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Short Communication

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Evolutionary relationship analysis of Middle East respiratory syndrome coronavirus 4a and 4b protein coding sequences

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ABSTRACT

The 4a and 4b proteins of the Middle East respiratory syndrome coronavirus (MERS-CoV) have been described for their antagonism on host innate immunity. However, unlike clustering patterns of the complete gene sequences of human and camel MERS-CoVs, the 4a and 4b protein coding regions did not constitute species-specific phylogenetic groups. Moreover, given the estimated evolutionary rates of the complete, 4a, and 4b gene sequences, the 4a and 4b proteins might be less affected by species-specific innate immune pressures. These results suggest that the 4a and 4b proteins of MERS-CoV may function against host innate immunity in a manner independent of host species and/or evolutionary clustering patterns.

Keywords: Middle East respiratory syndrome coronavirus; molecular evolution; phylogeny; zoonoses

Human infection with the Middle East respiratory syndrome coronavirus (MERS-CoV), a positive-sense single-stranded RNA virus of the family *Coronaviridae*, was first reported in 2012 [1]. Since then, more than 2,200 laboratory-confirmed cases have been described with an estimated 35.5% case-fatality rate [2]. Middle East countries appeared to be mainly affected by the life-threatening pathogenicity of the virus, but some exported cases were also reported in Asia, Europe, and the United States of America [1,3]. Given zoonotic transmission of the virus from dromedary camels to humans and its lethality in humans [1], vaccines and/or antivirals should be prepared, but no approved medical countermeasures are available, yet.

In line with the pathogenic mechanisms reported with MERS-CoV [4], such as acute respiratory symptoms and local immune responses, viral antagonism against host innate immune responses may be of great significance in terms of the pandemic potential and host range restriction of the virus. Viral antagonism had been already described for MERS-CoV [5], and, of the MERS-CoV proteins, protein 4a was suggested responsible for its role against RNA-dependent protein kinase-mediated antiviral immunity and activation of retinoic acid-inducible gene I and Melanoma Differentiation-Associated protein 5 of host cells [6,7]. The antagonistic effects of MERS-CoV protein 4b were also demonstrated against nuclear factor-κB-dependent immunity and RNase L activation of host cells [8,9]. As shown for influenza and Zika viruses [10,11], the species-specific effects of viral antagonism of MERS-CoV on

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Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

Conceptualization: Park MS; Data curation: Kim JI, Park MS; Formal analysis: Kim JI, Park S, Bae JY; Funding acquisition: Park MS; Supervision: Park MS; Validation: Kim JI, Park MS; Visualization: Kim JI, Park S, Bae JY; Writing - original draft: Kim JI; Writing - review & editing: Kim JI, Park MS.

host innate immunity can be revealed by analysis of the evolutionary relationship of the 4a and 4b genes between human and camel MERS-CoV strains. To this end, we investigated the evolutionary history and rates of MERS-CoV 4a and 4b protein coding regions as well as those of MERS-CoV complete gene sequences using a time-framed Bayesian inference method (BEAST package, v1.10.0; BEAST, New Zealand) [12] with individual settings of molecular clock (for complete gene, lognormal relaxed clock model and for 4a and 4b coding regions, strict clock model), nucleotide substitution (based on the estimated results of best-fit substitution model using jModeltest, v2.1.10: for complete gene, GTR + I + γ; for 4a, TN93 + I; and for 4b, TN93 + γ) [13], and Markov chain Monte Carlo runs (2×10^8 chain length with every 2×10^5 iterations after a 10% burn-in). The phylogenetic relationship of MERS-CoV complete gene sequences was reconstructed using a total of 260 sequences (human strains, n = 126 and camel strains, n = 134; sequence sets are available upon request) that were downloaded from the Virus Variation Resource database of National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/genome/viruses/variation/), and their maximum clade credibility trees were visualized using FigTree (v1.4.3; Figtree Systems Pty, Australia). Given the phylogenetic relationship of the complete gene sequences, most camel sequences constituted 5 different genetic groups (camel G1–G5), except for some distantly-related sequences, and the camel G2, G3, and G4 strains appeared to evolve exclusively from human strains. Transmission of MERS-CoV between humans and camels appeared to be identified only in the camel G1 and G5 groups (**Fig. 1**).

