WHOLE GENOME SEQUENCING SHOWS INCREASING HIV-1 SUBTYPE COMPLEXITY AMONG MSM IN THE UK

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BACKGROUND

Co-infection with two genetically distinct HIV-1 strains can result in recombination and does signify a high risk behaviour. HIV whole genome sequencing (WGS) allows comprehensive characterization and a better understanding of the emergence of recombinant strains, virus evolution and transmission dynamics. This could help inform the implementation of public health intervention strategies.

The aims of this study were to:

1. Determine the prevalence and distribution of HIV-1 complex recombinant strains among men-who-have-sex-with-men (MSM) in the UK.
2. Characterize circulating HIV-1 complex recombinant strains among MSM in the UK by performing whole genome sequencing (WGS) of samples from recently infected individuals.
3. Undertake phylogenetic analyses to better understand emergence of the complex recombinants and transmission dynamics.

METHODS

- HIV-1 WGS was undertaken on 382 samples from MSM collected between 2000-2006 (n=201) and 2015-2016 (n=181). The former consisted of chronic (n=157) and recent (n=44) infections whereas the latter of recent infections only.
- Recency of infection was determined using a recent infection testing algorithm (RITA) which includes an avidity assay and clinical information.
- More than 110,000 partial *pol* gene sequences from routine HIV-1 genotyping in the UK were obtained from the UK HIV Drug Resistance Database (UK-HDRD).
- Subtyping was performed using REGA HIV subtyping tool
- Transmission clusters were identified using Cluster Picker software with a clade support >90% and maximum genetic distances of 1.5% or 4.5%.
- TreeTime and BEAST software were used for reconstruction of time-stamped phylogeny and PastML for ancestral state reconstruction
- Linked clinical and demographic data were extracted from the HIV and AIDS Reporting System at PHE.

RESULTS

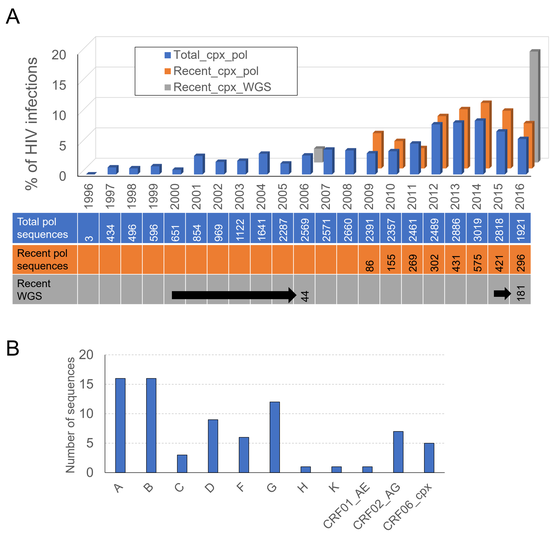


Figure 1. *Increasing prevalence of HIV-1 complex recombinant strains among MSM in the UK.*
The trends in distribution of HIV-1 complex recombinant strains between 1996 and 2016 were estimated using all partial *pol* sequencing data from routine genotyping in the UK (blue bar) and for those determined to have a recent infection since 2009 (red bar) (panel A). The data shows a gradual increase in diagnosed infections involving complex recombinants from 1.2% in 1997 to a nadir of 8.8% in 2014 (10.7% in recently infected). WGS data (grey bar) shows a higher proportion of recent infections involving complex recombinants in 2015-2016 at 18.1%. This indicates that routine HIV-1 partial *pol* sequencing does significantly underestimate the prevalence and complexity of circulating recombinant strains among MSM in the UK.
The most common subtypes making up the complex recombinants were either subtype B, which is the most common pure subtype among MSM in the UK making up ~73% of total infections in 2016, or subtype A (panel B).

