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Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak

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In its largest outbreak, Ebola virus disease is spreading through Guinea, Liberia, Sierra Leone, and Nigeria. We sequenced 99 Ebola virus genomes from 78 patients in Sierra Leone to ~2000× coverage. We observed a rapid accumulation of interhost and intrahost genetic variation, allowing us to characterize patterns of viral transmission over the initial weeks of the epidemic. This West African variant likely diverged from central African genetic variation, allowing us to characterize patterns of viral transmission over the initial weeks of the epidemic. This West African variant likely diverged from central African genetic variation, allowing us to characterize patterns of viral transmission over the initial weeks of the epidemic. This West African variant likely diverged from central African genetic variation, allowing us to characterize patterns of viral transmission over the initial weeks of the epidemic. This West African variant likely diverged from central African genetic variation, allowing us to characterize patterns of viral transmission over the initial weeks of the epidemic. This West African variant likely diverged from central African genetic variation, allowing us to characterize patterns of viral transmission over the initial weeks of the epidemic. This West African variant likely diverged from central African genetic variation, allowing us to characterize patterns of viral transmission over the initial weeks of the epidemic. This West African variant likely diverged from central African genetic variation, allowing us to characterize patterns of viral transmission over the initial weeks of the epidemic. This West African variant likely diverged from central African genetic variation, allowing us to characterize patterns of viral transmission over the initial weeks of the epidemic. This West African variant likely diverged from central African genetic variation, allowing us to characterize patterns of viral transmission over the initial weeks of the epidemic. This West African variant likely diverged from central African genetic variation, allowing us to characterize patterns of viral transmission over the initial weeks of the epidemic. This West African variant likely diverged from central African genetic variation, allowing us to characterize patterns of viral transmission over the initial weeks of the epidemic. This West African variant likely diverged from central African genetic variation, allowing us to characterize patterns of viral transmission over the initial weeks of the epidemic. This West African variant likely diverged from central African genetic variation, allowing us to characterize patterns of viral transmission over the initial weeks of the epidemic. This West African variant likely diverged from central African genetic variation, allowing us to characterize patterns of viral transmission over the initial weeks of the epidemic. This West African variant likely diverged from central African genetic variation, allowing us to characterize patterns of viral transmission over the initial weeks of the epidemic. This West African variant likely diverged from central African genetic variation, allowing us to characterize patterns of viral transmission over the initial weeks of the epidemic.

Ebola virus (EBOV; formerly Zaire ebolavirus), one of five ebolaviruses, is a lethal human pathogen, causing Ebola virus disease (EVD) with an average case fatality rate of 70% (7). Previous EVD outbreaks were confined to remote regions of central Africa; the largest, in 1976, had 318 cases (2) (Fig. 1A). The current outbreak started in 2014 in Guinea, West Africa (3) and spread into Liberia in March, Sierra Leone in May, and Nigeria in late July. It is the largest known EVD outbreak and is expanding exponentially, with a doubling period of 34.8 days (Fig. 1B). As of 19 August 2014, 2240 cases and 1229 deaths have been documented (4, 5). Its emergence in the major cities of Conakry (Guinea), Freetown (Sierra Leone), Monrovia (Liberia), and Lagos (Nigeria) raises the specter of increasing local and international dissemination.

In an ongoing public health crisis, where accurate and timely information is crucial, new genomics technologies can provide near–real-time insights into the pathogen’s origin, transmission dynamics, and evolution. We used massively parallel viral sequencing to understand how and when EBOV entered human populations in the 2014 West African outbreak, whether the outbreak is continuing to be fed by new transmissions from its natural reservoir, and how the virus changed, both before and after its recent jump to humans.

In March 2014, Kenema Government Hospital (KGH) established EBOV surveillance in Kenema, Sierra Leone, near the origin of the 2014 outbreak (Fig. 1C and fig. S1) (6). Following standards for field-based tests in previous (7) and current (3) outbreaks, KGH performed conventional polymerase chain reaction (PCR)–based EBOV diagnostics (8) (fig. S2); all tests were negative through early May. On 25 May, KGH scientists confirmed the first case of EVD in Sierra Leone. Investigation by the Ministry of Health and Sanitation (MoHS) uncovered an epidemiological link between this case and the burial of a traditional healer who had treated EVD patients from Guinea. Tracing led to 13 additional cases—all females who attended the burial. We obtained ethical approval from MoHS, the Sierra Leone Ethics and Scientific Review Committee, and our U.S. institutions to sequence patient samples in the United States according to approved safety standards (6).

We evaluated four independent library preparation methods and two sequencing platforms.

Fig. 1. Ebola outbreaks, historical and current. (A) Historical EVD outbreaks, colored by decade. Circle area represents total number of cases (RC = Republic of the Congo; DRC = Democratic Republic of Congo). (B) 2014 outbreak growth (confirmed, probable, and suspected cases). (C) Spread of EVD in Sierra Leone by district. The gradient denotes number of cases; the arrow depicts likely direction. (D) EBOV samples from 78 patients were sequenced in two batches, totaling 99 viral genomes [replication = technical replicates (6)]. Mean coverage and median depth of coverage with range are shown. (E) Combined coverage (normalized to the sample average) across sequenced EBOV genomes.

†These authors contributed equally to this work. §These authors jointly supervised this work.
We also sequenced 35 samples from suspected EVD cases that tested negative for EBOV; genomic analysis identified other known pathogens, including Lassa virus, HIV-1, enterovirus A, and malaria parasites (fig. S3).

