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Short communication

Transmission of HIV drug resistance and non-B subtype distribution in the Spanish cohort of antiretroviral treatment naïve HIV-infected individuals (CoRIS)

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ABSTRACT

CoRIS is an open multicentre cohort of HIV seroprevalent ARV-naïve subjects who began treatment at 32 Spanish healthcare centres from January 2004. Up to November 2008, a total of 683 FASTA format sequences, encoding the HIV protease and reverse transcriptase (RT) derived from plasma samples at entry into the cohort, had been obtained for examination of transmitted drug resistance (TDR) and HIV clade. TDR was found in 8.5% of the patients (4.4% NRTIs, 4% NNRTIs, 2.2% PIs). The most prevalent resistance mutations were: T215 revertants (3.8%), D67NG (1.3%), K219QENR (1.2%) and M41L (1%), for NRTIs; K103N (3.2%), for NNRTIs; I54VLMSAT, M46I and L90M (0.7%), for PIs. Non-B subtypes were recognized in 104 patients (15.2%) and were more common in Sub-Saharan Africans (15/17, 88.2%), Eastern Europeans (7/12, 58.3%) and Northern Africans (8/16, 50%) than among Spaniards (53/479, 11%) ($p < 0.001$). The most prevalent non-B subtype was CRF02_AG (4.4%), followed by subtype D (1.9%), CRF03_AB (1.5%), CRF07_BC and subtype F1 (1%). A trend was observed for the transmission of non-B subtypes to increase and for TDR to decrease.

Sequence data from resistance testing offer unique opportunities to characterize the structure of HIV infection epidemics, in two ways: via the transmission of HIV drug resistance mutations (Truong et al., 2006) and via the distribution of non-B subtypes (Gifford et al., 2006). In the USA and Europe, the overall prevalence of primary drug resistance in recent years is around 10–15%, with some differences among regions and time periods (Bannister et al., 2008; Weinstock et al., 2004; Yerly et al., 2007). Two large studies, the SPREAD study (Vercauteren et al., 2009), and the Eurocord–CHAIN joint project (Wittkop et al., 2011), have consistently shown an overall prevalence of transmitted drug resistance (TDR) of around 9% across European countries. Several studies have reported the recent introduction of HIV-1 non-B subtypes into Western Europe following immigration from other regions (Fox et al., 2010; Galimand et al., 2010; Lai et al., 2010), with a prevalence of 13–30%, depending on the country, the period and the population studied.

Genotypic testing for treatment naïve individuals was first introduced in Spain following international recommendations in 2005 (Sax et al., 2005) and nowadays is almost universal. The present study characterises the molecular epidemiology of HIV infection in Spain and the transmission of drug resistance mutations and non-B subtypes, within one of the largest HIV cohorts of seroprevalent individuals analysed in this country, that of naïve HIV infected individuals (CoRIS) (Caro-Murillo et al., 2007), and describes the trends observed over time.

CoRIS is an open, prospective, multi-centre cohort of HIV-positive subjects, aged over 13 years, who initiated care for the first time after 1 January, 2004 while naïve for antiretroviral therapy at entry. CoRIS is a joint activity administered by the Research Network of Excellence (AIDS research network, RIS). A total of 32 centres in 12 different regions in Spain participate in CoRIS. By November 2008, 3351 HIV-positive HAART-naïve patients were

