GENETIC INTRA-HOST VARIABILITY OF FULL-LENGTH MPXV GENOMES IN MULTIPLE TISSUES OVER TIME

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BACKGROUND

The international mpox outbreak, recognized in May 2022, revealed sexual contact as a new transmission route for mpox virus (MPXV), and the genital tract represented the main target of viral lesions. Despite this, other districts are permissive to the infection, and the virus can be found widespread in the body of an infected host. There is no evidence for MPXV genome intra-host variability or genetic evolution over time. Here, we investigated this wide-spectrum tropism of MPXV by studying the genetic intra-host variability of full-length genomes in multiple tissues.

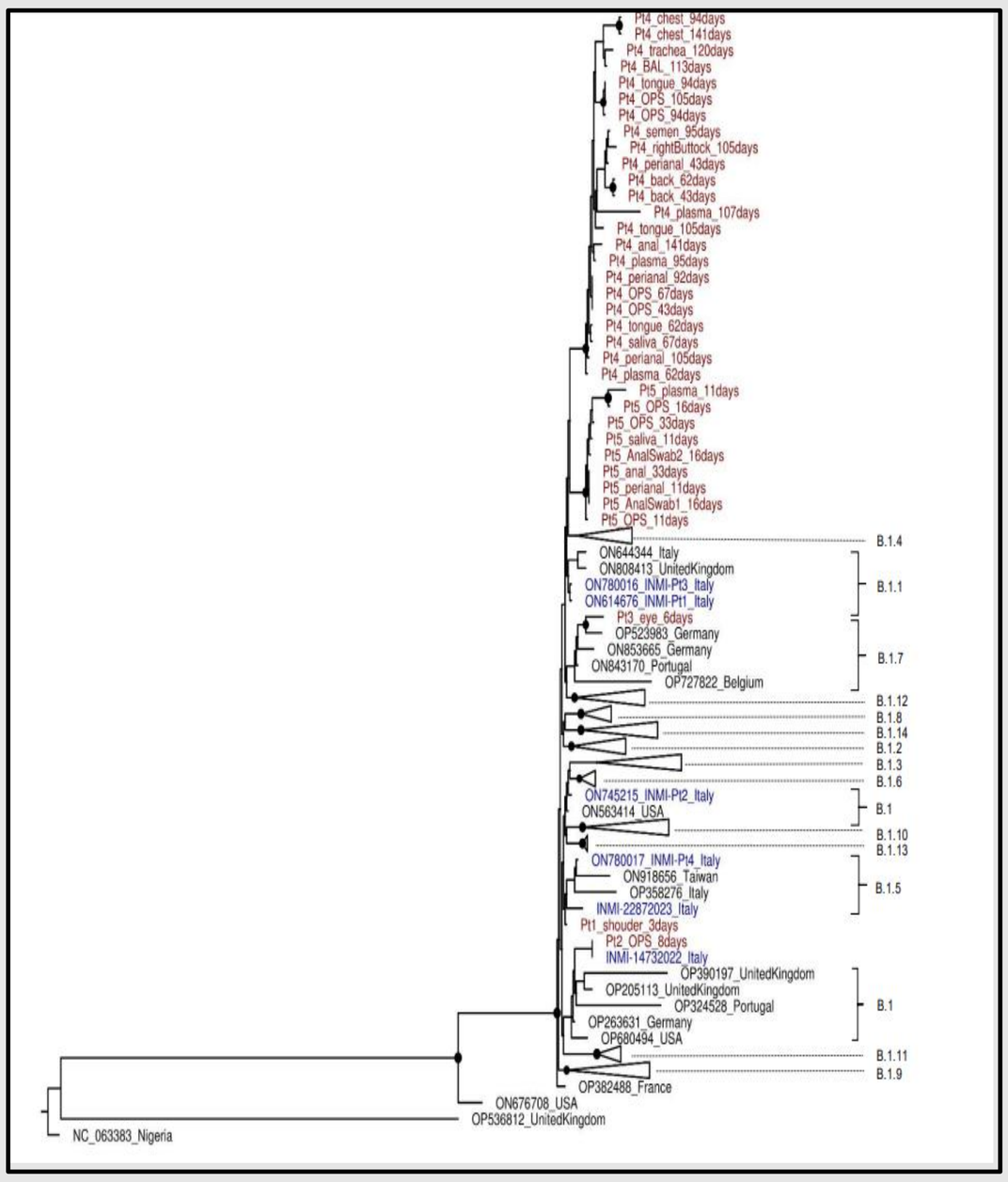
METHODS

- Two pools of primers for a total of 163 amplicons with a medium length of 2000 bps were used to amplify MPXV genomes.
- Libraries were prepared starting from 10-100 ng of amplified DNA, and sequencing was performed on the Gene Studio S5 Prime Sequencer to obtain 1 million reads/sample.
- Reads longer than 50 nucleotides and with a mean Phred score of at least 20 were mapped on the MPXV genome USA-2022-MA001 using the bwa-mem aligner software. Consensus sequences were then reconstructed by a homemade script.
