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Temporal trend of drug-resistance and APOBEC editing in PBMC genotypic resistance tests from HIV-1 infected virologically suppressed individuals

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ABSTRACT

Background: We aimed at evaluating the temporal trend of drug-resistance and APOBEC editing from HIV-DNA genotypic resistance tests (GRT) in virologically suppressed individuals.
Material and methods: Major resistance mutations (MRM), genotypic susceptibility score (GSS) for the current regimen and APOBEC-related mutations (APO-M) were evaluated. Potential changes in trends of MRM and APO-M over-time were assessed and predictors of MRM detection or sub-optimal GSS (GSS<2) at HIV-DNA-GRT were estimated through logistic regression analyses.
Results: Among the 1126 individuals included, 396 (35.2%) harboured at least one MRM (23.4% to NRTI, 18.8% to NNRTI, 7.7% to PI and 1.4% to INSTI [N=724]); 132 (12.3%) individuals showed a GSS <2. APO-M and stop codons were found in 229 (20.3%) and 105 (9.3%) individuals, respectively. APO-DRMs were found in 16.8% of individuals and were more likely observed in those individuals with stop codons (40.0%) compared to those without (14.4%, P<0.001). From 2010 to 2021 no significant changes of resistance or APO-M were found. Positive predictors of MRM detection at HIV-DNA GRT were drug abuse, subtype B infection, and a prolonged and complex treatment history. Perinatal infection and having at least 2 stop codons were associated with a current suboptimal regimen.
Conclusions: In virologically suppressed individuals, resistance in HIV-DNA and the extent of APOBEC editing were generally stable in the last decade. A careful evaluation of APOBEC editing might be helpful to improve the reliability of HIV-DNA GRT. Further investigations are required to understand how to apply the estimation of APOBEC editing in refining genotypic evaluation.

1. Introduction

In spite of the extraordinary success of combined antiretroviral therapy (cART) [1], HIV-1 infected virologically suppressed individuals often require changes to their treatment to avoid drug toxicity, intolerance, drug-drug interactions, and to improve adherence [2]. Given that it is fundamental to maintain virological suppression (VS) without jeopardizing future treatment options, a proper assessment of resistance history should always be considered before switch treatment [3,4]. In this context, standard genotypic resistance testing (GRT) of

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plasma virus is not possible and HIV-1 DNA GRT of peripheral blood mononuclear cells (PBMCs) has increasingly been used in individuals with VS or low plasma virus levels to estimate resistance [5,6]. In fact, treatment guidelines suggest that genotype testing on HIV-DNA may be useful in virologically suppressed persons with multiple virological failures, unavailable resistance history or low-level viremia at the time of switch [3,4]. However, this test is still not recommended due to some concerns about the sensitivity of GRT from HIV-DNA in detecting drug-resistance [3–6]. It is well known that resistance in HIV-DNA might be underestimated [7,8] due to potential dilution of resistant strains in viral reservoir. Moreover, Sanger technique can detect variants only with a frequency >15-20% in viral populations, while using next generation sequencing (NGS) improves detection of historical archived resistance in virologically suppressed individuals [5,9]. However, NGS on HIV-DNA is still not commonly used.

Another point to take into account is the fact that HIV-DNA GRT reliability might be affected by the activity of the host cytidine deaminases APOBEC3F and 3G [5,6,10–12]. These enzymes might introduce G to A nucleotide mutations that can impair crucial enzymatic sites or generate stop codons that reduce the amount of replication competent proviruses [10–12].

In this context, some resistance mutations detected in HIV-DNA might be marginally relevant being present in defective proviruses. Moreover some DRMs in HIV-DNA might arise not under antiretroviral pressure but due to APOBEC activity that can occur at positions potentially associated with drug resistance, thus these substitutions should be regarded with caution [5]. Despite these concerns on HIV-DNA GRT, so far the extent of this phenomena is unknown due to the lack of studies that estimate resistance in DNA together with the level of APOBEC editing in a large population of virologically suppressed individuals. Studies like this might be important to estimate the proportion of individuals that are virologically suppressed but receiving suboptimal cART and to verify the impact of APOBEC editing on maintaining virological control despite resistance.

Based on these considerations, the aim of this study is to estimate, taking into account APOBEC editing, HIV-DNA resistance prevalence and its temporal trend from 2010 to 2021 in a large cohort of virologically suppressed individuals followed for clinical routine in Italy.

2. Study design

2.1. Study population

This retrospective study included 1126 HIV-1 drug-experienced virologically suppressed subjects with an available HIV-DNA GRT over the period 2010-2021 performed for routine clinical practice in several reference centers in Italy.

2.2. HIV-1 DNA extraction and genotyping

HIV-1 DNA was obtained from whole-blood (N=180) or lymphomonocytic cells (N=936) after separation from peripheral blood from HIV-1-infected patients with a Ficoll-Hypaque gradient, as described previously [13]. HIV-1 DNA was extracted from PBMCs by using a commercially available kit (QIAampDNAViral minikit; Qiagen), according to the product specifications. Sanger sequencing was performed as previously described [14,15].

Resistance interpretation and estimation of APOBEC editing in HIV-DNA were made through Stanford algorithm (HIVdb version 9.1, https://hivdb.stanford.edu/). A sequence was considered affected by APOBEC editing when at least one APOBEC related mutation (APO-M) or a stop codon (APO-stop) was detected in protease/reverse transcriptase (PR/RT) or integrase. The presence of the 18 APOBEC context drug resistance mutations (APO-DRM) according to Stanford HIVdb algorithm was also evaluated.

For individuals with complete treatment information, the genotypic susceptibility score (GSS) of the current regimen was carried out using the Stanford algorithm. A regimen with GSS<2 was defined as suboptimal considering that it is recommended to switch to a new regimen which includes at least two fully active ARV drugs [3,4].

The prevalence of resistance was evaluated on the overall population and according to the presence/absence of APO-M or stop codons. The prevalence trends of resistance mutations and APOBEC editing were also

Table 1
Patients' characteristics.

