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Characterization of CRF56_cpx, a new circulating B/CRF02/G recombinant form identified in MSM in France

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Several B/CRF02_AG Unique Recombinant Forms (URFs) have previously been identified in France. Here we show that one of them (URF5_B/02/G) is emerging in MSM, a high-risk population where HIV incidence and number of superinfections are increasing. We describe this new Circulating Recombinant Form, CRF56_cpx, estimate the time to its most recent common ancestor, investigate its origins and show that it probably shares common ancestors with strains from the East Mediterranean.

HIV type 1 group M (HIV-M) shows a high genetic diversity and has been subdivided into nine subtypes (A–D, F–H, J, K). Recombination, which is frequent during HIV replication, produced intersubtype recombinant forms, at least 55 of which have spread and been described as Circulating Recombinant Forms (CRFs) [1]. The reasons why a particular recombinant form can spread into a population are probably diverse, including epidemiological opportunities, fitness and ability to escape diverse host pressures.

The geographical distribution of HIV-M subtypes and CRFs has first reflected the epidemiological history of the pandemic, as for subtype B predominance in North America and Western Europe [2], but travelling and mixing of populations makes the molecular epidemiology of HIV-M constantly evolving [3]. In France, subtype B now accounts for only 50% of new diagnosed infections, and CRF02_AG has become the most prevalent non-B form [4]. Subtype B and CRF02_AG were first associated with different risk groups (MSM and Sub Saharan migrants, respectively), but they are now cocirculating in the MSM population, a high-risk group in which we recently described B/CRF02 Unique Recombinant Forms (URFs) [5]. Here, we show that one of these, URF5 (CRF02/B/G), was identified in three new patients living in Paris, allowing us to define the new CRF56_cpx.

Patients A (previously reported as infected by URF5), B, C and D were MSM, aged to 21–32 years when sampled in Infectious Diseases units of three hospitals in Paris. They were diagnosed recently (years 2009 to 2011), three of them during Primary HIV Infection (supplementary Table 1, http://links.lww.com/QAD/A365). Plasma samples of the new patients, showing the same recombination pattern as URF5 based on routine resistance sequencing of the Protease and Reverse Transcriptase, were analyzed.

Single Genome Analysis (SGA) was performed on the Protease and partial Reverse Transcriptase regions (963 bp) as previously described [5] for identifying putative parental strains, and comparing the quasi-species circulating in the patients. The three new strains were characterized by sequencing the near-full-length genomes, using an overlapping nested reverse transcriptase-PCR strategy as previously described [5].

Intrapatient and mean interpatient genetic distances were estimated between SGA sequences, and interpatient distances between the near-full-length sequences (supplementary methods, http://links.lww.com/QAD/A365). Subtyping, recombination, phylogenetic and evolutionary analyses were performed using Los Alamos National Laboratory HIV Blast and highlighter (http://www.hiv.lanl.gov/), NCBI genotyping (http://www.ncbi.nlm.nih.gov/), simplot 3.5.1[6], MEGA5 [7] and BEAST v1.7 [8] (supplementary methods, http://links.lww.com/QAD/A365).

The SGA analysis showed no parental strain in any sample, demonstrating the absence of superinfection and suggesting a direct transmission of the recombinant strain to each patient. Phylogenetic analysis showed that the sequences from patients A, B and C formed individual clusters nested within the patient D sequences (supplementary Figure 1a, http://links.lww.com/QAD/A365), and quasi-species analysis showed patient-specific mutations (supplementary Figure 1b, http://links.lww.com/QAD/A365), demonstrating distinguishable strains and intrapatient evolution. Intrapatient genetic distances ranged from 0.001 to 0.004, consistent with short duration of infections (supplementary Table 1, http://links.lww.com/QAD/A365). Interpatient mean distances ranged from 0.003 to 0.008 for the SGA sequences, and from 0.006 to 0.008 for the near-full-length genomes. Taking into account the recent infections (2009–2011) and the absence of direct link between the four patients, these results suggest a recent diffusion of this new form.

