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CASE SERIES

# Investigation of One Familial Cluster of COVID-19 in Taiwan: Differentiation of Genetic Variation Among Isolates and Implications for Epidemiological Investigation and Surveillance by Genomic Assay

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**Abstract:** The COVID-19 pandemic has caused a global public health crisis. Taiwan experienced two waves of imported cases of coronavirus disease 2019 (COVID-19), first from China in January to late February, 2020 then from other countries starting in early March. As of Dec 14, 2020, 733 cases have been reported in Taiwan, with cases of entire families being infected. This study aimed to investigate the clinical characteristics and differentiation of genetic variation among isolates from a cluster of familial COVID-19 infection. The parents had pneumonia (Case 14, father, and Case 15, mother), the elder son (Case 17) had mild cough, and the younger son (Case 18) was asymptomatic. In this study, four full viral genomes were sequenced by Illumina sequencing directly from specimens. Phylogenetic tree analysis revealed that these sequences came from Italy, not China, indicating that no major strain has been circulating in Taiwan. Several novel mutations were observed in the asymptomatic patient, such as nsp2, nsp12, and nsp14. These mutations may be associated with the severity of COVID-19 infection.

**Keywords:** COVID-19, SARS-CoV-2, imported family infections, whole-genome sequencing, phylogenetic analysis

## Introduction

Coronavirus disease (COVID-19) has spread globally, mainly via person–person transmission; it poses a major public health concern.[1] An unknown etiology of novel coronavirus (SARS-CoV-2) infection was reported in December 2019.[2] The disease caused by this virus has been called novel coronavirus disease 2019 (COVID-19). On March 11, 2020, the World Health Organization declared the worldwide spread of COVID-19 a pandemic.[3] As of Dec 14, 733 cases have been reported in Taiwan. Here, we report the first four-member familial cluster of COVID-19 in Taiwan. Two patients presented with pneumonia following admission to our hospital (Tri-Service General Hospital). The other two patients were admitted based on positive results of RT-PCR tests for SARS-CoV-2 viral nucleic acid. All patients were successfully treated with oseltamivir, piperacillin/tazobactam, and clarithromycin as empirical therapy, and were eventually discharged. Previous

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studies have identified positive COVID-19 cases via nucleic acid tests, where some patients remained afebrile and asymptomatic.[4,5] However, the viral shedding pattern and genomic characteristics of COVID-19 remain poorly understood. Up-to-date information on viral genomics is crucial in understanding the global dispersion characteristics of the virus and providing insights into its viral pathogenicity and transmission. In this study, we performed whole-genome sequencing of four clinical specimens of SARS-CoV-2 taken from the same infected family. We compared the genomes obtained with those of other strains from the GISAID database to understand their evolutionary trajectory. We also assessed the number of nucleotide substitutions in one family member who was asymptomatic. Through genome-wide association analysis on SARS-CoV-2 genomes, we sought to identify genetic variations that might be associated with the severity of COVID-19 infection. SARS-CoV-2 was confirmed by RT-PCR as described previously[6] and re-confirmed by the Taiwan Centers for Disease Control. Extracted viral RNA was stored at −80°C for further analysis. Data such as demographic information, underlying illnesses, clinical manifestations, and outcomes were collected from medical records. The case definition for COVID-19 was in accordance with Taiwan Centers for Disease Control's recommendations.