Unlike the phylogenetic clustering patterns of the complete gene sequences (**Fig. 1**), human and camel strains appeared to constitute common genetic groups in the 4a and 4b phylogenetic trees (**Fig. 2**). Neither human-specific nor camel-specific clustering patterns were observed in the 4a and 4b phylogenetic trees. Rather, the camel G2, G3, and G4 sequences, which constituted the camel-only phylogenetic groups in the complete gene sequence analysis (**Fig. 1**), were mixed with other camel sequences of different phylogenetic groups and with human sequences, and, from camel G1 to G5, all camel sequences were grouped together with human sequences (**Fig. 2**). These groupings may imply little or no species-specific antagonism of the human and camel MERS-CoV 4a and 4b proteins against host innate immune responses. Of course, not phylogenetic placements between different host viruses but certain genetic determinants may determine the viral antagonistic mechanism(s) in hosts. The other viral proteins or genetic mutations on the other viral proteins may also compensate for a lack of sufficient viral antagonism [14]. However, results of serological and epidemiological analyses indicate dromedary camels are one of the susceptible hosts of MERS-CoV, even though disease severity of MERS is quite different between humans and camels [1], and, given that the interaction mechanism(s) between viral surface protein and cellular receptors of hosts may also determine host range restriction [15], the effects of species-specific viral antagonism on host innate immunity might not be associated with the sustained dissemination of MERS-CoV in humans. Rather, a lack of sufficient viral antagonism of MERS-CoV in humans may be associated with the transmission of the virus from camels to humans, as indicated by the almost 2-fold increased evolutionary rate (1.42×10^{-3} substitutions/site/year) of the complete gene sequences of human and camel MERS-CoV strains (**Table 1**), compared with the previous study result (0.74×10^{-3} substitutions/site/year) reported by Kim et al. [3], which assessed only the human strain sequences. In contrast, the evolutionary rates of the 4a (1.89×10^{-3} substitutions/site/year) and 4b (1.44×10^{-3} substitutions/site/year) protein-coding regions of human and camel MERS-CoV strains were estimated to be almost in similar ranges with those (1.26×10^{-3} and 1.27×10^{-3} substitutions/site/year for the 4a and 4b protein coding regions, respectively)

