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CASE REPORT

Open-Reading Frame 1a (ORF1a) Gene Target Failure with Omicron BA.5.2.1 Caused by Partial Deletion of the ORF1a Gene

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SUMMARY

Background: **We identified two cases of Omicron BA.5.2.1 that exhibited characteristics of open-reading frame 1a (ORF1a) gene target failure (OGTF) in RNA detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The purpose of this study was to determine the cause of the OGTF in the two samples.**
Method: **Samples were specimens obtained from a 37-year-old male and a 43-year-old female who were suspected to have Coronavirus disease 2019. SARS-CoV-2 RNA detection using µTAS Wako g1 system (FUJIFILM Wako Pure Chemical, Osaka, Japan) was performed and it detected S gene but did not detect ORF1a gene.**
Results: **The results of whole genome sequencing of these samples showed that SARS-CoV-2 genome in these two samples matched to Omicron BA.5.2.1. SARS-CoV-2 genomes in the two samples had a deletion of 15 bases and a deletion of 9 bases, so that they overlapped with the 3'-terminal side of the primer binding region in ORF1a gene, and these deletions were considered to be the cause of OGTF.**
Conclusions: **Since such a gene target failure due to base deletion is not considered rare, it was considered desirable to implement a multi-target detection in SARS-CoV-2 RNA molecular diagnostic system to address the mutation or deletion of the viral gene.**
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KEYWORDS

ORF1a gene, OGTF, SARS-CoV-2, COVID-19, NAAT, µTAS

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative virus of Coronavirus disease 2019 (COVID-19), changes viral characteristics through repeated mutations of genome in a short period of time. As a result, new variants emerged, leading to a delay in the convergence of the pandemic [1,2]. To make a diagnosis of COVID-19, a nucleic acid amplification test (NAAT) targeting one or more regions among the envelope (E) gene, spike (S) gene, open reading frame (ORF) gene, and RNA-dependent RNA polymerase gene of the genome of SARS-CoV-2 has

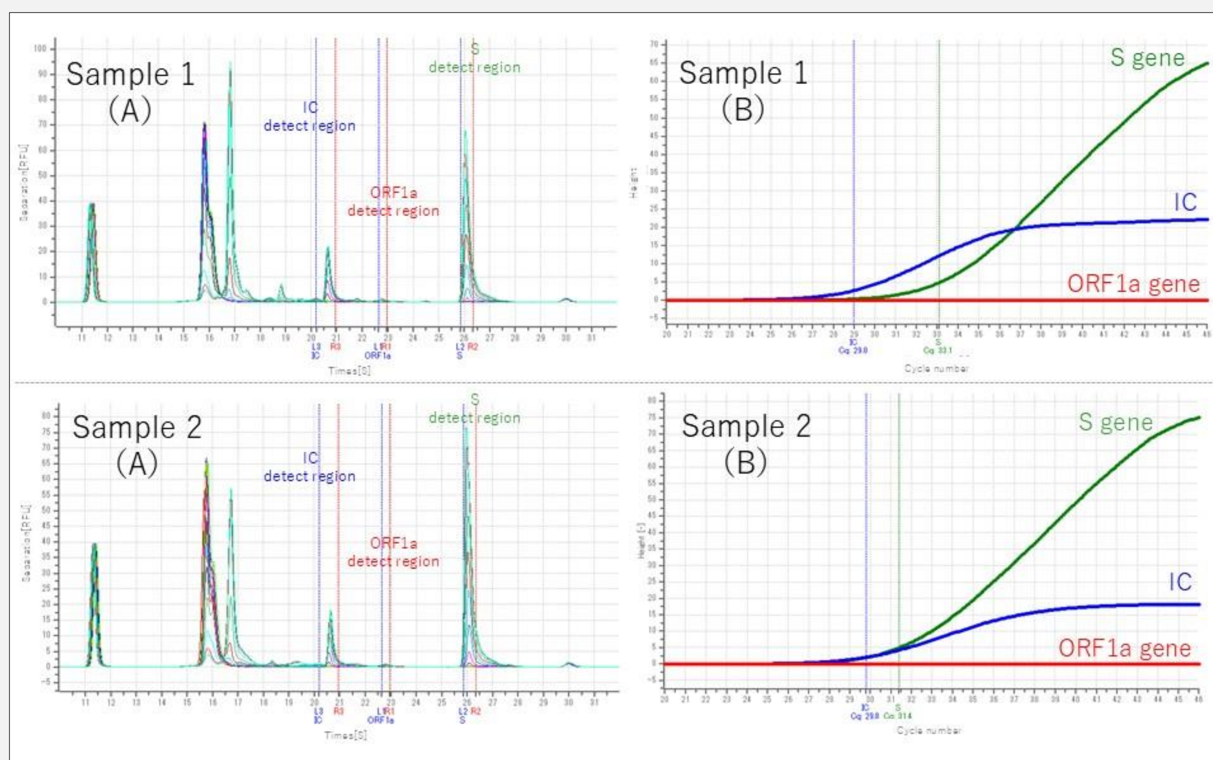

Figure 1. Fluorescence intensity per amplification in microdevice electrophoresis (A) and amplification curves (B) using a µTAS Wako g1 system.