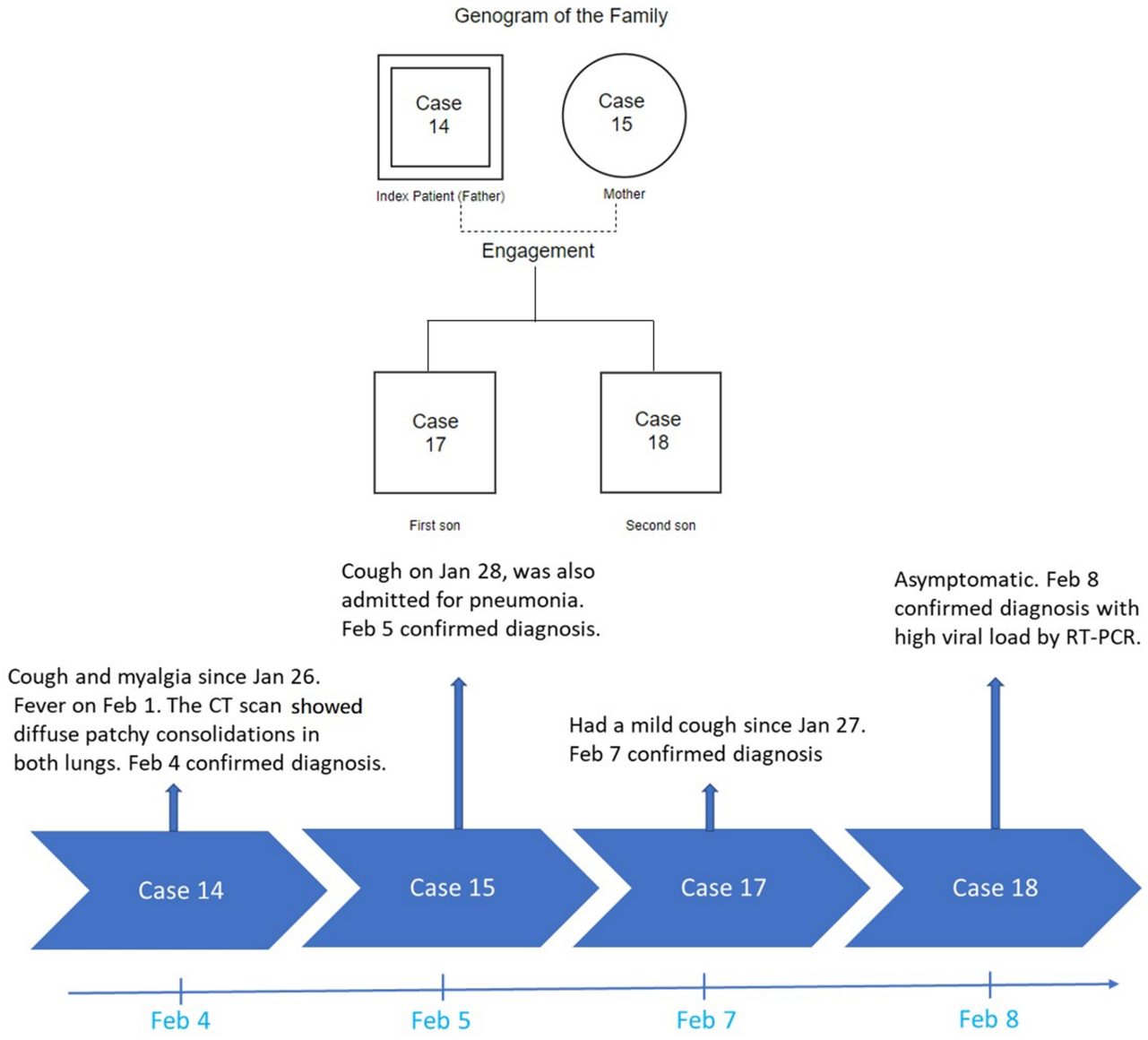


**Figure 1** Genogram of family members infected with COVID-19 who returned to Taiwan from Italy, including their clinical courses.

## Case Report

Confirmed case number information came from the content of the Taiwan's Central Epidemic Command Center (CECC) press conference and a press release from the Taiwan Centers for Disease Control . A 53-year-old Taiwanese man (Case 14), his wife (Case 15, 53 years old), and two sons (Cases 17 and 18, 24 and 22 years old, respectively) departed by air on January 22, 2020 from Taiwan, transferred at Hong Kong (where they stayed at the airport for 1 h), and arrived in Italy on January 23; they returned to Taiwan on February 1 (Figure 1). Case 17 had a mild cough since January 27, and case 18 reported no discomfort. Cases 17 and 18 were quarantined, admitted to the hospital on February 7 and 8, and discharged from the hospital on February 27 and March 6, respectively. Case 18 was the first asymptomatic diagnosis in Taiwan and the viral load detected in the body was high. Although the younger son had no symptoms, the other three family members were diagnosed successively (laboratory findings shown in Table 1). Exposure to the same source of infection could have been from contact with another infected person while on an airplane, in an airport, or in Italy.

