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High burden of transmitted HIV-1 drug resistance in Italian patients carrying F1 subtype

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Background: Transmitted drug resistance (TDR) is mainly restricted to individuals carrying B subtype, with low prevalence among non-B subtypes when grouped together. Subtype F1 is the most frequent non-B variant found in subjects living in Italy, allowing a specific assessment of TDR associated with this clade.

Methods: We analysed *pol* sequences of HIV-1-positive individuals carrying the F1 variant included in the Antiretroviral Resistance Cohort Analysis database in the 1998–2009 period. Mutations were analysed with the Surveillance Drug Resistance Mutation and the International AIDS Society lists for naive and treated patients, respectively.

Results: Among 343 HIV-1-infected patients carrying an F1 subtype, resistance was evaluated in a subset of 221 patients whose treatment status was known (169 drug naive and 52 drug experienced). The prevalence of TDR was 15.4% (11.8% for nucleoside/nucleotide reverse transcriptase inhibitors, 6.5% for non-nucleoside reverse transcriptase inhibitors and 7.1% for protease inhibitors). Among the 169 naive patients, 75.1%, 10.1% and 7.1% were Italians, South Americans and Romanians, respectively. Heterosexuals were prevalent among Italians and Romanians, while men who have sex with men were predominant among South Americans. The overall frequency of TDR declined from 21.4% to 7.1% in the 1998–2009 period. Although no statistical difference was detected, the frequency of TDR was higher in South Americans (23.5%) compared with Italian and Romanian naive patients (15% and 8.3%, respectively).

Discussion: Our study shows a remarkable frequency of TDR in the F1 subtype-infected population. The high prevalence of TDR detected in South American subjects is linked to the homosexual route of infection. However, TDR was considerably high also in Italian subjects harbouring the F1 subtype, deserving careful monitoring.

Keywords: HIV-1 subtypes, antiretroviral therapy, resistance epidemiology

Introduction

Transmitted drug resistance (TDR) is an important concern in HIV-1 treatment, representing the major factor that may compromise the efficacy of the initial highly active antiretroviral therapy (HAART) regimen.[1] Therefore, international and national guidelines recommend the detection of baseline drug susceptibility to guide an optimal treatment choice (http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf). Although some reports have shown a decreasing or stabilizing prevalence of fTDR in recent years,[2,3] more data are needed to confirm this trend.

Studies performed in several European countries have shown that TDR is mainly restricted to individuals carrying the B subtype; however, the strength of this association may vary according to the spread of non-B clades in different countries at the time of survey.[4–7] Despite the considerable waves of migration from Africa, Central and South America, and South-East Asia, TDR in patients with non-B subtypes was reported at low frequency in Europe until recent years. This prevalence was shown to fluctuate

from 6% in the 2001–03 period to 3.2% in 2006 in the UK,[4] while surveillance studies reported a prevalence of 11% in France.[5] We previously reported a TDR prevalence of 5% in individuals harbouring a non-B clade virus from 1992 to 2005 in Italy,[6] whilst another Italian study, conducted in the period 1996–2006, showed a TDR prevalence of 7%, which was significantly lower than that found in B subtype-carrying patients (17%).[7]

European surveillance studies have so far considered TDR in non-B clades grouped together. However, TDR analysis in specific non-B clades may provide interesting data if related with epidemiological correlates, such as the country of origin and the modality of infection.

Subtype F1 is the most frequent non-B variant (44.3%) found in subjects living in Italy,[8] thus allowing a specific assessment of TDR associated with this clade. This subtype, both as a 'pure' strain or as part of recombinant forms, is circulating at high prevalence in South America, Central Africa and Eastern Europe. In South America it circulates at high frequency among intravenous drug users (IDUs) and heterosexuals (HETs),[9] whereas it has become highly prevalent in Romania (>70%) among adults and institutionalized children.[10]

The aim of this study was to determine the prevalence of drug resistance and TDR among F1 subtype HIV-1-infected patients, whose genotype and clinical data have been recorded in a national database since 1998. Furthermore, we investigated the relationship between drug resistance and the main demographic variables, such as ethnicity, route of infection and gender among naive patients.