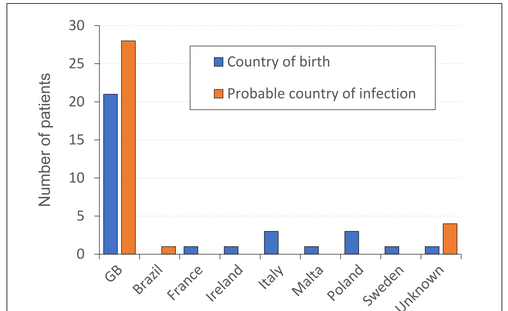


Figure 2. *Most recent infections involving complex recombinant strains were acquired in UK.*
In 2015-2016, 63.6% (21/33) MSM recently infected with HIV-1 complex recombinants were born in the UK and the probable country where the infection was acquired was the UK in 84.8% (28/33). This suggests that the recombinant strains either may have originated in the UK or may have been circulating in the UK for some time.

Table 1. *Transmission cluster analysis of complex recombinant strains in the UK.*

Cluster no.	Total no. of sequences in cluster (WGS)	Subtypes comprising the recombinant	*pol* subtyping	tMRCA
3	3 (1)	B, D, A1, G	cpx	2009
10	2 (1)	B, D, A1	B	2013
42	5 (2)	C, B	cpx	2011
93	3 (1)	B, D, A1	B	2013
175	9 (4)	CRF02_AG, G	CRF02_AG	2010

Transmission cluster analysis using the *pol* gene of the 33 WGS complex recombinants sampled in 2015-2016 and >110,000 routine genotyping data from the UK-HDRD. The analysis was performed using a maximum genetic distance threshold of 1.5% to capture 'recent transmission events' and identified 27.3% (9/33) in clusters (using a maximum genetic distance of 4.5% resulted in 75.8% (25/33) sequences in clusters). The time to most recent common ancestor (tMRCA) showed the complex recombinant strains to have been circulating in the UK for up to 7 years.

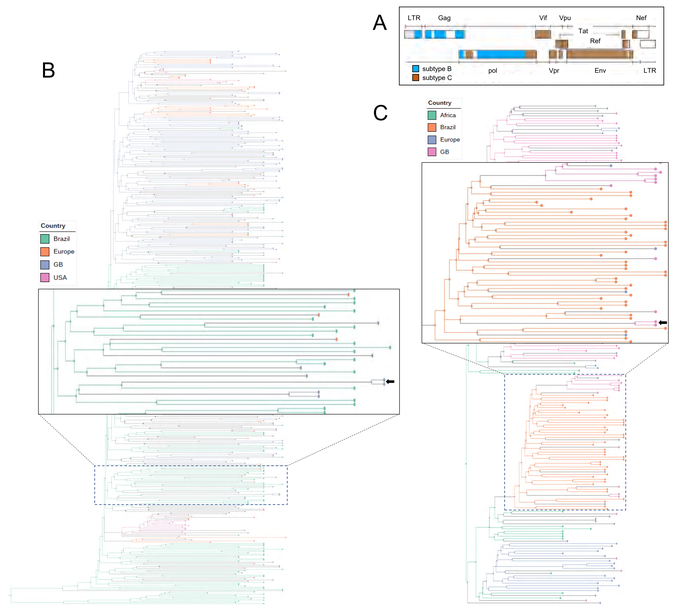


Figure 3. *Determining the origins of the complex recombinant strains.*
Ancestral state reconstruction was performed to determine the origins of the two complex recombinants in cluster #42 in **Table 1**. The complex recombinants consisted of subtypes B and C (panel A). Ancestral state reconstruction was performed using the WGS generated during this study and other HIV-1 sequences downloaded from public databases (NCBI and Los Alamos HIV Database) with known sampling date and country. The analysis was performed for Gag and Env regions (panels B and C, respectively). This showed that the ancestral state of the node subtending the sequences from the two complex recombinants was Brazil. This suggests that either the recombinant or the parental strains have origins in Brazil.

- 1 in 5 HIV-1 recent infections among MSM in the UK involve a complex recombinant strain, up from <1 in 100 two decades ago.
- 4 in 5 HIV-1 recent infections involving complex recombinants were acquired in the UK.

CONCLUSIONS

- Routine HIV-1 genotyping significantly underestimates the prevalence and complexity of circulating recombinant strains among MSM in the UK.
- 18% of recent infections among MSM in the UK involve a complex recombinant of which 85% are acquired in the UK.
- Evidence shows that at least 27% of the complex recombinants have been circulating in the UK for up to 7 years.
- In-depth analysis of one cluster suggests importation rather than emergence of the recombinant in the UK.

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