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included in CoRIS. For the purposes of this study (CoRIS Primary Resistance Analyses), contributing centres were asked to retrieve FASTA format sequences from routine clinical care diagnosis for the HIV drug resistance test (population sequencing), encoding the HIV protease and RT derived from plasma samples at the moment of inclusion in the cohort (baseline). A total of 18 centres in 7 regions agreed to participate and 683 FASTA sequences (38 obtained in 2004, 55 in 2005, 111 in 2006, 304 in 2007 and 175 in 2008) were sent to the coordinating centre. Transmitted Drug Resistance (TDR) associated mutations were evaluated following the surveillance drug resistance mutation list updated in 2009 (Bennett et al., 2009). The HIV subtype was ascribed using the protease gene, and phylogenetic analyses were carried out using the Therapy Edge™ (ABL, Luxembourg) subtyping tool. Molecular evolutionary analyses were conducted using the MEGA v4 program (Tamura et al., 2007), and clusters were defined considering bootstrap values >90. TDR and subtype were merged with the clinical, epidemiologic, virologic and immunologic information available from these patients in the CoRIS database for analysis. Descriptive analyses are presented using absolute numbers and percentages for categoric variables. Otherwise, the median and interquartilic range is shown. All tests were two sided and a significance level of 5% was used. Chi-square tests were used to assess differences in proportions, and to reveal trends. Maentel–Haenszel crude odds ratios (OR) of association with TDR according to the HIV-subtype were obtained. Statistical analyses were performed using Stata10® software (Texas, USA).

Table 1 describes the baseline characteristics of the study population. The prevalence of TDR during the whole study period (2004–2008) was 8.5% (95% CI: 6.6–10.8). By drug classes, the highest prevalence was found for nucleoside/tide reverse transcriptase inhibitors (NRTIs) with a prevalence of 4.4% (95% CI: 3.0–6.1), followed by non-nucleoside reverse transcriptase inhibitors (NNRTIs) with 4% (95% CI: 2.7–5.6) and protease inhibitors with 2.2% (95% CI: 1.3–3.5). We also recorded a calendar-year declining trend in TDR prevalence from 2004 to 2008, from 15.8% (95% CI: 6.7–30.0) in 2004 to 6.8% (95% CI: 3.8–11.4) in 2008 (p for trend = 0.09), mainly due to a decrease in the transmission rate of NRTI mutations between 2004 and 2008, from 13.2% (95% CI: 5.0–26.8) to 4% (95% CI: 1.1–6.2), respectively (p for trend = 0.04) (Table 2). As resistance testing in naïve patients was not recommended in Spanish guidelines until early 2007 (Expert Committee of GESIDA, 2007), this finding may reflect the selection of patients from higher risk groups in earlier calendar years. Ten patients (1.5%; 95% CI: 0.8–2.6) presented resistance mutations associated with resistance to two drug classes, and only two patients (0.3%; 95% CI: 0.0–1.0) presented resistance to all three drug classes evaluated. These data demonstrate that the risk of transmission of multidrug-resistant genotypes remains low in Spain.

Table 1
Demographic and baseline characteristics of the study population, by year of inclusion in CoRIS.

>No.	2004 38		2005 55		2006 111		2007 304		2008 175		Total 683	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Gender												
Male	30	78.9	41	74.5	91	81.2	279	91.7	145	82.8	586	85.9
Risk category												
IDU	3	7.9	6	10.9	9	8.1	26	8.6	21	12	65	9.5
MSM	18	47.3	30	54.5	61	54.9	207	68.1	102	58.2	418	61.2
Heterosexual	16	42.1	17	31	40	36.1	57	18.7	50	28.6	180	26.4
Other	1	2.7	2	3.6	1	0.9	14	4.6	2	1.2	20	2.9
Median age (years)	33 (26–37.5)		35 (28–43)		36.5 (30–41)		33 (29–41)		36 (29–42.5)		34 (29–41)	
Country of origin												
Spain	32	84.3	43	78.2	95	85.6	183	60.2	126	72	479	70.1
Western Europe	0	0	2	3.6	2	1.8	12	3.9	0	0	17	2.5
Eastern Europe	1	2.6	1	1.8	1	0.9	4	1.3	5	2.8	12	1.8
Sub-Saharan Africa	0	0	2	3.6	1	0.9	9	3	5	2.8	17	2.5
North Africa	1	2.6	1	1.8	0	0	6	2	8	4.7	16	2.3
Latin America	3	7.9	5	9.2	12	10.8	86	28.3	26	14.9	132	19.3
Others	1	2.6	1	1.8	0	0	4	1.3	5	2.8	10	1.5
Median viral load at entry (Log copies/mL)	4.7 (4–5.1)		4.4 (3.9–4.9)		4.4 (4–5.1)		4.5 (4–5)		4.5 (3.9–5)		4.5 (4–5)	
Median CD4 counts (cells/mm^3)	502 (361.5–729.9)		405 (271.5–568.5)		355 (209–602)		399 (245–618)		361 (210–571)		395 (239–616)	
Non-B subtypes	2	5.3	10	18.2	13	11.7	42	13.8	37	21.1	104	15.2