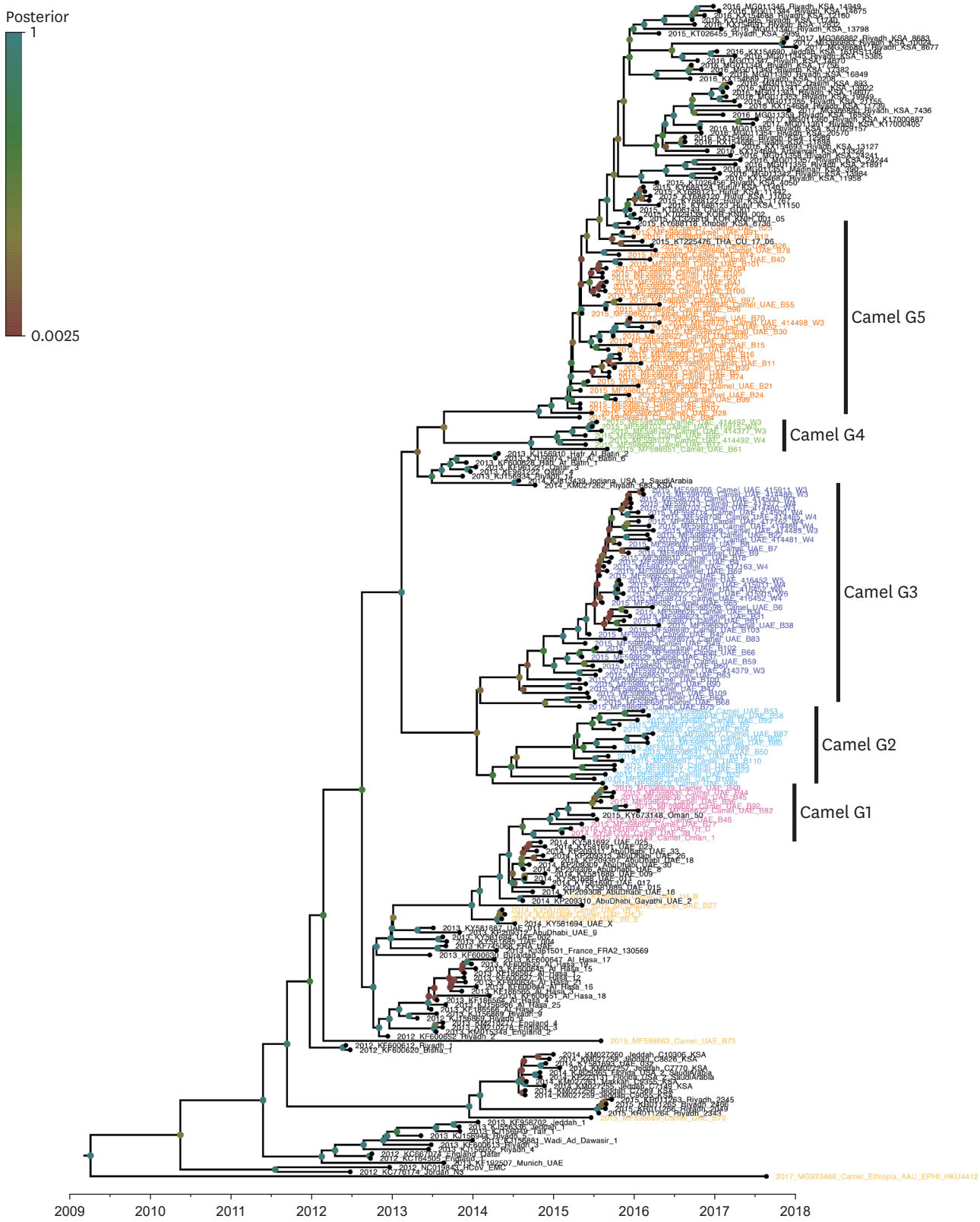

Fig. 1. Phylogenetic relationship of the complete genes of human and camel MERS-CoV strains. The complete gene sequences of human and camel MERS-CoV strains were evaluated for their evolutionary relationships. Human strain sequences were colored black, whereas camel sequences were colored differently based on their individual distinct groups (camel G1, magenta; G2, sky blue; G3, purple; G4, light green; and G5, orange). The other camel strains not included in the camel G1–G5 groups were colored cantaloupe. The color of each node indicates its posterior probability score (0.0025–1).
MERS-CoV, Middle East respiratory syndrome coronavirus.

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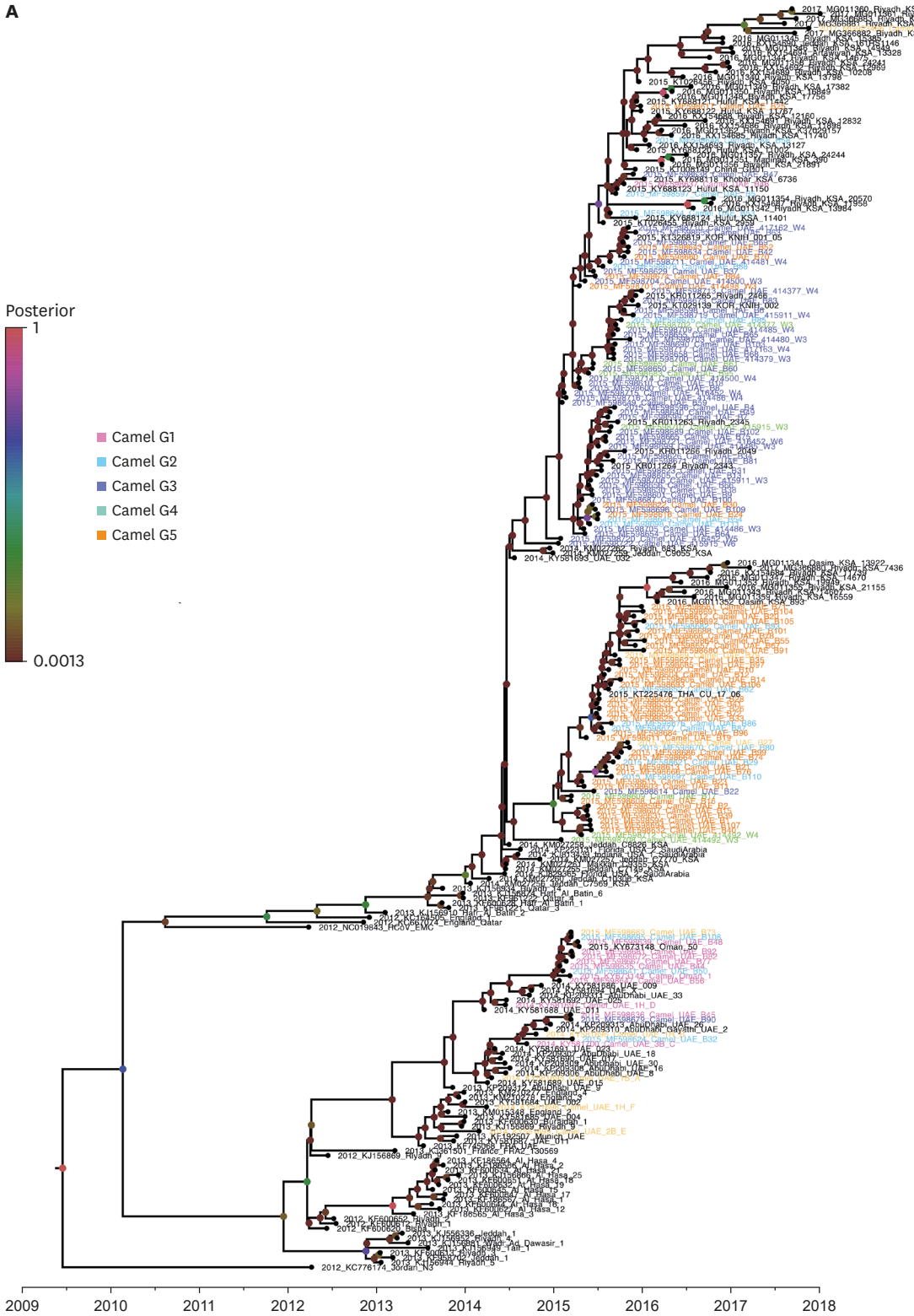

