Evolution patterns of the Middle East respiratory syndrome coronavirus (MERS-CoV) obtained from MERS patients in early 2015.

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In a collaborative effort between the Kingdom of Saudi Arabia (KSA) Ministry of Health, King Saud University and its affiliated academic hospital: King Khalid University Hospital (KKUH), Riyadh, the U.S. CDC, and the Wellcome Trust Sanger Institute, we have continued to monitor the evolution and transmission patterns of MERS-CoV.

**Summary.** A series of MERS-CoV infections occurred in February and March 2015 at King Khalid University Hospital (KKUH), Riyadh, KSA. As part of the outbreak investigation, upper respiratory samples from confirmed MERS cases and PCR positive contacts were amplified and subjected to deep sequencing with paired 149 nt Illumina MiSeq data followed by *de novo* assembly (see (1, 2)). 21 samples were processed with 18 samples yielding phylogenetically-useful sequences and 8 yielding full MERS-CoV genomes (>28000 nt). The novel 2015 MERS-CoV sequences are available at the following link.
**(http://tinyurl.com/MERS-CoV-4Jun15)**

**Disclaimer.** These sequence data are being made publically available with the hope that they are useful for other scientists and the caveat that they are preliminary and still undergoing quality control. A manuscript analyzing the phylogenetic patterns in more detail is in preparation. If investigators would like to use these sequence data for publication prior to release of our paper, please contact us.

**Phylogenetic patterns.** The 2015 MERS-CoV sequences cluster within a novel lineage related to the Hafr-Al-Batin_1 clade (Figure 1). The genetic distance from other known MERS-CoV sequences is consistent with the novel lineage stemming from a separate zoonotic event.

**Spike protein changes.** It is important to monitor evolution in the coronavirus spike region. Changes in the spike protein accompanied the evolution of the SARS coronavirus and may be

an important marker of increased transmissibility. None of the amino acid changes previously observed in the MERS-CoV spike protein in previous years have persisted and the proteins encoded by the recent Riyadh viruses are similar to the clade B ancestral sequence (Figure 2). This suggests that at least in the current samples, there is no evidence that a spike variant has emerged in human infections that provides a selective advantage over the variants circulating in the animal reservoir.

**Evolutionary rate.** The updated inferred evolutionary rate of the virus is $9.29x10^{-4}$ (95% CI: $7.19x10^{-4}$, $1.15x10^{-3}$). From the time-resolved phylogeny, the root of the tree (last common ancestor between human MERS and the Egyptian camels) is October 2010 (95% CI: January 2010, June 2011). The last common ancestor between clade A (EMC/2012 and Jordan-N3/2012) and B (rest of human isolates) is December 2010 (95% CI: March 2010, July 2011) and the root of clade B is December 2011(95% CI: July 2011, April 2012).

