We evaluated genetic variation in Middle East respiratory syndrome coronavirus (MERS-CoV) imported to South Korea in 2018 using specimens from a patient and isolates from infected Caco-2 cells. The MERS-CoV strain in this study was genetically similar to a strain isolated in Riyadh, Saudi Arabia, in 2017.

Between 2012 and the end of August 2018, a total of 2,248 laboratory-confirmed cases of Middle East respiratory syndrome coronavirus (MERS-CoV) infection and 798 associated deaths (case-fatality rate 35.3%) were reported in 27 countries (1). Most of these cases (83%) were reported in Saudi Arabia (1,871 cases, including 724 related deaths, for a case-fatality rate of 38.7%).

The 2015 MERS-CoV outbreak in South Korea resulted in 186 laboratory-confirmed cases and 38 deaths. MERS-CoV spread via intrahospital and interhospital transmission at 16 clinics and hospitals across South Korea (2). This outbreak was recorded as the largest MERS outbreak outside of the Middle East (3).

In August 2018, eight laboratory-confirmed cases of MERS-CoV infection were reported in Saudi Arabia, with 4 associated deaths; 1 case in a person who had travel history to the Arabian Peninsula was reported in the United Kingdom (4). In September 2018, a man in South Korea with a history of travel to the Middle East became ill and was suspected of having MERS-CoV infection. We report the investigation of this case and genetic characterization of the virus on the basis of variation and phylogenetic analyses of the spike (S) gene and the full-length genome from patient specimens and isolates from Caco-2 cells.

The Study
On September 7, 2018, a 61-year-old man who had moved from Kuwait to South Korea visited the hospital with diarrhea and fever (5). The patient had a history of visiting the Middle East and was suspected of MERS-CoV infection; accordingly, he was transferred to a hospital with a national isolation ward.

On September 8, we collected nasopharyngeal swab and sputum samples from the patient and transported them to the Seoul Provincial Institute of Public Health and Environment (Seoul, South Korea). Real-time reverse transcription PCR showed that the sputum was positive for the upstream regions of the E protein (upE) and orf1a genes (6). Later the same day, we collected nasopharyngeal swab, throat swab, and sputum samples from the patient for further confirmation. The Korea Centers for Disease Control and Prevention (KCDC) confirmed that throat swabs and sputum samples were positive for upE and orf1a.

We added throat swab and sputum samples from the patient to a monolayer of Caco-2, Huh 7, and Vero E6 cells, which we incubated at 37°C in 5% CO₂. We observed a cytopathic effect on the third day after inoculation in Caco-2 cells and confirmed viral replication of MERS-CoV in the supernatant of the infected cells by real-time reverse transcription PCR.

We extracted viral RNA from the specimens and the supernatant of the Caco-2 cells using the Viral RNA Mini Kit (QIAGEN, http://www.qiagen.com). We performed cDNA synthesis using the Superscript IV First-Strand synthesis system (Thermo Fisher Scientific, http://www.thermofisher.com) with random hexamers. We amplified cDNA by overlapping PCR to generate products of 600–1,100 bp covering the entire S gene and the full-length genome; we sequenced the resulting PCR amplicons by Sanger sequencing using an ABI 3730 Analyzer (Applied Biosystems, http://www.thermofisher.com).

We used ClustalW (http://www.clustal.org) to align the S gene sequences of the isolates from the patient, MERS-CoV/KOR/KCDC/001_2018/SP-1 (from sputum sample), TS-1 (from throat swab sample), and TSVi (from Caco-2 processing), with those of 157 other MERS-CoV strains from GenBank and the MERS-CoV sequence database (https://www.ncbi.nlm.nih.gov/
We sequenced and analyzed the full-length genome of MERS-CoV/KOR/KCDC/001_2018/TSVi and 39 other MERS-CoV strains. We constructed phylogenetic trees by the maximum-likelihood method with 1,000 bootstrap replicates using MEGA7 (https://www.megasoftware.net) and RAxML (https://github.com/stamatak/standard-RAxML). We constructed phylogenetic trees from the MERS-CoV S genes (4,062 bp) (Figure 1) and the full-length genome (30,150 bp) of the virus (Figure 2) obtained from the patient in this study and the most similar human MERS-CoV sequences from other countries (4).

