Env mutation A539V is prevalent for people newly diagnosed with HIV in the UK and is associated with suboptimal virological outcomes

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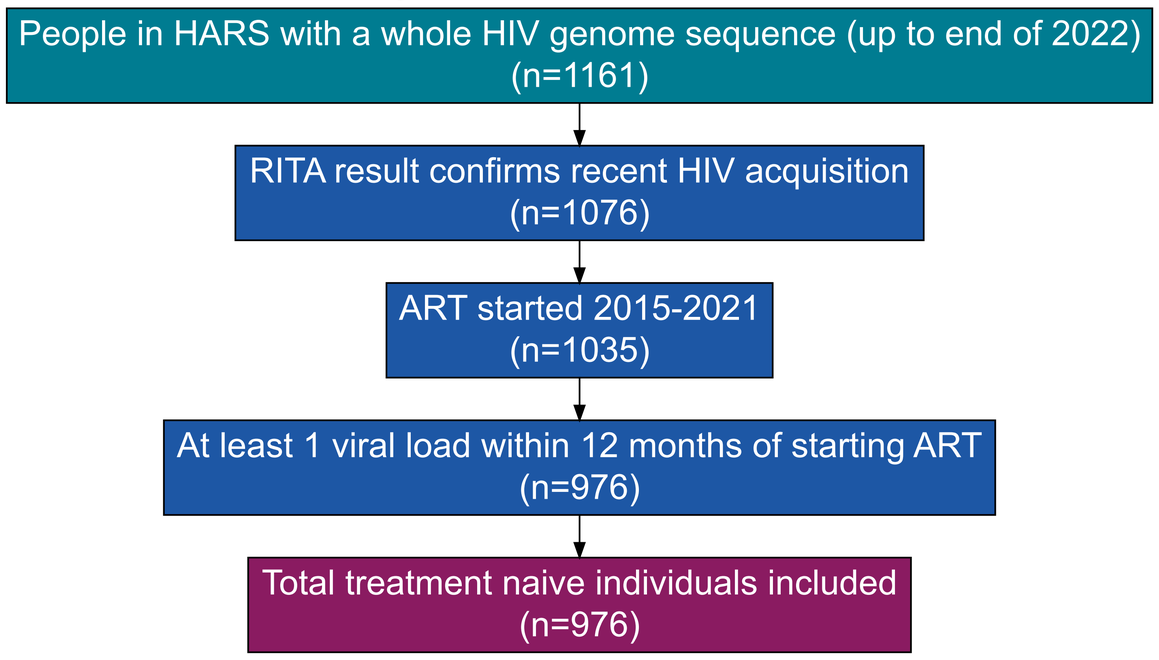
Introduction

- Integrase strand transfer inhibitors (INSTIs) are mainstays of antiretroviral treatment globally.
- Virological breakthrough is uncommon, but tends to manifest as low level viraemia.
- Non-integrase mutations that have been implicated include mutations in *env*[1] and the 3'polypurine tract in *nef*[2].
- Here, we define the prevalence of these mutations in an ART-naïve population and explore relationships with virological outcomes.

Methods

- Since 2015 UKHSA has applied whole genome sequencing to viral samples from people newly diagnosed with recently acquired HIV-1 (<4 months).
- Resistance mutations previously identified *in vitro* within *env*[1] and *nef*[2] were selected from literature and identified at a variant frequency threshold of >20%.
- Enzyme related drug resistance mutations were identified by querying sequences against the Stanford HIV drug resistance database[3].
- Linkage to HARS was used to provide information on demographic and clinical details such as gender, ethnic group, and viral load over time.
- Primary outcome was viral load <50 copies/mL by 12 months post-ART initiation (VS12).
- All patients who had any prior evidence of treatment, indication of not having acquired HIV recently, or insufficient follow-up to identify outcomes within 12 months of treatment initiation were excluded **(Figure 1).**
- Fisher's and the Wilcoxon test were used to assess associations between the presence of mutations of interest and patient characteristics.
- Univariable and multivariable logistic regression was used to analyse variables associated with VS12 and identify those with an association significant at the $p \leq 0.05$ level.

