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EFFECT OF HIV/HCV CO-INFECTION ON THE PROTEASE EVOLUTION OF HIV-1B INFECTING PEDIATRIC POPULATIONS

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The Madrid cohort of HIV-infected children and adolescents, ascribed to the cohort of the Spanish Paediatric HIV Network (CoRISpe), is one of largest paediatric HIV-infected cohorts in Europe with 561 patients registered since the beginning of the epidemic until Dec 2015, all of them with available follow-up epidemiological-clinical-virological information. It includes almost all infected children and adolescents infected since the beginning of HIV epidemic and born since 1981, which are under clinical follow-up in nine public hospitals in Madrid. This currently represents 48% (561/1165) of the HIV-1B infected children and adolescents in Spain. By March 2016, there were 35 (6.2%) patients HIV-1/HCV coinfected in the Madrid cohort, and 15 (46.8%) of them had available HIV-1 *pol* sequences. From this cohort, a total of 71 patients were enrolled in this pilot study: 15 HIV-1B/HCV coinfected and 56 HIV-1B monoinfected pediatric patients. **Table S1** summarizes the clinical, epidemiological and virological features of the study population. At sampling time, both groups presented similar HIV-1 viremia, immunological profile, time without ART until HIV diagnosis, time under antiretroviral therapy (ART), and antiretroviral (ARV) experience to nucleoside reverse transcriptase inhibitors (NRTI) or protease inhibitors (PI). In contrast, non-nucleoside reverse transcriptase inhibitors (NNRTI) experience was lower in HIV-1B/HCV coinfected than monoinfected patients. Time under monotherapy, under combined ART (cART) or under high-active ART (HAART) was also similar across groups (**Table S1**).

41 **Table S1**: Clinical-virological features of study population at sampling time.

	HIV-1B/HCV Coinfected n (%)	HIV-1B Monoinfected n (%)	*p*-value
Demographics			
Number of patients	15	56	
Female gender	7 (46.7)	31 (55.4)	0.549
Age (median, [IQR])	11.03 [12.52-4.35]	10.86 [14.34-7.35]	0.923
Birth date			
1984-1992	6 (40)	28 (50)	1.00
1993-2001	9 (60)	28 (50)	
HIV-1 transmission			
Perinatal	14 (93.3)	55 (98.2)	0.380
Blood transfusion	1 (6.7)	1 (1.8)	
HIV-1 diagnosis year			
1986-1995	12 (80)	25 (52.1)	0.372
1996-2005	3 (20)	23 (47.9)	
Time under HIV-1 infection(y, [IQR])	11.3 [9.8-13.1]	10.9 [7.5-21.8]	0.773
Year of first sequence			
1993-1999	5 (33.3)	20 (35.7)	1.00
2000-2004	5 (33.3)	34 (60.7)	0.323
2005-2009	5 (33.3)	2 (3.6)	0.140
Virologic features			
Viral load (median RNA-HIV-1 cp/mL, [IQR])	5,820 [319-35820]	3,700 [200-52160]	0.346
Years under HIV exposure (mean)	11.26 ± 4.85	11.06 ± 4.99	0.641
Immunologic status			
CD4+ T cells % (median, [IQR])	26 [20.31-37]	32 [23.75-38.08]	0.375
CD4+ T cells mm3 (median, [IQR])	665 [504.5-1159]	719 [559.2-821]	0.941
CD8+ T cells % (median, [IQR])	42 [34-51]	38 [30.50-49.50]	0.234
CD8+ T cells mm3 (median, [IQR])	1,077 [460, 2639]	970 [50, 2754]	0.172
ART status			
Drug-naïve	1 (6.7)	8 (14.3)	1.00
Under ART	14 (93.3)	48 (85.7)	
Years under ART			
<5 years	5 (35.7)	23 (41.8)	
≥5 years	9 (64.3)	32 (58.2)	0.677
Unknown	0	1 (2.1)	
Ever without ART[D]			
No	0	0	1.000
Yes	15 (100)	56 (100)	
Time without ART[D]			
Months (median,[IQR])	34 [5.4-70.5]	37.6 [5.8-62.5]	0.822