Variables	Statistics (N=1126)
Male, n (%)	826 (73.4)
Age, years, median (IQR)	50 (43-56)
Risk factor, n (%)	
Homosexual	307 (27.3)
Heterosexual	412 (36.6)
Drug abuser	241 (21.4)
Sexual[a]	55 (4.9)
Perinatal/Iatrogenic	19 (1.7)
Unknown	92 (8.2)
Subtype B, n (%)	897 (79.7)
Nationality, n (%)	
Italian	911 (80.9)
Foreigner	215 (19.1)
Year of genotyping, median (IQR)	2018 (2015-2019)
Nadir CD4 count, n (%)	
≤200 cells/mm^3	477 (42.4)
>200 cells/mm^3	417 (37.0)
Unknown	232 (20.6)
CD4 count at HIV-DNA GRT, median (IQR), cells/mm^3	631 (439-830)
Zenit viremia, n (%)	
<100,000 copies/mL	336 (29.8)
100,000-500,000 copies/mL	331 (29.4)
>500,000 copies/mL	227 (20.2)
Unknown	232 (20.6)
Time under virological suppression before GRT, years, median (IQR)	3.7 (0.3-8.0)
Virological suppression achieved under last regimen, n (%)	530 (47.1)
Years under cART, n (%)	
<5	269 (23.9)
5-10	242 (21.5)
11-15	196 (17.4)
>15	386 (34.3)
Unknown	33 (2.9)
Number of previous regimens before GRT, n (%)	
1	191 (17.0)
2	444 (39.4)
3	349 (31.0)
≥4	121 (10.7)
Unknown	21 (1.9)
Duration of current cART, median (IQR), years	2.1 (0.8-4.3)
Type of current cART, n (%)	
PIb + 2 NRTI	273 (24.2)
NNRTI + 2 NRTI	270 (24.0)
INSTI 2nd gen. + 2 NRTI	137 (12.2)
INSTI 1st gen. + 2 NRTI	64 (5.7)
Dual INSTI 2st gen.	64 (5.7)
Dual INSTI 1st gen.	57 (5.1)
Dual PIb	62 (5.5)
Mono PIb	31 (2.8)
Other	111 (9.9)
Unknown	57 (5.1)
Number of previous antiretroviral classes experienced, n (%)	
2	383 (34.0)
3	414 (36.8)
4	236 (21.0)
≥5	69 (6.1)
Unknown	24 (2.1)

[a] Includes: bisexuals, transsexuals and people for whom their sexual behaviour was not specified. cART: combined antiretroviral therapy; EI: entry inhibitors (enfuvirtide, maraviroc); INSTI: integrase strand-transfer inhibitors; IQR: interquartile range; NRTI: nucleos(t)ide reverse transcriptase inhibitors; NNRTI: non NRTI; PIb: ritonavir/cobicistat boosted protease inhibitors.

Table 2
Prevalence of resistance according to ABOBEC editing in HIV-DNA GRT in virologically suppressed individuals.

Sequences data	Overall prevalence (N=1126)	APOBEC editing associated substitutions					
		APOBEC related mutations			Stop codons		
		No (N=897)	Yes (N=229)	P Value	No (N=1021)	Yes (N=105)	P value
Class resistance							
None	730 (64.8)	588 (65.6)	142 (62)	0.316	664 (65)	66 (62.9)	0.656
One	256 (22.7)	201 (22.4)	55 (24)	0.604	232 (22.7)	24 (22.9)	0.975
Two	104 (9.2)	82 (9.1)	22 (9.6)	0.828	96 (9.4)	8 (7.6)	0.548
At least three	37 (3.3)	27 (3.0)	10 (4.4)	0.304	30 (2.9)	7 (6.7)	0.074
Resistance mutations							
Any major	396 (35.2)	309 (34.4)	87 (38)	0.316	357 (35)	39 (37.1)	0.656
PI major	87 (7.7)	68 (7.6)	19 (8.3)	0.717	75 (7.3)	12 (11.4)	0.136
NRTI major	264 (23.4)	209 (23.3)	55 (24.0)	0.819	237 (23.2)	27 (25.7)	0.564
NNRTI major	212 (18.8)	162 (18.1)	50 (21.8)	0.192	193 (18.9)	19 (18.1)	0.840
INSTI major[a]	11 (1.5)	**1 (0.2)**	**10 (7.9)**	**<0.001**	8 (1.2)	3 (4.1)	0.090
INSTI accessory[a]	120 (16.6)	**86 (14.4)**	**34 (26.8)**	**0.001**	107 (16.4)	13 (17.8)	0.765
APOBEC context drug resistance mutations							
Any	189 (16.8)	**121 (13.5)**	**68 (29.7)**	**<0.001**	**147 (14.4)**	**63 (40.0)**	**<0.001**
PI	42 (3.7)	**28 (3.1)**	**14 (6.1)**	**0.033**	**33 (3.2)**	**9 (8.6)**	**0.012**
NRTI	109 (9.7)	81 (9.0)	28 (12.2)	0.144	**93 (9.1)**	**16 (15.2)**	**0.043**
NNRTI	42 (3.7)	**9 (1.0)**	**33 (14.4)**	**<0.001**	**22 (2.2)**	**20 (19.0)**	**<0.001**
INSTI[a]	32 (4.4)	**0 (0.0)**	**32 (25.2)**	**<0.001**	**22 (3.4)**	**10 (13.7)**	**0.001**
Suboptimal GSS of current regimen (GSS <2)[b]	132 (12.3)	98 (11.5)	34 (15.5)	0.109	**113 (11.7)**	**19 (18.8)**	**0.038**

The analyses were performed on 724 GRT spanning protease/reverse-transcriptase/integrase HIV-DNA sequence and on 402 GRT spanning protease/reverse-transcriptase sequence.

[a] Available for 724 individuals.

[b] Available for 1069 individuals with complete treatment information. The sum of scores retrieved per each drug included in the regimen administered at the moment of HIV-DNA GRT was used to calculate the genotypic susceptibility score (GSS); individuals receiving INSTIs for whom an integrase HIV-DNA GRT was not available were considered infected with viruses susceptible to this class, if they never failed or were never previously exposed to INSTIs. Entry and fusion inhibitors were not considered in the GSS calculation.

estimated from 2010 to 2021.