The waveforms depicted in panels (A) illustrate fluorescence intensity per amplification observed via microdevice electrophoresis. L1 to R1 delineates the detection region for the ORF1a gene, L2 to R2 denotes the detection region for the S gene, and L3 to R3 represents the detection region for the internal control (IC). While amplification signals are detected in both the IC and S gene detection regions via microdevice electrophoresis, they are notably absent in the ORF1a gene detection region.
Panels (B) present amplification curves illustrating the fluorescence intensity increases for the IC, S gene, and ORF1a gene. Fluorescence intensities shows discernible rises for both the IC and S gene, whereas no corresponding increase is observed for the ORF1a gene.
ORF1a - Open-reading frame 1a, S - Spike, IC - Internal control, Cq - Quantitation Cycle.

been widely used [3]. It is known that gene mutations in the genome of viruses affect even the results of NAAT, and that mutation or deletion in the target gene at the region to which a primer binds causes the failure or delay in detection of the target gene [4,5]. Thermo Fisher Scientific TaqPath COVID-19 assay was reported to have resulted in S-gene target failure (SGTF) for Alpha variant (B1.1.7) and Omicron variants (B.1.1.529, BA.1, BA.4, and BA.5) of SARS-CoV-2 [6]. Furthermore, it has been reported that in cobas SARS-CoV-2 and cobas SARS-CoV-2 & influenza A/B, differences in the cycle threshold appear between E gene and open-reading frame 1ab (ORF1ab) gene in multi-target detection as a result of partial mutation in ORF1ab gene of Omicron variant (BA.2.12.1) [7]. However, these reports have not revealed whether the viral mutations or deletions exist in the primer binding region of the target gene by sequence analysis.

In this study, for Omicron BA.5.2.1 associated with failure in detection of the ORF1a gene using µTAS Wako g1 system (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), a fully automated genetic analyzer incorporating multi-target detection for open-reading frame 1a (ORF1a) gene and S gene [8], we demonstrated a partial deletion at the primer binding region of the ORF1a gene, which was the cause of the ORF1a gene target failure (OGTF).

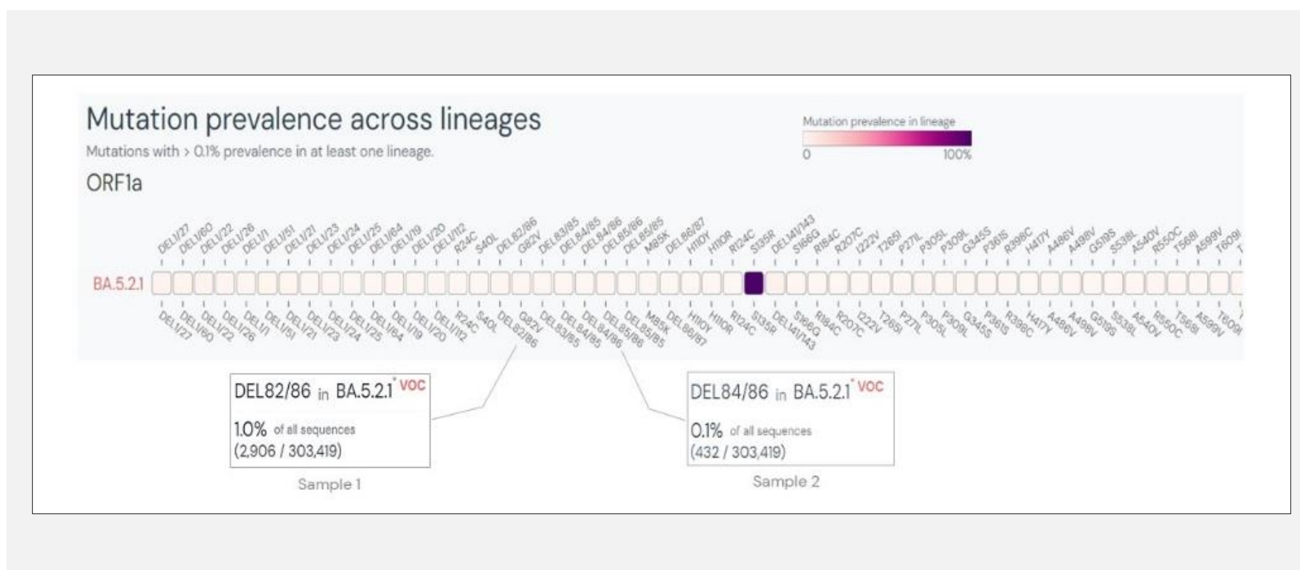

Figure 2. ORF1a gene mutation prevalence across lineages with Omicron BA.5.2.1.

