

Impact of HIV-1 subtype on genotypic resistance to protease inhibitors in the UK.



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BACKGROUND

- This increasing genetic diversity has implications for potential differences in transmissibility, pathogenesis and clinical management - particularly monitoring of therapeutic response and detection of resistance (3-6).
- The recent epidemiology of HIV-1 infection in the UK and western European countries has been characterized by a marked increase in non-B subtypes among newly diagnosed cases (1,2).
- To date, most studies have failed to demonstrate a difference in the rate of disease progression, or in the response to antiretroviral therapy between B and non-B subtypes (5,6).
- A few studies have shown that when drug resistance does emerge, there are differences across subtypes. However, these have been limited by a small sample size, or the lack of reliable antiretroviral treatment histories (7-12).

OBJECTIVE

To identify whether mutations occurring in the protease (PR) gene of individuals with HIV-1 non-B subtypes exposed to selective drug pressure are the same as those occurring in subtype B infections and whether novel mutations emerge in non-B subtype viruses of patients virologically failing their first protease inhibitor (PI) regimen.

METHODS

PR sequence data from the UK HIV Drug Resistance Database were merged with antiretroviral therapy data from the UK Collaborative HIV Cohort Study (UK CHIC).

Eligible patients

- PI naïve or exposed to a single PI only for ≥ 28 days
- Genotype result available within 60 days of stopping the first PI.

The UK Collaborative HIV Cohort Study

- An observational cohort of patients seen at any of six large HIV treatment centers in London and Brighton, UK (13).
- Centers contribute demographic- and routine clinical data (antiretroviral start and stop dates, CD4 counts, viral loads, AIDS events and deaths) on all patients attending since 1 January 1996.
- This analysis is based on approximately 16,500 patients who received HIV care between 1996-2002. 9525 of these received HAART.

UK HIV Drug Resistance Database

- This contains results of resistance tests performed since 1996 on behalf of most HIV clinics in the UK using a variety of in-house and commercial systems.
- All sequences include at least codons 4-99 of the PR gene and 34-234 of the RT gene.

Subtype assignment

- Subtypes were assigned using the STAR algorithm(14). STAR compares query sequences of unknown subtype to subtype- specific profiles which were created by generating position-specific scoring matrices from multiple amino acid alignments of HIV-1 sequence data from Genbank, phylogenetically divided into subtypes.

Statistical analysis

- Mutations were defined as differences between PI exposed and naïve sequences within the same subtype rather than differences between PI exposed non-B sequences and wild-type consensus B sequence.
- PR sequences for individuals who had been on a single first PI for ≥ 28 days were compared with those from PI-naïve patients within the same subtype.
- Multiple chi square tests (Fisher's exact, where appropriate) were performed to detect significant differences in the prevalence of amino-acids (AA) at specific positions between the PI exposed and naïve groups for each subtype.
- The analysis was based on exposure to saquinavir, nelfinavir, indinavir, ritonavir and combinations of ritonavir/ saquinavir and ritonavir/ indinavir. Data was not available to distinguish between the use of ritonavir in treatment or boosting doses when used with another PI.
- Any differences with $p \leq 0.01$ were considered potentially relevant and compared with known IAS mutations (15) and with findings of a recent large study examining the impact of HIV-1 subtype on protease genotype in treatment exposed patients (16).
- Significance tests for interaction between PI exposure and subtype were also performed using generalized linear models.

RESULTS

- 15,624 gnotypes were successfully subtyped. 11,692 were subtype-B. The most frequent non-B subtypes were C (n=2,043), A (n=815), D (n=428) and AG (n=322). After linkage with treatment data, 2,505 sequences were classified as from PI naïve and 1155 from patients exposed to PI ≥ 28 days.
- Table 1 shows the numbers of naïve- and exposed individuals in the analysis, by subtype.

Table 1: Numbers of protease inhibitor naïve and -exposed sequences by subtype.

Drug	Subtype					Total
	A	AG	B	C	D	
Naïve	98	28	2,024	230	33	2,483
Saquinavir (SQV)	14	1	17	15	15	58
Indinavir (IDV)	14	1	17	15	15	58
Ritonavir (RTI)	14	1	17	15	15	58
Nelfinavir (NFV)	14	1	17	15	15	58
RTI/SQV	14	1	17	15	15	58
RTI/IDV	14	1	17	15	15	58
Total PI exposed	174	74	832	824	85	3,669

Table 2: Significant differences (p<0.01) detected between protease inhibitor exposed and -naïve sequences for non-B subtypes at positions with known resistance mutations (15) and at novel positions.