2.3. Statistical analyses

All analyses were executed using the SPSS v.26.0 software pack-age for Windows (SPSS,Inc., Chicago, IL). Potential difference in resistance prevalence according to APOBEC editing was carried out using either Fisher's exact test or Chi-Squared test for categorical variables when appropriate. Chi-Squared test for trend was used to evaluate potential changes in temporal trends of resistance and APOBEC editing in HIV-DNA sequences. Demographical, viro-immunological and therapeutic variables (listed in Table 5) were investigated as potential factors associated with the presence of at least one MRM in HIV-DNA GRT or with suboptimal GSS of current regimen by logistic regression models.

3. Results

3.1. Patients' characteristics

At HIV-DNA GRT, individuals were under virological suppression for a median (IQR) of 3.7 (0.3-8.0) years, and were mainly under triple therapy (66.1%) based on PI, NNRTI or INSTI plus 2 NRTIs (Table 1). Around half of them (47.1%) achieved virological suppression under the last regimen and 44.3% had previously received a regimen containing an INSTI.

3.2. Resistance and APOBEC-editing evaluation

An overview of resistance and APOBEC-editing detected at HIV-DNA GRT is reported in Table 2. In HIV-DNA GRT, 396 (35.2%) individuals showed at least one MRM. APOBEC editing substitutions were found in 246 (21.8%) individuals; 229 (20.3%) and 105 (9.3%) individuals showed at least one APO-M or stop codon, respectively. Most people showed resistance to one class (22.7%), while a minority harboured strains resistant to at least 3 drug classes (3.3%) among PI, NRTI, NNRTI and INSTI. Resistance was mainly related to NRTI (23.4%) and NNRTI (18.8%), while resistance to PI and INSTI was low (7.7%) and marginal (1.4%), respectively. INSTI ARM were found in 16.5% of individuals. Both INSTI MRM and INSTI ARM were more likely observed in sequences with stop codons ($P<0.05$). Concerning genotypic susceptibility of current regimen, 132 (12.3%) individuals were under a suboptimal regimen (GSS<2) according to HIV-DNA GRT.

The highest prevalence of APO-DRM was related to NRTI (9.7%), followed by INSTI (4.4%), PI (3.7%) and NNRTI (3.7%). The proportion of individuals harbouring viral strains with APO-DRM was significantly higher in sequence with APO-M or stop codons ($P<0.05$, Table 2).

The proportion of individuals receiving a suboptimal regimen was significantly higher among those with sequences containing stop codons (18.8%) compared to those without stop codons (11.7%, P=0.038).

Concerning specific APO-DRM, 10 out 18 mutations (PI: G73S, D30N; NRTI: M184I; NNRTI: M230I, E138K, G190E/S INSTI: G163R, G140R, E138K) were more likely observed in sequences with APOBEC editing (Table 3). Importantly, among MRM also listed as APO-DRM, M46I in PR, D67N in RT and G140S, G118R and R263K were not associated with presence of APOBEC editing.

3.3. Trends of resistance and APOBEC editing over the period 2010-2021

Apart from some random fluctuations, from 2010 to 2021 no significant changes were found in the proportion of individuals harbouring MRM to any drug-class (from 35.7% to 30.8%), to PI (from 5.0% to 6.7%), to NRTI (from 17.5% to 17.9%), to NNRTI (from 25.0% to 16.5%), to INI (from 0% to 1.6% for MRM; from 11.1% to 16.5% for ARM, Table 4). The proportion of individuals receiving a suboptimal regimen was moderately low and did not significantly change over time (Table 4).

From 2010 to 2021 an increase in the proportion of individuals harbouring APO-M (from 7.5% to 20.5%, P=0.055) was found. This increase was mostly observed from 2010 to 2015 (from 7.5% to 21.1%, P=0.010) and it was followed by a stable trend from 2016 to 2021 (from 23.3% to 20.5%, P=0.462). Differently, from 2010 to 2021, no significant changes in the proportion of individuals with stop codons (from 7.5% to 4.0%) or APO-DRM to any class both in PR/RT and integrase sequences were observed over time (Table 4).

Table 3
Prevalence of APOBEC context DRMs detected in PBMC GRT according to APOBEC editing.

APO-DRM	Overall prevalence (N=1126)	APOBEC editing associated substitutions					
		APOBEC related mutations		P value	Stop codons		P value
		No (N=1056)	Yes (N=70)		No (N=1056)	Yes (N=70)	
PI							
M46I	31 (2.8)	23 (2.6)	8 (3.5)	0.443	27 (2.6)	4 (3.8)	0.524
G73S	14 (1.2)	10 (1.1)	4 (1.7)	0.501	**8 (0.8)**	**6 (5.7)**	**0.001**
D30N	**7 (0.6)**	**1 (0.1)**	**6 (2.6)**	**<0.001**	**3 (0.3)**	**4 (3.8)**	**0.002**
NRTI							
D67N	94 (8.3)	79 (8.8)	15 (6.6)	0.270	87 (8.5)	7 (6.7)	0.513
M184I	**17 (1.5)**	**3 (0.3)**	**14 (6.1)**	**<0.001**	**7 (0.7)**	**10 (9.5)**	**<0.001**
NNRTI							
M230I	**20 (1.8)**	**0 (0.0)**	**20 (8.7)**	**<0.001**	**7 (0.7)**	**13 (12.4)**	**<0.001**
E138K	**12 (1.1)**	**6 (0.7)**	**6 (2.6)**	**0.020**	**8 (0.8)**	**4 (3.8)**	**0.020**
G190E	**9 (0.8)**	**2 (0.2)**	**7 (3.1)**	**<0.001**	**4 (0.4)**	**5 (4.8)**	**0.001**
G190S	**5 (0.4)**	**1 (0.1)**	**4 (1.7)**	**0.001**	5 (0.5)	0 (0.0)	1.000
INSTI[a]							
G163R	14 (1.9)	10 (1.8)	4 (2.3)	0.750	**10 (1.5)**	**4 (5.5)**	**0.044**
G163K	6 (0.8)	6 (1.1)	0 (0.0)	0.345	6 (0.9)	0 (0.0)	1.000
G140R	**4 (0.6)**	**0 (0.0)**	**4 (2.3)**	**0.003**	**1 (0.2)**	**3 (4.1)**	**0.004**
D232N	4 (0.6)	3 (0.5)	1 (0.6)	1.000	3 (0.5)	1 (1.4)	0.347
E138K	**3 (0.4)**	**0 (0.0)**	**3 (1.8)**	**0.013**	2 (0.3)	1 (1.4)	0.273
G140S	3 (0.4)	3 (0.5)	0 (0.0)	1.000	3 (0.5)	0 (0.0)	1.000
G118R	2 (0.3)	1 (0.2)	1 (0.6)	0.417	1 (0.2)	1 (1.4)	0.192
R263K	1 (0.1)	0 (0.0)	1 (0.6)	0.236	0 (0.0)	1 (1.4)	0.101