Table 2
Prevalence of transmitted drug resistance during the study period.

Drug resistance	2004 *n* = 38		2005 *n* = 55		2006 *n* = 111		2007 *n* = 304		2008 *n* = 175		Total *n* = 683		*p* (trend)
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	
Any class	6	15.8	4	7.3	8	7.2	28	9.3	12	6.8	58	8.5	0.09
NRTI	5	13.2	3	5.5	4	3.6	11	3.6	7	4	30	4.4	0.04
NNRTI	3	7.9	2	3.6	4	3.6	14	4.6	4	2.3	27	4	0.21
PI	1	2.6	1	1.8	4	3.6	6	2	3	1.7	15	2.2	0.77
Two classes											10	1.5	
Three classes											2	0.3	

Table 3
Prevalence of mutations proposed by the World Health Organization for surveillance of transmitted drug resistance.

NRTI mutations			NNRTI mutations			PI mutations		
Mutation	*N*	(%)	Mutation	*N*	(%)	Mutation	*N*	
M41L	7	(1.02)	L100I	1	(0.15)	V32I	3	(0.44)
D67NG	9	(1.32)	K101EP	4	(0.58)	M46IL	7	(1.02)
T69D	1	(0.15)	K103N	22	(3.24)	I47V	2	(0.30)
K70ER	2	(0.30)	V106M	1	(0.15)	I50L	1	(0.15)
L74V	1	(0.15)	Y181C	1	(0.15)	F53L	2	(0.30)
V75M	1	(0.15)	G190A	5	(0.74)	I54LVT	5	(0.74)
Q151M	1	(0.15)				G73AS	2	(0.30)
M184IV	3	(0.44)				L76V	1	(0.15)
L210W	5	(0.74)				V82A	3	(0.44)
T215Rev*	26	(3.80)				N83D	1	(0.15)
T215F	2	(0.30)				I84V	1	(0.15)
K219QENR	8	(1.18)				N88S	1	(0.15)

NRTI, nucleotide reverse transcriptase inhibitors; NNRTI, non nucleotide reverse transcriptase inhibitors; PI, protease inhibitors. Amino acid abbreviations: A, alanine; C, cysteine; D, aspartate; E, glutamate; F, phenylalanine, G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.
* =C/D/E/N/I/V/S.

