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ORIGINAL RESEARCH

Emergence of an HIV-1 cluster harbouring the major protease L90M mutation among treatment-naïve patients in Tel Aviv, Israel

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Objective

Drug resistance-associated mutations (DRMs) among HIV-1 treatment-naïve patients have increased in recent years. Their incidence and prevalence in various exposure risk categories (ERCs) were evaluated.

Design

Plasma samples of HIV-1 treatment-naïve patients diagnosed between 2001 and 2009 at the Tel Aviv Medical Center were screened for DRMs.

Methods

Samples obtained from patients following the HIV diagnosis were analysed retrospectively. Genotyping was carried out using the Trugene HIV-1 genotype kit (Siemens, Berkeley, CA, USA). Phylogenetic relationships among viral sequences were estimated using the maximum likelihood method.

Results

Thirty-eight of the 266 analysed sequences (14.3%) had DRMs, all occurring exclusively in the group of men who have sex with men (MSM). The rate of DRMs has constantly risen, reaching a peak of 21.9% in 2009. Notably, protease inhibitor (PI) DRMs became the most frequent DRMs in 2009. Phylogenetic analysis showed a tight cluster comprising 13 of 14 viruses harbouring the L90M major PI resistance mutation, suggesting a single infection source.

Conclusion

There was an unexpectedly high rate of the major L90M PI resistance mutation in the MSM group. The clustered transmission of this mutation might be related to a high-risk sexual behaviour. Added to nonnucleoside reverse transcriptase inhibitor and nucleoside reverse transcriptase inhibitor resistance mutations, such a PI mutation may limit future therapeutic options for this particular patient population.

Keywords: HIV, drug resistance transmission, protease inhibitor L90M mutation

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Introduction

The HIV-1-infected population in Israel is unique in its diversity of exposure risk categories (ERCs) and HIV-1 subtypes [1] as a consequence of a wave of immigration from Ethiopia and the former Soviet Union, as well as an influx of numerous worker immigrants (WIs) from Africa. However, the distribution of ERCs in Tel Aviv, one of the country's most populated cities, is similar to that in other industrialized countries. The incidence and prevalence of HIV infections in these countries have risen in the era of combination antiretroviral therapy (cART), particularly

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among men who have sex with men (MSM) [2–4]. The rate of drug resistance-associated mutations (DRMs), mainly those associated with resistance to nonnucleoside reverse transcriptase inhibitors (NNRTIs), among HIV-1 treatment-naïve patients has also increased [5]. In accordance with these trends, we have been observing an increase in the number of new HIV-infected patients in our clinic at the Tel Aviv Medical Center, mainly among MSM. Thus, the objective of this study was to check for DRMs among HIV-1 treatment-naïve patients.

Methods

The first blood samples collected from treatment-naïve patients after the diagnosis of HIV infection were retrospectively analysed. The Trugene HIV-1 genotyping assay (Siemens, Berkeley, CA, USA) was used to sequence the protease (PR) and reverse transcriptase (RT) regions. Phylogenetic relationships among these sequenced RT and PR viral regions were estimated using the maximum likelihood method [6]. The years 2001–2005 were grouped together because of a relatively low number of documented sequences during that time period. Transmitted DRMs were defined according to the criteria suggested by Bennett *et al.* [7]. Isolates were subtyped based on the Stanford database (Stanford database Version 6.0.10; http://hivdb.stanford.edu/). Obtained sequences were aligned using the MAFFT software version v6.821b [8]. A maximum-likelihood tree search [6] was conducted using the PhyML web server [9,10], assuming the HKY substitution model [11]. Edge reliability was estimated using the bootstrap sampling approach with 100 replicates [12]. χ^2 and Fisher tests were applied to statistically compare resistance rates. Ethical approval for the study was granted by the institutional ethics committee.

Results

A total of 266 sequences from patients diagnosed between 2001 and 2009 were analysed. The patients' characteristics are summarized in Table 1. Altogether, 195 patients (73.3%) in the tested population belonged to the MSM ERC. Almost three-quarters of the sequences (n = 198; 74.4%) were subtype B, with a higher rate in the MSM group (n = 183; 93.8%) (Table 1).