Near-full-length characterizations confirmed a complex recombination pattern, involving subtypes B, G and CRF02_AG with 16 breakpoints (Fig. 1a), common to

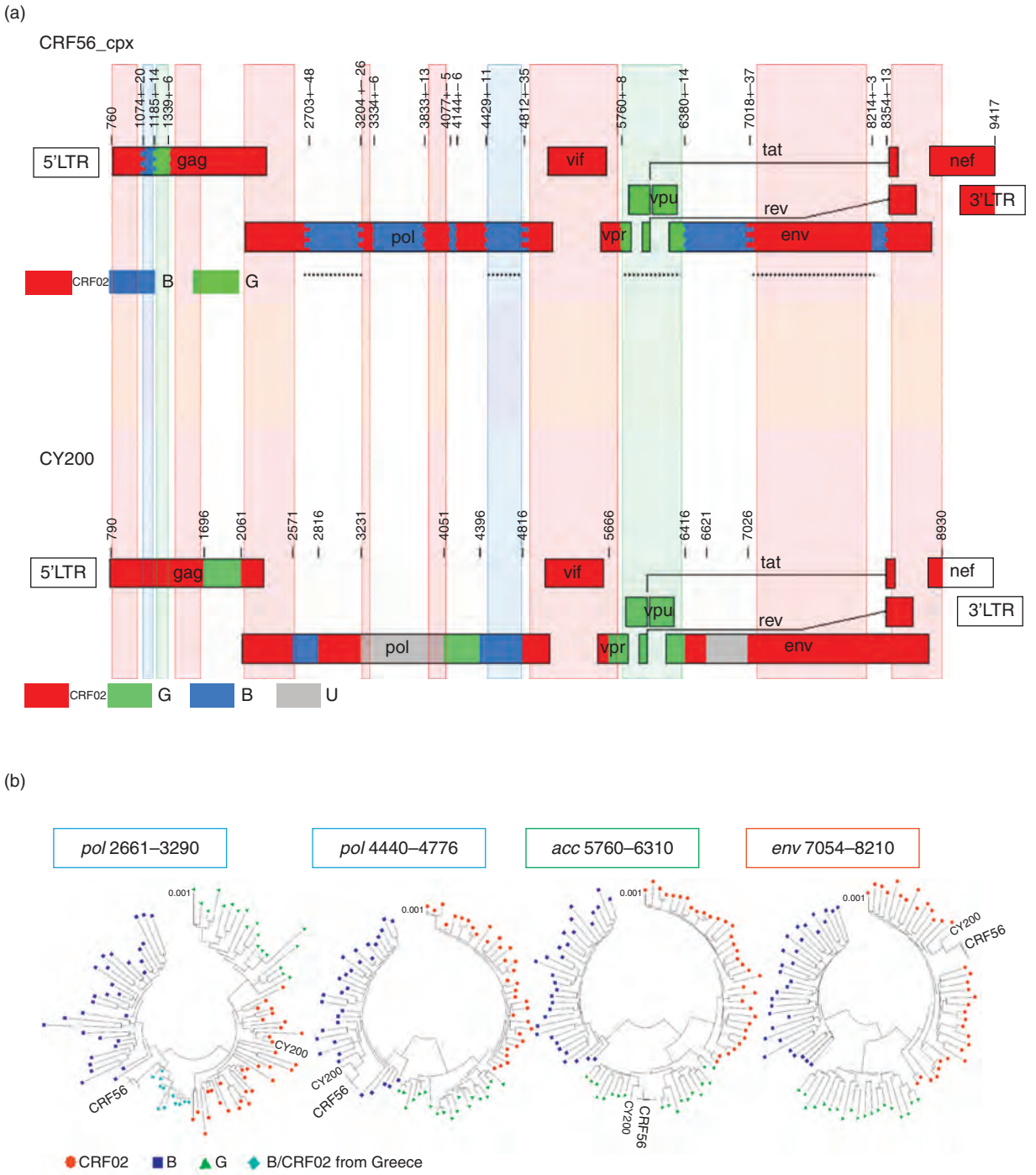

Fig. 1. Recombination pattern and phylogenetic relationships of CRF56_cpx to closely related strains from Cyprus and Greece. (a) Recombination patterns of CRF56_cpx, and of CY200 as described previously [10]. The subtypes and breakpoints were mapped using LANL Recombinant HIV-1 Drawing tool (http://www.hiv.lanl.gov/). The regions where these two recombinant forms cluster together are highlighted by coloured boxes. Note that subtype assignment in the CY200 pattern were sometimes imprecise. The dotted lines represent the regions involved in the phylogenetic trees in (b). (b) Maximum Likelihood trees representing CRF56_cpx, CY200 and a set of reference sequences from subtypes B (blue square), G (green triangle) and CRF02 (red round) in four regions of the genome, defined by the gene and the positions studied according to HXB2 numbering. In *pol* 2661–3290, 12 partial sequences of CRF02/B recombinant strains from Greece are included in the analysis. Bootstrap values for CRF56_cpx clustering with CY200 are: 64% in *pol* 4440-4776; 69% in *acc* 5760-6310; and 100% in *env* 7054-8210.

the four strains. Thus, we proposed to name this new Circulating Recombinant Form CRF56_cpx, according to the international HIV nomenclature guidelines [9]. Time to the Most Recent Common Ancestor of the four strains was estimated in regions deriving from subtype B, G or CRF02 (supplementary Figure 2a, http://links.lww.com/QAD/A365), with a mean result around 2007 consistent with recent diffusion.

Interestingly, using HIV Blast as a screening tool for possible related strains, we found CRF56_cpx to be close to CY200, a CRF02/B/G URF sampled in 2007 in a MSM patient infected in Cyprus [10]. They shared at least five breakpoints and significantly clustered together in regions belonging either to subtype B, G or CRF02 (Fig. 1, and Bayesian Markov Chain Monte Carlo analysis, not shown). Still, CY200 and CRF56_cpx also presented unshared breakpoints, so it seems unlikely that one could have been a direct ancestor for the other. They would rather share a CRF02/B/G common ancestor, which would be dating from the 1990s (supplementary Figure 2b, http://links.lww.com/QAD/A365). Whether this putative parental recombinant strain emerged in France, Cyprus or elsewhere is uncertain.