The following 18 APOBEC context drug resistance mutations were considered according with HIVdb algorithm:: PR:30N, PR:46I, PR:48S, PR:73S, RT:67N, RT:138K, RT:184I, RT:190E, RT:190S, RT:230I, IN:118R, IN:138K, IN:140R, IN:140S, IN:163K, IN:163R, IN:232N, IN:263K. Mutations underlined were also listed as major resistance mutations.

[a] Available for 724 individuals.

3.4. Predictors of resistance at HIV-DNA genotyping

By multivariable logistic regression models, drug abuse, infection with subtype B strains, and a prolonged and complex treatment history (longer time under cART, higher number of regimens, higher number of antiretroviral classes experienced) were independent predictors of having at least one MRM at HIV-DNA GRT (Table 5). Concerning the GSS of the current regimen, individuals with a perinatal/iatrogenic risk transmission factor and having at least 2 stop codons detected in HIV-DNA had an increased risk to be under a suboptimal regimen. Conversely, people who achieved virological suppression under the last regimen had a significantly decreased risk to be under a suboptimal treatment (Table 5).

Table 4
Trends of prevalence of major resistance mutations and APOBEC context mutations over 2010-2021.

Sequences data	Overall (N=1126)	Trends over 2010-2021						P Value
		2010-2011 (N=40)	2012-2013 (N=89)	2014-2015 (N=194)	2016-2017 (N=231)	2018-2019 (N=348)	2020-2021 (N=224)	
Major resistance mutations								
Any	396 (35.2)	15 (37.5)	31 (34.8)	69 (35.6)	77 (33.3)	135 (38.8)	69 (30.8)	0.648
PI	87 (7.7)	2 (5.0)	8 (9.0)	16 (8.2)	20 (8.7)	26 (7.5)	15 (6.7)	0.652
NRTI	264 (23.4)	7 (17.5)	23 (25.8)	51 (26.3)	53 (22.9)	90 (25.9)	40 (17.9)	0.318
NNRTI	212 (18.8)	10 (25.0)	10 (11.2)	32 (16.5)	43 (18.6)	80 (23.0)	37 (16.5)	0.407
INSTI[a]	11 (1.5)	-	0 (0.0)	2 (3.4)	2 (1.2)	4 (1.4)	3 (1.6)	0.921
INSTI Accessory[a]	120 (16.5)	-	3 (11.1)	9 (15.5)	33 (19.4)	42 (15.2)	33 (17.2)	0.848
APOBEC context mutations[b]								
Any APO-M	229 (20.3)	**3 (7.5)**	**10 (11.2)**	**41 (21.1)**[c]	54 (23.4)	75 (21.6)	46 (20.5)	0.055
Any stop	105 (9.3)	3 (7.5)	4 (4.5)	18 (9.3)	17 (7.4)	43 (12.4)	20 (8.9)	0.138
Any APO-DRM	160 (14.2)	6 (15.0)	11 (12.4)	33 (17.0)	32 (13.9)	54 (15.5)	24 (10.7)	0.372
PI APO-DRM	41 (3.7)	2 (5.0)	2 (2.2)	12 (6.2)	9 (3.9)	12 (3.4)	5 (2.2)	0.189
NRTI APO-DRM	109 (9.7)	3 (7.5)	10 (11.2)	18 (9.3)	21 (9.1)	37 (10.6)	20 (8.9)	0.966
NNRTI APO-DRM	42 (3.7)	3 (7.5)	3 (3.4)	10 (5.2)	5 (2.2)	17 (4.9)	4 (1.8)	0.189
INSTI APO-DRM[c]	32 (4.4)	-	1 (3.7)	3 (5.2)	6 (3.5)	9 (3.2)	13 (6.8)	0.323
Suboptimal GSS of current regimen (GSS <2)[d]	132 (12.3)	2 (5.1)	10 (12.7)	28 (15.2)	33 (14.7)	46 (13.7)	13 (6.3)	0.213

[a] Available for 724 individuals.

[b] According to HIVdb algorithm that includes also the following 18 APOBEC context drug resistance mutations: PR:30N, PR:46I, PR:48S, PR:73S, RT:67N, RT:138K, RT:184I, RT:190E, RT:190S, RT:230I, IN:118R, IN:138K, IN:140R, IN:140S, IN:163K, IN:163R, IN:232N, IN:263K.

[c] A significant increase was detected from 2010 to 2015 (P=0.010).

[d] Available for 1069 individuals with complete treatment information.

Table 5
Factors associated with the presence of resistance in HIV-DNA in virologically suppressed individuals.