	HIV-1B/HCV Coinfected n (%)	HIV-1B Monoinfected n (%)	*p*-value
Ever under monotherapy[D]			
No	4 (26.7)	31 (55.4)	0.051
Yes	11 (73.3)	25 (44.6)	
Time under monotherapy			
Months (medians[IQR])	31.9 [15.5-47.3]	20.4 [9.3-42.6]	0.525
Ever under cART			
No	4 (26.7)	23 (41.1)	0.307
Yes	11 (73.3)	33 (58.9)	
Time under cART			
Months (medians[IQR])	8.7 [4.9-10.8]	8.2 [4.4-17.2]	0.674
Ever with HAART			
No	10 (66.7)	44 (78.6)	0.331
Yes	5 (33.3)	12 (21.4)	
Time on HAART			
Months (medians[IQR])	65.4 [47.8-97.8]	49.8 [8.8-78.9]	0.191
ARV experience			
NRTIs	**12/14 (85.7)**	**36/37 (97.3)**	0.892
AZT	12 (100)	30 (83.3)	0.553
3TC	8 (66.67)	28 (77.8)	0.300
DDI	11 (91.67)	30 (83.3)	0.553
D4T	11 (91.67)	31 (86.1)	0.584
ABC	2 (16.67)	9 (25)	1.00
TDF	0 (0)	2 (5.6)	1.00
ddC	0	1 (2.8)	1.00
NNRTIs	**3/13 (23.1)**	**22/37 (59.5)**	**0.024**
EFV	3 (100)	17 (77.3)	0.331
NVP	0 (0)	9 (40.9)	0.588
RPV	0	1 (4.5)	0.647
PIs	**10/13 (76.9)**	**41/48 (14.6)**	0.112
LPV/r	2 (20)	9 (21.9)	1.00
NFV	7 (70)	20 (48.8)	0.550
IDV	0	9 (21.9)	0.450
SQV	1(10)	9 (21.9)	0.594
Available HIV-1 *pol* sequences			
PR	15 (100)*	56 (100)	1.00
RT	13 (86.7)	46 (82.1)	0.963
PR sequences from treated patients	14 (93.3)	48 (85.7)	0.955
RT sequences from treated patients	13 (92.9)	37 (77.1)	0.647

Significance threshold: *P*-value<0.05; IQR, interquartile range; ART, antiretroviral therapy; ARV, antiretroviral; DRM, drug resistance mutation; NRTI, nucleoside reverse transcriptase

inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitors. ATV, atazanavir; DRV, darunavir; FPV, fosamprenavir; IDV, indinavir; NFV, nelfinavir; SQV, saquinavir; 3TC, lamivudine; ABC, abacavir; AZT, zidovudine; d4T, estavudine; ddI, didanosine; FTC, emtricitabine; TDF, tenofovir; EFV, efavirenz; ETV, etravirine; NVP, nevirapine; RPV, rilpivirine. PR, HIV-1B protease; RT, HIV-1B retrotranscriptase; D: until HIV diagnosis; cART: combined antiretroviral therapy (two combined drugs); HAART: highly active ART (two combined family drugs in one regimen). * One record from coinfected patients was a resistance profile. Regarding PR, we only analyzed 285 of the 297 aa sites of HIV-1B PR sequences, as they were available in most coinfected patients under study.

Table S2. Genbank accession numbers from HIV-1 in the study groups.