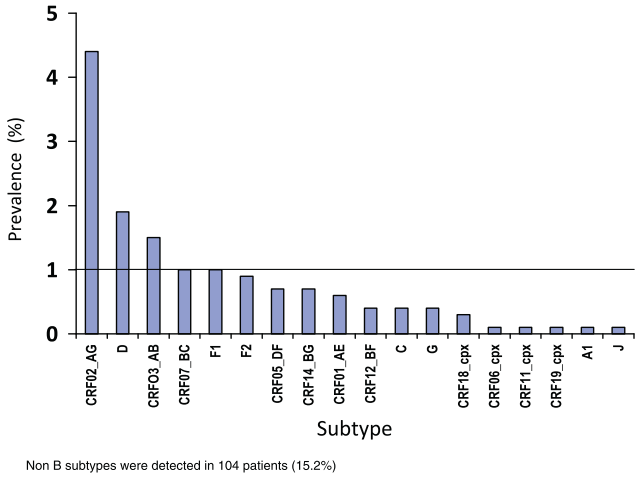
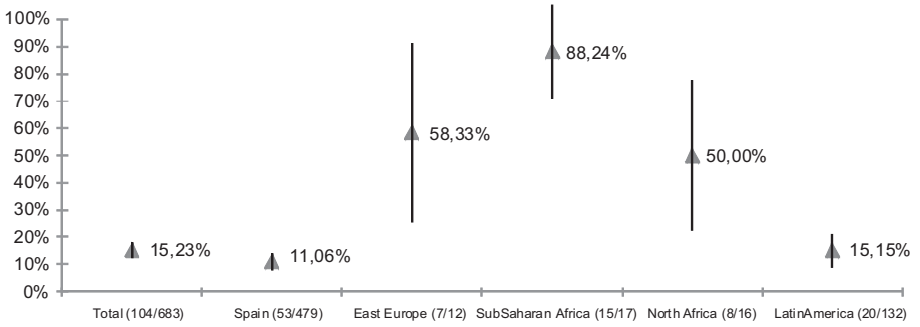


Fig. 1. Non-B subtype distribution in CoRIs.

No association was observed between TDR and gender [OR for TDR 0.53 (0.27–1.03), $p = 0.06$]; risk category for HIV infection [Intravenous Drug Users, 0.6 (0.2–1.87); Men who have sex with men, 0.82 (0.45–1.49), $p = 0.74$ vs heterosexuals-HTX]; country of origin [Western Europe, 0.66 (0.08–5.12); Eastern Europe, 0.9 (0.11–7.14); Sub-Saharan Africa, 0.62 (0.08–4.78); North Africa, 1.41 (0.31–6.43); Latin America, 0.72 (0.34–1.52); $p = 0.88$ vs Spanish]; age, viral load or median CD4 count at entry. As in other European studies (Vercauteren et al., 2009; Wittkop et al., 2011), T215 Revertants (3.8%; 95% CI: 2.6–3.5) and K103 N (3.2%; 95%CI: 2.1–4.8) were the most prevalent mutations responsible for NRTI and NNRTI resistance, respectively. In Spain, where efavirenz is one of the drugs most widely used as first-line therapy, the presence of K103N represents a problem for first-line antiretroviral therapy. Therefore, in accordance with current treatment guidelines (doi:10.1016/j.eimc.2010.12.0004), primary resistance testing is mandatory before initiating first-line therapy. Other prevalent mutations (Table 3) found in our study were the thymidine-associated mutations (TAMs) D67NG, (1.3%; 95% CI: 0.6–2.4); K219QENR (1.18%; 95% CI: 0.5–2.2) and M41L, (1.02%; 95% CI: 0.4–2.0). For PIs, no individual mutation associated with TDR was detected at a level exceeding 1%.

The distribution of non-B subtypes and their association with demographic factors, over the course of the HIV epidemic in a region, provide useful information in terms of clinical, epidemiologic and prevention strategies. In our study, subtype B accounted for 84.8% (95% CI: 81.9–87.3) of HIV-1 infections in CoRIS, and 104 patients (15.2%; 95% CI: 12.7–18.1) harboured non-B strains. CRF02_AG was the most prevalent non-B (4.4%, 95% CI: 3.0–6.1), followed by subtype D (1.9%; 95% CI: 1.1–3.2), CRF03_AB (1.5%; 95% CI: 0.7–2.6), CRF07_BC and subtype F1 (1%; 95% CI: 0.4–2.0); other subtypes included subtype F2 (0.9%; 95% CI: 0.4–1.8), CRF05_DF & CRF14_BG (0.7; 95% CI: 0.3–1.6), CRF01_AE (0.6%; 95% CI: 0.2–1.4), CRF12_BF, subtype C, and subtype G (0.4; 95% CI: 0.1–1.2), CRF18_cpx (0.3; 95% CI: 0.1–1.0), CRF06_cpx, CRF11_cpx, CRF19_cpx,A1 subtype A1, and subtype J (0.1%; CI95: 0.0–0.7) (Fig. 1). As other authors have reported (de Mendoza et al., 2005) non-B subtypes are noticeably present in newly-diagnosed patients in Spain, constituting over 15% during the study period and 21% in 2008.

