Preplanned Studies

Emergence of Lineage E.4 and Structural Plasticity of A28L Protein in Mpox Virus: Characterization of LCR7 Length Polymorphisms Across Lineages — Shenzhen City, Guangdong Province, China, 2023–2025

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Summary

What is already known about this topic?

The A28L protein is involved in mpox virus (MPXV) attachment and fusion, is a major target of neutralizing antibodies, and contains regions prone to genetic variation.

What is added by this report?

Analysis of 6,834 global and 260 Shenzhen MPXV genomes identified lineage-specific length polymorphisms in the A28L low-complexity region (LCR7). Emerging E.4 viruses exhibited complex deletion patterns and a characteristic OPG153_E293Q substitution, indicating ongoing structural diversification.

What are the implications for public health practice?

Structural variation in A28L provides additional insight into MPXV evolution beyond conventional single-nucleotide analyses. Continued surveillance of these genomic features may improve monitoring of viral transmission and the emergence of new variants.

ABSTRACT

Introduction: A28L, a virion membrane protein involved in mpox virus (MPXV) attachment and fusion, is a major target of neutralizing antibodies and a hotspot for genetic variation. This study investigated variations in its low-complexity region (LCR7) among MPXV strains circulating in Shenzhen, China.

Methods: A total of 6,834 publicly available MPXV clade IIb genomes from the Global Initiative on Sharing All Influenza Data (GISAID) were analyzed, and whole-genome sequencing was performed on 260 clinical isolates collected in Shenzhen during 2023–2025. LCR7 polymorphisms in OPG153/A28L and associated amino acid substitutions were characterized.

Results: Global genomic analysis identified a conserved four-residue deletion (D382–D385) in LCR7 that was prevalent in lineage A viruses. In contrast, the dominant B.1 lineage and its derivatives, including all Shenzhen isolates, exhibited heterogeneous truncations, most commonly a single D385 deletion. Among locally circulating E.4 viruses, complex deletion patterns (e.g., D383–D385 and D384–D385) and a characteristic amino acid substitution, OPG153_E293Q, were identified, the latter representing a lineage-defining mutation.

Conclusion: MPXV evolution in Shenzhen was characterized by LCR7 structural plasticity and the accumulation of lineage-specific amino acid substitutions, supporting the genomic accordion model of viral adaptation. Continued surveillance of these genomic features is essential for monitoring local transmission and informing public health interventions.

Mpox virus (MPXV), a double-stranded DNA virus belonging to the *Orthopoxvirus* genus, has emerged as a significant public health threat since 2022 (*1*). Historically endemic to Central and West Africa, the virus has demonstrated an unprecedented capacity for human-to-human transmission, leading the World Health Organization to declare a Public Health Emergency of International Concern (PHEIC). The causative agent of the 2022 epidemic, classified as subclade IIb, is a descendant of an MPXV lineage traced back to Nigeria in 1971. A key feature of this outbreak has been a higher capacity for person-to-person transmission compared with clade I or subclade IIa MPXV, suggesting underlying genomic adaptations that enhance viral fitness in human populations (*2*–*3*).

A key to understanding orthopoxvirus evolution lies in their use of "genomic accordion" strategies (*4*). These viruses adapt rapidly to selective pressures through genome expansions and contractions, which can occur via gene amplification or single-nucleotide insertions/deletions within homopolymer stretches. Recent evidence suggests that low-complexity regions (LCRs), including short tandem repeats (STRs) and homopolymers, represent a third form of genomic accordion. These regions, often difficult to sequence and historically considered uninformative, exhibit higher entropy than single-nucleotide polymorphisms (SNPs) and are non-randomly distributed across the MPXV genome (*4*–*6*).

The A28L protein, encoded by the OPG153 gene, is a key component of the mature virion membrane. It mediates virion attachment to laminin, regulates viral egress, and functions in membrane fusion. A28L is also a major target of broadly neutralizing antibodies following MPXV infection or vaccination (*7*–*9*). During the evolution of MPXV clades I and II, mutations have been observed at specific sites in the A28L gene. In addition, the low-complexity region 7 (LCR7), which encodes a poly-aspartic acid tract within the coding sequence, shows variable truncation. This suggests that LCR7 may act as a rapid evolutionary mechanism potentially affecting gene expression, protein stability, or function in a manner consistent with the genomic accordion model (*4*,*6*–*8*).

