

HIV-1 diversity in France, 1999–2001: molecular characterization of “ non B ” HIV-1 subtypes and potential impact on ARV susceptibility

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Background

Little information is available on the impact of HIV-1 non-B genetic diversity on antiretroviral (ARV) susceptibility.

- Our aims were (i) to characterize HIV-1 non-B strains circulating in France in a population with a known date of infection (ii) to assess the prevalence of recombination (iii) to study the polymorphism of reverse transcriptase (RT) and protease (prot) genes in terms of potential drug resistance.

Methods

All adults tested HIV-1 positive on Western Blot for the first time between 1999 and 2001 were eligible. Data on age, sex, country of birth, HIV transmission group, dates of the last negative and first positive HIV tests (time limit \leq 24 months) and clinical stages were collected. Serotyping was performed with a peptide subtype-specific enzyme immunoassay on each plasma sample. All non-B samples were selected, and a genotypic study was then carried out. The nucleotide sequences of the RT, prot and env C1/V1 or env C2/V3 genes were studied from PBMC samples. HIV-1 subtype was determined by phylogenetic analysis. Polymorphism and ARV drug resistance mutations were noted in the RT and prot genes.

Table 1 Distribution of non-B and B samples

Subtype	Number of samples
B	59
CRF01_AE	3
CRF02_AG	1
D	2
A	3
CRF01_BE	6
CRF07_BC	5
CRF15_01	1
CRF15_02	1
CRF15_03	1
CRF15_04	1
CRF15_05	1
CRF15_06	1
CRF15_07	1
CRF15_08	1
CRF15_09	1
CRF15_10	1
CRF15_11	1
CRF15_12	1
CRF15_13	1
CRF15_14	1
CRF15_15	1
CRF15_16	1
CRF15_17	1
CRF15_18	1
CRF15_19	1
CRF15_20	1
CRF15_21	1
CRF15_22	1
CRF15_23	1
CRF15_24	1
CRF15_25	1
CRF15_26	1
CRF15_27	1
CRF15_28	1
CRF15_29	1
CRF15_30	1
CRF15_31	1
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CRF15_90	1
CRF15_91	1
CRF15_92	1
CRF15_93	1
CRF15_94	1
CRF15_95	1
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CRF15_97	1
CRF15_98	1
CRF15_99	1
CRF15_100	1

Figure 1 Neighbor joining phylogenetic tree of RT nucleotide sequences

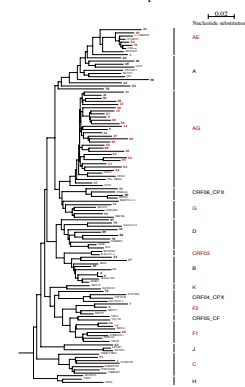


Figure 2 Neighbor joining phylogenetic tree of prot nucleotide sequences

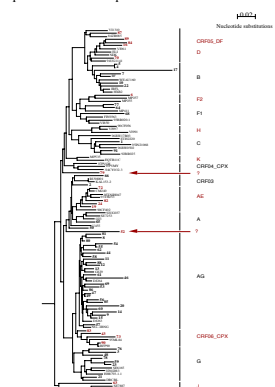


Figure 3 Aminoacid alignment of prot sequences of subtype B consensus and B and non-B isolates.

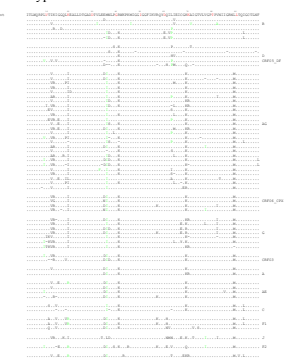
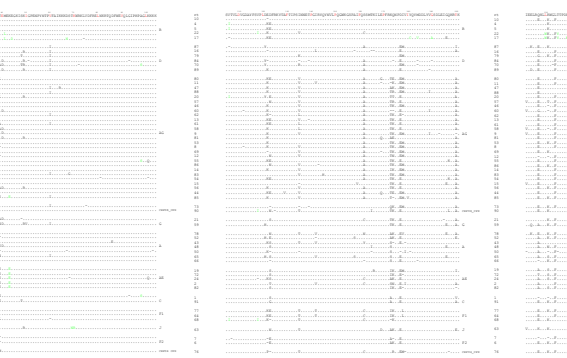


Figure 4 Aminoacid alignment of RT sequences of subtype B consensus and B and non-B isolates.



Results

Among 64 samples classified non-B or undetermined by serotyping, 59 were confirmed to be non-B by the phylogenetic analysis. These 59 non-B were distributed as shown in Table 1 : 23 CRF02_AG, 1 D, 2 A, 3 CRF01_AE, 3 F1, 6 intersubtype recombinants ; 5 isolates seemed even more complex and 16 could not be classified. 5 samples were B subtypes classified undetermined by serotyping ; the sequence data are presented to enable a comparison with B consensus and non-B subtypes.

Prot sequences of non-B samples. No primary resistance mutation to PI was detected (Figure 3). The amino acid substitutions K20I and M36I were observed in most of the AG, CRF06 and G sequences at codons involved into PI resistance ; the V82I substitution was predominant in G sequences. Few isolates contained the amino acid change R57K related to a worse response to first line PI-containing therapy. We found very few substitutions L63P. Also, the following polymorphisms were noted at high frequencies : I13V, R41K, H69K and L89M.

RT sequences of non-B samples. No major resistance mutation to NRTI and NNRTI was observed (Figure 4). The following polymorphisms were noted in most of the samples : V35T, E36D, T39A/K, E122K, D123E, I135V/T, S162A/E, K173T, Q174K, D177E, I178M, Q207E and R211E. Amino acid substitutions K70R and V106I were exceptionally found. Numerous samples contained the substitution L214F which is suspected to play a role in dual resistance to AZT and 3TC. Also, most of the non-B isolates presented the amino acid change V245Q involved in low level resistance to NNRTI.

Prot and RT sequences of B samples.

Compared to B consensus sequence, there was no particular characteristics of the prot sequences. Considering RT, TAMs (particularly M41L, L210W and T215Y) could be observed in samples 22 and 17 ; it was confirmed that the corresponding patients were or had been receiving NRTI.

Conclusion

There is an extensive polymorphism of prot and RT genes of non-B subtypes compared to B consensus sequences; we have no genotypic data indicating that some of these non-B isolates may have a reduced sensitivity to ARV; however the phenotypic consequences of some substitutions deserve more attention. It is clear from this study that there is a high genetic diversity of non-B isolates, including intersubtype recombinants, within the French population.

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