

## GENETIC VARIABILITY OF HIV-1 ISOLATES FROM MINAS GERAIS, BRAZIL

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### SHORT COMMUNICATION

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#### ABSTRACT

We report results of nucleotide sequencing and phylogenetic analysis of the *env* gene of 11 HIV-1 isolates, in Belo Horizonte, Brazil. Ten isolates belonged to HIV-1 subtype B and one was a probable B/F mosaic. This putative B/F recombinant is similar but not identical in its nucleotide sequence to other B/F mosaics described in Brazil.

**Key words:** HIV-1, genome, PCR, AIDS, hemophiliacs.

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Human immunodeficiency virus establishes persistent infections in humans, in most cases leading to AIDS. HIV-1 has been genetically classified into major (M) and outlier (O) groups. The M group is further divided into nine subtypes (A-I), based on sequence diversity, where members of the same subtype differ in general by less than 10%, and those of different subtypes by 30% or more, if *env* gene is analyzed (2,4,6). Variation of HIV-1 can result in a spectrum of viruses exhibiting differences in cell tropism, replication, transmission rates and cytopathogenicity. At present there is considerable effort in genotyping viruses recovered from infected individuals to gain insights as to the types of viruses being transmitted in different geographical regions (6). Belo Horizonte is the third largest city in Brazil, with over 3 million inhabitants in the metropolitan area and the fourth in absolute number of AIDS cases

in Brazil, as of March 1997, with over 2,400 cumulative cases (7). The objective of this work was to examine the genetic variation and the phylogenetic analysis the *env* gene of HIV-1 isolates of seropositive patients with history of use of clotting factor concentrates (n=6), multiple transfusions (n=1), heterosexual transmission (n=3) and homosexual contact (n=1) in Belo Horizonte, Brazil.

After informed consent, samples of peripheral blood of 11 HIV-1 seropositive patients were collected in EDTA. PBMCs (peripheral blood mononuclear cells) were separated using a Ficoll-Hypaque® gradient (Pharmacia, Sweden). Crude lysates were obtained and PCR was performed using primers encompassing the C2-C3 region of the *env* gene of HIV-1 (nested reaction). Primers were based on isolate HXB2 genome (GenBank accession number KO3455), and were provided by the World

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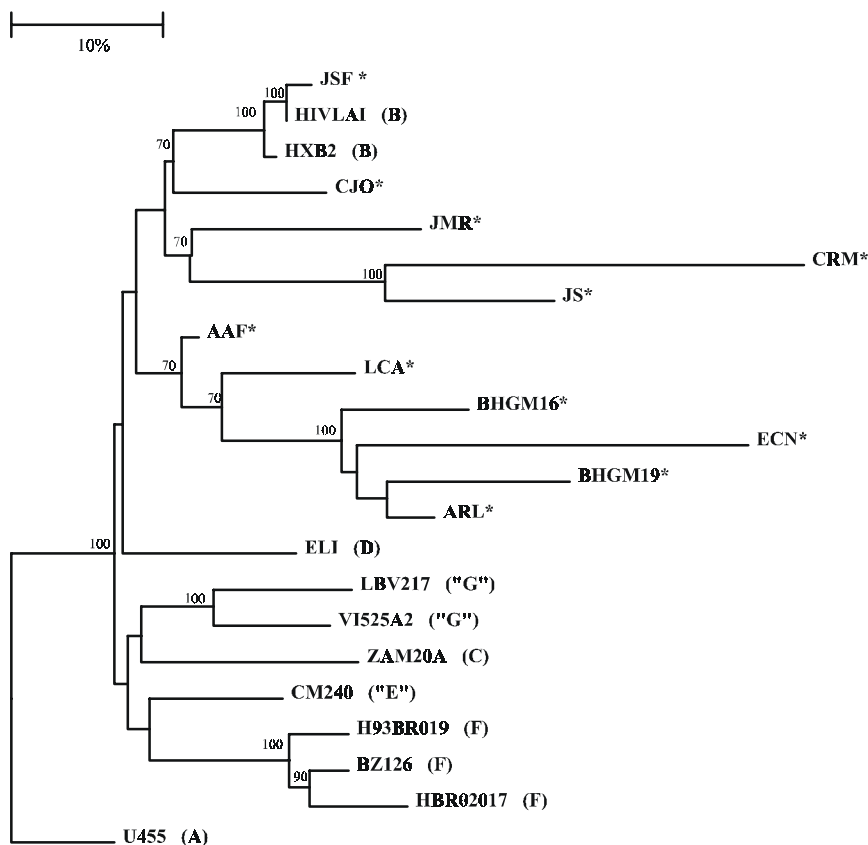
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Health Organization (WHO). The first round reactions of the *env* gene were performed using primers ED3/ED14, corresponding to positions 5956-5985 and 7960-7931, which amplified a 2.0 Kb fragment. The nested reactions were done with primers ED31/ ED33, corresponding to positions 6816-6844 and 7359-7380, producing a 0.5 Kb fragment (region C2-C3). Nucleotide sequencing (Sequenase®, USB, USA) was performed directly on DNA amplified by PCR of the PBMC lysates, using the primers ED31, ED33 and ES7 (C2-V3 region). Nucleotide sequences were obtained from both strands and each was done at least twice, using the dideoxi termination method (9). Sequences were aligned using Clustal W. Pairwise distance methods, using the Jukes and Cantor correction and bootstrap analysis, were performed for phylogenetic inference, with the package Treecon 3.1 for Windows (10).