In Western Europe the increase in non-B subtypes is related mainly to immigration (Yerly et al., 2007). Thus, in our study the patients from Sub-Saharan Africa (15/17, 88.2%), Eastern Europe (7/12, 58.3%) and North Africa (8/16, 50%) showed a higher prevalence of non-B subtypes than did Spaniards (53/479, 11%, $p < 0.01$), (Fig. 2). Circulating recombinant forms (CRFs) accounted for 2/3 of non-B subtypes (66.3%; 95% CI: 56.9–75.0) and for 1/10 (10.1%; 95% CI: 8.0–10.5) of all the patients studied. As for all non-B subtypes, CRFs were more prevalent in Sub-Saharan Africans (64.7%; 95% CI: 40.5–84.3), North Africans (37.5%; 95% CI: 16.8–62.4) and Eastern Europeans (33.3%; 95% CI: 11.6–62.3) than among Spaniards (6.9%; 95% CI: 4.9–9.4) or Latin Americans (7.5%; 95% CI: 4.2–12.5). An increase in the prevalence of non-B subtypes



p<0.001 (Chi square) Sub-Saharan Africans, Eastern Europeans and North Africans vs Spaniards and p=0.086 vs Latin Americans. Vertical bars represent 95%CI.

Fig. 2. Prevalence of non-B subtypes by geographical origin in CoRIS.

was observed during the study period, from 5.4% (95% CI: 0.9–16.3) in 2004 to 21% (95% CI: 15.6–27.7) in 2008 (p for trend <0.05). However, compared with our neighbouring countries, France (Galimand et al., 2010), Italy (Lai et al., 2010) and Portugal (Palma et al., 2007), where non-B subtypes in newly diagnosed patients may account for 30%, the proportion of non-B subtypes in Spain seems to be low.

TDR was no higher for non-B subtype than for B subtype infected patients in CoRIS. Resistance mutations were detected in 8.6% (CI 95: 4.3–15.3) of the non-Bs [OR for TDR 1.18 (0.57–2.41), p = 0.65]. This was also true for CRF02_AG and subtype D, the most prevalent non-B subtypes in our cohort. These data are in contrast to those of Vercauteren et al., 2009, who found that infection with a B subtype was the strongest predictor for TDR. Different study periods and different populations may account for the differences, and thus there is a need for further studies to evaluate TDR and subtypes.

Among recent HIV infections, clusters ranging from 2 to 25 or more members are frequently identified (Ragonnet-Cronin et al., 2010; Recordon-Pinson et al., 2009). The segregation into clusters suggests the existence of forward transmission events and has implications for the presence of drug resistance mutations and non-B subtypes in the newly-infected population. However, in our study only two clusters, involving 5 patients with TDR, were found.

There are some obvious limitations to our study. First, the number of patients included per year, which may explain why we found no significant differences over time. Second, as we studied 683 patients from 18 healthcare centres in 7 Spanish regions, the population studied was not always representative of the whole Spanish HIV-infected population. Nevertheless, this is the largest survey of TDR and HIV subtypes conducted so far in Spain.

In conclusion, this paper describes the largest Spanish survey to date to address HIV molecular epidemiology in antiretroviral naïve patients during 2004–2008, and we present the first Spanish report in this respect following international guidelines. The prevalence of non-B subtypes, with a high representation of recombinants, was relatively high in 2008, mainly due to the impact of the immigrant population. These observations may have important implications from the epidemiological point of view and for the clinical management of HIV-infected patients in Spain.

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Transparency declarations

None to declare.

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