Subtype	Known resistance positions	Drug*	Novel positions	Drug*
A	10	RTI/IDV	74	RTI/SQV
	46	NFV	89	PI: NFV/SQV, RTI
	71	NFV/SQV		
	82	RTI, RTI/IDV		
AG	71	PI: NFV/RTI/SQV	41	RTI/SQV
			89	PI: NFV/RTI/SQV
	20	PI	18	NFV
	30	PI/NFV	18	NFV/RTI/SQV
C	36	RTI/SQV	37	SQV
	71	PI, RTI/IDV		
	82	PI, SAQ, RTI/SQV		
	90	PI, NFV, RTI/IDV, SAQ, RTI/SQV		
D	10	IND	33	RTI, RTI/IDV
	30	PI, NFV, RTI/IDV	57	RTI/IDV
	46	IDV	68	RTI
	63	IDV	72	RTI/IDV
82	NFV			

*# Denotes the specific drug associated with significant amino acid change; PI: Overall group with PI exposure.

*A significant (p<0.01) interaction was found between subtype and PI exposure at positions 12, 63, 82 and 89. Table 3 shows the change from the predominant wild type amino acid by subtype at these positions following drug exposure.

*Statistically significant associations with PI exposure were found at novel position 89 for subtypes A (any PI; NFV/SQV; RTI) and AG (any PI; NFV/RTI/SQV).

Table 3: Prevalence of the predominant amino-acid in PI naïve and -exposed sequences by subtype for positions 12, 63, 82 and 89 where PI/subtype interaction was statistically significant (p<0.01)

Position	Subtype	Amino acid	Prevalence in naïve individuals (%)	Prevalence in exposed individuals (%)	Formal test for subtype/PI interaction
12	A	T	100	94.7	p<0.01
	B	T	91.1	82.1	
	C	T	29.5	43.5	
	D	T	98.0	92.7	
63	A	P	15.9	12.0	p<0.01
	AG	P	14.3	12.5	
	B	P	53.9	65.3	
	C	P	29.3	19.9	
82	A	V	38.8	40.0	p<0.001
	B	V	97.9	88.6	
	AG	V	93.0	100	
	B	V	98.4	90.0	
89	A	V	96.5	98.0	p<0.001
	D	V	96.1	95.0	
	AG	M	100	95.1	
	C	M	95.6	71.4	
D	M	95.0	76.7		
	D	M	5.7		4.9

*Methionine (M) did not occur at position 89 in any wildtype subtype B samples but was the most prevalent amino acid in subtypes A, AG and C.

Table 4: Prevalence of amino acids in PI naïve- and -exposed sequences with subtypes A, B and AG at Position 89.

Subtype	Amino Acid	PI naïve (%) (N)	PI exposed (%) (N)	p-value
A	I	0 (0)	5.4 (4)	p<0.004
	M	100 (96)	85.1 (63)	
	T	0 (0)	4.1 (3)	
	V	0 (0)	4.1 (3)	
	Other	0 (0)	1.4 (1)	
B	L	99 (1,978)	98.9 (890)	p=0.786
	Other	1 (20)	1.1 (10)	
AG	L	3.5 (2)	0 (0)	p=0.000
	I	0 (0)	7.1 (1)	
	M	96.8 (56)	71.4 (10)	
T	0 (0)	21.4 (3)		

CONCLUSIONS

- Differences in the amino acids between PI naïve and -exposed sequences (p<0.01) were detected for non-B subtypes at the following positions known to be associated with resistance in subtype B viruses: 10, 20, 30, 36, 46, 63, 71, 82 and 89.
- Apparently novel PR mutations in non-B subtypes were rare. Such mutations were detected at positions: 13, 16, 33, 37, 41, 57, 65, 72, 74 and 89. However, significant associations with PI exposure (p<0.01) were found for >1 non-B subtype only at positions 89.
- The substitution M89I/V has recently been described in association with treatment failure in patients infected with subtypes C, F and G (17). The association for subtypes A and AG has not been described.
- Our data support the finding of others (17) that the comparison of PI exposed non-B sequences with wild-type consensus B (as used by Kantor et al.(16)) may miss mutations at positions where the consensus wild type codon differs between B and non-B subtypes.
- Whereas Kantor et al regarded any differences from the consensus B sequence in non-B subtypes as mutations(16), we distinguished between different amino acid substitutions at the same position, and identified significant PI-subtype interactions at positions 12, 63, 82 and 89.
- Despite utilising the largest clinical- and resistance databases in the UK, we had limited power to examine the effect of specific PIs on the development of treatment associated mutations in non-B subtypes. Novel findings from this study therefore require confirmation in other large resistance databases.