Figure 1. Patient cohort



Results

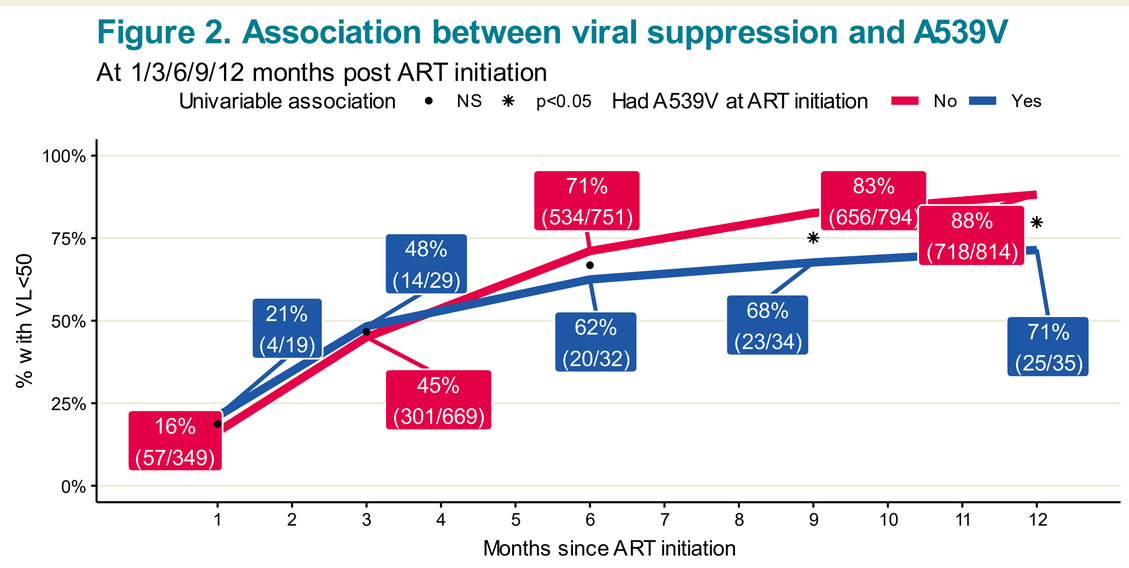
Mutation prevalence

- Three of the mutations identified in the literature were identified at a prevalence over 1%, one nucleotide polymorphism (*nef*-9053), and two amino acid polymorphisms (*env* Y61H and A539V).
- Excluding patients with ambiguous reads at the relevant sites, *nef*-9053 was found in 4.5% of patients (41/911), *env*-Y61H in 11% of patients (92/846), and *env*-A539V in 4.1% of patients (35/849).

Mutation associations

- Only *env*-539V was significantly associated with not reaching viral suppression by 12 months after starting ART (odds ratio (OR) 0.33, 95% confidence interval (CI) 0.16-0.75), and so became the focus of subsequent analysis.
- This association was observed from 9 months of treatment onwards **(Figure 2).**

Figure 2. Association between viral suppression and A539V



Characteristics associated with *env*-A539V

- The presence of *env*-A539V (n=35) was significantly associated with age, ethnic group, presence of any accessory INSTI resistance, region of birth, and HIV subtype **(Table 2)**.
- Compared to those whose HIV did not have the *env*-A539V mutation, those that did were:
  - Typically older
  - More commonly of black African, black Caribbean or black other ethnic groups.
  - More commonly born in Africa, Asia or Europe.
- In addition, their HIV more commonly:
  - Had at least one accessory INSTI resistance mutation.
  - Was of the CRF02_AG subtype.