**Table 1** Clinical and Laboratory Characteristics of the Familial Cluster Infection Cases

| Variables | Case 14 | Case 15 | Case 17 | Case 18 |
|---|---|---|---|---|
| Age | 53 | 53 | 24 | 22 |
| Gender | Male | Female | Male | Male |
| **Laboratory findings** | | | | |
| White-cell count (per μL) | 5030 | 4200 | 6940 | 5880 |
| Hemoglobin (g/dl) | 15.5 | 12.1 | 16.3 | 15.9 |
| Platelet (per μL) | 194,000 | 187,000 | 282,000 | 277,000 |
| Differential count (%) | | | | |
| Neutrophils | 77.7 | 65.3 | 60.8 | 65.4 |
| Lymphocytes | 15.9 | 24.5 | 33.9 | 26.9 |
| Sodium (mmol/L) | 137 | 134 | 141 | 140 |
| Creatinine (mg/dl) | 0.9 | 0.7 | 0.9 | 0.9 |
| AST (U/L) | 48 | 18 | 19 | 16 |
| ALT (U/L) | 46 | 8 | 25 | 15 |
| C-reactive protein (mg/l) | 8.60 | 1.37 | <0.10 | <0.10 |
| Influenza screen (A &B) | Negative | Negative | Negative | Negative |
| SARS-Cov-2 RT-PCR | Positive | Positive | Positive | Positive |
| Ct-values | 33 | 24 | 26 | 27 |
| **Signs and symptoms** | | | | |
| Cough | Yes | Yes | Yes | No |
| Sore throat | Yes | Yes | Yes | No |
| Dyspnea | Yes | No | No | No |
| Fever | Yes | Yes | Yes | No |
| Chest radiograph | Abnormal | Abnormal | Normal | Normal |

**Abbreviations:** AST, aspartate aminotransferase; ALT, alanine aminotransferase.

## Whole-Genome Sequencing of SARS-CoV-2

Whole genome sequences of SARS-CoV-2 were obtained using the Illumina TruSeq Stranded mRNA Library Prep Kit protocol to enrich SARS-CoV-2 cDNA using multiplex RT-PCR amplicons. The NovaSeq 6000 platform (Illumina, San Diego, USA) was used for sequencing of the indexed libraries. To determine the full viral genome sequences, the Next Generation Sequencing reads were first mapped to the human genome (hg38), and unmapped reads were compared to the SARS-CoV-2 Wuhan-Hu-1 reference genome sequence (29.9kb ss-RNA; GenBank ID: NC_045512.2) using SPAdes v3.13.2 with the parameter "–trusted-contigs" to assemble the specimen-specific SARS-CoV- 2 genome sequence. All viral assemblies were uploaded to GISAID (Supplementary Table S1).

## Phylogenetic Relationship Analysis

SARS-CoV-2 genome sequences from GISAID and those of the four cases were aligned using Clustal Omega.[7] Aligned nucleic acid sequences were used to construct phylogenetic trees based on the neighbor-joining tree algorithms provided by PHYLIP.[8] The tree file created by PHYLIP was drawn and managed in MEGA X.[9] Different COVID-19 sample collection times and phylogenetic trees created by viral genome sequencing with branch lengths were analyzed using the RelTime with Dated Tips (RTDT) method[10,11] supported by MEGA X.[9]