Fig. 2. Phylogenetic relationship of the 4a and 4b coding regions of human and camel MERS-CoV strains. The 4a (A) and 4b (B) coding region sequences of human and camel MERS-CoV strains were evaluated for their evolutionary relationships. Human strain sequences were colored black, and camel sequences were colored differently based on their corresponding complete gene sequence group, as indicated and as in **Fig. 1**. The color of each node indicates its posterior probability score (0.0013–1).
MERS-CoV, Middle East respiratory syndrome coronavirus.

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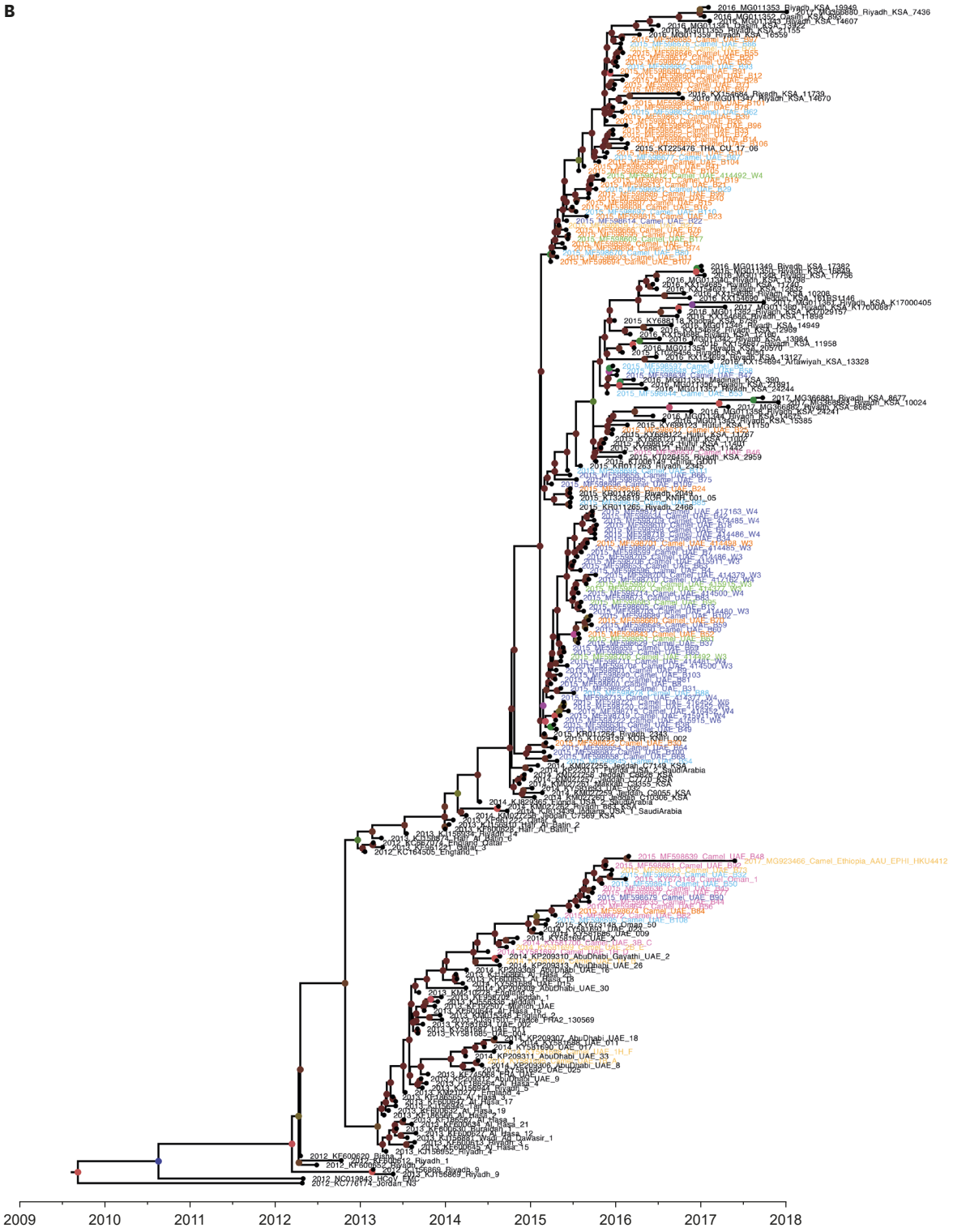

