Detection of numerous HIV-1/MO recombinants in France: implications for biological and clinical practice

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Supplementary Data

Alternative RT-PCRs

Alternative RT-PCRs were performed as follows: viral RNA was extracted from 200 µl of plasma using EZ1 DSP Virus Kit, eluted in 60 µL elution buffer and 10 µl of extract were used in a 50 µL RT-PCR using SuperScript™ III One-Step RT-PCR System with Platinum® Taq DNA polymerase (Invitrogen), 20 pmol of each primer (supplementary Table 2) and 1.5 mM $MgSO_4$. Two microliters of amplification product were used in a 50 µl nested PCR using HotStar Taq Master Mix (Qiagen), 20 pmol of each primer (supplementary Table 2) and 1.5 mM $MgCl_2$. (RT-)PCRs were run on a PerkinElmer Gene Amp 9700 thermal cycler and cycling conditions are described in Supplementary Table 2.

Phylogenetic analyses

Phylogenetic analyses were performed using the neighbor-joining method. Genetic distances were calculated with the Kimura two-parameter method and 1000-replicate parametric bootstrap analysis was performed to assess the reliability of branching order. Sequences of available co-infections HIV-1/M+O and recombinants HIV-1/MO previously described were included. All the accession numbers of the previously and newly described sequences are listed in Supplementary Table 3.

Supplementary Table 1. Patient's characteristics

Patients	Gender	Age	CD4/mm^3	Year of sampling	Origin
RBF216	F	35	25	2009	Cameroon
BCF212	F	61	231	2006	Cameroon
RBF221	F	62	N/A	2010	Cameroon
BCF204	F	55	N/A	2013	Cameroon
BCF174	F	30	363	2005	Cameroon
RBF222	M	45	306	2010	Cameroon
RBF237	F	37	399	2013	Cameroon
RBF240	M	35	390	2016	Cameroon
RBF243	F	44	130	2017	Cameroon
RBF235	F	46	84	2013	Chad

N/A: Not Available

Supplementary Table 2. Alternative primers and PCR conditions used to characterize HIV-1/MO recombinants

Sample	Genomic region	Step	Primer	Primer sequence (5'-3')	Fragment size (bp)	Cycling conditions		
						AT[3] (°C)	ET[4]	Cycles
RBF235	RT-PT	RT PCR	PROTO (S[1])	TTCAAYTGTGGRAAAGAGGGAC	1392	55	1' 30"	35
			MJ4 (AS[2])	CTGTTAGTGCTTTGGTTCCTCT				
		NESTED	PROT4 (S)	CAGCCCCACCRATGGAGG	1163	50	1' 30"	35
			NE1 (AS)	CCTACTAACTTCTGTATGTCATTGACAGTCCAGCT				
RBF240	RT-PT	RT PCR	40U (S)	GGYTGYTGGAAATGTGG	1761	55	2'	35
			90L (AS)	GAATCCAGGTRGCYTGCC				
		NESTED	43U (S)	ATGTGGAMAGGAAGGWCAYCA	1334	50	1'30"	35
			85L (AS)	CCTGGATARATYTGACTTGC				
RBF221 (REC A)	ACC	RT PCR	127U (S)	GGGTYTATTACAGRGACAGCAGAG	1561	50	2"	35
			167L (AS)	GGGTCTGTGGGWACACAGGC				
		NESTED	127U (S)	GGGTYTATTACAGRGACAGCAGAG	1240	50	1'30"	35
			MVPU (AS)	TACTATRGTCCACACAACTATKGCT				
RBF221 (REC B)	ACC	RT PCR	127U (S)	GGGTYTATTACAGRGACAGCAGAG	1561	50	2'	35
			167L (AS)	GGGTCTGTGGGWACACAGGC				
		NESTED	128U (S)	AGAGAYCCWATTTGGAAAGGACC	1514	55	2'	35
			307L (AS)	TTGTGMTGCCCAAATATTATG				

[1] sense primer [2] antisense primer [3] annealing temperature [4] extension time

Supplementary Table 3. GenBank accession numbers of previously described and new HIV-1/MO recombinants and HIV-1/M+O dual infections

Sample	GenBank accession number	Reference
97CA.MP645MO	AJ239083	Peeters *et al*. 1999
DSC1320	AY489738	Yamaguchi *et al*.2004
RBF140_GPO	KY359367	Plantier *et al.*2004
RBF140_GPM	KY359368	
RBF140_polM	KY359375	
RBF140_polO	KY359376	
RBF208	GQ351296	Vessière *et al.*2010
REC003	KM438031	Ngoupo *et al.*2016
REC024	KM438032	
YBF301_polM	KX398152	De Oliveira *et al.* 2017
YBF301_polO	KX398153	
YBF320_polM	KX398154	
YBF320_polO	KX398155	
YBF211_polO	KX398156	
YBF211_polM	KX398157	
YBF212_polO	KX398158	
YBF212_polM	KX398159	
YBF205_polO	KX398160	
YBF205_polM	KX398161	
YBF298_polM	KX398162	
YBF301_GPO	KX398163	
YBF301_GPM	KX398164	
YBF320_GPO	KX398165	
YBF320_GPM	KX398166	
YBF211_GPO	KX398167	
YBF211_GPM	KX398168	
YBF212_GPM	KX398169	
YBF212_GPO	KX398170	
YBF205_GPO	KX398171	
YBF205_GPM	KX398172	
YBF298_GPM	KX398173	
YBF282_polM	KX398174	
YBF282_GPO	KX398175	
YBF280_polO	KX398176	
YBF280_polM	KX398177	
YBF280_GPO	KX398178	
YBF280_GPM	KX398179	
YBF274_polO	KX398180	
YBF274_GPO	KX398181	
YBF274_GPM	KX398182	
YBF221_polO	KX398183	
YBF221_polM	KX398184	
YBF221_GPO	KX398185	
YBF221_GPM	KX398186	
RBF235_GPO	KY359362	New submissions
RBF235_GPM	KY359363	
BCF174_GPO	KY359364	
RBF221_GPO	KY359365	
RBF221_GPM	KY359366	
RBF216_GPO	KY359369	
RBF216_GPM	KY359370	
RBF235_polOM	KY359371	
RBF235_polO	KY359372	
BCF174_polM	KY359373	
RBF221_polM	KY359374	
RBF216_polM	KY359377	
RBF216_polO	KY359378	
BCF212_GPO	KY359379	
BCF212	KY359380	
RBF237	KY359381	
RBF222	KY359382	
BCF174	KY359383	
RBF240	KY359384	
BCF204	KY359385	
RBF243	KY995542	