Variables	Risk of detect at least one major resistance mutation at DNA-GRT				Risk of having suboptimal GSS of current regimen at DNA-GRT[b]			
	Crude		Adjusted[a]		Crude		Adjusted[a]	
	OR (95% C.I.)	P Value	OR (95% C.I.)	P Value	OR (95% C.I.)	P Value	OR (95% C.I.)	P Value
Gender (female vs. Male)	1 (0.8-1.3)	0.943			0.9 (0.6-1.3)	0.439		
Age (per five years higher)	**1.1 (1.0-1.2)**	**<0.001**	1.0 (0.9-1.1)	0.630	**1.1 (1.0-1.2)**	**0.002**	1.0 (0.6-1.7)	0.969
Risk factor								
Homosexual[c]	1.0				1.0			
Heterosexual	1.0 (0.7-1.3)	0.88	0.9 (0.6-1.2)	0.402	1.0 (0.6-1.7)	0.917	1.0 (0.6-1.7)	0.969
Drug abuser	**2.5 (1.7-3.5)**	**<0.001**	**1.5 (1.1-2.2)**	**0.042**	**2.1 (1.3-3.4)**	**0.004**	1.3 (0.7-2.3)	0.354
Sexual	0.9 (0.5-1.7)	0.821	0.9 (0.5-1.7)	0.730	0.2 (0.0-1.4)	0.096	0.2 (0.0-1.6)	0.127
Perinatal/Iatrogenic	**3.9 (1.5-10.2)**	**0.006**	2.8 (0.9-7.4)	0.085	**4.4 (1.6-12.7)**	**0.005**	**4.8 (1.4-16.2)**	**0.012**
Unknown	0.8 (0.5-1.4)	0.404	0.8 (0.4-1.3)	0.316	1.3 (0.6-2.8)	0.441	1.8 (0.8-4.0)	0.156
Subtype (B vs non-B)	**2.5 (1.8-3.6)**	**<0.001**	**1.8 (1.2-2.8)**	**0.008**	**3.1 (1.6-5.8)**	**0.001**	1.5 (0.7-3.2)	0.330
Nationality (Italian vs. foreigner)	**1.6 (1.1-2.2)**	**0.009**	0.8 (0.5-1.3)	0.388	**2.8 (1.5-5.3)**	**0.002**	1.7 (0.8-3.5)	0.330
Nadir CD4 count (cells/mm^3)								
≥200[c]	1.0				1.0			
<200	**1.8 (1.3-2.3)**	**<0.001**	1.3 (1.0-1.8)	0.070	1.3 (0.9-2.0)	0.193		
Unknown	1.0 (0.7-1.4)	0.999	1.1 (0.7-1.6)	0.452	0.9 (0.6-1.6)	0.844		
Zenith viremia (copies/mL)								
<100,000[c]	1.0				1.0			
100,000-500,000	1.0 (0.7-1.3)	0.860			1.0 (0.6-1.5)	0.894		
>500,000	1.0 (0.7-1.5)	0.840			0.6 (0.4-1.1)	0.110		
Unknown	0.7 (0.5-1.0)	0.090			0.7 (0.4-1.2)	0.240		
Time under virological suppression before GRT (per 1 year higher)	**1.0 (1.0-1.1)**	**0.046**	1.0 (1.0-1.0)	0.283	**1.1 (1.1-1.1)**	**<0.001**	1.0 (1.0-1.1)	0.348
Virological suppression achieved under last regimen	1.0 (0.8-1.3)	0.862			**0.4 (0.3-0.6)**	**<0.001**	**0.5 (0.3-0.9)**	**0.019**
Years under cART								
<5	1.0		1.0		1.0		1.0	
6-10	0.9 (0.6-1.3)	0.574	0.8 (0.5-1.3)	0.415	0.7 (0.3-1.4)	0.298	0.4 (0.2-1.0)	0.058
11-15	1.1 (0.7-1.7)	0.582	0.9 (0.6-1.5)	0.743	1.8 (0.9-3.4)	0.081	1.0 (0.4-2.3)	0.978
>15	**3.3 (2.4-4.7)**	**<0.001**	**1.8 (1.0-2.9)**	**0.035**	**3.6 (2.1-6.2)**	**<0.001**	1.4 (0.6-3.4)	0.425
Unknown	1.9 (0.9-4.1)	0.088	2.5 (1.0-6.5)	0.053	1.4 (0.3-6.5)	0.665	0.6 (0.1-3.1)	0.503
Number of previous regimens								
1[c]	1.0		1.0		1.0		1.0	
2-4	0.9 (0.6-1.3)	0.496	0.7 (0.4-1.1)	0.146	1.3 (0.7-2.7)	0.418	0.9 (0.4-2.2)	0.857
5-9	**1.7 (1.1-2.5)**	**0.008**	0.7 (0.4-1.4)	0.352	**2.9 (1.5-5.7)**	**0.002**	1.1 (0.4-3.4)	0.805
≥10	**6.2 (3.8-10.3)**	**<0.001**	1.6 (0.8-3.5)	0.210	**5.8 (2.8-12.1)**	**<0.001**	1.6 (0.5-5.2)	0.472
Unknown	0.4 (0.1-1.5)	0.181	0.2 (0.0-3.2)	0.246				
Number of antiretroviral classes experienced before GRT								
2[c]	1.0		1.0		1.0		1.0	
3	**1.4 (1.1-2)**	**0.021**	1.2 (0.8-1.8)	0.318	1.4 (0.9-2.3)	0.179	1.0 (0.5-1.9)	0.963
4	**2.7 (1.9-3.8)**	**<0.001**	**1.8 (1.1-2.8)**	**0.020**	**2.4 (1.5-4.0)**	**0.001**	1.3 (0.6-2.6)	0.518
≥5	**5.1 (3.0-8.8)**	**<0.001**	**2.3 (1.1-4.5)**	**0.020**	**4.2 (2.2-8.1)**	**<0.001**	1.9 (0.8-4.6)	0.154
Unknown	0.6 (0.2-1.7)	0.333	1.3 (0.1-15.6)	0.840				
Number of stop codons detected at HIV-DNA GRT								
None[c]	1.0				1.0			
1	1.0 (0.6-1.8)	0.874			1.1 (0.5-2.4)	0.771	1.0 (0.4-2.6)	0.928
≥2	1.2 (0.6-2.3)	0.594			**3.0 (1.4-6.1)**	**0.003**	**3.5 (1.2-10.1)**	**0.022**
Number of APO-M detected at HIV-DNA GRT								
None[c]					1.0		1	
1	1.2 (0.8-1.7)	0.480			1.1 (0.6-2.0)	0.797	0.9 (0.5-1.8)	0.837
≥2	1.2 (0.8-1.7)	0.430			**1.7 (1.0-2.9)**	**0.038**	1.4 (0.6-3.1)	0.384