We also found 12 CRF02/B sequences, encompassing the Protease and partial Reverse Transcriptase, to be close to our sequences. All 12 were sampled in Greece in 2002–2005 from newly infected patients [11]. Phylogenetic analyses confirmed that they clustered together in the Reverse Transcriptase region, deriving from subtype B (Fig. 1b), but in the Protease, the Greek recombinant sequences involved various CRF02_AG parental strains with no link to that of CRF56_cpx, suggesting multiple recombination events. CRF56_cpx could not be related directly to one of the recombinant forms described in Greece yet, but would rather be linked to the Greek parental subtype B strain.

The distribution of HIV subtypes and CRFs across the world is constantly evolving [3], leading to an increase of strains cocirculation and recombination. Recombinant forms become more frequent and complex, and now dominate the epidemic in some parts of the world [12]. The growing diversity resulting from recombination must be taken into account as numerous factors can be impacted, from viral properties to host–virus interactions, patients monitoring or vaccine design. Here, we describe a new recombinant form, complex (involving more than two subtypes) and of second generation (involving at least a recombinant parental strain), which is closely related to several recombinant forms from the Eastern Mediterranean region.

Now that bridges have been established between different risk groups, HIV diversity and recombination are increasing in French MSM [13,14]. This could favour the emergence of CRFs in this population, as reported before in England [15]. Whether the recent diffusion of CRF56_cpx in our patients was the result of a favourable epidemiologic context, or particular fitness or adaptation properties is to be investigated. The small intrapatient and interpatient distances are compatible with the hypothesis of a transmission cluster in the MSM population of Paris, which is particularly at-risk, but no direct links were identified between the patients. Moreover, patient A, even if linked to Paris, lives in South Eastern France, leading to a risk of a larger geographical spread.