DRMs were found in a total of 38 patients among the 266 sequences tested (14.3%). There was a constant increase in mutation rate ($P = 0.001$ for trend): while there were no resistance mutations between 2001 and 2005 ($n = 35$), there were 14.3% in 2006 ($n = 14$), 9.5% in 2007 ($n = 42$), 11.4% in 2008 ($n = 61$) and 21.9% in 2009 ($n = 114$). Resistance mutations were exclusively from the MSM ERC. Excluding two subtype A viruses, all DRMs were subtype B viruses. Within the mutated viruses, 18 (6.8%) harboured nonnucleoside reverse transcriptase inhibitor (NNRTI)-associated resistance mutations, with K103N being the most abundant; 15 (5.6%) had protease inhibitor (PI)-associated mutations; and three (1.1%) had nucleoside reverse transcriptase inhibitor (NRTI)-associated mutations. One virus had two classes (NNRTI and PI) and another virus harboured three classes of associated resistance mutations. Although not statistically significant ($P = 0.66$), in 2009 we documented a switch in the abundance of mutations as PI DRMs became more frequent than NNRTI DRMs (11.4% *vs.* 8.7%, respectively).

Phylogenetic analysis carried out on a total of 198 subtype B sequences identified two major clusters of DRMs (Fig. 1a). One of the identified clusters included 13 of the 14 viruses harbouring the L90M major PI-resistance mutation grouped together with a bootstrap support of 100%. Eleven patients within this cluster were diagnosed in 2009, one in 2008 and one in 2006. The low evolutionary distance between these sequences and their pattern of segregation suggest a single source of infection (Fig. 1b). The second cluster included 12 of 17 viruses harbouring the K103N NNRTI-associated resistance mutation (Fig. 1c).

Table 1 Characteristics of patients and viral subtypes

Total (n)	266
Gender (n)	
Male	241
Female	25
Age (years) [range (median)]	19–65 (34)
CD4 count (cells/μL) [range (median)]	4–2780 (350)
Viral load (copies/ml)[§] (range)	546–100 000
ERC [n (%)]	
MSM	195 (73.3)
Heterosexual	35 (13.1)
IDU	23 (8.6)
ETHJ	7 (2.6)
WI	6 (2.2)
Country of origin [n (%)]	
Israel	163 (61.2)
Ex-SU	71 (26.6)
South America	12 (4.5)
Ethiopia	9 (3.3)
Sub-Saharan Africa*	5 (1.9)
Other[†]	6 (2.2)
Subtype	
B	198 (74.4)
A/AE[†]	46 (17.2)
C	11 (4.1)
Others	11 (4.1)

DRM, drug resistance mutation; ERC, exposure risk category; ETHJ, Ethiopian Jews; IDU, injecting drug user; MSM, men who have sex with men; SU, Soviet Union; WI, worker immigrants.
*Africa, except Ethiopia.
[†]CRF01_AE.
[†]Canada, Egypt, Iran, Jordan, Thailand and Yemen.
[§]Range of detection 50–100 000 copies/ml (Cobas Amplicor HIV-1 Monitor test; Roche, Branchburg, NJ, USA).