[a] Only variables significant at univariable analysis (P<0.05) were retained in multivariable models.
[b] Models built on 1069 individuals with complete treatment information.
[c] Reference (dummy). APO-M: APOBEC related mutation; cART: combined antiretroviral therapy; GRT: genotypic resistance test

4. Discussion

In the context of the increasing interest on PBMC genotyping, as far as we know, the present study is the largest available HIV-DNA resistance survey performed on HIV-1 infected virologically suppressed individuals followed for clinical routine. Moreover, for the first time temporal trend of archived resistance and the extent of APOBEC editing in HIV-DNA were evaluated. Specifically, in our population, 35% of individuals harboured viral strains with drug-resistance in HIV-DNA, mainly related to NRTI and NNRTI. It is challenging to compare resistance prevalence with other previous studies. In fact, resistance detected in HIV-DNA was variable due to the fact that most of the available studies were performed in virologically suppressed individuals with heterogenous treatment histories [7,8,16,17]. Moreover, comparisons are challenging because resistance might be underestimated in HIV-DNA GRT at several degrees based on number and duration of past virological failures [5,6]. We found that only 12% of individuals were receiving a suboptimal regimen according with GSS retrieved from HIV-DNA GRT. This is an important information that underlines the fact that, even though few, virologically suppressed individuals might have a certain frailty related to their current treatment and deserve particular attention.

In light of concerns regarding reliability of HIV-DNA GRT related to potential APOBEC activity, we estimated this phenomenon and we found that, around 20% of individuals tested in this study showed APOBEC editing. Again, it is challenging to compare these findings with those observed in previous studies. In fact, few studies, focused on APOBEC editing estimation, are available and, most importantly, different sequencing platforms and heterogeneous criteria to identify APOBEC editing in HIV-DNA sequences were used [5,18–21]. Nevertheless, studies based on Sanger sequencing indicated a prevalence of hypermutations or stop codons ranging from 6% to 34% [19–21].

Concerning the role of APOBEC editing on resistance onset, we found that around 17% of individuals showed at least one APO-DRM. However, this apparently high prevalence is the summatory of several APO-DRM that are not always related with APOBEC editing. In fact, we found that among the 18 APO DRMs listed in the Stanford database only 8 were associated with the presence of stop codons (Table 3). Among them, mutations such as G73S in PR and M184I, E138K and M230I in RT were already described as related to APOBEC activity [22–24], thus considering a priori these mutations might cause an overestimation of resistance. On the other hand, other APO-DRM detected with considerable prevalence such as D67N (8.3%) in RT and M46I (2.8%) in PR were not associated with APO-M or stop codons; consequently not considering these mutations might underestimate resistance assessment. Prevalence of INSTI APO-DRM was too low to considerably affect GRT reliability, but G163R and G140R were effectively detected quite often with stop codons.

These results suggest that the reliability of HIV-DNA GRT might be affected by APOBEC editing, but not all APO-DRM are really associated with APOBEC editing, thus not consider *"a priori"* APO-DRMs in resistance assessment might affect final evaluation.

We also evaluated the temporal trends of both resistance, genotypic susceptibility and APOBEC editing. In general, apart from random fluctuations, no significant differences in temporal trends were observed from 2010 to 2021. The presence of APO-M increased from 2010 to 2015 and later the proportion of individuals with them remained stable until 2021. This might be associated with some bias. In particular, before 2014 a lower number of HIV-DNA GRT was collected. Furthermore, at that time probably, *"a priori",* sequences with APOBEC editing mutations were considered with poor reliability and therefore discarded.

So far no data regarding temporal trends of resistance in HIV-DNA are available, but this stability is in line with those observed in our previous studies in which resistance prevalence observed in plasma GRT remained stable after 2010 [25,26].

In the present study we found that the risk to detect MRM in HIV-DNA is associated with drug abuse and a prolonged/complex previous history, as previously described [14]. Interestingly, concerning predictors of treatment susceptibility, people who achieved virological suppression during their last regimen showed a decreased risk of receiving a suboptimal regimen. This is expected in a good clinical practice in which individuals reach a suppressed viremia after switching to a regimen chosen according with treatment guidelines [3,4]. Differently, people perinatally infected and those with at least 2 stop codons detected in HIV-DNA sequence had an increased risk of receiving suboptimal current treatment. This is not surprising considering that perinatally infected individuals had accumulated so much resistance during their long treatment history that it is challenging to build a fully active regimen for them [26–28]. Anyway, thanks to the modern potent ARVs at high genetic barrier available, they might maintain viral suppression despite the considerable resistance accumulated [29–31]. Concerning the role of stop codons in increasing the risk of being under a suboptimal treatment, we might presume that individuals with high level of resistance but more than 2 stop codons can maintain viral suppression because resistant strains are not replication competent. Thus the presence of stop codons might be a marker of "inactive" resistance. In this regard, the presence of stop codons was already associated with an increased proportion of resistance mutations [20,32] and association of stop codons with lower viral reservoir [18] or positive effect on resistant individuals [33] are already described.

This study might have some limitations. Firstly, HIV-DNA GRT was performed through Sanger technology. This test has a fairly limited sensitivity but was the technique used in clinical routine during the time period described. Secondly, we also included individuals without poor treatment history, for whom HIV-DNA GRT information was requested after clinicians' decisions, thus potential biases related to missing data typical of observational studies might affect the results obtained. Moreover, the lack of HIV-DNA quantification or the estimation of size of replication competent proviral DNA (still not recommended and not widely used in clinical practice) did not allow to completely explain the findings about APOBEC editing.

In conclusion, HIV-DNA sequences collected until 2021 from virologically suppressed individuals, showed that resistance in HIV-DNA is considerable and stable over time. However, only a limited group of individuals was under a suboptimal regimen despite suppression, demonstrating a good clinical practice based on genotype tailored treatment. In this context, a careful evaluation of APOBEC editing might be helpful to improve the reliability of HIV-DNA GRT. Further investigations are required to understand how to apply the estimation of APOBEC editing in refining genotypic evaluation.

