Circulation of Multiple Patterns of Unique Recombinant Forms B/CRF02_AG in France

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Precursor Signs of the Emergence of an Upcoming Circulating Recombinant Form CRF_B/02_AG?

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BACKGROUND

HIV-1 group M is characterised by substantial genetic diversity, and includes nine subtypes, more than 45 CRFs, and numerous Unique Recombinant Forms (URFs).

In France, the epidemic is characterised by :

-predominance of subtype B strains,

- increasing prevalence of non-B subtypes (fig.1), CRF02_AG being the most prevalent,

- increasing at-risk behaviour in the MSM population.

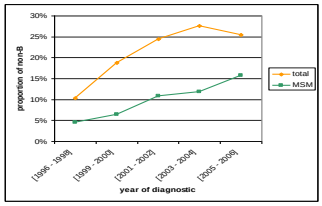


Fig.1: evolution of the proportion of non-B strains among primo-infections in France (adapted from Chaix et al, 2009)

The high prevalence and co-circulation of B and CRF02_AG strains in this population raise the possibility that recombinant forms might emerge and spread.

METHODS

Screening for subtyping discordances in different regions of the genome

↓

Near full length sequencing and pattern characterization

↓

Searching for parental strains or minor recombinants using Single Genome Analysis

↓

Analysis of the phylogenetic relationships between the B/CRF02 recombinants

RESULTS

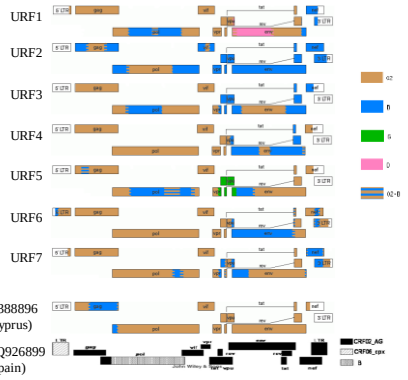
Seven samples were selected from seven patients, of whom five are MSM (tab.1)

patient	sex	age (years)	origin	risk group	year of diagnosis of HIV infection	year of sampling	therapeutic status	CD4 (cells/mm3)	RNA viral load Log copies/mL	URF[b] and size (bp)	nb of SGA sequences[c]
P1	M	67	France (caucasian)	homosexual	2008	2008	naive	490	5.8	URF1 : 8659	8
P2	F	46	France (caucasian)	IDU	2004	2009	naive	504	3.7	URF2 : 8891	11
P3	M	38	France (caucasian)	homosexual	2006	2009	treated	549	5	URF3 : 8295	8
P4	M	-[a]	-[a]	-[a]	2002	2002	-[a]	-[a]	5.1	URF4 : 8424	10
P5	M	25	France (caucasian)	homosexual	2010	2010	naive	159	5.1	URF5 : 8396	10
P6	M	39	France (caucasian)	homosexual	2006	2006	naive	391	5.2	URF6 : 9608	NA
P7	M	32	France (caucasian)	homosexual	2006	2006	naive	397	6.2	URF7 : 9158	NA

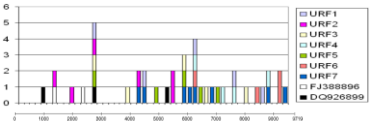
[a]: not available
[b]: URF identified in the patient
[c]: number for a given URF of sequences obtained by SGA for breakpoint confirmation
NA: not applicable

Tab.1: Characteristics of the seven patients from whom the samples were selected, and of the corresponding URFs characterized

a)



b)



Two of the seven new URFs involve a 3rd subtype (fig. 2.a).
The seven recombination patterns are distinct from each other (fig. 2.a), and from the only two previously described B/CRF02 URFs from Cyprus (Kousiappa et al., 2009) and Spain (Holguin et al., 2008).

The breakpoints are distributed all along the genome (fig. 2.b) with hotspots in *pol* at the protease-to-Reverse Transcriptase border, and in *vpu* where the overlapping start of *env* is located.

Fig.2: Recombination patterns of the 7 new URFs and the 2 previously described (a) and breakpoints distribution along the genome (b)

There was no evidence of parental or minor recombinant strains circulation, although polymorphism was observed for all the strains tested (fig. 3).

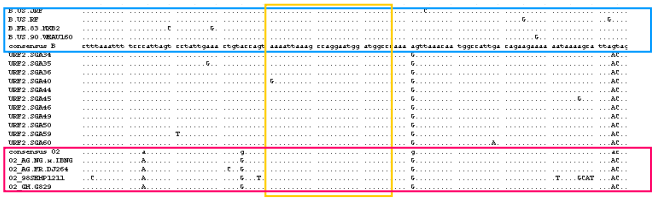
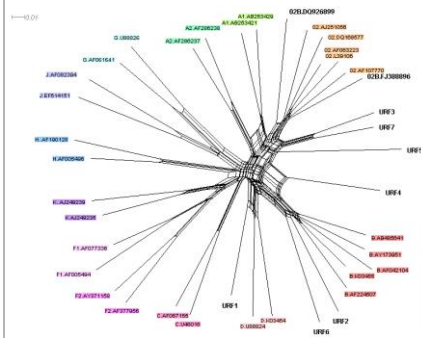


Fig.3: Example of SGA results for URF2. The 11 sequences obtained are aligned with a set of subtype B (blue box) and CRF02 (red box) reference sequences to determine their recombination patterns; the yellow box indicates the region were recombination occured for these SGA sequences.



In the *env* gp120 region, URF3 and URF7 appeared to be linked despite their different recombination patterns.

No link was observed between any URF in the *pol* pr-RT region (not shown)

Fig.4: Phylogenetic network representing the relationships between the env gp120 sequences of the 9 URFs and a set of reference sequences.

CONCLUSIONS

The **complexity of the molecular epidemiology** is growing in France due to the **raise of non-B strains** prevalence and to subsequent **recombinations**
The predominant forms, **subtype B and CRF02**, are co-circulating in the MSM population and frequent co-infections led to local **emergence of several URFs**, one of those (URF7) being **linked with a cluster** of CRF02_AG sequences identified in the MSM population of Paris
The absence of parental strains suggests **direct transmission** of these strains, indicating that they could spread in particular within this population
A surveillance is needed to determine if this dynamic could lead to the **genesis of a new CRF_B/CRF02_AG** in France