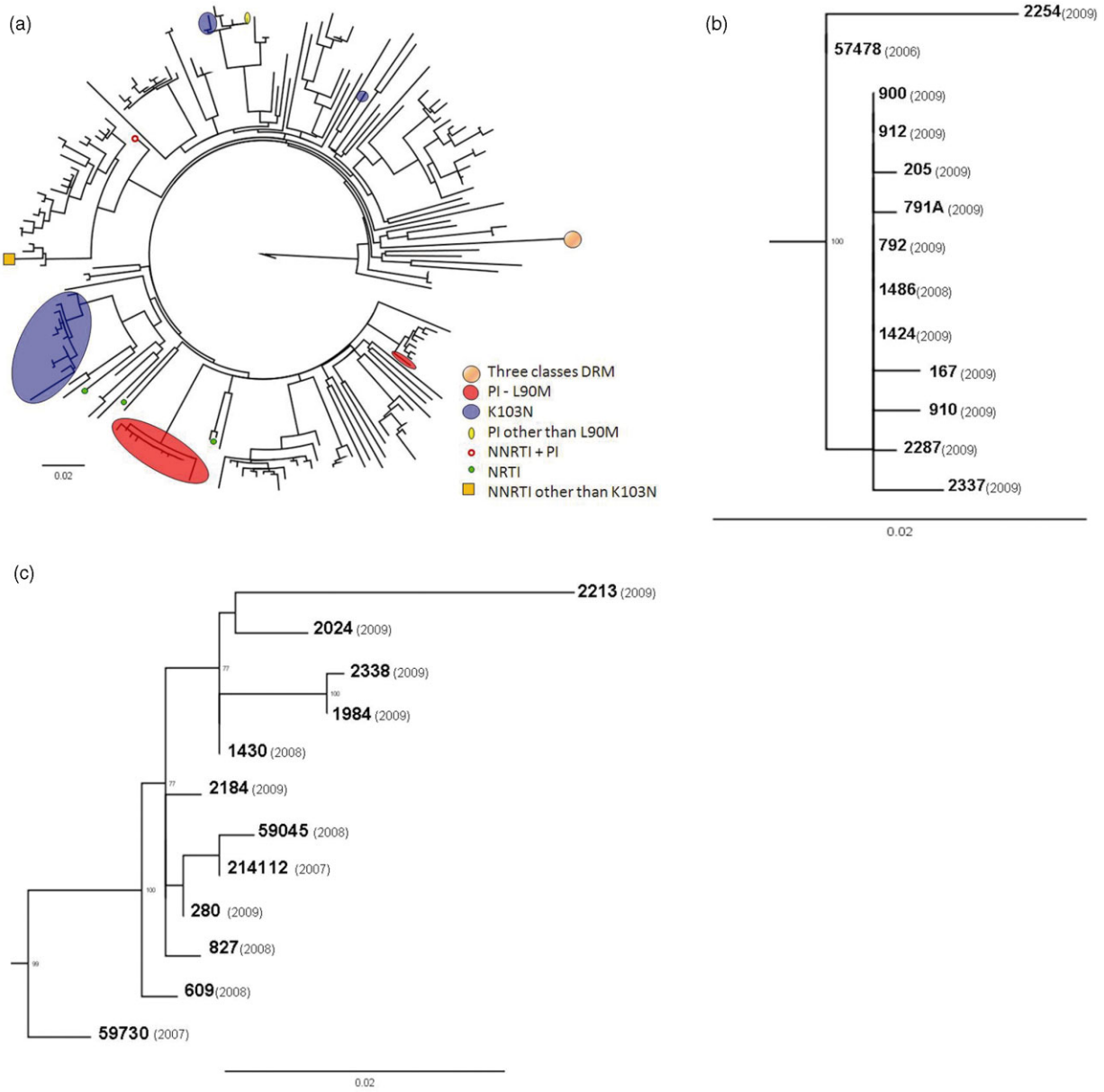

Fig. 1 Phylogenetic trees. (a) Phylogenetic tree describing the evolutionary relationships among 198 subtype B HIV-1 [reverse transcriptase (RT) and protease (PR)] sequences from naïve patients. Two major clusters of drug resistance mutations (DRMs) are demonstrated, including the L90M major protease inhibitor (PI) and K103N nonnucleoside reverse transcriptase inhibitor (NNRTI) mutations. (b) Phylogenetic tree showing the cluster pattern of 13 RT and PR sequences of viruses harbouring L90M in the protease region and the year the blood was drawn. Bootstrap supports are indicated at the base of the corresponding branches. (c) Phylogenetic tree describing the cluster pattern of 12 RT and PR sequences of viruses harbouring K103N in the RT region and the year the blood was drawn. Bootstrap supports are indicated at the base of the corresponding branches. NRTI, nucleoside reverse transcriptase inhibitor.

