogeneous. Illustrating this homogeneity, pairwise nucleotide comparisons revealed a 7.4% difference within this subcluster, as compared to an 11.5% difference within the remaining sequences. The latter are more related to HIV-1 subtype C sequences from different countries (Fig. 1). Statistical comparisons, using a previously published method,12 revealed significant sequence differences between the main group and the subcluster at positions 270, 272, 278, 286, and 294 (Fig. 2). The presence of a subcluster of the C subtype may suggest an independent introduction of the HIV subtype C virus in Addis Ababa. However, the subcluster could not be detected with significant preference in

### Table 1. Study Subjects, Number of Sera Sequenced, and Year of Sample Collection

<table>
<thead>
<tr>
<th>Year</th>
<th>Subjects</th>
<th>Samples tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989</td>
<td>Commercial sex workers</td>
<td>7</td>
</tr>
<tr>
<td>1990</td>
<td>Commercial sex workers</td>
<td>6</td>
</tr>
<tr>
<td>1992</td>
<td>Commercial sex workers</td>
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<tr>
<td>1995</td>
<td>Commercial sex workers</td>
<td>19</td>
</tr>
<tr>
<td>1995</td>
<td>Pregnant women</td>
<td>9</td>
</tr>
<tr>
<td>1995</td>
<td>Blood donors</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Total:</td>
<td>94</td>
</tr>
</tbody>
</table>

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FIG. 1. Phylogenetic tree analysis of 94 Ethiopian HIV-1 sequences by neighbor-joining algorithm. The main group and a subcluster are indicated; in addition, consensus sequences for HIV-1 A–E subtypes and different C-subtype sequences from various geographical areas are shown. Sequence names have been indicated by codes: 2589 (...), CSW’s 1989; AS90 (...), CSW’s 1990; KAZS92 (...), CSW’s 1992; TKS92 (...), CSW’s 1992; TP95 (...), PW 1995; KP95 (...), PW 1995; KS95 (...), CSW’s 1995; TS95 (...), CSW’s 1995; B95 (...). BD 1995. Numbers by the branches represent bootstrap values out of a 100 replications.
 Predicted amino acid sequences of the V3 region of gp120 of the 94 Ethiopian HIV-1 envelope protein sequences, as compared to a derived Ethiopian consensus sequence. The subtype A sequence is shown first, followed by the main group (62 sequences) and the subcluster (32 sequences). Dashes indicate amino acid identity with the consensus sequence; dots indicate gaps introduced for the purpose of alignment; X, unreadable nucleotides (N); asterisks indicate nucleotide positions with significant differences between the main group and the subcluster. Sequence names have been indicated by codes as described in Fig 1.

Figure 2 presents the amino acid sequence alignment of the Ethiopian samples, subdivided into main group and subcluster. All V3 loops are 35 amino acids long and show the characteristic C-subtype pattern of GPGQT at the apex. The single Ethiopian A-subtype sample shows at the apex a C-like GPGQT motif, instead of the consensus A subtype apex (GPGQA). The Ethiopian C-subtype sequences show a high frequency of methionine at position 306, as compared to other C isolates. This is particularly true for the subcluster (30 of 32 sequences, 94%) as compared to the main group (26 of 62 sequences, 42%). The biological consequences of this mutation remain to be elucidated. On the basis of previous studies the amino acid positions 305 and 319 determine the non-syncytium-inducing (NSI) versus syncytium-inducing (SI) phenotype of HIV-1 strains.

According to the amino acids predicted at these positions in the sequenced Ethiopian isolates, 93 of them would be NSI and possibly 1 would be SI (B95032). Because the majority of samples collected in a certain year, risk group, or site, giving no information regarding possible independent introductions of these sequences.
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REFERENCES


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