Our findings confirm the growing complexity of HIV-1 molecular epidemiology and underline the need for maintaining surveillance and reinforcing prevention of HIV transmission in MSM. Despite the global HIV incidence decrease in other populations and large use of antiretroviral drugs, HIV prevalence and incidence remains high in this population [16], as well as the level of sexual risk behaviours [17], which at least two of our patients reported. Similar trends have been observed in many countries [18], underlining the risk of high transmission bursts in MSM. Current French molecular surveillance systems [13,19,20] will, thus, be useful to monitor the spread of CRF56_cpx or other emerging forms in the population.

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Conflicts of interest

There are no conflicts of interest.

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Improvements in virological control among women conceiving on combination antiretroviral therapy in Western Europe

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Among 396 HIV-infected women conceiving on combination antiretroviral therapy and enrolled in the European Collaborative Study in 2000–2011, the proportion with virological failure (>200 copies/ml after ≥24 weeks of treatment) declined substantially from 34% in 2000–2001 to 3% in 2010–2011. In adjusted analyses, younger women and those with at least two children were at increased risk of virological failure, highlighting the importance of close monitoring and adherence support.

Increasing numbers of HIV-positive women are having children in Europe, most likely reflecting improvements in HIV treatment and care and the very low rates of mother-to-child transmission (MTCT) now achievable [1–3]. A recent UK study demonstrated a significant increase in pregnancy incidence among HIV-positive women over 2000–2009 after adjusting for changing sociodemographic characteristics [4]. HIV-positive pregnant women are increasingly aware of their diagnosis before conception, due to previous diagnosis in antenatal care [5] or another setting [6,7]. Thus, the proportion of HIV-positive pregnant women already on combination antiretroviral therapy (cART) at conception is increasing in Western Europe [8,9]. Virological failure among these

women has implications not only for their own health [10,11], but also for risk of MTCT, including potential for transmission of drug-resistant virus.

We investigated virological failure in pregnancy among women conceiving on cART in the European Collaborative Study (ECS), a cohort enrolling HIV-positive pregnant women in nine Western European countries, in 2000–2011 [12]. Eligibility criteria for this analysis were receipt of at least 28 days of cART by conception and the availability of a viral load measure after at least 24 weeks of uninterrupted treatment (taken during pregnancy or in the month preceding conception). Poisson regression models with robust variance estimators were fitted to investigate factors associated with virological failure, defined as a viral load of more than 200 copies/ml.

Of 396 women included, median age was 33 years (interquartile range 30–37), 56% (218/393) were from sub-Saharan Africa [increasing from 45% (120/269) in 2000–2005 to 79% (98/124) in 2006–2011, $\chi^2 = 40.72$ $P < 0.01$] and 14% (56/394) had a history of IDU [declining from 20% (53/271) in 2000–2005 to 2% (3/123) in 2006–2011, $\chi^2 = 20.33$ $P < 0.01$]. The proportion with at least one previous live birth in the ECS was 29% (114/396); 7% (27/396) had at least two. Half (206/396) were on a protease inhibitor-based regimen, most commonly nelfinavir ($n = 78$), and 38% ($n = 152$) were on a nonnucleoside reverse transcriptase inhibitor (NNRTI)-based regimen. Nonboosted protease inhibitors accounted for 83% (74/89) of protease inhibitor-based regimens in 2000–2001, declining to 8% (2/26) in 2010–2011 (trend $P < 0.01$). Women enrolling in 2010–2011 had been diagnosed as HIV-positive a median of 2.8 years before conception, compared with 1.7 years for those enrolling in 2004–2005 and 1.1 years for those enrolling in 2000–2001 (trend $P < 0.01$).