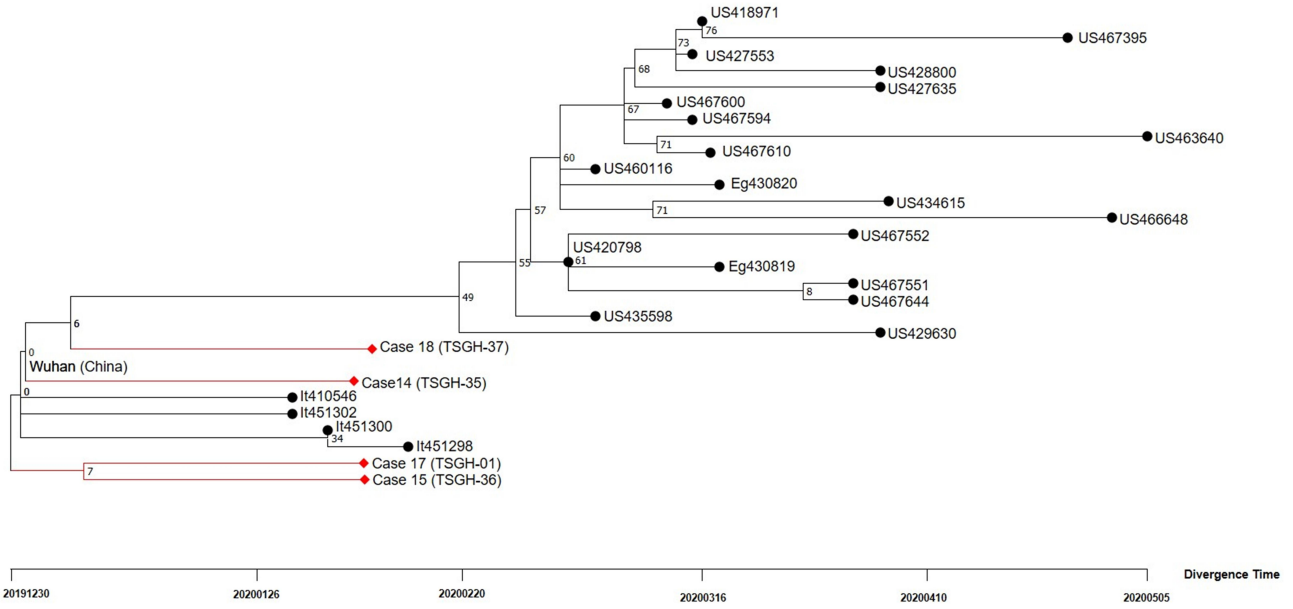


**Figure 2** Estimating TSGH strains using TimeTree with MEGA X. Strain names: Wuhan, severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1; TSGH, this study. Numbers associated with strain names are GISAID Accession IDs.
**Abbreviations:** Eg, Egypt; It, Italy; US, United States of America.

Bootstrap analysis was calculated with 1000 replicates and analysis values were shown next to the branch nodes.[12]

## Discussion

Four full viral genomes were resolved in this study directly from specimens. Phylogenetic tree analysis showed that these four cases were imported from Italy, not China (Figure 2), indicating that the outbreak of COVID-19 in Italy may have occurred earlier than the end of January, 2020. One of the sequences (case 14, strain name: TSGH-35) lies near the Wuhan SARS-CoV-2 reference sequence representing the early virus strains as an index patient, also mapping the divergence time of late-December 2019 to mid-January 2020 as the origin of the Wuhan strain (Wuhan-Hu-1). To decipher the time point when the family could have been infected, another 75 strains were aligned; the closest area found was Wuhan (Figure 3), indicating that the early Wuhan virus strain spread to Italy. None of the four genomes harbored the D614G mutation, which has been linked to higher mortality in COVID-19.[13] A previous study reported that COVID-19 cases were more likely to have comorbidities than non-severe cases, with host contributing factors including hypertension, diabetes mellitus, etc.[14] Davies et al also showed that the age disparities in observed cases could be explained by children having lower susceptibility to infection and a lower propensity to showing clinical symptoms.[15] This may explain why Case 17 and 18 in our study were paucisymptomatic or asymptomatic. We further investigated the asymptomatic case, in which some novel nucleotide substitutions were observed (Figure 4), such as nsp12 (RdRp) with the P378L mutation, nsp14 with the A23S mutation, nsp2 with the M141L mutation, and N protein with the P309L and F363V mutations. It has been suggested that the number of asymptomatic infections of COVID-19 has been underestimated due to an increasing number of infected people being found who have not traveled to epidemic hotspots or been linked to known COVID-19 patients.[16] Previous studies have identified COVID-19 transmission caused by asymptomatic carriers who had normal chest CT findings, but were found to have a viral load similar to that of a symptomatic patient.[17,18] RNA viruses show variation in their genomes due to nucleotide substitutions generated by the low fidelity of RNA-dependent RNA polymerase during replication. Further studies are required to investigate the mutation sites correlated with the function of SARS-CoV-2 in asymptomatic cases.