Pediatric Group	HIV-1BPR GenBank accession numbers
HIV-1B/HCV coinfected (n= 14)	HQ426719, HQ426726, HQ426728, HQ426733, HQ426734, HQ426781, HQ426807, HQ426818, HQ426838, HQ426843, HQ426858, HQ426863, KP881489 and KP881494
HIV-1B monoinfected (n= 54)	HQ426717, HQ426720, HQ426722, HQ426727, HQ426737, HQ426748, HQ426749, HQ426752, HQ426758-HQ426763, HQ426770, HQ426783, HQ426784, HQ426790, HQ426800, HQ426806, HQ426809, HQ426814, HQ426815, HQ426819-HQ426821, HQ426823, HQ426825, HQ426826, HQ426840, HQ426844, HQ426849, HQ426850, HQ426854, HQ426856, HQ426861, HQ426866, HQ426871, HQ426878, HQ426884, HQ426886, HQ426891, JQ351956, JQ351961, JQ351967, JQ351989, JQ351992, JQ351997, JQ352003, KP881484, KP881488, KP881492, KX607058, KX607067, KX607070, KX607077.

58 **Table S3**. Protease inhibitor experience and resistance mutations at HIV-1B protease.

	HIV-1B/HCV Coinfected N=15 (%)	**HIV-1B Monoinfected N=56 (%)**	***p*-value**
Experience to PI	10/14 (71.4)	41/48 (14.6)	0.112
LPV/r	2 (20)	9 (21.9)	1.00
NFV	7 (70)	20 (48.8)	0.550
IDV	0	9 (21.9)	0.450
SQV	1(10)	9 (21.9)	0.594
Prevalence of DRM to PI			
	5/15(33.3)	28/56(50%)	0.065
DRM to PI (major)			
D30N	4(80)	11(39.3)	0.640
M46I	0	11(39.3)	0.144
Q58E	0	1(3.6)	1.00
T74P	0	1(3.6)	1.00
L76V	0	1(3.6)	1.00
V82A	1(20)	7(25)	1.00
I84V	0	1(3.6)	1.00
N88S	0	2(7.1)	0.931
L90M	0	2(7.1)	0.931

59 PI, protease inhibitor; DRM, drug resistance mutations.

Table S4. Results from ten bootstrapped replicates (n=11) from 41 sequences of HIV monoinfected group.

	Genetic distances		**Non-synonymous mutations**		**Synonymous mutations**		**Selection Pressure**	
	~~1.~~ π	Comparison HIV/HCV	d_N	Comparison HIV/HCV	d_S	Comparison HIV/HCV	d_N- d_S	Comparison HIV/HCV
HIV+/HCV+ (n=11)	0.052±0.01	-	0.045±0.01	-	0.074±0.03	-	-0.026±0.018	-
HIV+/HCV- (n=41)	[0.035,0.055]	NS	0.025±0.01	*	[0.038,0.118]	NS	[-0.066,-0.04]	*
HIV+/HCV-Rep.1	[0.046,0.064]	NS	[0.011,0.031]	*	[0.037,0.077]	NS	[-0.06,-0.02]	*
HIV+/HCV-Rep.2	[0.041,0.059]	NS	[0.015,0.035]	*	[0.047,0.091]	NS	[-0.024, 0.022]	*
HIV+/HCV-Rep.3	[0.05,0.066]	NS	[0.013,0.022]	*	[0.035,0.079]	NS	[-0.021,0.031]	*
HIV+/HCV-Rep.4	[0.051,0.069]	NS	[0.015,0.035]	*	[0.037,0.117]	NS	[-0.068,-0.048]	*
HIV+/HCV-Rep.5	[0.069,0.089]	*	[0.061,0.087]	*$	[0.053,0.113]	NS	[-0.032, 0.014]	NS
HIV+/HCV-Rep.6	[0.041,0.059]	NS	[0.014,0.036]	*	[0.047,0.091]	NS	[-0.022, 0.024]	*
HIV+/HCV-Rep.7	[0.053,0.071]	NS	[0.019,0.038]	*	[0.041,0.107]	NS	[-0.058,-0.038]	*
HIV+/HCV-Rep.8	[0.043,0.057]	NS	[0.041,0.059]	NS	[0.019,0.051]	*$	[-0.005,0.035]	*$
HIV+/HCV-Rep.9	[0.05,0.068]	NS	[0.012,0.036]	*	[0.037,0.077]	NS	[-0.071,-0.031]	*
HIV+/HCV-Rep.10	[0.04,0.054]	NS	[0.037,0.055]	NS	[0.032,0.088]	NS	[-0.044,0.018]	NS

Significance was calculated according to 95% confidence intervals. NS: not significant; *, significant; *$: significant and different from the original set. Only two replicates presented differences with the real original data.