One fifth (74/396) of women had virological failure (median viral load 1370 copies/ml, interquartile range 400–6000), declining from 34% (32/93) in 2000–2001 to 3% (1/33) in 2010–2011 (trend $P < 0.01$), a median of 18.5 months after initiation of their current cART regimen. Viral load measurements were taken at median of 13.3 weeks of gestation for the suppressed group and 12.9 weeks of gestation for the nonsuppressed group (Wilcoxon–Mann–Whitney test $P = 0.74$), indicating similar access to antenatal care. In analyses adjusting *a priori* for country, year of delivery and cART duration, factors positively associated with virological failure were: at least two previous ECS live births (vs. none), nonboosted protease inhibitor (vs. NNRTI-based) regimen, younger maternal age, longer time since HIV diagnosis and earlier calendar year of delivery (Table 1). Of 74 nonsuppressed women, 65 had at least one viral load measure available later in pregnancy, of whom 63% (41/65) attained suppression (≤200 copies/ml). This proportion increased from 36% (10/28) in 2000–2001 to 80% (8/10) in 2006–2011 (trend $P < 0.01$). Of the 291 women virologically suppressed at first measure and with

Table 1. Factors associated with virological failure among women who conceived on combination antiretroviral therapy, 2000–2011.

	Proportion with viral load >200 copies/ml at first measure	Crude prevalence ratio (95% CI)	Adjusted prevalence ratio (multivariable model)[a], $n = 380$
Sub-Saharan African origin			
No	30.4% (52/171)	1	
Yes	10.2% (22/215)	0.34 (0.21–0.53) $P < 0.01$	
Previous ECS live birth			
No	18.3% (51/278)	1	1
Yes – one	18.8% (16/85)	1.03 (0.62–1.70) $P = 0.92$	1.47 (0.89–2.44) $P = 0.13$
Yes – two or more	26.9% (7/26)	1.47 (0.74–2.90) $P = 0.27$	3.08 (1.49–6.36) $P < 0.01$
IDU history			
No	15.9% (53/333)	1	
Yes	38.9% (21/54)	2.44 (1.61–3.70) $P < 0.01$	
Type of cART[b]			
NNRTI-based	12.4% (18/145)	1	1
PI-based (nonboosted)	34.7% (35/101)	2.79 (1.68–4.65) $P < 0.01$	2.26 (1.28–4.00) $P < 0.01$
PI-based (boosted with ritonavir)	15.3% (15/98)	1.23 (0.65–2.33) $P = 0.52$	1.90 (0.96–3.78) $P = 0.07$
Triple NRTI/NtRTI	13.3% (6/45)	1.07 (0.45–2.54) $P = 0.87$	1.27 (0.52–3.12) $P = 0.60$
Maternal age (per increasing year)		0.97 (0.93–1.01) $P = 0.18$	0.95 (0.91–0.99) $P = 0.02$
Time from HIV diagnosis to conception (per increasing year)		1.06 (1.02–1.11) $P < 0.01$	1.06 (1.01–1.10) $P < 0.01$
Duration of current cART regimen (per increasing year)		0.88 (0.75–1.03) $P = 0.11$	1.01 (0.83–1.23) $P = 0.92$
Year of delivery (per increasing year)		0.81 (0.74–0.88) $P < 0.01$	0.80 (0.72–0.89) $P < 0.01$

CI, confidence interval; ECS, European Collaborative Study; cART, combination antiretroviral therapy; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucloside reverse transcriptase inhibitor; NtRTI, nucleotide reverse transcriptase inhibitor.
[a]Adjusted *a priori* for country of delivery (not shown), year of delivery and duration of current cART regimen. Other variables were assessed for their contribution to the multivariable model's goodness-of-fit using Wald's test, significance level <0.01.
[b]Seven women on both a protease inhibitor and an NNRTI were excluded (all of whom were virologically suppressed).

another measure 4 weeks or less before delivery, 94% (271/291) remained suppressed.

Our study shows substantial improvements over calendar time in virological control among women conceiving on cART, as shown in cohorts of treated HIV-positive individuals [13,14]. The association between virological failure and younger age is consistent with poorer cART adherence among younger people reported elsewhere [15,16]. Our finding of an increased risk of virological failure among treated women who already had at least two children may be partly explained by challenges adhering to cART while caring for a family. This is important given the context of increasing sequential pregnancies among HIV-positive women in Europe [5] and also has potential implications for the risk–benefit of an Option B+ strategy [17]. Our results indicate that younger pregnant women and treatment-experienced multiparous mothers may require close monitoring and additional adherence support.

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Conflicts of interest

There are no conflicts of interest.

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