**References**

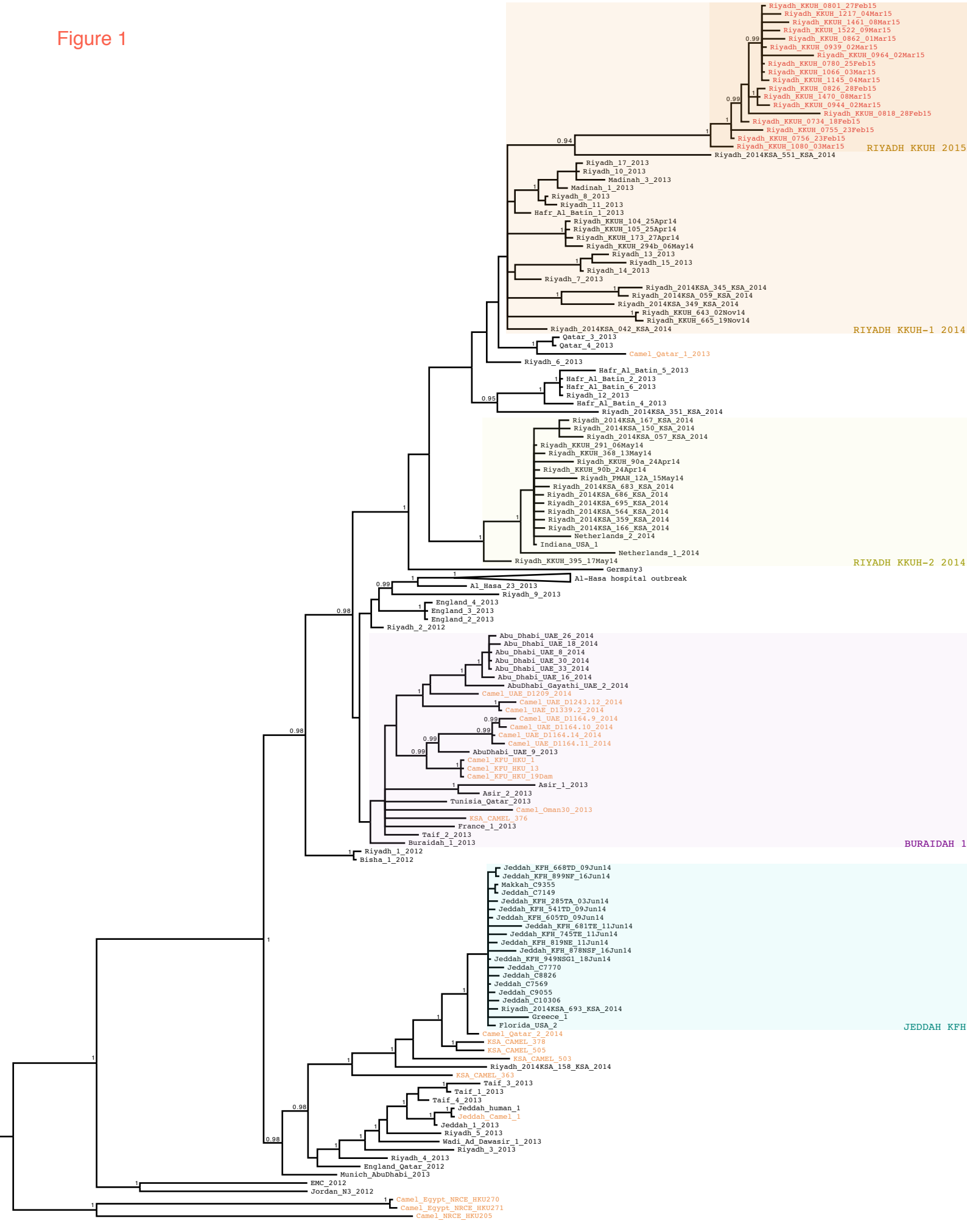

1. **Cotten M, Lam TT, Watson SJ, Palser AL, Petrova V, Grant P, Pybus OG, Rambaut A, Guan Y, Pillay D, Kellam P, Nastouli E.** 2013. Full-genome deep sequencing and phylogenetic analysis of novel human betacoronavirus. Emerg Infect Dis **19:**736-742B.
2. **Cotten M WS, Zumla AI, Makhdoom HQ, Palser AL, Ong SH, Al Rabeeah AA, Alhakeem RF, Assiri A, Al-Tawfiq JA, Albarrak A, Barry M, Shibl A, Alrabeah FA, Hajjar S, Balkhy HH, Flemban H, Rambaut A, Kellam P, Memish ZA.** 2014. Spread, circulation, and evolution of the Middle East respiratory syndrome coronavirus. mBio **5:**e01062-01013.
3. **Huelsenbeck JP, Ronquist F.** 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics **17:**754-755.


**Figure Legends**

**Figure 1.** Bayesian phylogeny of 170 MERS-CoV isolates inferred from a multiple sequence alignment of 30055 nucleotides. The phylogeny was generated using MrBayes v3.2.4 (3), running 3 Markov chain Monte Carlo runs for 2,000,000 states under a GTR+Γ substitution model. The 18 new sequences from 2015 are marked in red, while those isolated from camels are in orange. The Al-Hasa hospital outbreak sequences are collapsed to a single node for clarity. Viral clades (or lineages, for HAB_1) are indicated in colored boxes. Posterior probabilities are shown for nodes with a support >0.9. The scale bar indicates the genetic distance, in substitutions/site.

**Figure 2.** Protein changes in the spike encoded by recent MERS-CoV. All recent MERS-CoV spike ORFs were translated, the proteins were aligned and amino acid differences from the reconstructed ancestral Clade B protein were determined and marked by vertical colored bars and the changes observed in more than one genome are marked within the figure. Functional domains of the spike protein are indicated in the top panel and include the N-terminal domain, the receptor binding domain, the fusion domain (Fusion), the heptad repeats 1 and 2 (HR1 and HR2), the transmembrane (TM) and cytoplasmic (Endo) domain.