Methods

We analysed HIV-1-positive individuals carrying the F1 variant, who had been referred to 50 clinical centres in 13 Italian regions in the 1998–2009 period. Patients received a genotypic resistance test at diagnosis or prior to the start of therapy, or at treatment failure. Patients were included in the Antiretroviral Resistance Cohort Analysis database and provided informed consent to have their anonymized data stored on a central server (www.hivarca.net). For each patient included in the analysis, the earliest available HIV-1 genotype was evaluated. Demographic data (gender, risk category, country of origin, date of diagnosis and age) were recorded in the database together with virological, immunological and clinical information.

Subtyping was based on a partial HIV-1 *pol* sequence of ~1000–1280 nucleotides long, depending on the sequencing protocol used at the contributing laboratory.

Non-B sequences were aligned to the most recent reference dataset from the Los Alamos National Laboratory web site (http://hiv.lanl.gov/) using BioEdit 7.0.5 and ClustalX 1.83. The resulting alignment was analysed with Phylip package version 3.67 (http://evolution.genetics.washington.edu/phylip.html), building a neighbour-joining tree based on the Kimura two-parameter substitution model. The reliability of the tree topology was assessed through bootstrapping using 1000 replicate datasets.

Mutations related to drug resistance were analysed with the Surveillance Drug Resistance Mutation[11] and the International AIDS Society lists[12] for naive and treated patients. The distribution of study subjects with regard to categorical parameters was compared using χ^2 or Fisher's exact tests. Standard non-parametric methods (Kruskal–Wallis test) were used to compare the median age, HIV-1 RNA levels and CD4 counts. Categorical data were analysed using linear-by-linear associations for trends over time periods. For all analyses, an α error of 5% was considered. Statistical calculations were performed using the statistical software package SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

Results

We studied 343 HIV-1-infected patients enrolled in the 1998–2009 period carrying the F1 subtype. Resistance was evaluated in a subset of 221 patients whose treatment status was known, of whom 169 were drug naive.

Among naive patients, 75.1% ($n=127$), 10.1% ($n=17$) and 7.1% ($n=12$) were Italians, South Americans and Romanians, respectively. HETs, men who have sex with men (MSM) and IDUs accounted for 63.4% ($n=83$), 31.3% ($n=41$) and 5.3% ($n=7$) of the patients. Three out of four subjects were males ($n=129$, 76.3%).

The median HIV-1 viral load was 4.71 log copies/mL (IQR=3.85–5.30 log copies/mL) and the median CD4 count was 347 cells/mm^3 (IQR=127–555 cells/mm^3). The median age was 38 years (IQR=31–46 years).

Table 1 shows the distribution of demographic characteristics and viro-immunological parameters in Italian, South American and Romanian naive patients. HETs were prevalent among Italians and Romanians, while MSM were predominant in South Americans. Male gender accounted for the majority of Italians and South Americans. The median age was 40, 33 and 25 years in Italians, South Americans and Romanians, respectively. Thus, risk factor, gender and age were differently distributed according to the country of origin ($P<0.001$ for all comparisons).

Overall, drug resistance was documented in 25.3% (56/221) of our study population. The prevalence of any resistance was 15.4% [26/169; 11.8% ($n=20$) for nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), 6.5% ($n=11$) for non-nucleoside reverse transcriptase inhibitors (NNRTIs) and 7.1% ($n=12$) for protease inhibitors (PIs)] in naive individuals.

The frequency of TDR was higher in South Americans (23.5%) compared with Italian and Romanian naive patients (15% and 8.3%, respectively), even though no statistical difference was detected.

TDR appeared equally distributed among MSM, HETs and IDUs (19.5%, 14.5% and 14.3%, respectively) and between males and females (15.5% versus 15.4%, respectively). Table 1 shows the proportions of TDR class resistance according to the origin of the patients.