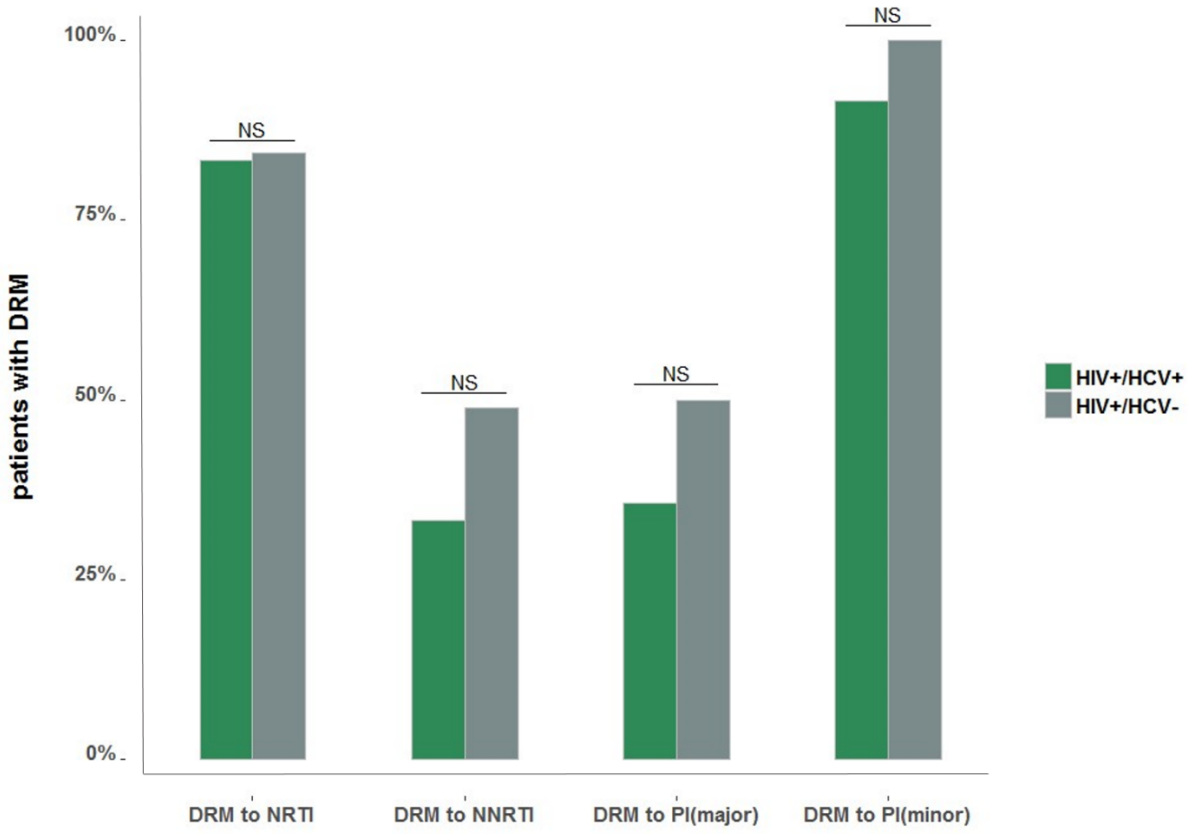

Figure S1. Prevalence of DRM to ARV families between 14 coinfected and 48 monoinfected pediatric patients under ART with available resistance information. DRM, Drug Resistance Mutation; NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitors (major, primary mutations to PI; minor, secondary mutations to PI); NS: Not Significant *p*-value>0.05. Some DRM to NRTI at RT were more frequent among coinfected patients compared to monoinfected patients: D67N (80% *vs.* 37%; X^2=4.15, *p*=0.030), K219QE (60% *vs*. 