We further looked into the laboratory characteristics and response to cART of patients infected with the L90M viruses. A large range of viral loads and CD4 counts were found at baseline (989–100 000 HIV-1 RNA copies/ml and 150–760 cells/μl, respectively). Seven of the clustered L90M-infected patients started cART. One of the three patients who were treated with efavirenz and tenofovir/emtricitabine failed to suppress the viral load and rapidly developed the K103N resistance mutation in RT despite good adherence. In contrast, two others responded well to

the same regimen. Four patients were given a higher genetic barrier regimen, for example darunavir. Three of them maintained their viral load below 40 copies/ml, but one failed to suppress the viral load below 40 copies/ml.

Discussion

Similar to previous reports from other industrialized countries and Israel [4,13–16], the data presented herein demonstrate an increasing rate of DRMs in the treatment-naïve population in Tel Aviv, mainly in the MSM ERC. Interestingly, in contrast to reports from Europe and America, we noted in 2009 a high rate of clustered transmission of the major PI mutation L90M. As has been shown in studies using minor population techniques, the actual rate of transmission could be even higher than that found here [17]. As data regarding the time of infection were lacking for most patients, viruses that might have undergone de-selection in untreated patients from the time of infection until the HIV diagnosis could have been missed.

This high rate of the L90M mutation is unexpected in our study group as this mutation is mainly selected by saquinavir and nelfinavir [18], but saquinavir was less frequently prescribed in Israel than other PIs, and nelfinavir has not been prescribed at all in Tel Aviv since 2008. However, as shown in Figure 1b, the transmitter source could date to as early as 2006. One can speculate that the high transmission rate of the L90M PI mutation reflects an exceptionally high-risk sexual behaviour in the MSM group. The phylogenetic analysis presented in Figure 1b, which illustrates the genetic similarities between the L90M-harbouring viruses, supports the clustered transmission of this mutation from a single infective source. It is noteworthy that all the viruses in this cluster also shared several other minor genetic features (e.g. minor mutations and polymorphisms in RT and PR; data not shown). Attempts to establish the speculated high-risk sexual behaviour among the L90M clustered patients proved unsuccessful, as these patients were unwilling to disclose information about their partners or about their sexual behaviour.

The role of DRMs in clustered transmission has been discussed previously. In a Swiss cohort study, which described the effect of clusters on transmitted DRMs, clusters were more frequent among transmitted DRMs than among sensitive viruses, and the L90M mutation was also detected among these clusters [15]. Brenner *et al.* studied the role of clustering in the transmission of drug-resistant viruses in Quebec, Canada and demonstrated an association between clustering and increased transmission of viruses harbouring NNRTI DRMs. They suggested that sexual behaviour, mainly of MSM, could be the reason for such transmission. Interestingly, clusters of PI DRMs were limited to viruses harbouring the L90M mutation [13]. Others have also attributed transmission clustering to sexual behaviour, mainly that of MSM [19].

The question arises as to whether the fitness of these viruses plays a role in their frequent transmission. Attempts at addressing this issue yielded conflicting data regarding viral loads and mutant representation among patients infected with DRMs harbouring viruses (mostly NNRTI DRMs [20,21] and the NTRI M184I/V [22,23] DRM).

Concerning the fitness of PI resistance mutations, results from *in vitro* studies performed by van Maarseveen *et al.* failed to show that higher replication capacity was responsible for maintenance of the DRM. The virus harbouring the PI-associated mutation I84V did not reverse in the absence of PIs, although a non-increase in replication capacity was shown [24]. Our study could not address this issue as the study population was too small and there was a large range of viral loads among patients with viruses harbouring the L90M mutation.

Another concern is the significance of the L90M mutation in choosing a therapeutic regimen in naïve patients. A recent study showed that a single transmitted DRM is not an indicator for transmission of a more extensive resistance profile [25], but further investigations evaluating the efficacy of various regiments in treating L90M-harbouring patients are needed.

In conclusion, this study provides data on transmitted viruses harbouring DRMs in Tel Aviv, Israel. All patients with transmitted DRMs were from the MSM ERC. In contrast to the findings of other studies from industrialized countries, there was a high rate of PI-associated DRMs. Clustering was shown to possibly facilitate the spread of viruses harbouring these mutations. Questions regarding viral fitness and therapeutic strategy remain open and call for a larger prospective investigation of this unique patient group.

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