Ethics committee approval

This study was approved by the ethics committee of Tor Vergata Hospital (Ethics Approval No. 77/21, 21 April 2021). The research was conducted on anonymous samples in accordance with the principles of the Declaration of Helsinki and the Italian Ministry of Health. All information, including virological and clinical data, was recorded in an anonymized database.

Authors' contributions

D.A., M.M.S. and F.C.S. carried out study conception and design, analysis and interpretation of data. D.A. and M.M.S. carried out the drafting of the manuscript. C.A., F.F, L.F., A.B., M.B., W.G. contributed to sequencing and collection of virological data; R.G.,V.B, A.V, S.C.,V.M contributed to the collection of the data. C.A, V.S., V.C., M.A., C.M., A. A., C.F.P. contributed to the study conception and design. All authors approved the final version of the manuscript for submission.

Declaration of Competing Interest

The authors have no conflicts of interest related to this manuscript.

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References

[1] T.R. Kemnic, PG. Gulick, HIV Antiretroviral Therapy, StatPearls, 2021.
[2] D. Chastain, M. Badowski, E. Huesgen, N.S. Pandit, A. Pallotta, S. Michienzi, Optimizing antiretroviral therapy in treatment-experienced patients living with HIV: a critical review of switch and simplification strategies. An opinion of the HIV practice and research network of the American college of clinical pharmacy, J. Int. Assoc. Provid AIDS Care 18 (2019), https://doi.org/10.1177/2325958219867325.
[3] Panel on Antiretroviral Guidelines for Adults and Adolescents, Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents with HIV, Department of Health and Human Services, 2023 last update Mar 23, https://clinicalinfo.hiv.gov/sites/default/files/guidelines/documents/adult-adolescent-arv/guidelines-adult-adolescent-arv.pdf.
[4] European AIDS Clinical Society. Guidelines - version 11.1 2022. https://www.eacsociety.org/media/guidelines-11.1_final_09-10.pdf.
[5] C. Chu, D. Armenia, C. Walworth, M.M. Santoro, R.W. Shafer, Genotypic resistance testing of HIV-1 DNA in peripheral blood mononuclear cells, Clin. Microbiol. Rev. (2022), https://doi.org/10.1128/cmr.00052-22.
[6] A.M. Geretti, J.L. Blanco, A.G. Marcelin, C.F. Perno, H.J. Stellbrink, D. Turner, et al., HIV DNA sequencing to detect archived antiretroviral drug resistance, Infect. Dis. Ther. 11 (2022) 1793–1803, https://doi.org/10.1007/S40121-022-00676-Y.
[7] M. Wirden, C. Soulie, M.A. Valantin, S. Fourati, A. Simon, S. Lambert-Niclot, et al., Historical HIV-RNA resistance test results are more informative than proviral DNA genotyping in cases of suppressed or residual viraemia, J. Antimicrob. Chemother. 66 (2011) 709–712, https://doi.org/10.1093/jac/dkq544.
[8] C. Delaugerre, J. Braun, I. Charreau, S. Delarue, M.L. Nere, N. de Castro, et al., Comparison of resistance mutation patterns in historical plasma HIV RNA genotypes with those in current proviral HIV DNA genotypes among extensively treated patients with suppressed replication, HIV Med. 13 (2012) 517–525, https://doi.org/10.1111/j.1468-1293.2012.01002.x.
[9] C. Rodriguez, M.L. Nere, V. Demontant, I. Charreau, M. Mercier-Darty, H. Delagreverie, et al., Ultra-deep sequencing improves the detection of drug resistance in cellular DNA from HIV-infected patients on ART with suppressed viraemia, J. Antimicrob. Chemother. 73 (2018) 3122–3128, https://doi.org/10.1093/JAC/DKY315.
[10] B. Mangeat, P. Turelli, G. Caron, M. Friedli, L. Perrin, D. Trono, Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts, Nature 424 (2003) 99–103, https://doi.org/10.1038/nature01709.
[11] A.E. Armitage, K. Deforche, C.C. hao, E. Wee, B. Kramer, J.J. Welch, et al., APOBEC3G-induced hypermutation of human immunodeficiency virus type-1 is typically a discrete “all or nothing” phenomenon, PLos Genet. 8 (2012) 22–24, https://doi.org/10.1371/journal.pgen.1002550.
[12] R.A. Russell, M.D. Moore, W.S. Hu, VK. Pathak, APOBEC3G induces a hypermutation gradient: purifying selection at multiple steps during HIV-1 replication results in levels of G-to-A mutations that are high in DNA, intermediate in cellular viral RNA, and low in virion RNA, Retrovirology 6 (2009) 16, https://doi.org/10.1186/1742-4690-6-16.
[13] L. Fabeni, G. Berno, V. Svicher, F. Ceccherini-Silberstein, C. Gori, A. Bertoli, et al., Genotypic tropism testing in HIV-1 proviral DNA can provide useful information at low-level viremia, J. Clin. Microbiol. 53 (2015) 2935–2941, https://doi.org/10.1128/JCM.00893-15.
[14] M. Zaccarelli, M.M. Santoro, D. Armenia, V. Borghi, W. Gennari, C. Gori, et al., Genotypic resistance test in proviral DNA can identify resistance mutations never detected in historical genotypic test in patients with low level or undetectable HIV-RNA, J. Clin. Virol. 82 (2016) 94–100, https://doi.org/10.1016/j.jcv.2016.07.007.
[15] D. Armenia, M. Zaccarelli, V. Borghi, W. Gennari, D. di Carlo, A. Giannetti, et al., Resistance detected in PBMCs predicts virological rebound in HIV-1 suppressed patients switching treatment, J. Clin. Virol. 104 (2018) 61–64, https://doi.org/10.1016/j.jcv.2018.04.001.