We submitted viral S gene sequences MERS-CoV/KOR/KCDC/001_2018_SP-1, TS-1, TSVi from the patient to GenBank under accession nos. MH978886, MH978887, and MH978888. MERS-CoV/KOR/KCDC/001_2018 SP-1, TS, and TSVi were most closely related (99.85%–99.90% nucleotide identity) to a human MERS-CoV strain isolated in Riyadh, Saudi Arabia, in 2017 (Hu-Riyadh-KSA-9730_2017; GenBank accession no. MG912608).
KOR/KCDC/2018 SP-1 and TS had 5 nucleotide substitutions (C309T, T519C, C2928T, T3375C, and T3598C) compared with a MERS-CoV strain found in Riyadh in 2017, whereas MERS-CoV/KOR/KCDC/001_2018 TSVi had 3 nucleotide substitutions (C309T, C2928T, and T3375C) compared with the same strain. There were no substitutions in the N terminal domain (NTD) and receptor-binding domain (RBD) of the S gene in the same comparison (Table). We submitted the full-length genome sequences from MERS-CoV/KOR/KCDC/001_2018_TSVi, obtained from cell isolates, to GenBank under accession no. MK129253. In a phylogenetic analysis, the virus was located in a lineage of locally endemic 2016 and 2017 Saudi Arabia strains (lineage 5 in Figure 2) but was distinguished from 2015 South Korea strains.

**Conclusions**

We characterized the S gene and full-length genome of MERS-CoV imported to South Korea from the Middle East using sputum and throat swab specimens from

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**Figure 2.** Maximum-likelihood tree showing MERS-CoV isolates from South Korea, September 2018 (black dot), and reference MERS-CoV genomes. Tree estimated using RAxML values (https://github.com/stamatak/standard-RAxML) on branches are shown as percentages based on 1,000 bootstrap replicates from the nucleotide sequences. MERS-CoV, Middle East respiratory syndrome coronavirus. Scale bar indicates nucleotide substitutions per site.
To improve our understanding of the evolution of MERS-CoV and highlight the need for enhanced surveillance, especially for persons traveling to the Middle East.

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About the Author

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Table. Comparison of spike glycoprotein gene sequence variants for a MERS-CoV isolates from a patient in South Korea, 2018, and reference strains*

| MERS-CoV strain | NTD | RBD | Other regions |
|----------------|-----|-----|--------------|
|                | 183 | 258 | 409          | 1586 | 1848 | 1886 | 3177 | 3267 |
|                | (61) | (86) | (137)       | (529) | (530) | (616) | (629) | (1059) | (1089) |
| JX869509_HCoV-EMC_2012 | C (G) | T (V) | A (S) | T (I) | G (V) | T (V) | G (R) | T (D) | C (S) |
| KRO11266_KSA_Hu/Riyadh-KSA-2049/2015 | C (V) | | | | | | | | |
| KRO11264_KSA_Hu/Riyadh-KSA-2345/2015 | C (V) | | | | | | | | |
| KRO11265_KSA_Hu/Riyadh-KSA-2346/2015 | C (V) | | | | | | | | |
| KT026453_KSA_Hu/Riyadh-KSA-2959/2015 | C (V) | | | | | | | | |
| KT026454_KSA_Hu/Riyadh_KSA-4050/2015 | C (V) | | | | | | | | |
| KT026469_Hu_Jeddah-KSA-161RS1146_2016 | C (V) | | | | | | | | |
| KT026488_Hu-Riyadh-KSA-17739_2016 | C (V) | | | | | | | | |
| KT026469_Hu-Artawiyah-KSA-13328_2016 | C (V) | | | | | | | | |
| MG011361_Hu-Riyadh-KSA-K17000405_2017 | C (V) | | | | | | | | |
| MG011360_Hu-Riyadh-KSA-K17000887_2017 | C (V) | | | | | | | | |
| MG012608_Hu-Riyadh-KSA-9730_2017 | C (V) | | | | | | | | |
| MERS-CoV/KOR/KNIH/001_05_2015 | C (V) | | | | | | | | |
| KT009139-MERS-CoV/KOR/KNIH/002_05_2015 | C (V) | C (R) | | C (D) | T (S) | | | | |
| MERS-CoV/KOR/KNIH/009_05_2015 | C (V) | C (V) | | C (D) | T (S) | | | | |
| KT006149-ChinaGD01_2015 | G (G) | C (V) | | C (D) | T (S) | | | | |
| MERS-CoV/KOR/KNIH/012_05_2015 | C (V) | C (T) | | C (D) | T (S) | | | | |
| MERS-CoV/KOR/KNIH/013_05_2015 | C (V) | C (T) | | C (D) | T (S) | | | | |
| MERS-CoV/KOR/KNIH/015_05_2015 | C (V) | C (T) | | C (D) | T (S) | | | | |
| MERS-CoV/KOR/KNIH/042_04_2015 | C (V) | | | | | | | | |
| MERS-CoV/KOR/KCDC/001_2018_2SP-1 | C (V) | | | | | | | | |
| MERS-CoV/KOR/KCDC/001_2018_4TS | C (V) | | | | | | | | |
| MERS-CoV/KOR/KCDC/001_2018_4TSVI | C (V) | | | | | | | | |

*Only variations from the reference strain are shown. Boldface indicates the South Korea isolates and the most similar strain identified in this study. MERS-CoV, Middle East respiratory syndrome coronavirus; NTD, N-terminal domain; RBD, receptor-binding domain.

†Reference MERS-CoV strain.
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