After the first mpox case was reported in the United Kingdom in May 2022, the disease spread rapidly worldwide. In 2022, MPXV cases were detected in at least 110 countries. The predominant circulating strains belonged to clade IIb B.1 and its descendant lineages. As a major international port megacity in southern China, Shenzhen reported its first locally transmitted mpox case in June 2023. Subsequently, the city experienced rapid local transmission, with a shift from the B.1 lineage to C.1.1. By late 2024 and early 2025, additional lineages such as E.3 and E.4 emerged (data source: https://nextstrain.org/mpox/clade-IIb, accessed December 29, 2025). Previous studies have focused mainly on site-specific mutations in the MPXV genome, while often overlooking variation in LCRs (*2*–*3*). In A28L, LCR7 is located within the coding region (the LCR7 $[ATC]_{14}TATGAT[ATC]_3$ motif spans positions 136513–136569, reference: NC_063383). Truncation of LCR7 may represent an adaptive evolutionary strategy. Therefore, enhanced surveillance of variation in this gene is warranted.

To systematically investigate variation in A28L among MPXV subclade IIb strains, all publicly available high-coverage subclade IIb genomes were retrieved from the Global Initiative on Sharing All Influenza Data (GISAID) database (accessed January 11, 2026). After quality filtering and lineage assignment using Nextclade CLI (v3.9.0) (*10*), 6,834 genomes were included. Of these, 381 (5.57%) belonged to lineage A and its descendants, while 6,453 (94.43%) belonged to lineage B.1 and its descendants. Initial screening identified a polymorphic low-complexity region (LCR7) within OPG153, showing structural variation relative to the reference strain (NC_063383.1). This region, containing an [ATC]n motif corresponding to amino acid residues 367–385, showed a conserved deletion spanning D382–D385 in lineage A strains, whereas lineage B.1 and its descendants predominantly exhibited truncation at D385 only. The prevalence of full-length LCR7 was significantly lower in lineage B.1 than in lineage A strains (Figure 1).

As of May 2025, the Shenzhen Center for Disease Control and Prevention (Shenzhen CDC) had detected at least 260 mpox cases in the region. Whole-genome sequencing showed that all viruses belonged to lineage B.1 or its descendants. The lineage distribution comprised B.1 (1/260), C.1 (10/260), C.1.1 (142/260), C.1.3 (5/260), E.1 (1/260), E.3 (47/260), E.4 (51/260), and F.2 (3/260). The single D385 deletion was identified only in C.1 (n=1) and C.1.1 (n=3) strains. Among E.4 viruses, deletions of D383–D385 (n=2) and D384–D385 (n=2) were observed (Figure 2).

Phylogenetic analysis of 260 local viral genomes showed that E.3 and E.4 cluster within a larger clade with C.1.1, suggesting local persistence alongside repeated importations. Both E.3 and E.4 lineages appear to have evolved from C.1.1. Genomic analysis identified characteristic amino acid substitutions in these sub-lineages (Figure 3). In all E.4 strains, mutations including OPG023_R280K, OPG061_H27Y, OPG092_E155K, OPG137_R107C, and OPG153_E293Q were nearly ubiquitous (Figure 3). Additional divergence within E.4 included mutations such as OPG015_S3L, OPG043_P139L, OPG059_E30K, OPG087_D39N, and OPG122_N133D, which were present only in subsets of strains. Notably, OPG153_E293Q, located in the linker region between two α-helices within the N-terminal globular domain of A28L (*7*), requires further functional investigation.