Table 2. Env-A539V

Characteristic[1]	FALSE, N = 814[2]	TRUE, N = 35[2]	p-value[3]
Age	37 (31, 47)	43 (36, 52)	0.016
Ethnic group			0.030
White	595 (97%)	19 (3.1%)	
Black African	44 (90%)	5 (10%)	
Black Caribbean	18 (90%)	2 (10%)	
Black other	6 (86%)	1 (14%)	
Asian	54 (96%)	2 (3.6%)	
Other/Mixed	64 (96%)	3 (4.5%)	
Not Stated	33 (92%)	3 (8.3%)	
Accessory INSTI resistance			0.001
FALSE	766 (97%)	27 (3.4%)	
TRUE	48 (86%)	8 (14%)	
Region of birth			0.014
United Kingdom	494 (97%)	13 (2.6%)	
Africa	46 (88%)	6 (12%)	
Americas	36 (95%)	2 (5.3%)	
Asia	47 (92%)	4 (7.8%)	
Europe	123 (94%)	8 (6.1%)	
Oceania	6 (100%)	0 (0%)	
Unknown	62	2	
HIV subtype			<0.001
B	458 (99%)	4 (0.9%)	
A1	21 (100%)	0 (0%)	
A1 + D	12 (100%)	0 (0%)	
A6	13 (100%)	0 (0%)	
C	77 (99%)	1 (1.3%)	
CRF01_AE	61 (100%)	0 (0%)	
CRF02_AG	65 (76%)	20 (24%)	
CRF06_cpx	19 (86%)	3 (14%)	
F	41 (100%)	0 (0%)	
Other subtype	47 (87%)	7 (13%)	

[1] Colour indicates notable associations
[2] Median (IQR); n (%)
[3] Wilcoxon rank sum test; Fisher's exact test

Multivariable analysis

- Univariable logistic regression analysis was used to identify associations between VS12 and gender, age, ethnic group, region of birth, probable route of exposure, baseline viral load, viral subtype, treatment with integrase inhibitors, year of starting ART, and the presence of any resistance mutations for each major drug class, as well as *env*-A539V.
- Of these, ethnic group, viral subtype, year of starting ART, presence of *env*-A539V, and the presence of at least one accessory INSTI resistance mutation showed indication of association with VS12 ($p<0.1$), and so were included in the multivariable model **(Table 3)**.