In total, we generated 99 EBOV genome sequences from 78 confirmed EVD patients, representing more than 70% of the EVD patients diagnosed in Sierra Leone from late May to mid-June; we used multiple extraction methods or time points for 13 patients (table S2). Median coverage was >2000×, spanning more than 99.9% of EBOV coding regions (Fig. 1, D and E, and table S2).

We combined the 78 Sierra Leonean sequences with three published Guinean samples (3) [correcting 21 likely sequencing errors in the latter (6)] to obtain a data set of 81 sequences. They
reveal 341 fixed substitutions (35 nonsynonymous, 173 synonymous, and 133 noncoding) between the 2014 EBOV and all previously published EBOV sequences, with an additional 55 single-nucleotide polymorphisms (SNPs; 15 nonsynonymous, 25 synonymous, and 15 noncoding), fixed within individual patients, within the West African outbreak. Notably, the Sierra Leonean genomes differ from PCR probes for four separate assays used for EBOV and pan-filovirus diagnostics (table S3).

Deep-sequencing coverage allowed identification of 263 iSNVs (73 nonsynonymous, 108 synonymous, 70 noncoding, and 12 frameshift) in the Sierra Leone patients (6). For all patients with multiple time points, consensus sequences were identical and iSNV frequencies remained stable (fig. S4). One notable intrahost variation is the RNA editing site of the glycoprotein (GP) gene (fig. S5A) which we characterized in patients (6).

Phylogenetic comparison to all 20 genomes from earlier outbreaks suggests that the 2014 West African virus likely spread from central Africa within the past decade. Rooting the phylogeny using divergence from other ebolavirus genomes is problematic (Fig. 2A and fig. S6) (6, 17).

Fig. 4. Viral dynamics during the 2014 outbreak. (A) Mutations, one patient sample per row; beige blocks indicate identity with the Kissidougou Guinean sequence (GenBank accession KJ660346). The top row shows the type of mutation (green, synonymous; pink, nonsynonymous; gray, intergenic), with genomic locations indicated above. Cluster assignments are shown at the left. (B) Number of EVDbased patients per day, colored by cluster. Arrow indicates the first appearance of the derived allele at position 10,218, distinguishing clusters 2 and 3. (C) Intra host frequency of SNP 10,218 in all 78 patients (absent in 28 patients, polymorphic in 12, fixed in 38). (D and E) Twelve patients carrying iSNV 10,218 cluster geographically and temporally (HCW-A = unsequenced health care worker; Driver drove HCW-A from Kissi Tenga to Jawie, then continued alone to Mambolo; HCW-B treated HCW-A). KGH = location of Kenema Government Hospital. (F) Substitution rates within the 2014 outbreak and between all EVD outbreaks. (G) Proportion of nonsynonymous changes observed on different time scales (green, synonymous; pink, nonsynonymous). (H) Acquisition of genetic variation over time. Fifty mutational events (short dashes) and 29 new viral lineages (long dashes) were observed (intrahost variants not included).
However, rooting the tree on the oldest outbreak reveals a strong correlation between sample date and root-to-tip distance, with a substitution rate of $8 \times 10^{-5}$ per site per year (Fig. 2B and fig. S7) (25). This suggests that the lineages of the three most recent outbreaks all diverged from a common ancestor at roughly the same time, around 2004 (Fig. 2C and Fig. 3A), which supports the hypothesis that each outbreak represents an independent zoonotic event from the same genetically diverse viral population in its natural reservoir.

Genetic similarity across the sequenced 2014 samples suggests a single transmission from the natural reservoir, followed by human-to-human transmission during the outbreak. Molecular dating places the common ancestor of all sequenced Guinea and Sierra Leone lineages around late February 2014 (Fig. 3B), 3 months after the earliest suspected cases in Guinea (Fig. 3B), predating their co-appearance in Sierra Leone (Fig. 4G). Similar findings have been seen previously (15) and are consistent with expectations from incomplete purifying selection (16–18). Determining whether individual mutations are deleterious, or even adaptive, would require functional analysis; however, the rate of non-synonymous mutations suggests that continued progression of this epidemic could afford an opportunity for viral adaptation (Fig. 4H), underscoring the need for rapid containment.

As in every EVD outbreak, the 2014 EBOV variant carries a number of genetic changes distinct to this lineage; our data do not address whether these differences are related to the severity of the outbreak. However, the catalog of 395 mutations, including 50 fixed nonsynonymous changes with 8 at positions with high levels of conservation across ebolaviruses, provides a starting point for such studies (table S4).

To aid in relief efforts and facilitate rapid global research, we have immediately released all sequence data as it is generated. Ongoing epidemiological and genomic surveillance is imperative to identify viral determinants of transmission dynamics, monitor viral changes and adaptation, ensure accurate diagnosis, guide research on therapeutic targets, and refine public health strategies. It is our hope that this work will aid the multidisciplinary international efforts to understand and contain this expanding epidemic. In memoriam: Tragically, five co-authors, who contributed greatly to public health and research efforts in Sierra Leone, contracted EVD and lost their battle with the disease before this manuscript could be published: Mohamed Pullah, Mbaatu Fonnie, Alex Moigboi, Alice Kovoma, and S. Humayr Khan. We wish to honor their memory.

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SUPPLEMENTARY MATERIALS
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