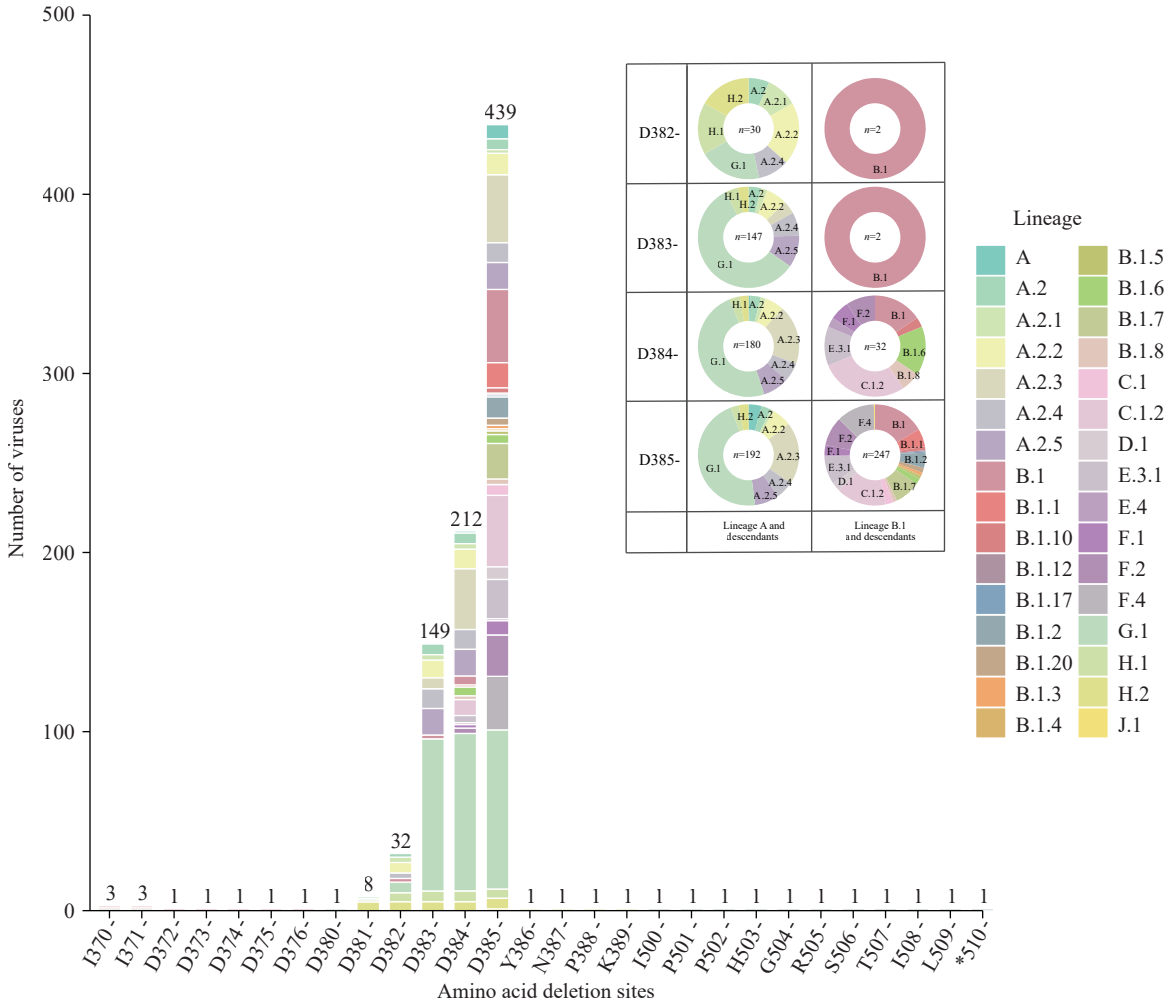

FIGURE 1. Deletion patterns in the A28L LCR7 region among clade IIb MPXV genomes from GISAID.
Note: A total of 6,834 clade IIb MPXV genomes were retrieved from the GISAID database. Following lineage assignment and variant analysis using Nextclade CLI (v3.9.0, https://github.com/nextstrain/nextclade) with the reference strain NC_063383.1, deletion mutations in A28L were identified and visualized. The x-axis shows amino acid deletion sites in A28L, and the y-axis shows the number of genomes carrying each deletion. Colors indicate sub-lineages. The LCR7 region corresponds to amino acid positions 367−385 of A28L. In the inset, genomes with deletions in the D382−D385 region are grouped as either Lineage A and its descendants (*n*=381) or Lineage B.1 and its descendants (*n*=6,453).
Abbreviation: LCR7=low-complexity region 7; MPXV=mpox virus; GISAID=Global Initiative on Sharing All Influenza Data.

