

When phylogenetic analysis complements the epidemiological investigation: a case of HIV-2 infection, Italy

Massimo Ciccozzi¹, Annapaola Callegaro², Alessandra Lo Presti¹, Eleonora Cella¹, Marta Giovanetti¹, Romina Salpini³, Muhammed Babakir-Mina⁴, Claudio Farina⁵, Franco Maggiolo⁵, Carlo Federico Perno^{3,4}, Marco Ciotti⁴

¹Department of Infectious, Parasitic and Immunomediated Diseases, National Institute of Health, Rome, Italy;

²Microbiology and Virology, Ospedali Riuniti di Bergamo, Italy;

³Department of Experimental Medicine and Biochemical Sciences, University of Tor Vergata, Rome, Italy;

⁴Laboratory of Molecular Virology, Foundation Policlinic Tor Vergata, Rome, Italy;

⁵Division of Infectious Diseases, Ospedali Riuniti di Bergamo, Italy

SUMMARY

Human immunodeficiency virus type 2 (HIV-2) infection is geographically restricted, affecting West African countries such as Guinea-Bissau and Cape Verde. We describe a recent case of HIV-2 infection in an Italian patient. Phylogenetic analysis of the V3 region of HIV-2 indicated that the Italian patient was infected by HIV-2 subtype A2. The sequence obtained from the Italian patient clustered significantly with a sequence isolated from Senegal. A phylogenetic doubt may arise from a Guinea Bissau sequence because it was in a major clade with the Italian and Senegal sequences, but was not statistically significant. The discovery of another Italian case over a short time frame stresses the importance of strengthening the surveillance system for HIV-2 because of the increase in migration from endemic areas to Italy.

KEY WORDS: HIV-2, Italian patient, Phylogenesis.

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CASE REPORT

Human immunodeficiency virus type 2 (HIV-2) infection is geographically restricted, affecting West African countries such as Guinea-Bissau and Cape Verde (Barin *et al.*, 1985; Clavel *et al.*, 1986). In Europe the prevalence of HIV-2 is high in those countries that have socioeconomic relationships with this African region, such as Portugal and France (Soriano *et al.*, 2000; Damond *et al.*, 2001; Gomes *et al.*, 2003). However, increased migration towards west Europe and international travel might increase the risk of spreading the virus in Europe (Ciccozzi *et al.*,

2011). Indeed, we recently described a case of HIV-2 infection in an Italian patient whose strain belonged to the subtype A (Ciccozzi *et al.*, 2011). The present report describes a new case. The patient is a 44-year-old Italian woman who contracted the infection in Italy after having a sexual relationship with a man from Senegal. The woman was admitted to the Infectious Disease Unit at Bergamo Hospital because of acute symptoms such as fever, lymphadenopathy, etc. No travel history to West Africa was referred by the patient. HIV-2 infection was tested by Western blot and confirmed by PCR. The genetic analysis of the *env* (V3) region confirmed that the viral strain originated from West Africa.

Two data sets were built: the first included the *env* (V3) region of the Italian patient with reference sequences downloaded from the Los Alamos database (<http://www.hiv.lanl.gov/content/index>), while the second included sequences downloaded from the NCBI database with a similarity >90%

Corresponding author

Massimo Ciccozzi

Department of Infectious

Parasitic and Immunomediated Diseases

National Institute of Health

Viale Regina Elena, 299 - 00161 Roma, Italy

E-mail: massimo.ciccozzi@iss.it - ciccozzi@iss.it

(<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The sequences were selected on the basis of the following criteria:

- 1) sequences published in peer-reviewed journals (except for the new sequence described below);
- 2) no uncertainty about the subtype assignment of each sequence with the exclusion of recombinants;
- 3) the city/state of origin were known and clearly established in the original publication. The HIV-2 *env* sequence was first analyzed using the REGA HIV-1 Subtyping Tool (De Oliveira *et al.*, 2005).

All the sequences in both data sets were aligned using CLUSTAL X v. 2; then the sequences were manually edited with the Bioedit program and

gaps were removed from the final alignment. Maximum likelihood phylogenetic trees were generated with the GTR + I + G model of nucleotide substitution as already described (Ciccozzi *et al.*, 2011). The evolutionary model was chosen as the best-fitting nucleotide substitution model in accordance with the results of the hierarchical likelihood ratio test (HLRT) implemented in MODELTEST software version 3.6 (Posada and Buckley, 2004). The statistical robustness and reliability of the branching order within the phylogenetic trees were confirmed with the bootstrap analysis; all calculations were performed using Phyml v 3.0 (Guindon *et al.*, 2010).