We then evaluated the trends of any and class-specific TDR over three time intervals (1998–2001, 2002–05 and 2006–09). A decline of both any and NRTI-associated TDR was observed over time: from 21.4%, to 23.9% and then to 7.1% ($P=0.010$), and from 14.3%, to 21.1% and then to 3.6% ($P=0.007$), respectively. There was a low prevalence of both NNRTI- and PI-associated mutations in our dataset, ranging from 0% to 11.3% and then to 3.6% and from 7.1% to 12.7% and then to 2.4%, respectively. No significant trend could be seen in the TDR for these classes of antiretrovirals.

Discussion

Studies of the last decade showed a limited prevalence of TDR among individuals carrying a non-B clade with a range of 3.2%–11% in Europe, depending on the area and time of survey.[5–9] These data may be related to a low exposure to treatment of individuals harbouring non-B subtypes, since they migrated in recent years from countries where antiretroviral

Table 1. Demographic characteristics and viro-immunological parameters among Italian, South American and Romanian drug-naive patients harbouring subtype F1 HIV-1

	Patient geographical origin[a]			
	Italy ($n=127$)	South America ($n=17$)	Romania ($n=12$)	*P*
Risk factor, % (*n*)				<0.001
HETs	51.9 (66)	5.9 (1)	66.6 (8)	
MSM	19.7 (25)	82.4 (14)	0.0 (0)	
IDUs	5.5 (7)	0.0 (0)	0.0 (0)	
other[b] or unknown	22.8 (29)	11.8 (2)	33.3 (4)	
Male, % (*n*)	82.6 (105)	94.1 (16)	25.0 (3)	<0.001
Age (years), median (IQR)	40 (34–47)	33 (28–40)	25 (19–33)	<0.001
HIV-RNA (log copies/mL), median (IQR)	4.67 (3.72–5.27)	4.09 (3.63–4.70)	4.98 (4.75–5.57)	0.168
CD4 (cells/mm^3), median (IQR)	357 (182–565)	384 (118–573)	153 (80–323)	NS
Overall TDR, % (*n*)	15.0 (19)	23.5 (4)	8.3 (1)	NS
NRTI resistance	11.8 (15)	17.6 (3)	—	NS
NNRTI resistance	7.1 (9)	5.9 (1)	8.3 (1)	NS
PI resistance	7.9 (10)	5.9 (1)	—	NS

NS, not significant.
[a]Thirteen individuals carrying F1 subtypes had other or unknown origin.
[b]Other: professional risk, transfusions and vertical transmission.

drugs were not widely available for the treatment of HIV infection. Therefore, a continuous monitoring of TDR prevalence among non-B subtypes is needed to understand the interactions between ethnic groups that may influence the rate and trend of TDR, and thus impact the response to treatment.[1]

Previous TDR evaluations have considered non-B clades grouped together.[4–7] However, differences in TDR distribution may be detected among different subtypes as possible consequences of their specific spread in various populations of different countries. A large survey conducted in Italy detected a significantly higher rate of TDR in subtype B-infected versus non-B-infected subjects.[7] However, our study shows a remarkable frequency (15.4%) of TDR in the subtype F1-infected population. This figure is comparable to the TDR rate in subtype B-infected patients in Italy.[6,7]

The high prevalence of TDR (23%) detected in South American subjects is linked to the homosexual route of infection (82%), which is predominant in these individuals and, consequently, to the male gender (94%). On the other hand, TDR was less prevalent (8%) among the Romanian population living in our country, in which the HET route of infection is predominant, with the proportion of females being much higher. These results are in agreement with the epidemiology of HIV infection among people coming from Romania, where the F1 subtype initially spread through infected blood products and unsterilized needles.[10]

Interestingly, TDR was considerably high (15%) also in the Italian subtype F1-infected population, deserving careful monitoring. Among these subjects, the sexual route of transmission was predominant together with the male gender. Through a phylogenetic analysis, we recently reported that the majority of transmission events of the F1 subtype among Italians involved South American MSM.[13] Therefore, the spread of this subtype in the Italian population may be related to sexual intercourse, likely through homosexual contacts or transgender prostitution. Indeed, we found a comparable frequency of TDR both in Italians and South Americans as a possible consequence of the linkage of these groups of patients.