HIV-1 *env* sequences obtained from Belo Horizonte isolates clustered with isolates of subtype

B in the phylogenetic analysis performed (Fig. 1). These results are in concordance with *env* heteroduplex mobility assays (HMA) (1) results of the same samples, except for an isolate from an asymptomatic blood donor (BHGM19), whose HMA results showed F subtype mobility, but whose C2V3 *env* sequences clustered consistently with subtype B. This discordant branching has been reported before in an isolate from São Paulo, Brazil (93BR019), which was a mosaic of subtypes B and F (8).

In nine of eleven *env* amino acid sequences, the tetrameric subdomain at the crown of V3 loop (Table 1) had the sequence GPGR, including the B/F isolate (BHGM19, blood donor, heterosexual transmission), whose crown had the sequence HIGPGRAF, identical to previously described and independently collected F subtype isolates from other cities in Brazil (3,5). One isolate (JS, hemophiliac patient) had the GPGQ sequence, which is common in subtypes A,



**Figure 1** - Phylogenetic relationships of the sequences of the C2V3 region of HIV-1 *env* gene of isolates from Minas Gerais (asterisks), compared to sequences representative of subtypes A-G. Isolate U455 was chosen arbitrarily to root the tree. Quotes indicate "mosaic" subtypes. Values at the nodes represent bootstraps analysis (shown only values above 75%).

C and E isolates and one (JMR, heterosexual transmission) had the sequence GPGK, rarely reported in subtype B. The five cysteine residues of the C2-V3 region were conserved in all isolates, including the ones at the base of V3 loop (C1 and C35).

**Table 1** - Aminoacid sequences of the V3 crown of HIV-1 *env* gene and risk factor in 11 seropositive patients, Belo Horizonte, Minas Gerais, 1997.

Patient	V3 Loop Crown Sequences	Probable mode of transmission
ECN	GPGR	Hemophilic patient <sup>1</sup>
CJO	GPGR	Hemophilic patient <sup>1</sup>
JSF	GPGR	Homosexual transmission
CRM	GPGR	Hemophilic patient <sup>1</sup>
AAF	GPGR	Transfusion <sup>2</sup>
ARL	GPGR	Hemophilic patient <sup>1</sup>
GM16	GWGR	Heterosexual transmission
GM19	GPGR	Heterosexual transmission
JS	GPGQ	Hemophilic patient <sup>1</sup>
JMR	GPGK	Heterosexual transmission
LCA	GPGR	Hemophilic patient <sup>1</sup>

<sup>1</sup> Use of clotting factor concentrates

<sup>2</sup> Use of packed red blood cells

All the isolates in this study, with one exception, belonged to HIV-1 subtype B, which is the most prevalent in the United States and Europe. The exception was a probable B/F mosaic. This putative B/F recombinant from Belo Horizonte (BHGM19) is similar, but not identical in its nucleotide sequence to other B/F mosaics described in Brazil (3,5,8). Sequencing of additional regions of these viruses may help to understand the recombination events that took place to generate these chimeric genomes.

**GeneBank accession numbers:** AF025918 to AF025926; U46210; U46122

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## RESUMO

### Variabilidade genética de isolados de HIV-1 em Minas Gerais, Brasil

Relatamos resultados do estudo de seqüência de nucleotídeos e análise filogenética do gene *env* 11 isolados HIV-1 em Belo Horizonte, Brasil. Dez isolados pertenciam ao subtipo B e um era provavelmente um mosaico B/F. Este possível recombinante B/F é similar, mas não idêntico, em sua seqüência de nucleotídeos, aos demais mosaicos B/F descritos no Brasil.

**Palavras-chave:** HIV-1, genoma, PCR, AIDS, hemofílicos.

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