MEGA 5 software (Tamura *et al.*, 2011) allowed the calculation of the genetic distances among dif-

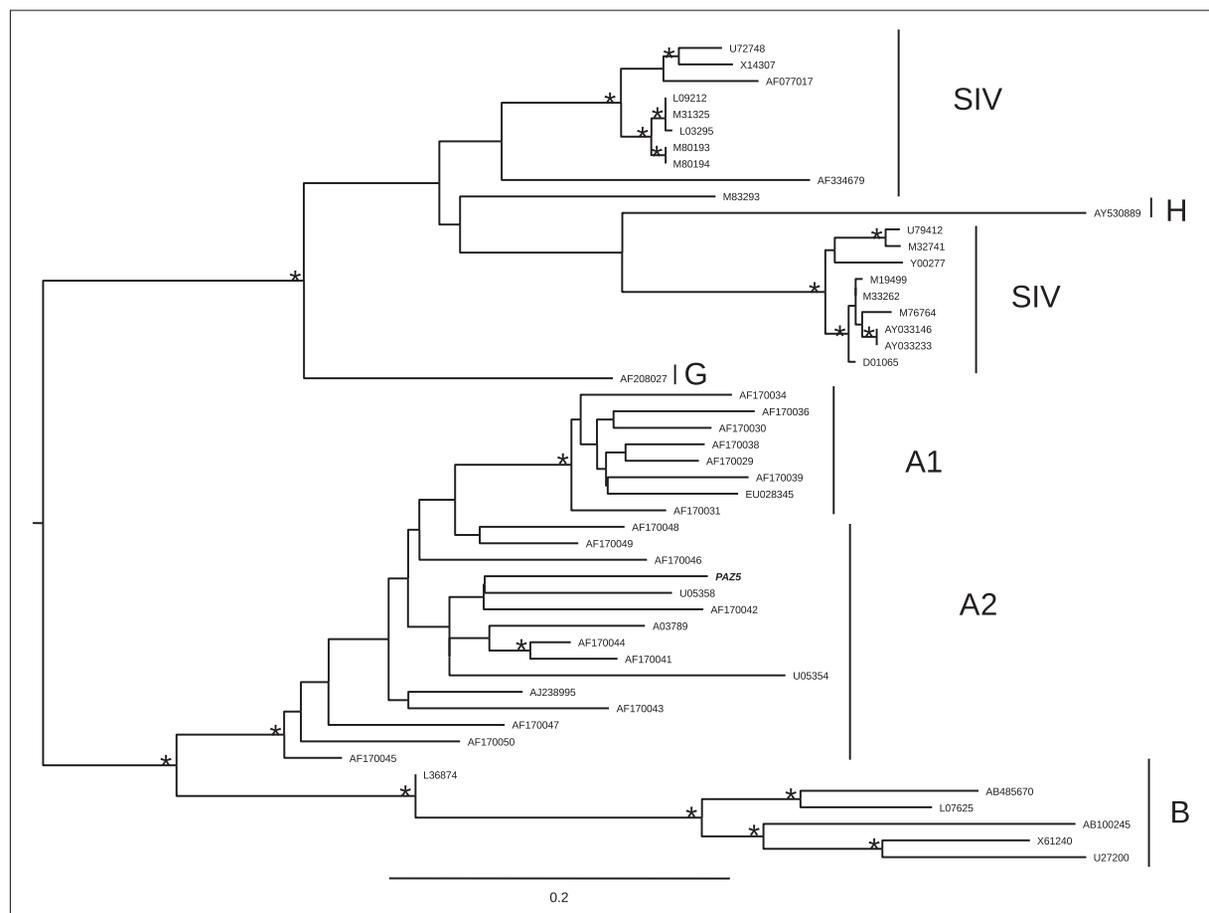


FIGURE 1 - Phylogenetic relationships of the Italian HIV-2 isolates with the subtype-specific reference sequences downloaded from the Los Alamos sequence database (www.hiv.lanl.gov/content/index). The Italian sequence named PAZ5 (Italian patient) is in italics and bold. The asterisks (*) along a branch represent significant statistical support for the clade subtending that branch (bootstrap support >70%). The scale bar indicates 0.2 nucleotide sequence divergence.

ferent clades using the model of nucleotide maximum likelihood (ML) method including transitions and transversions. The substitution rates were set equal among sites but different among lineages. Genetic distance was established grouping the sequences by country of origin: Senegal, Italy and Guinea Bissau. The nucleotide sequence obtained with this study will be deposited in GenBank after acceptance.

Phylogenetic analysis of the V3 region of HIV-2 indicated that the Italian patient was infected by HIV-2 subtype A2 (Figure 1). The sequence obtained from the Italian patient (PAZ5) clustered significantly (bootstrap value >70%) with a sequence isolated from Senegal, although a sequence from Guinea Bissau also seems involved

in a major clade but not statistically supported (Figure 2). The nucleotide genetic distance between and within groups showed that the Guinea Bissau sequences were more distant from the Italian sequence compared to the Senegal sequences (0.12 vs 0.10).