Also worthy of note, a declining trend of transmitted resistance to any drug class and NRTIs was highlighted in our study. These data are consistent with other observations that indicated that new potent combinations of antiretrovirals may positively impact on TDR.[2] No significant trends of NNRTI- and PI-associated TDR could be detected in our population, probably due to the observed biphasic pattern and/or the limited proportions of mutations for drugs of these classes.

We report here that primary resistance in patients with the F1 subtype may largely exceed that of non-B clades in Italy.[7] Since a relevant prevalence of TDR was not previously found in a population infected with non-B subtype, this issue deserves further investigation. The contact tracing of resistant isolates in epidemiological clusters may clarify the impact of F1 spread on TDR and help in targeting public interventions to limit the spread of primary resistance to antiretrovirals.

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Transparency declarations

None to declare.

References

1 Wittkop L, Günthard HF, de Wolf F *et al*. Effect of transmitted drug resistance on virological and immunological response to initial combination antiretroviral therapy for HIV (EuroCoord–CHAIN joint project): a European multicohort study. *Lancet Infect Dis* 2011; **11**: 363–71.

2 UK Collaborative Group on HIV Drug Resistance; UK Collaborative HIV Cohort Study; UK Register of HIV Seroconverters. Evidence of a decline in transmitted HIV-1 drug resistance in the United Kingdom. *AIDS* 2007; **21**: 1035–9.

3 Vercauteren J, Wensing AM, van de Vijver DA *et al*. Transmission of drug-resistant HIV-1 is stabilizing in Europe. *J Infect Dis* 2009; **200**: 1503–8.

4 Chilton DN, Castro H, Lattimore S *et al*. HIV type-1 drug resistance in antiretroviral treatment-naive adults infected with non-B subtype virus in the United Kingdom. *Antivir Ther* 2010; **15**: 985–91.

5 Descamps D, Chaix ML, Montes B *et al*. Increasing prevalence of transmitted drug resistance mutations and non-B subtype circulation in antiretroviral-naive chronically HIV-infected patients from 2001 to 2006/2007 in France. *J Antimicrob Chemother* 2010; **65**: 2620–7.

6 Riva C, Lai A, Caramma I *et al*. Transmitted HIV type 1 drug resistance and non-B subtypes prevalence among seroconverters and newly diagnosed patients from 1992 to 2005 in Italy. *AIDS Res Hum Retroviruses* 2010; **26**: 41–9.

7 Bracciale L, Colafigli M, Zazzi M *et al*. Prevalence of transmitted HIV-1 drug resistance in HIV-1-infected patients in Italy: evolution over 12 years and predictors. *J Antimicrob Chemother* 2009; **64**: 607–15.

8 Lai A, Riva C, Marconi A *et al*. Changing patterns in HIV-1 non-B clade prevalence and diversity in Italy over three decades. *HIV Med* 2010; **11**: 593–602.

9 Montano SM, Sanchez JL, Laguna-Torres A *et al*. Prevalences, genotypes, and risk factors for HIV transmission in South America. *J Acquir Immune Defic Syndr* 2005; **40**: 57–64.

10 Dumitrescu O, Kalish ML, Kliks SC *et al*. Characterization of human immunodeficiency virus type 1 isolates from children in Romania: identification of a new envelope subtype. *J Infect Dis* 1994; **169**: 281–8.

11 Bennett DE, Camacho RJ, Otelea D *et al*. Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. *PLoS ONE* 2009; **4**: e4724.

12 Johnson VA, Calvez V, Gunthard HF *et al*. 2011 update of the drug resistance mutations in HIV-1. *Top Antivir Med* 2011; **19**: 156–64.

13 Lai A, Zehender G, Franzetti M *et al*. High proportion of HIV-1 subtype F1 epidemiological networks among Italian heterosexual males associated with introduction events from South America and high proportion of resistance within clusters. *Antivir Ther* 2011; **16** Suppl 1: A154.