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Genetic subtypes of human immunodeficiency virus type 1 (HIV-1) in Istanbul, Turkey

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Summary
Background: Epidemiological surveillance of HIV-1 subtypes is an important and ongoing element of preparation for global antiviral interventions.
Objective: To assess the molecular epidemiology of HIV-1 in Istanbul, Turkey.
Study design: 27 HIV/AIDS patients were investigated. Data on age, sex, country of birth, and HIV acquisition route were collected. Following amplification with PCR the sequences of the gp41 region of the *env* gene were determined using a 310 DNA sequencer (ABI prism, Foster City, USA) and phylogenetically analyzed.
Results: Among the 27 patients (26 adults and 1 infant), 22 were male, born in Turkey, and 20 infected through heterosexual contact. Two patients acquired the virus through blood and/or blood transfusion and one infant by vertical transmission. The distribution of the subtypes was as follows: four were subtype A, 19 subtype B, one subtype C, one subtype D, and two subtype F1. According to our results, although the B subtype is still predominant, non-B subtypes are also present, even though the number of registered HIV/AIDS patients is low.
Conclusion: These are the first subtyped HIV-1 strains in Turkey where a low level of HIV prevalence has been observed since the first reported case in 1985. These findings and Turkey's specific geographic localization indicate the need for a nationwide surveillance to detect all subtypes including the new recombinant ones.

Introduction

Human immunodeficiency virus type 1 (HIV-1) is characterized by a very high genetic diversity. This genetic heterogeneity is due to the error-prone reverse transcriptase, the

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rapid turnover of HIV-1 in vivo, recombination, and selective immune pressures by the host. It is divided into three main groups: M (major), O (outlier), and N (non-M, non-O-new). Group M, which includes the great majority of HIV isolates is divided into at least nine genetic subtypes (A–D, F–H, J and K) and at least 15 major circulating recombinant forms (CRFs). The second group, O, is composed of highly divergent HIV-1 strains. New strains isolated from Cameroonian patients have recently been classified in the group N.

The extraordinary variability of HIV has led to the development and geographical distribution of various distinctive clades or subtypes of the virus. Although data are limited, HIV clades and subtypes may have inherent resistance against certain classes of antiretroviral drugs. Viral fitness and therefore viral kinetics may vary between HIV subtypes and may also influence drug sensitivity of the virus. Some subtypes have been found to be associated with rapid progression to resistance under antiretroviral therapy. So the importance of the epidemiological surveillance of HIV-1 subtypes cannot be denied.[1–7] The objective of the study was to assess the molecular epidemiology of HIV-1 in Istanbul, Turkey.

Material and methods

We investigated 27 HIV/AIDS patients. Data on age, sex, country of birth, and HIV acquisition route were collected. DNA was extracted from uncultured peripheral blood lymphocytes by using a QIAmp DNA mini kit according to the manufacturer's protocol (Qiagen, Hilden, Germany).

Nested PCR with the CDC HIV-1 primer sets (gp40F1, gp41R1 and gp46F2, gp47R2) for HIV-1 group M, N, and O strains were used to amplify a 460 bp-long part of the gp41 region of the *env* gene. For reverse transcription and primary PCR the primers were gp40F1 (forward primer: 5′tcttaggagcagcaggaagcactatggg) and gp41R1 (reverse primer: 5′aacgacaaaggtgagtatccctgcctaa). For the nested PCR the primers were gp46F2 (forward primer: 5′acaattattgtctggtatagtgcaacagca) and gp47R2 (reverse primer: 5′ttaaacctatcaagcctcctactatcatta). After initial denaturation at 94 °C for 2 min, 35 cycles of PCR were performed. Each cycle consisted of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s and extension at 72 °C for 60 s, with a final extension at 72 °C for 5 min. For nested PCR the PCR mixtures were subjected to 35 cycles under the same conditions as the primary PCR.[8–11]

The PCR product was subjected to cycle-sequencing using a big-dye terminator kit and sequenced bidirectionally using an ABI prism 310 sequencer (Applied Biosystems, Foster City, USA). Traces were assembled and edited on GeneStudio Pro Contig editor (GeneStudio Inc., GA, USA). Sequences were aligned by using Clustal W 1.83. Gaps and ambiguous positions were removed resulting in an alignment of 366 nt sequences and phylogenetic trees were constructed with the neighbor-joining method. Distances for neighbor-joining trees were computed with the correction for multiple substitutions using a maximum-likelihood model implemented in Phylip version 3.5 c contained in the GeneStudio package.[12] Recombination analysis was performed using Simplot 2.5 software[13] along with comparisons to recombinant sequences. The standard sequences were obtained from the NIH full-length sequences.