DISCUSSION

The circulation of HIV-2 is mainly restricted to countries of West Africa. Immigration from this African region has led to the spread of the virus to other continents and countries like Italy. In Italy few reports have been published on this topic (Ciccozzi *et al.*, 2011; Ciccaglione *et al.*, 2010;

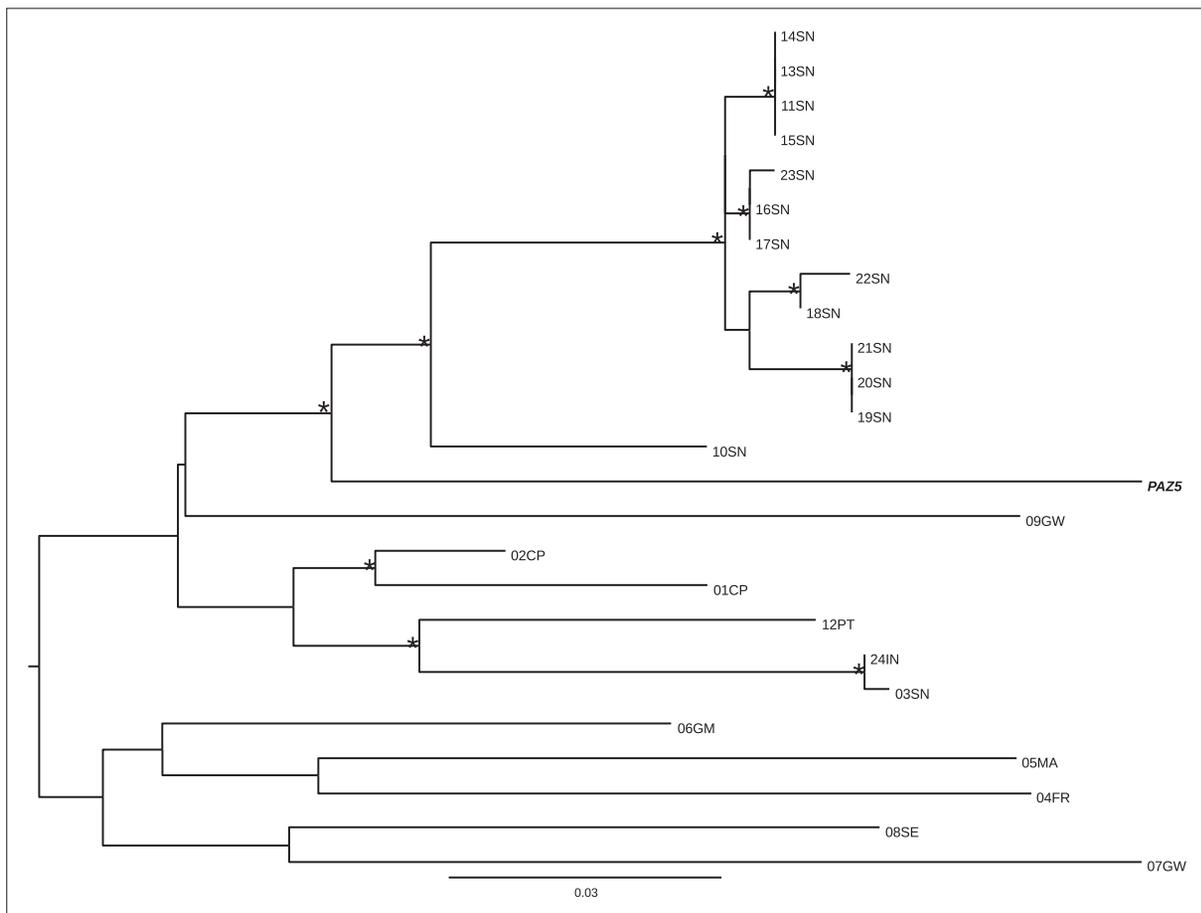


FIGURE 2 - Phylogenetic relationships of the Italian HIV-2 isolates with the subtype-specific selected sequences from the NCBI database with a similarity >90% and known country of origin (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The Italian sequence named PAZ5 (Italian patient) is in italics and bold. The asterisks (*) along a branch represent significant statistical support for the clade subtending that branch (bootstrap support >70%). The scale bar indicates 0.03 nucleotide sequence divergence.

Costarelli *et al.*, 2008; Quiros-Roldan *et al.*, 2001) emphasizing the scant interest in this neglected infection.

Here, we describe the case of an Italian woman infected by HIV-2 where the phylogenetic analysis supported the epidemiological investigation. Our sequence shares the same source of infection as sequences from Senegal. A sequence from Guinea Bissau also seems involved since it shares a major clade with the Italian and Senegal sequences but it was not statistically significant. Moreover epidemiological investigation revealed that the woman contracted the infection after having a sexual relationship with a man from Senegal.

Furthermore, the genetic distance between the Italian strain and those from Senegal was lower than that of Guinea Bissau strains. Taken together, these data suggest that the Guinea Bissau strain cannot be considered a candidate as a recent common ancestor.

Phylogenetic analysis cannot indicate the direction of virus flow but when it is considered together with the epidemiological investigation it seems likely that the virus came to Italy and not the contrary. The discovery of another Italian case over a short time frame stresses the importance of strengthening the surveillance system for HIV-2 because of the increase in migration from endemic areas to Italy. This problem is clinically relevant because a correct diagnosis avoids the patient being treated with inappropriate drugs.

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