[16] S. Lambert-Niclot, C. Allavena, M. Grude, P. Flandre, S. Sayon, E. Andre, et al., Usefulness of an HIV DNA resistance genotypic test in patients who are candidates for a switch to the rilpivirine/emtricitabine/tenofovir disoproxil fumarate combination, J. Antimicrob. Chemother. (2016) 1–4, https://doi.org/10.1093/jac/dkw146.
[17] D.P. Porter, J. Toma, Y. Tan, O. Solberg, S. Cai, R. Kulkarni, et al., Clinical outcomes of virologically-suppressed patients with pre-existing HIV-1 drug resistance mutations switching to rilpivirine/emtricitabine/tenofovir disoproxil fumarate in the SPIRIT study, HIV Clin. Trials 17 (2016) 29–37, https://doi.org/10.1080/15284336.2015.1115585.
[18] B. Abdi, S. Lambert-Niclot, M. Wirden, A. Jary, E. Teyssou, S. Sayon, et al., Presence of HIV-1 G-to-A mutations linked to APOBEC editing is more prevalent in non-B HIV-1 subtypes and is associated with lower HIV-1 reservoir, J. Antimicrob. Chemother. 76 (2021) 2148–2152, https://doi.org/10.1093/JAC/DKAB123.
[19] J. Brice, M. Sylla, N. Desire, S. Sayon, F. Telly, D. Bocar-Fofana, et al., Characterization of drug resistance and the defective HIV reservoir in virally suppressed vertically infected children in Mali, J. Antimicrob. Chemother. 75 (2020) 1272–1279, https://doi.org/10.1093/JAC/DKAA002.
[20] E.K. Alidjinou, J. Deldalle, O. Robineau, C. Hallaert, A. Meybeck, T. Huleux, et al., Routine drug resistance testing in proviral HIV-1 DNA: Prevalence of stop codons and hypermutation, and associated factors, J. Med. Virol. 91 (2019) 1684–1687, https://doi.org/10.1002/JMV.25474.
[21] C. Allavena, A. Rodallec, A. Leplat, N. Hall, C. Luco, L. le Guen, et al., Interest of proviral HIV-1 DNA genotypic resistance testing in virologically suppressed patients candidate for maintenance therapy, J. Virol. Methods 251 (2018) 106–110, https://doi.org/10.1016/J.JVIROMET.2017.10.016.
[22] M. Noguera-Julian, A. Cozzi-Lepri, F. di Giallonardo, R. Schuurman, M. Däumer, S. Aitken, et al., Contribution of APOBEC3G/F activity to the development of low-abundance drug-resistant human immunodeficiency virus type 1 variants, Clin. Microbiol. Infect. 22 (2016) 191–200, https://doi.org/10.1016/J.CMI.2015.10.004.
[23] S. Fourati, I. Malet, S. Lambert, C. Soulie, M. Wirden, P. Flandre, et al., E138K and M184I mutations in HIV-1 reverse transcriptase coemerge as a result of APOBEC3 editing in the absence of drug exposure, AIDS 26 (2012) 1619–1624, https://doi.org/10.1097/QAD.0b013e3283560703.
[24] S. Fourati, S. Lambert-Niclot, C. Soulie, M. Wirden, I. Malet, M.A. Valantin, et al., Differential impact of APOBEC3-driven mutagenesis on HIV evolution in diverse anatomical compartments, AIDS 28 (2014) 487–491, https://doi.org/10.1097/QAD.0000000000000182.
[25] D. Armenia, D. di Carlo, P. Flandre, Y. Bouba, V. Borghi, F. Forbici, et al., HIV MDR is still a relevant issue despite its dramatic drop over the years, J. Antimicrob. Chemother. (2020) 1301–1310, https://doi.org/10.1093/jac/dkz554.
[26] F. Lombardi, A. Giacomelli, D. Armenia, A. Lai, A. Dusina, A. Bezenchek, et al., Prevalence and factors associated with HIV-1 multidrug resistance over the past two decades in the Italian Arca database, Int. J. Antimicrob. Agents (2020), 106252, https://doi.org/10.1016/j.ijantimicag.2020.106252.
[27] A. Judd, R. Lodwick, A. Noguera-Julian, D.M. Gibb, K. Butler, D. Costagliola, et al., Higher rates of triple-class virological failure in perinatally HIV-infected teenagers compared with heterosexually infected young adults in Europe, HIV Med. 18 (2017) 171–180, https://doi.org/10.1111/HIV.12411.
[28] R. Ungaro, L. Taramasso, B. Bruzzone, I. Vicenti, L. Galli, V. Borghi, et al., Prevalence of acquired resistance mutations in a large cohort of perinatally infected HIV-1 patients, Clin. Microbiol. Infect. 25 (2019) 1443–1446, https://doi.org/10.1016/J.CMI.2019.07.004.
[29] J. Navarro, A. González-Cordón, J.L. Casado, J.I. Bernardino, P. Domingo, J. Portilla, et al., Effectiveness of boosted darunavir plus rilpivirine in patients with long-lasting HIV-1 infection: DARIL study, J. Antimicrob. Chemother. 75 (2020) 1955–1960, https://doi.org/10.1093/JAC/DKAA072.
[30] Andreatta K., Willkom M., Martin R., Chang S., Wei L., Liu H., et al. Switching to bictegravir/emtricitabine/tenofovir alafenamide maintained HIV-1 RNA suppression in participants with antiretroviral resistance including M184V/I 2023 10.1093/jac/dkz347.
[31] S. Rusconi, F. Adorni, P. Tau, V. Borghi, M. Pecorari, R. Maserati, et al., Dolutegravir (DTG)-containing regimens after receiving raltegravir (RAL) or elvitegravir (EVG): Durability and virological response in a large Italian HIV drug resistance network (ARCA), J. Clin. Virol. 105 (2018) 112–117, https://doi.org/10.1016/J.JCV.2018.06.012.
[32] Y. Li, B. Etemad, R. Dele-Oni, R. Sharaf, C. Gao, M. Lichterfeld, et al., Drug resistance mutations in HIV provirus are associated with defective proviral genomes with hypermutation, AIDS 35 (2021) 1015–1020, https://doi.org/10.1097/QAD.0000000000002850.
[33] D. Armenia, M.M. Santoro, M.C. Bellocchi, L. Carioti, L. Galli, A. Galli, et al., Viral resistance burden and APOBEC editing correlate with virological response in heavily treatment-experienced people living with multi-drug resistant HIV, Int. J. Antimicrob. Agents 59 (2022), 106492, https://doi.org/10.1016/j.ijantimicag.2021.106492.