23%, X^2=3.4; *p*-value=0.040). No differences were found in DRM to NNRTI comparing both groups, and K103N was the most prevalent substitution at RT. Among the major DRM to PI considered (30, 32, 46-48, 47, 48, 50, 53, 54, 58, 74, 76, 82, 83, 84, 88, and 90), D30N and V82A at HIV-1B PR were the only found in the coinfected group and was present in 80% of treated patients. L90M at HIV-1B PR was the most prevalent DRM to PI among the HIV-1 monoinfected group (52%), and was absent in the coinfected group.

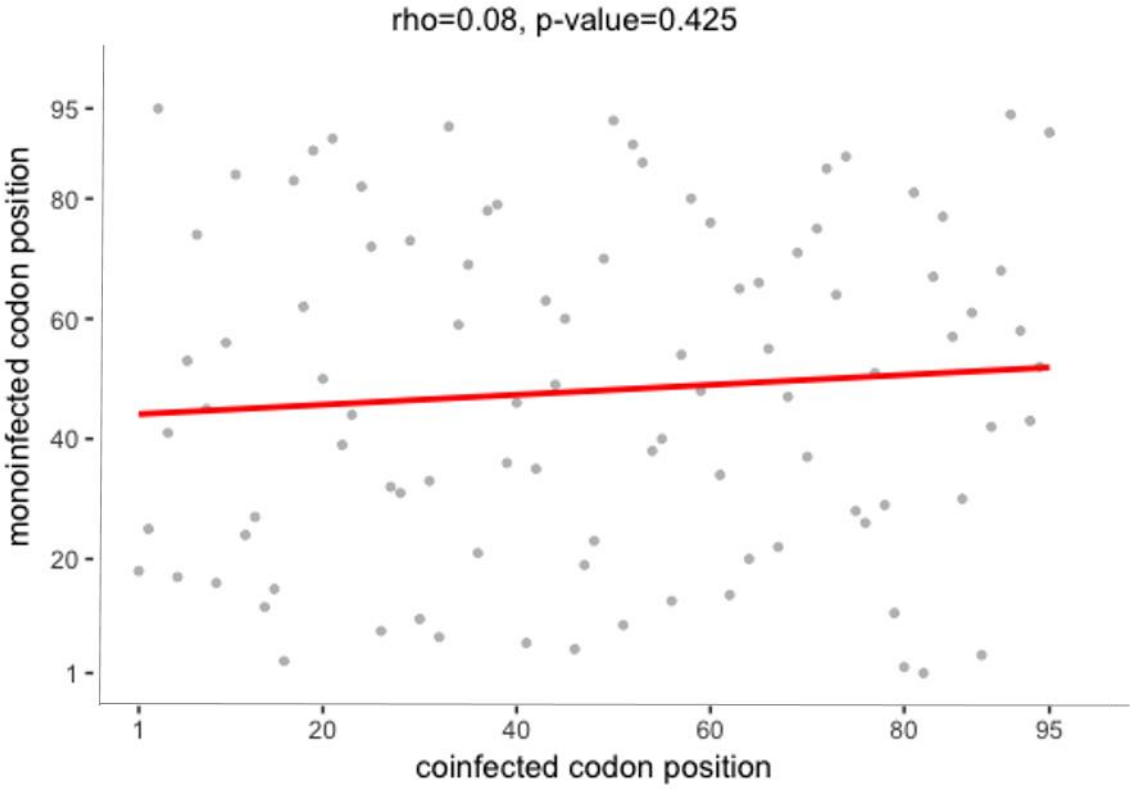

Figure S2: Spearman rank correlation of codon-specific d_N-d_S at the HIV-1BPR from monoinfected patients onto codon-specific d_N-d_S at the HIV-1BPR from coinfected patients.