Table 3. Univariable and multivariable logistic regression - VS12

	VS12		*Univariable analysis*			*Multivariable analysis*		
Characteristic[1]	**FALSE**, N = 118[2]	**TRUE**, N = 858[2]	**N**	**OR**[3]	**95% CI**[3]	**p-value**[4]	**OR**[3]	**95% CI**[3]	**p-value**[4]
Year ART started			976						
2015	19 (11%)	160 (89%)		—	—		—	—	
2016	28 (22%)	98 (78%)		0.42	0.22, 0.78	0.007*	0.43	0.21, 0.88	0.022*
2017	18 (11%)	145 (89%)		0.96	0.48, 1.91	0.9	1.28	0.60, 2.76	0.5
2018	16 (9.0%)	162 (91%)		1.20	0.60, 2.45	0.6	1.57	0.73, 3.44	0.2
2019	24 (14%)	148 (86%)		0.73	0.38, 1.39	0.3	0.98	0.47, 1.99	>0.9
2020	6 (6.7%)	84 (93%)		1.66	0.67, 4.71	0.3	2.41	0.91, 7.26	0.092
2021	7 (10%)	61 (90%)		1.03	0.43, 2.76	>0.9	1.59	0.60, 4.62	0.4
Env-A539V			849						
FALSE	96 (12%)	718 (88%)		—	—		—	—	
TRUE	10 (29%)	25 (71%)		0.33	0.16, 0.75	0.005*	0.23	0.09, 0.59	0.002*
Unknown	12	115							
Ethnic group			976						
White	87 (12%)	631 (88%)		—	—		—	—	
Black African	11 (21%)	42 (79%)		0.53	0.27, 1.11	0.073	0.55	0.25, 1.27	0.14
Black Caribbean and Other black	2 (6.9%)	27 (93%)		1.86	0.54, 11.7	0.4	1.96	0.53, 12.8	0.4
Asian	6 (10%)	54 (90%)		1.24	0.56, 3.30	0.6	1.11	0.47, 3.06	0.8
Other/Mixed	7 (9.2%)	69 (91%)		1.36	0.65, 3.34	0.5	1.30	0.56, 3.58	0.6
Not Stated	5 (13%)	35 (88%)		0.97	0.40, 2.87	>0.9	0.76	0.30, 2.34	0.6
HIV subtype			976						
B	59 (11%)	482 (89%)		—	—		—	—	
A1	5 (22%)	18 (78%)		0.44	0.17, 1.37	0.12	0.34	0.12, 1.11	0.052
A1 + D	1 (7.7%)	12 (92%)		1.47	0.28, 27.0	0.7	0.96	0.16, 18.4	>0.9
A6	5 (31%)	11 (69%)		0.27	0.09, 0.88	0.018*	0.23	0.06, 0.93	0.026*
C	14 (16%)	74 (84%)		0.65	0.35, 1.26	0.2	0.59	0.29, 1.23	0.14
CRF01_AE	6 (8.8%)	62 (91%)		1.26	0.56, 3.38	0.6	1.01	0.43, 2.79	>0.9
CRF02_AG	9 (9.6%)	85 (90%)		1.16	0.58, 2.58	0.7	2.47	1.01, 6.83	0.061
CRF06_cpx	4 (16%)	21 (84%)		0.64	0.23, 2.26	0.4	0.85	0.26, 3.43	0.8
F	5 (11%)	40 (89%)		0.98	0.40, 2.92	>0.9	0.99	0.37, 3.45	>0.9
Other subtype	10 (16%)	53 (84%)		0.65	0.33, 1.41	0.2	0.87	0.38, 2.24	0.8
Accessory INSTI resistance			976						
FALSE	105 (11%)	809 (89%)		—	—		—	—	
TRUE	13 (21%)	49 (79%)		0.49	0.26, 0.97	0.030*	0.51	0.24, 1.16	0.092

[1] Colour indicates associations with $p<0.1$ in univariable analysis
[2] n (%)
[3] OR = Odds Ratio, CI = Confidence Interval
[4] *$p<0.05$

- After adjustment, initiating ART in 2016, presence of the *env*-539V mutation, and HIV-subtype A6 were all associated with significantly lower odds of VS12.

Limitations

- The absolute number of patients whose HIV had the *env*-A539V mutation was small.
- Viral loads were not available at consistent timepoints, especially during COVID-19 emergency measures.
- Significant missingness exists within HARS ART data, making it difficult to investigate how *env*-A539V interacts with different regimens.

Conclusions

- *env*-A539V is a relatively common polymorphism in ART naïve populations in the UK and may be associated with adverse virological outcomes after ART initiation.
- It was seen more commonly in patients who were older, of black African, black Caribbean or black other ethnicity, born in Africa, and whose HIV had an accessory INSTI resistance mutation or was of the CRF02_AG subtype.
- A significant association between *env*-A539V and not reaching VS12 remained robust when controlling for patient demographics as well as other resistance mutations, and subtype.
- Further work is needed to examine the specific context in which this effect is seen and to identify if it holds clinical relevance.

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References

- [1]Van Duyne et al. 'Mutations in the HIV-1 Envelope Glycoprotein Can Broadly Rescue Blocks at Multiple Steps in the Virus Replication Cycle'. *Proceedings of the National Academy of Sciences of the United States of America* 116, no.18 (30 April 2019): 9040–49. https://doi.org/10.1073/pnas.1820333116.
- [2]Malet et al. 'Mutations Located Outside the Integrase Gene Can Confer Resistance to HIV-1 Integrase Strand Transfer Inhibitors'. *MBio* 8, no. 5 (26 September 2017): e00922-17. https://doi.org/10.1128/mBio.00922-17.
- [3]Liu et al. 'Web Resources for HIV Type 1 Genotypic-Resistance Test Interpretation'. *Clinical Infectious Diseases* 42, no. 11 (1 June 2006): 160818. https://doi.org/10.1086/503914.