

## Sequence Note

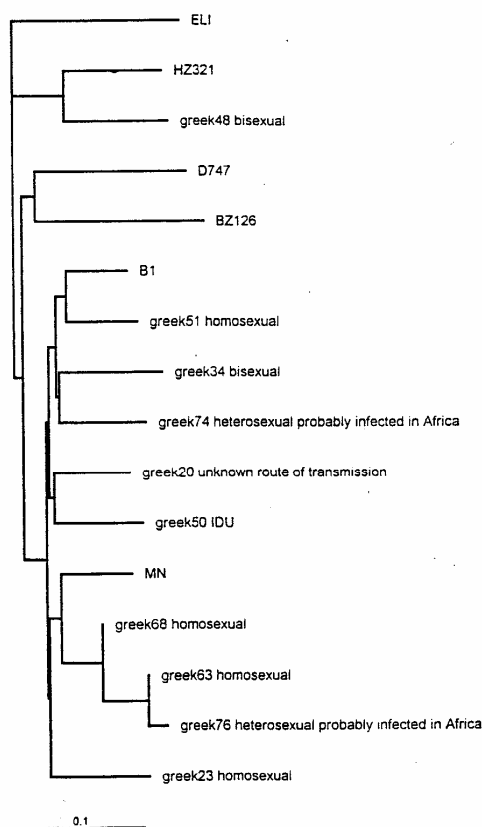
### The Subtypes of HIV Type 1 in Greece

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**S**TUDY OF THE DISTRIBUTION of the subtypes of HIV-1 throughout the world had shown that particular subtypes are, in general, associated with distinct geographical areas.<sup>1,2</sup> For instance, subtype B is the main HIV-1 subtype found in North America and Europe, and also in South America, but it is rarely found in Africa and Asia, where other subtypes (A, C, D, and E) predominate. However, the range of non-B subtypes described in Western countries has increased, mainly owing to individuals who have traveled to other continents or immigrated from them.<sup>3-10</sup> Subtype I was described in the Republic of Cyprus.<sup>11</sup> This subtype diversity has implications for the diagnosis, treatment, and prevention of HIV-1 infection.

To gain insight into the sequence diversity of HIV-1 in Greece, a country visited by many tourists, we have studied the genetic divergence of HIV-1 strains by polymerase chain reaction (PCR) amplification of HIV *env* sequences, followed by heteroduplex mobility assay (HMA) and sequencing. Provirial DNA was extracted from uncultured peripheral blood mononuclear cells obtained from 58 HIV-positive patients (45 men, 13 women) from different risk groups for HIV infection. All of the patients were living in northern Greece and were infected with HIV between 1991 and 1997. Fifteen of them were homosexual, 7 were bisexual, 16 were heterosexual, 7 were intravenous drug users (IDUs), 1 was multiply transfused, and for 10 the transmission route was unknown. Two patients who were believed to have been infected in Africa were also included in the study.

The 500-bp amplicon of *env*, containing the C2-V3 region, was amplified by nested PCR, using ED3 and ED14 as outer primers and ED31 and ED33 as inner primers. Heteroduplexes were formed as described<sup>12</sup> and the products of the HMA were analyzed on 3% MetaPhor XR agarose gels (FMC BioProducts, Philadelphia, PA).<sup>13</sup> From the total of 58 samples, 53 were characterized as subtype B and 1 as subtype A. Four could not be typed by HMA. These four samples, the subtype A sample, and five other samples were further characterized by sequencing and phylogenetic analysis. For this purpose, PCR products were purified from gel slices, using the Mermaid kit (BIO 101, La Jolla, CA). Direct sequencing of the purified PCR products was per-



**FIG. 1.** Phylogenetic tree based on a 398-nt *env* region containing the C2-V3 coding sequences of Greek specimens and representatives of each subtype from the Los Alamos HIV Sequence Database. The tree was constructed by the neighbor-joining method with Clustal W<sup>14</sup> and Phylip programs.<sup>15</sup> Horizontal distances are proportional to nucleotide differences (see bar scale).

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formed by dye termination cycle sequencing with an Applied Biosystems (Foster City, CA) 377 sequencer. Alignments of the DNA sequences were generated using Clustal W<sup>14</sup> and phylogenetic analysis was by neighbor joining, using Phylip programs.<sup>15</sup> A phylogenetic tree based on 398 aligned nucleotides was constructed from 10 sequences (Fig. 1). For comparison, existing sequence data were obtained from the GenBank Sequence Database.

Nine of the 10 sequences obtained (greek20, greek23, greek34, greek50, greek63, greek68, greek74, greek51, and greek76) clustered with subtype B sequences, and the other (greek48) with subtype A sequences. This subtype A sequence came from a bisexual male who had had many sexual partners of different nationalities. We believe he was infected in northern Greece. The two patients (greek74 and greek76) who had resided in Africa, and had probably acquired the virus heterosexually, had subtype B sequences. Greek74 is a native Greek who had lived for 15 years in Kenya, whereas greek76 is a black woman from Nigeria. Both patients were found to be HIV positive just when they came in Greece. The fact that they had subtype B sequences is unexpected and we do not know its significance.

In Greece, a total of 1699 HIV-1 infections has been reported up to the end of September 1997; of these, 1674 are adults and adolescents, and 25 are children. Of the 1674 adult cases, 74.3% were infected sexually, 4.3% by injecting drug use, 9.2% through multiple transfusions, and 12.2% by unknown routes.<sup>16</sup> Our study shows that in Greece subtype B is the most prevalent subtype and may be recovered from individuals in diverse risk groups. Subtype I was first described in Cyprus<sup>11</sup> and it is of note that we did not find any of this sequence among the patients we studied. Further molecular epidemiological surveillance of the sequence diversity of HIV-1 in Greece will be necessary to determine the extent to which other subtypes are being introduced into the country.

GenBank accession numbers are as follows: AJ224947 for greek50, AJ224948 for greek51, AJ224949 for greek63, AJ224950 for greek68, AJ224951 for greek74, AJ224952 for greek76, AJ224953 for greek20, AJ224954 for greek23, AJ224955 for greek34, and AJ224956 for greek48.

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