Table 1 Age distribution of the patients

Age	Number
Younger than 1 year	1
20–30 years	6
30–40 years	11
40–50 years	3
50–60 years	2
Older than 60 years	4

Table 2 Distribution of the 19 HIV/AIDS patients with genotype B according to HIV acquisition route

HIV acquisition route	Number
Heterosexual contact	11
HIV-positive partner	1
Infant of an infected mother	1
Blood transfusion	2
Not defined	4

Table 3 The HIV genotypes of 27 patients

Genotype	Number
A	4
B	19
C	1
D	1
F1	2[a]

[a] One Russian sex worker and one high-risk sexual contact with a foreign partner.

Results

Most of the patients (18 of 27) were under 40 years of age; the age distribution of patients is given in Table 1. Among the 27 patients (26 adults and 1 newborn), 22 were male, born in Turkey, and 20 were infected through heterosexual contact. Two patients acquired the virus through blood and/or blood transfusion and one was the child of an infected woman (Table 2). The distribution of the subtypes was as follows: four were subtype A, 19 subtype B, one subtype C, one subtype D and two subtype F1 (Table 3, Figure 1). Non-B subtypes were all Turkish except for one Russian who had acquired the infection through heterosexual contact.

Discussion

Developed countries conduct a systematic surveillance of HIV genetic variability to inform vaccine research and development and to determine whether currently approved assays for HIV are capable of detecting all circulating strains. Since the first reported cases of HIV/AIDS in the early 1980s, HIV has emerged as one of the most significant infectious agents infecting over 65 million people worldwide so far. Human immunodeficiency viruses are divided into two types, HIV-1 and HIV-2. The most widely spread, and the main causative agent of AIDS, is HIV-1.[14]

