

# 626 Natural polymorphism of HIV-1 subtypes B and non-B integrase gene

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## OBJECTIVES

To evaluate the natural polymorphism of integrase (IN) gene in clinical HIV-1 variants from B and non-B subtypes.

## METHODS

### Selection of clinical samples

Samples were selected among clinical isolates sent for genotypic resistance testing at Geneva Laboratory. Selection of non-B subtypes was based on RT and Pr sequences and plasma availability; subtype B samples were chosen at random. Patients with identical RT and Pr sequences or with bootstrap values > 99 were excluded from the analysis. None of the patients received IN inhibitors (INI).

### Sequencing

Extraction of viral RNA and reverse transcription were performed as described [1]. IN gene was amplified using primers: (outer) 5' CCA AAT GCC AGT CTC TTT CTC C; 5' TGG AGG AAA TGA ACA AGT AGA TAA ATT; (inner) 5' GGA AAA GGT CTA CCT GTC ATG GGT; 5' TCT TTC TGT TGT ATG CAG ACC C. PCR conditions: 95°C 3'; 40 cycles: 95°C 15", 60°C 15", 72°C 1'; 72°C 5'. Sequencing of IN gene was performed using inner PCR primers and ABI technology.

Subtypes and clusters were assessed by phylogenetic trees using the Neighbour-joining and Fitch-Margoliash methods with bootstrap analysis, respectively.

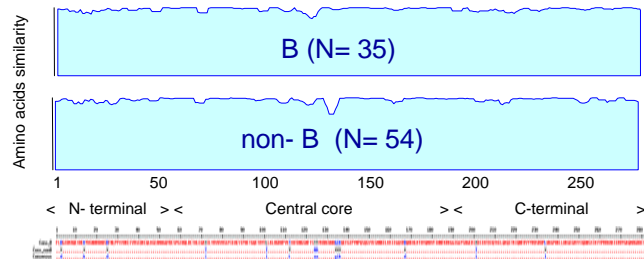
## CLINICAL SAMPLES

Subtypes (RT/Pr)	N	CRFs (RT/Pr)	N
A	5	CRF-01	3
B	35	CRF-02	15
C	8	CRF-06	1
D	2	CRF-09	1
F	3	CRF-10	1
G	1	CRF-11	8
J	1	CRF-13	2
K	1	CRF-14	1
		CRF-18	1

Prevalence of non-B subtypes is representative of circulating strains in Switzerland

## RESULTS

- ✓ Amino acids polymorphism:  
Overall: 48%; B: 32%; non-B: 40%
- ✓ Catalytic triad (DDE), HHCC motif 100% conserved
- ✓ Similar heterogeneity (aa, pairwise comparison):  
B: 5.6% (1-11%); non-B: 6.5% (0.2-11%)
- ✓ Similar variable regions in B and non-B subtypes (except for 134-136: highest variability in non-B)

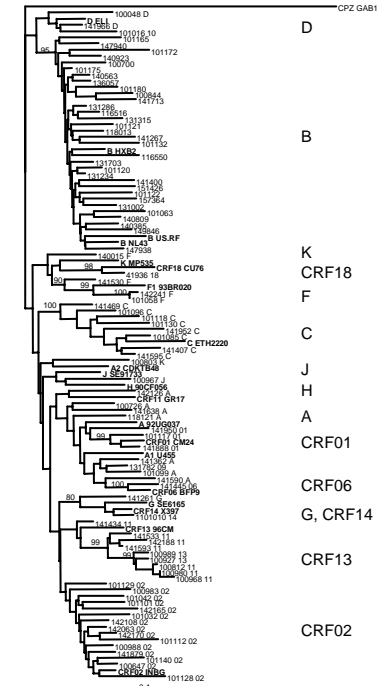


## Substitutions associated with INI resistance [2-6]

Codons	B (%)	Non-B (%)
V72I	17	19
L74M	0	4
T97A	0	4
F121Y	3	0
A128T	0	2
V151I	0	4
K156N	3	0
V165I	11	9
V201I	11	17
T206S	9	47
S230N (?)	14	2

- ✓ Not found as natural polymorphism: T66I, T125K, E138K, G140S, S153Y, M154I, 155H/S, V249I, S230R, C280Y
- ✓ No samples with > 1 substitution associated with resistance

## Phylogenetic tree of IN sequences



## CONCLUSIONS

- ✓ Catalytic triad and HHCC motif were highly conserved in both B and non-B subtypes.
- ✓ Genetic diversity was in the same range within B and non-B subtypes.
- ✓ Polymorphism at positions associated with INI resistance was present at the same level in both B and non-B subtypes.

### References:

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2. Hazuda D, et al. Science 2000;287:646-650.
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