- Phylogenetic analysis was performed with IQ-TREE26; HKY and FreeRate models with empirical base frequencies were selected with ModelFinder (5000 Bootstrap replicates).
- The hierarchical clustering analysis (HCA) was performed using all positions found mutated in at least one consensus sequence and including all minority variants present in the other samples of the same patient (with a minimum coverage of 50 reads; only positions present in at least 50% of the samples were considered).

RESULTS

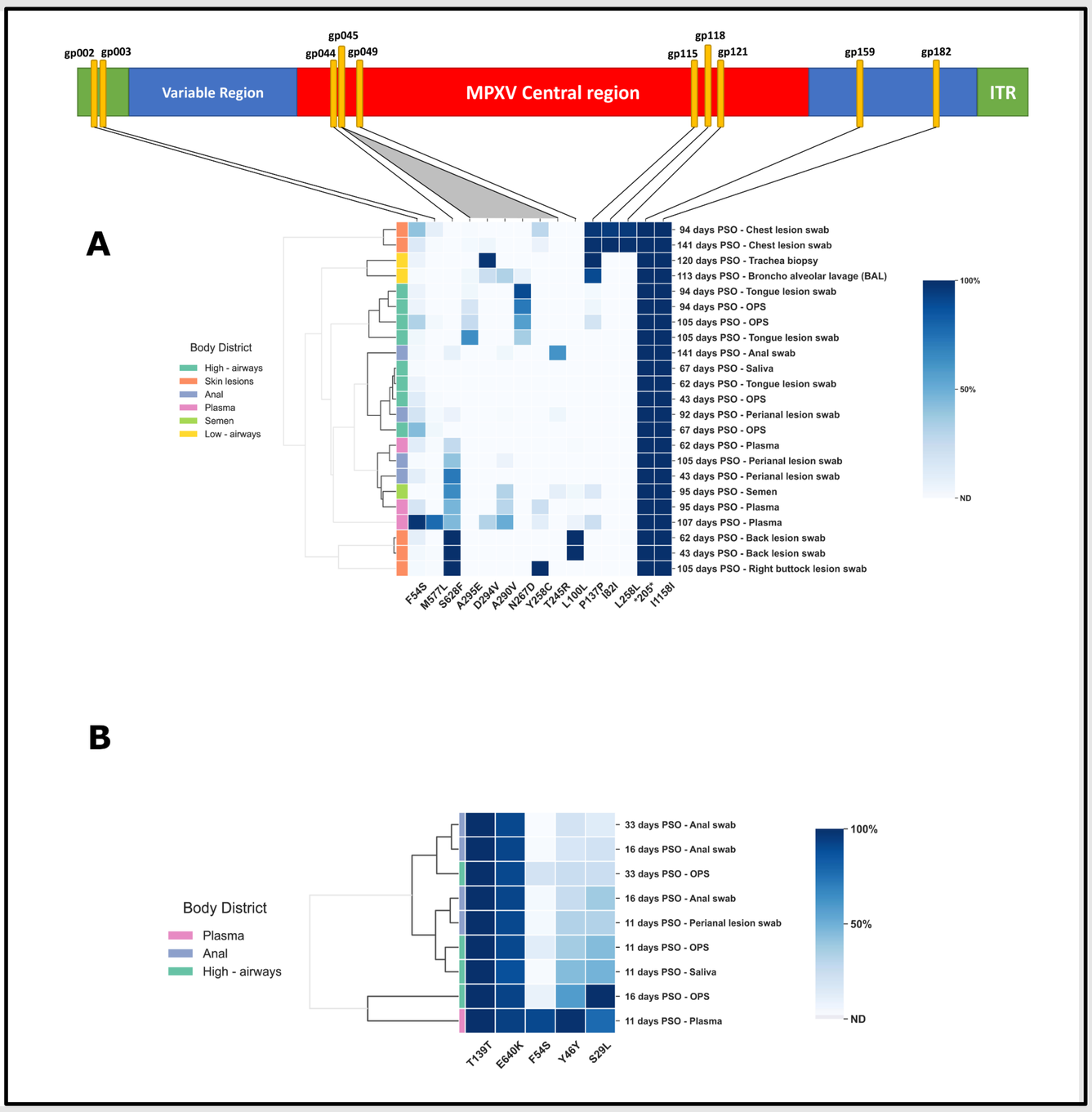
43 MPXV full-length genomes were obtained from 5 patients (three immunocompetent patients, Pt 1, Pt 2, and Pt 3; and two immunocompromised patients with HIV advanced infection and viral shedding of 141 and 33 days respectively, for Pt 4 and Pt 5): 7 genomes from Pt 1(4 from skin lesions, 1 from OPS, feces, and saliva), 2 from Pt 2 (2 OPS), 2 from Pt 3 (1 OPS, 1 ocular swab), 23 from Pt 4 (8 skin lesions, 4 anal swabs, 3 plasma, 4 OPS, 1 saliva, 1 semen, 1 BAL, 1 trachea biopsy), and 9 from Pt 5 (4 anal swabs, 3 OPS, 1 from plasma, 1 saliva).