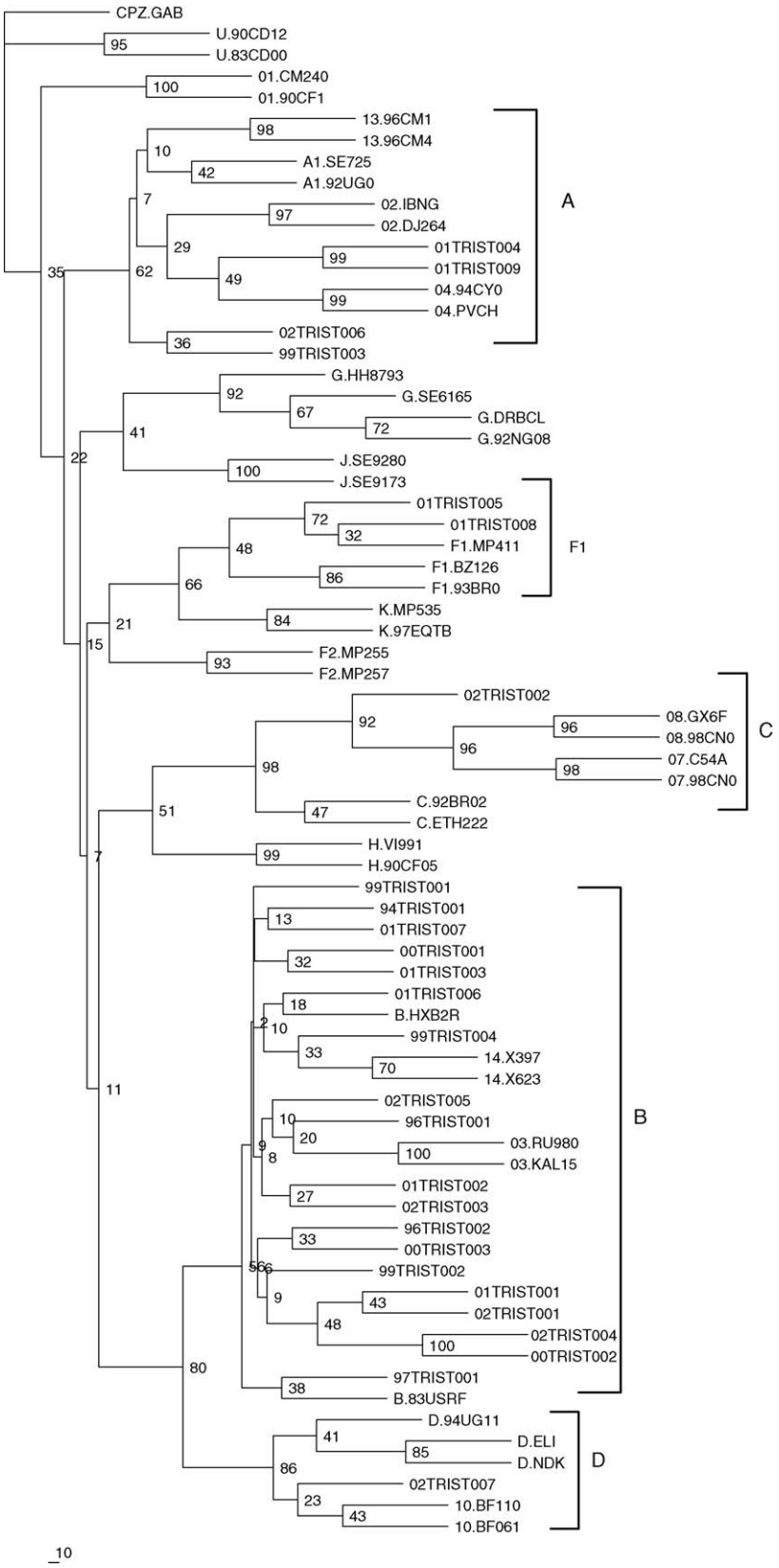

Figure 1 Phylogenetic analysis of HIV strains isolated in Istanbul. Phylogenetic trees were constructed by using the neighbor-joining method, which was implemented with the Phylip version 3.5 c package. Although the tree drawn using all our samples and prototypes has low bootstrap values, when each sample is analyzed separately along with the prototypes, bootstrap values were obtained that support the tree topology.

In 1985 the first AIDS case was reported in Turkey. The total number of reported HIV positive and AIDS cases reached 1515 in the period 1985 to the end of 2002. In fact, by considering patterns in other countries, HIV positivity is expected to be much higher than the reported cases. There has been one reported case of HIV-2 infection acquired as a result of a renal transplantation in Bombay.[15,16]

Geographically Turkey is in both the East European and Central Asian regions and borders countries where reported rates of curable STIs, and the probability of HIV infection being transmitted through unprotected sex, are high. Most of the HIV/AIDS cases reported so far are due to sexual transmission and intravenous drug use.[17] In our 27 genotyped patients, 20 of the HIV-1 cases were due to heterosexual contact.

Viruses of the M group are responsible for the AIDS pandemic. HIV-1 subtype B viruses were responsible from the first epidemic in North America, Europe, and Australia, and the metropolitan centers of other continents. Subtype E is the most prevalent in Thailand and Southern Asia. Subtype F has been found in Brazil and Romania and G is prevalent in Russia. Subtype D is limited to Africa. Almost all HIV-1 subtypes are found in sub-Saharan Africa with subtypes A, C, D, and CRFs (especially CRF02_AG) being the most prevalent in this continent. Subtype C is also frequently found in India. Another often-encountered CRF in Southeast Asia is CRF01_AE.

According to recent investigations, a substantial fraction of novel HIV-1 infections in various European countries is due to non-B subtypes and this fraction is likely to grow in the future.[1–7] Our results show that although subtype B is still predominant, non-B subtypes are also present even though the number of genotyped HIV/AIDS patients is low. Nineteen of the 27 patients were subtype B, whereas four were subtype A, one subtype C, one subtype D and two subtype F1.

Approximately 300 000 immigrants arrive annually in Turkey, especially from countries of the Middle East and the Balkans. Also the number of foreign students, especially from Africa, is not negligible. Turkey is an attractive country with regard to sex tourism for its northern neighbors where subtypes F and C are predominant. So the low proportion of subtypes other than B, especially C and F is somewhat surprising.

Viral divergence is created by point mutations and more strikingly by recombination. The co-existence of several genetic HIV-1 subtypes within a population increases the possibility of a dual infection and in this way the possibility for inter-subtype recombination. Large-scale screening and investigation of full-length genomes have led to the discovery of new subtypes as well as inter-subtype recombinant virus genomes. Consequences for the biological properties of the divergent viruses are not fully understood. Recombination is a frequent phenomenon especially in Africa. In Cyprus and Greece CRFs have been detected and in Greece they account for approximately 2% of the total HIV-1 infected individuals.[3–18] Although we performed analysis with Simplot, as only a small region of the gp41 was investigated in this study, we are not able to comment on the absence of CRFs.

Since in western countries the predominant subtype is B, current antiretroviral treatment regimens are designed and fine-tuned for use against this subtype. Transmission of subtype B in countries where antiretroviral therapy is widely used is declining, but resistant strains are emerging due to the selective pressure of therapy. Despite the predominance of subtype B, the presence of other subtypes in our small study population and the geographic bridging position of Turkey underline the need for regular, effective and smart nationwide surveillance. The scarcity of antiretroviral drugs and problems in therapy adherence may favor antiretroviral resistance in Turkey. Considering all of the above, and the possibility of diverse drug susceptibility patterns between HIV subtypes, the genotypic surveillance in our country is critically important.

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