

## **A case of Italian HIV-2 infection: a genetic analysis**

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**Running title:** HIV 2 infection in Italy

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## Abstract

Human immunodeficiency virus type 2 (HIV-2), originally restricted to Western Africa is now spreading to European Western countries because of migration from endemic areas. Therefore, it is mandatory to enforce the surveillance and improve the diagnostics of this neglected infection. In this report, we describe a case of HIV-2 infection affecting an Italian citizen along with 3 cases from India. Phylogenetic analysis showed that the viral strain identified in the Italian patient clustered with a strain isolated from an immigrant living in France. Of the 3 Indian strains, 2 clustered together and were statistically supported, whereas 1 clustered with a strain from Guinea Bissau. The description of the first case of HIV-2 infection in an Italian citizen tells us that the virus is spreading from endemic areas to countries involved in migration. A strict monitoring and the improvement of the diagnostic molecular tools are necessary in order to avoid misdiagnosis with relevant clinical consequences.

## Introduction

Human immunodeficiency virus type 2 (HIV-2) was first isolated from patients with AIDS originating from Cape Verde and Guinea-Bissau <sup>1,2</sup>. HIV-2 infections, geographically restricted to West Africa, have been also reported in European countries such as France, United Kingdom, and Portugal <sup>3-5</sup>. HIV-2 infection in West African countries is endemic <sup>6,7</sup> and the risk of transmission in Europe could become relevant because of the increase of migration and international travel.

The incidence of HIV-2 infection declined over the 1990-2000 years <sup>8,9</sup> with a number of infections mostly caused by groups A or B. Up to now, six groups of HIV 2 have been identified: A to F <sup>10,11</sup>.

This infection may be appreciable in countries with high number of foreign citizens like Italy where a considerable number of immigrants also come from western African countries (<http://epp.eurostat.ec>). In Italy, the use of HIV-2 specific diagnostic assays for the screening of blood donations became mandatory in 1992, but unfortunately the genetic variability of HIV still represents a challenge, especially in the initial phase of HIV-2 diagnosis. Moreover, the cross-reactivity between anti-HIV-2 antibodies and the envelope glycoproteins of HIV 1 may lead to a misclassification of the patient as HIV-2 negative. To reduce this risk, recently, two HIV-2 specific proteins (sgp105 and gp36) have been added to the strip used for western blot analysis (INNO-LIA™ HIV I/II Score, Innogenetics, Ghent, Belgium). Surveillance and correct diagnosis of HIV-2 infection may be important, for both an appropriate therapy and transmission risk reduction. Here, we describe four cases of suspected HIV-2 infection confirmed by western blot analysis, real-time PCR and phylogenetic analysis.

## Material and methods

### Samples collection

Plasma samples from one Italian and three Indian patients tested previously positive to HIV 1/2 antibodies were collected and stored at -80 °C until analysis. The Italian sample was collected in Rome, whereas the Indian samples were from the following cities: Bombay (Maharashtra State), Hubli (Karnataka State) and Lucknow (Uttar Pradesh State).

### Western blot analysis

To confirm and define the positivity to HIV antibodies, a western blot assay was carried out according to the manufacturer's instructions (INNO-LIA™ HIV I/II Score, Innogenetics, Ghent, Belgium).

### Real-time PCR and sequencing analysis

HIV-1 was searched by real-time PCR using the Abbott RealTime HIV-1 assay according to the manufacturer's instructions (Abbott molecular, Des Plaines, IL, USA). Plasma RNAs were run on the *m2000* system, a platform capable of automated RNA extraction and PCR set-up, followed by amplification/detection.

HIV-2 was instead detected by HIV 2 Real Time RT-PCR kit (Shanghai ZJ Bio-tech Co., Ltd., Shanghai, China). Plasma samples were extracted by QIAamp Viral RNA Mini Kit (Qiagen, Milan, Italy) and five µl added to the PCR mix according to the indications of the manufacturer.

Positive samples were further characterized by amplification and sequencing of a 546 bp fragment of the HIV-2 V3 region<sup>12</sup>.