DISCUSSION

The A28L protein, encoded by OPG153, is a pivotal structural and functional component of the MPXV life cycle (*8*). Located within the mature virion membrane, it mediates laminin-dependent attachment, facilitates virion egress, and drives membrane fusion during host cell entry, thereby contributing to infectivity and tropism. A28L is also a major target of broadly neutralizing antibodies following infection or vaccination (*7*–*8*), underscoring its importance in viral pathogenesis and immune recognition. Understanding its genetic variability is therefore essential for elucidating viral adaptation.

While Monzón et al. (*4*) proposed the genomic accordion model in global strains and Zhang et al. (*11*) characterized the emergence of C.1.1 in Shenzhen, this study reports lineage-specific structural variation in A28L, particularly in E.4. Two key patterns consistent with the genomic accordion model were observed: lineage-specific deletion patterns within LCR7 and the emergence of characteristic amino acid substitutions such as OPG153_E293Q in locally dominant lineages.

The transition from imported B.1 lineages to locally sustained sub-lineages such as C.1.1, E.3, and E.4 reflects ongoing viral adaptation in a non-endemic urban environment. Consistent with previous reports identifying D382–D385 as a hotspot, lineage A

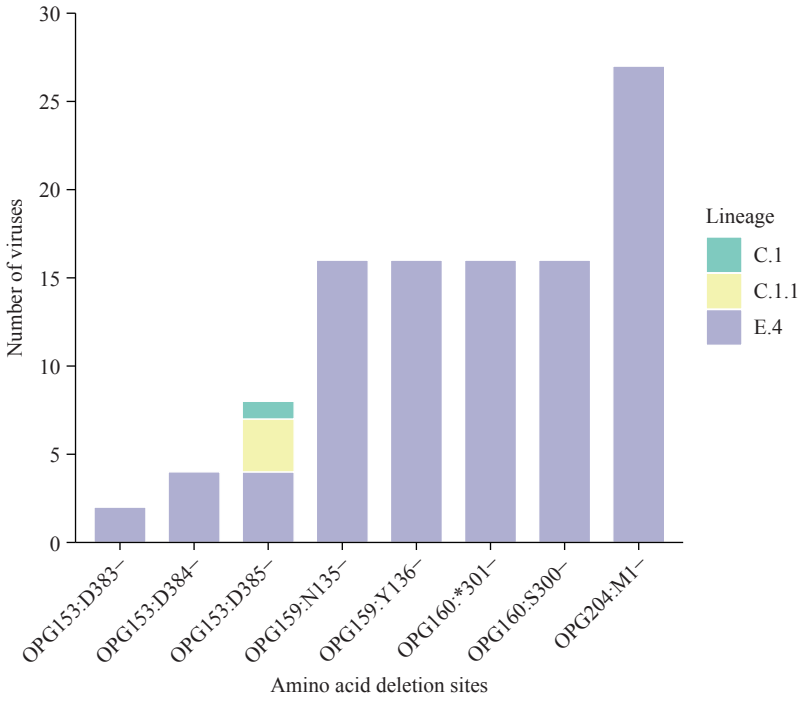

FIGURE 2. Deletion patterns in the A28L LCR7 region among MPXV genomes identified in Shenzhen, China, 2023−2025.
Note: A total of 260 whole-genome sequences obtained in Shenzhen during 2023−2025 were analyzed for deletion mutations in A28L. The x-axis shows amino acid deletion sites, and the y-axis shows the number of genomes carrying each deletion. Colors indicate sub-lineages.
Abbreviation: MPXV=mpox virus; LCR7=low-complexity region 7.

exhibited a conserved four-residue deletion (D382–D385), whereas B.1-derived strains, including all Shenzhen isolates, showed heterogeneous truncations primarily at D385. This plasticity within a homopolymer-rich region supports the genomic accordion model, in which LCRs function as tunable elements enabling rapid adaptation without accumulation of deleterious SNPs. The higher entropy of LCR7 relative to SNPs further supports its role in short-term evolution, likely influenced by host immune pressure and transmission bottlenecks (*4*). The more complex deletions observed in E.4 (D383–D385 and D384–D385) highlight ongoing diversification and suggest potential utility as a high-resolution marker for lineage tracking. These findings support the integration of LCR surveillance into routine genomic monitoring, as SNP-based approaches may miss rapidly emerging structural variation.