Fig. 2. (Continued) Phylogenetic relationship of the 4a and 4b coding regions of human and camel MERS-CoV strains. The 4a (A) and 4b (B) coding region sequences of human and camel MERS-CoV strains were evaluated for their evolutionary relationships. Human strain sequences were colored black, and camel sequences were colored differently based on their corresponding complete gene sequence group, as indicated and as in **Fig. 1**. The color of each node indicates its posterior probability score (0.0013–1).
MERS-CoV, Middle East respiratory syndrome coronavirus.

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Table 1. Estimated root years and evolutionary rates of the complete genes and the 4a and 4b coding regions of human and camel MERS-CoV strains

Gene (coding region)	tMRCA* (yr)		Evolutionary rate (10^{-3} substitutions/site/yr)	
	Mean	95% HPD†	Mean‡	95% HPD
Complete gene	2009.37	2006.35–2011.46	1.42 (0.74)	1.19–1.72
4a	2009.84	2006.67–2011.65	1.89 (1.26)	0.98–3.02
4b	2010.18	2008.17–2011.83	1.44 (1.27)	0.94–2.01

Values are presented as median (interquartile range) or number (%).
MERS-CoV, Middle East respiratory syndrome coronavirus; tMRCA, time to the most recent common ancestor; HPD, highest posterior density.
*The time of most recent common ancestor; †Lower and upper limits of 95% highest probability density; ‡Estimation results reported by Kim et al. [3] are indicated in parentheses.

reported by Kim et al. [3] (**Table 1**), which may suggest the absence of large immune-mediated pressure differences between human and camel MERS-CoV 4a and 4b proteins and no need for the adaptation of camel MERS-CoV strains prior to zoonotic dissemination to humans. As mentioned above, the effects of other viral proteins and molecular determinants of immune evasion mechanisms of MERS-CoVs on the zoonotic and reverse zoonotic transmission between humans and camels should be investigated further using *in vitro* and *in vivo* studies.

In this study, we investigated the evolutionary history and rates of the 4a and 4b protein coding sequences of human and camel MERS-CoV strains. By demonstrating that neither species-specific nor group-specific clustering patterns were observed in the 4a and 4b protein coding regions, we suggest that the antagonistic mechanism of MERS-CoV against host innate immunity might be mediated in a manner independent of host species and/or evolutionary clustering patterns. Given the disease severity and consequent public health threats of MERS, our results have expanded the knowledge of molecular evolution patterns and virus-host interactions of MERS-CoVs and provide information useful in the development of MERS-CoV vaccines.

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