Figure 1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Riyadh_KKUH_1080_03Mar15
RIYADH KKUH 2015
Riyadh_2014KSA_551_KSA_2014
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Riyadh_10_2013
Madinah_3_2013
Madinah_1_2013
Riyadh_8_2013
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Qatar_4_2013
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Riyadh_6_2013
Hafr_Al_Batin_5_2013
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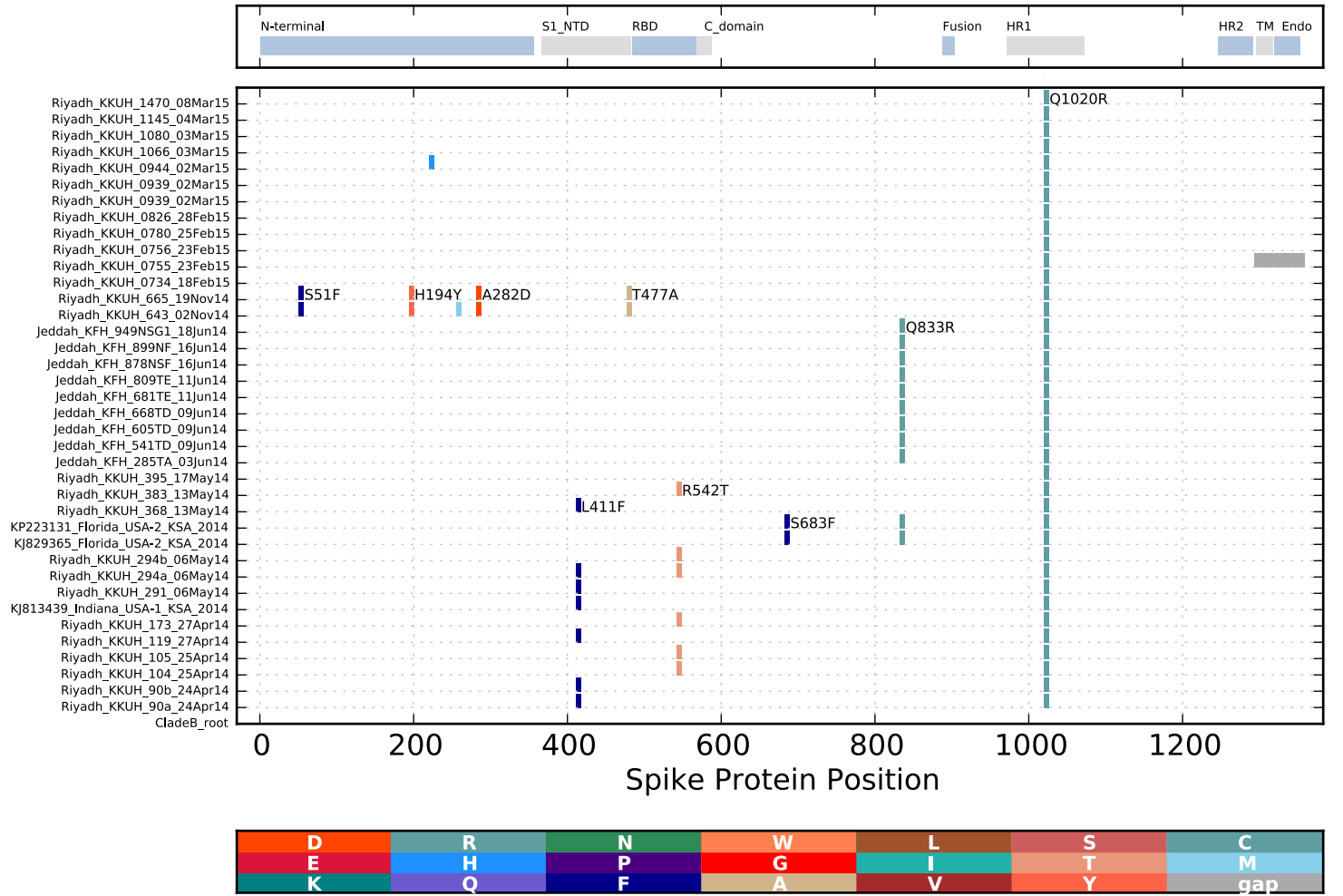
Figure 2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Spike Protein Position
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