Several limitations should be acknowledged. First, the functional consequences of LCR7 variation and A28L substitutions remain experimentally unverified. Second, the dataset is geographically restricted, and broader surveillance is needed to determine whether these patterns are local or widespread. Finally, interactions between A28L variation and other genomic elements remain unclear and warrant further study.

In conclusion, this study shows that MPXV evolution in Shenzhen is characterized by structural variation in the A28L LCR7 region and the accumulation of lineage-specific amino acid substitutions. These findings support genomic surveillance approaches that capture both SNPs and structural polymorphisms, particularly within LCRs, to improve monitoring of transmission dynamics and inform public health responses.

Conflicts of interest: No conflicts of interest.

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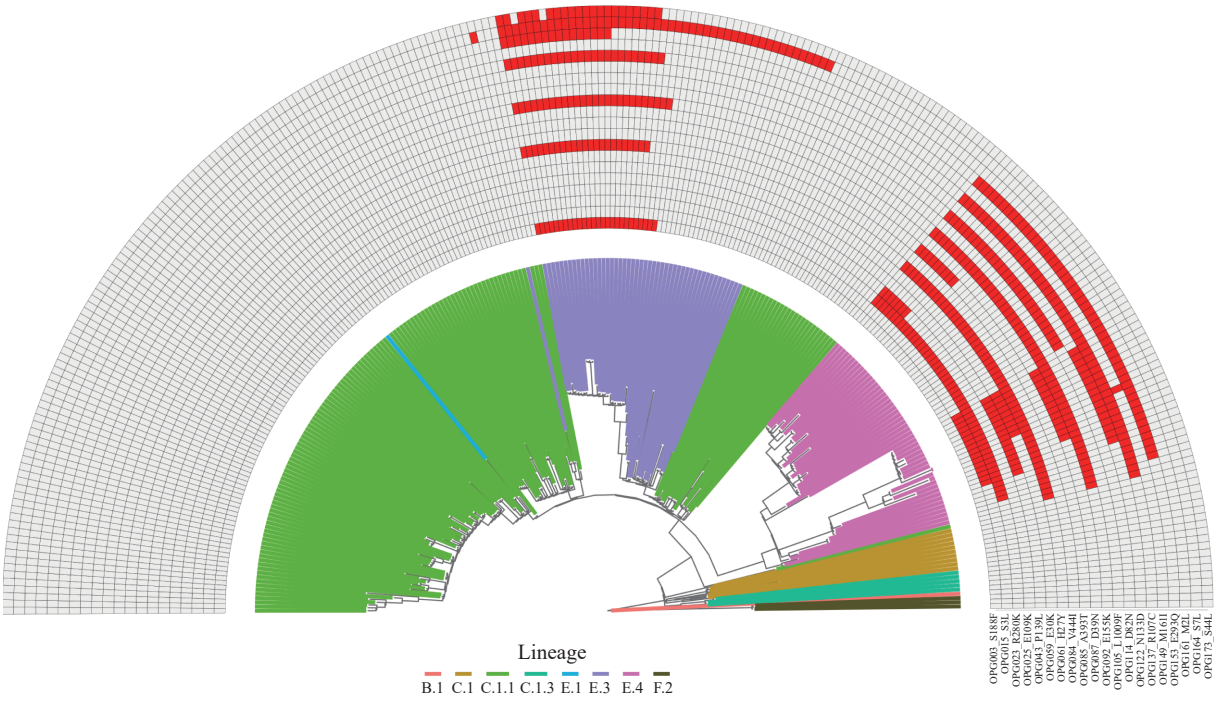

FIGURE 3. Maximum-likelihood phylogeny of MPXV based on whole-genome sequences.
Note: The phylogenetic tree was constructed from 260 MPXV genomes identified in Shenzhen, China, during 2023−2025 using IQ-TREE (v2.1.2, https://github.com/iqtree/iqtree2) under the GTR+F model with 1,000 bootstrap replicates. The scale bar represents nucleotide substitutions per site. Branch colors indicate viral sub-lineages. The heatmap above the tree shows characteristic amino acid substitutions identified in the E.3 and E.4 lineages. Red indicates the presence of a mutation and white indicates its absence.
Abbreviation: MPXV=mpox virus.

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