## Conclusion

Timely sequencing and sharing of whole genomes of SARS-CoV-2 from different locations is important for monitoring genetic changes in the virus that may be associated with viral spreading and clinical manifestations. Here, we determined the sequences of SARS-CoV-2 in

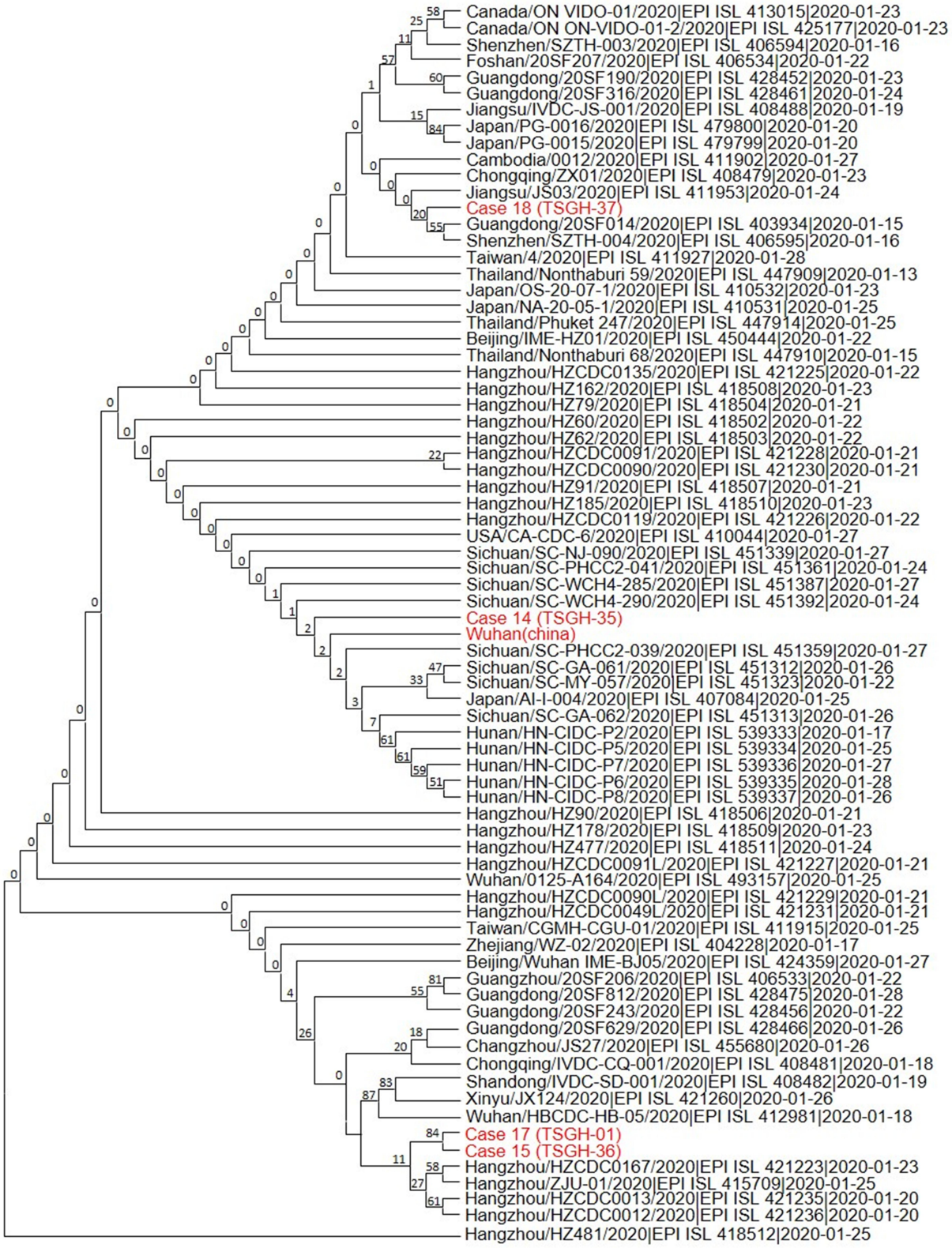


**Figure 3** Phylogenetic tree with 75 viral genome strains linking the four familial sequences.

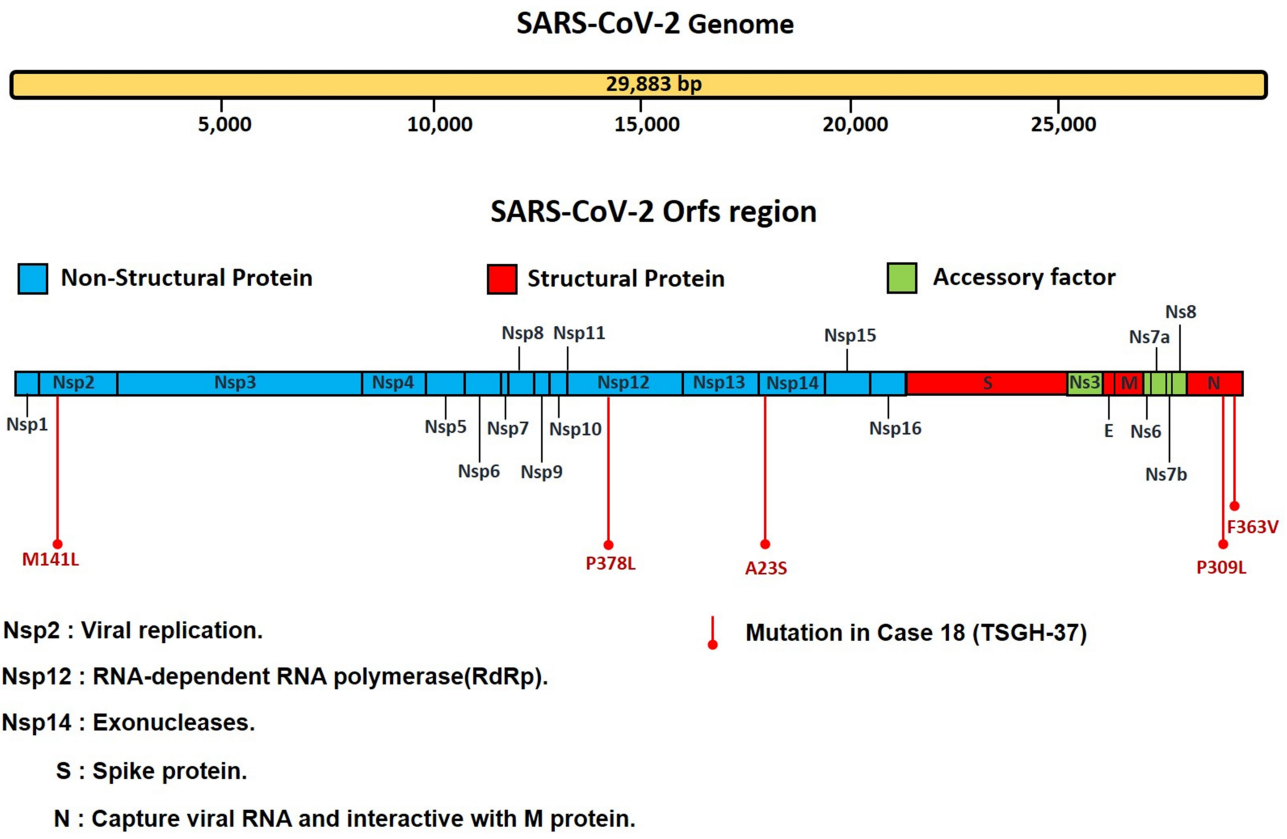


**Figure 4** Novel nucleotide substitutions found via whole-genome analysis of the asymptomatic case, Case 18.

one familial cluster of COVID-19 in Taiwan and showed that sequencing is a useful tool for tracing the infection source for this type of RNA virus. Moreover, the existence of fewer nucleotide changes in viral genomes from asymptomatic patients in clustered infections might help us elucidate the viral shedding pattern and replication model with SARS-CoV-2 infection.

## Ethics Statement

This study was approved by the Institutional Review Board, Tri-Service General Hospital (TSGHIRB No.: C202005041), registered on 20 March 2020. Written informed consent was obtained from the participants for the publication of the case report.

## Author Contributions

Ming-Jr Jian: Concepts, Design, Data analysis, Manuscript preparation; Hsing-Yi Chung, Chih-Kai Chang, Shan-Shan Hsieh, Kuo-Sheng Hung, Cherng-Lih Perng: Data analysis, Definition of intellectual content, Literature search; Jung-Chung Lin, Kuo-Ming Yeh, Chien-Wen Chen, Feng-Yee Chang, Sheng-Kang Chiu, Ming-Tsan Liu, Ji-Rong Yang: Concepts, Design, Definition of intellectual content; Hung-Sheng Shang: Manuscript editing, Manuscript review, Guarantor. All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed on submission to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work.

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## Disclosure

The authors report no conflicts of interest in this work.

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