Intra-patient genomes were identical in Pt 1 (7 sequences), Pt 2 (2 sequences), and Pt 3 (2 sequences) regardless of sample type and collection time. On the contrary, MPXV genomes sequenced from different samples of Pt 4 and Pt 5 presented differences in consensus sequences obtained from samples of a single patient: In each patient, 2 mutations were shared among all samples; in contrast, 14 and 3 substitutions (in Pt 4 and Pt 5, respectively) were distributed among different samples over time.



PHYLOGENETIC ANALYSIS

- All the MPXV sequences belong to the B.1 lineage, with those infecting Pt 3 clustered in the B.1.7 sub-lineage
- MPXV sequences from each patient clustered separately from those of the others and were interspersed among the foreign and Italian reference strains.
- Pt 4 and 5, which contained the greatest number of MPXV genomes, revealed distinct phylogenetic clusters of their sequences that branched off from a specific initial node with significant bootstrap and demonstrated clear intra-patient evolution.



HIERARCHICAL CLUSTER ANALYSIS

- HCA was performed for sequences from different Pt 4 (A) and 5 (B) samples with the goal of better characterizing the observed genetic intra-host variability and clustering the sequences based on mutational patterns.
- MPXV sequences from skin lesions (i.e., chest, back, and right buttock) revealed different sets of synonymous mutations that remain constant over time when considering the specific lesion localization.
- Other districts (upper and lower respiratory tracts, anal and perianal tracts, plasma): the analysis revealed genetic clusters based on the tissue compartmentalization of the sequences and their time of collection.
- Plasma: higher variability

CONCLUSION

Our results support the notion of structured compartmentalization of the viral populations circulating within an MPXV-infected host and they point out the role of the immunological status of the patient in favouring the evolution of such a phenomenon. Immunocompromised status and prolonged viral shedding led to a different evolution of MPXV in different body districts, and compartmentalized replication may be observed with the presence of specific mutations in lesion samples collected over time. Further investigations are needed to understand the possible clinical and therapeutic implications of the intra-host genomic variability of MPXV.