### Phylogenetic analysis.

The HIV-2 *env* sequence ( V3 region<sup>12</sup>) of four patients ( IT1, IN 1, 2 and 3) were aligned and compared using two different data sets: (i) reference sequences downloaded from Los

Alamos database (<http://www.hiv.lanl.gov/content/index>); (ii) sequences downloaded from NCBI database with similarity >90% (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). All the sequences in both data sets were aligned using CLUSTAL X<sup>13</sup>; then the sequences were manually edited with the Bioedit program and gaps removed from the final alignment. The accession numbers of the sequences used are listed in the phylogenetic tree of Fig. 1. The phylogenetic tree was constructed using the PAUP package<sup>13</sup>. We employed the general reversible model (HKY) of nucleotide substitution, incorporating maximum likelihood (ML) estimates of base composition and the shape parameter ( $\omega$ ) of a gamma distribution ( $\gamma$ ) model of among-site rate variation as it consistently gave much higher likelihood values using Modeltest v.3.7 implemented in PAUP<sup>13</sup>. The maximum likelihood tree was estimated under this model using tree bisection-reconnection (TBR) branch swapping. The statistical robustness and reliability of the branching order within each phylogenetic tree were confirmed through a bootstrap analysis using 1,000 replicates for the neighbor-joining (NJ) tree and through the zero branch length test for the ML tree<sup>13</sup>. The tree was rooted with a midpoint rooting.

**Nucleotide sequences accession number.** The nucleotide sequences obtained with this study have deposited in GeneBank under the following accession numbers: JF717827-JF717830.

## Results

Real-time PCR and western blot assays showed that the 4 patients were infected by HIV-2, while HIV-1 was not detected either at nucleic acid and antibodies level. In addition, phylogenetic analysis of the V3 region of HIV-2<sup>12</sup> indicated that the 4 patients were infected by HIV-2 subtype A, most likely subgroup A1, Fig. 1. The uncertainty about the subgroup is due to the fact that it was analyzed only a portion of the *env* gene and not the entire viral genome. The sequence obtained from the Italian patient (IT1) clustered with a sequence isolated from a patient living in France. Of the 3 Indian sequences, 1 (IN1) clustered with a sequence identified in a patient from Guinea Bissau, while the other 2 sequences (IN2 and IN3) clustered together and the cluster was statistically supported.

## Discussion

The circulation of HIV-2 is mainly restricted to countries of West Africa. However, the immigration from this African region has led to the spread of the virus also in other Continents with most of the cases diagnosed in France and Portugal<sup>14</sup>. In Italy, the attention towards this neglected infection increased recently especially among immigrants<sup>15</sup>. To our knowledge, however, no reports have been published on HIV-2 infection among Italian citizens. Here, we described the first Italian case of HIV-2 infection along with three cases from India. A phylogenetic analysis was carried out to determine the genetic features of these 4 HIV-2 strains. Of the 3 Indian strains, 2 were highly related suggesting that they shared the same source of infection. The third sequence clustered with a strain from Guinea Bissau. However, the similarity between this Indian strain and the Guinea Bissau strain is not suggestive of a recent common ancestor.

About the Italian strain, it clustered with a sequence isolated in France<sup>14</sup>. It seems that the common ancestor of these two viruses was in Africa with two importations into Europe, as it is that this clade of viruses has been evolving in France and Italy for decades. Likewise, the phylogeny cannot tell us the direction of travel, and it is as likely that the French virus came through Italy as the Italian virus coming through France.

In addition, the description of the first Italian case tells us of the importance to strength the surveillance system for HIV-2 because of the increase of migration from endemic areas to Italy. This problem is clinically relevant because patients whose HIV-2 serostatus is unknown may be treated with inappropriate drugs. Therefore it is important to have valid diagnostic tools , i.e. antibody and western blotting assays, able to distinguish between HIV-1 and 2 infection and valid real-time PCR to determine HIV-2 viral load.

### **Author Disclosure Statement**

No competing financial interests exist.



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### Figure legend:

**Figure 1.** Phylogenetic relationships of the Italian and Indian HIV-2 isolates with the subtype specific reference sequences downloaded from Los Alamos sequence database (<http://www.hiv.lanl.gov/content/index>). The reference sequences used in the analysis are shown in the tree with their original accession numbers. The \* along a branch represent significant statistical support for the clade subtending that branch (P= 0.001 in the zero-branch-length test; bootstrap support 75%). The scale bar indicates 0.09 nucleotide sequence divergence.

Patient	Sex	CD4+ level	HIV 2 viremia, cp/ml	Coinfection
IN 1	Male	30	1855	HBV
IN 2	Male	110	301849	No
IN 3	Male	950	NA	No

IT 1	Female	389	399221	No
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**Table 1.** Demographic and clinical characteristics of HIV 2 infected patients.