Square darkness correlates with prevalence of a particular mutation in the indicated lineage.
The 82 to 86 base deletions detected in Sample 1 were found to occur at a rate of 1.0% within BA.5.2.1, whereas the 84 to 86 base deletions detected in Sample 2 were observed at a rate of 0.1% within BA.5.2.1.
http://outbreak.info/compare-lineages Figure created February 11, 2023.
ORF1a - Open-reading frame 1a.

CASE PRESENTATION

Sample 1 was a nasopharyngeal mucus specimen obtained from a 37-year-old male with chief complaints of fever and sore throat in whom COVID-19 was clinically suspected. Sample 2 was a nasopharyngeal mucus specimen obtained from a 43-year-old female with chief complaints of sore throat, headache, cough, and rhinorrhea. NAAT of SARS-CoV-2 RNA in the nasal mucus was performed using µTAS Wako g1 system. PCR for verification of the S gene was performed using SARS-CoV-2 Direct Detection RT-qPCR Core Kit (Takara Bio, Shiga, Japan) and Primer/Probe L452R (SARS-CoV-2) Ver. 2 (Takara Bio, Shiga, Japan). For extraction and purification of viral RNA, QIAamp Viral RNA Mini kit (QIAGEN, Hilden, Germany) was used. As real-time PCR machine, cobas z480 system (Roche Diagnostics, Tokyo, Japan) was used. Whole genome sequencing (WGS) of SARS-CoV-2 viral RNA was outsourced to Takara Bio Inc. (Shiga, Japan). That is, the library for next generation sequencing (NGS) was prepared using primer (Alt_nCov 2019_primers/Primers/ver_N6) (https://github.com/nasasaki/Alt_nCov2019_primers/tree/master-/Primers/ver_N6) and QIAseq FX DNA Library UDI kit (QIAGEN, Hilden, Germany). WGS was performed using the MiSeq Reagent Kit v3 (Illumina, San Diego, CA, USA) and Illumina MiSeq (Illumina, San Diego, CA, USA). The consensus base sequence obtained was compared with the reference sequence of SARS-CoV-2 [GenBank No. MN908947.3] obtained from BLAST using Integrative Genomics Viewer Version 2.17.0 (https://igv.org/). Amino acid deletion was confirmed by checking the consensus sequence using the GISAID (https://gisaid.org/) website. The SARS-CoV-2 lineage was confirmed using the Pangolin website (https://cov-lineages.org/resources/pangolin.html).

Figure 1 shows waveforms obtained by capillary electrophoresis (A) and change curve of the fluorescence intensity (B) for each PCR cycle in µTAS Wako g1 system in 2 samples. The waveforms obtained by capillary electrophoresis (A) for each PCR cycle showed peak waveforms in the detection regions of internal control (IC) and S gene in both samples, but no peak waveform was observed in ORF1a detection region. As for changes in fluorescence intensity in each PCR cycle (B), IC showed Cq value of 29.0, ORF1a gene was not detected, and S gene showed Cq value of 33.1 in Sample 1. IC showed Cq value of 29.8, ORF1a gene was not detected, and S gene showed Cq value of 31.4 in Sample 2. The S gene of SARS-CoV-2 in both samples was negative for L452 wild type detection system and positive for L452R mutant detection system, indicating that the S gene in both samples had L452R mutation. WGS revealed a deletion of 15 bases at positions 508 to 522 and a deletion of 9 bases at positions 11,288 to 11,296 in the ORF1a gene region in Sample 1 and a deletion of 9 bases at positions 514 to 522 and a deletion of 10 bases at positions 11,288 to 11,297 in the ORF1a gene region in Sample 2. Deletions of amino acids encoded at

the deletion regions in ORF1a gene in Sample 1 were as follows: G82del, H83del, V84del, M85del, V86del, S106del, G107del and F108del; and those in Sample 2 were as follows: V84del, M85del, V86del, S106del, G107del, and F108del. The SARS-CoV-2 lineage in the two samples was Omicron BA.5.2.1.

DISCUSSION

This study showed that Omicron BA.5.2.1 that exhibited OGTF in NAAT of SARS-CoV-2 detection using µTAS Wako g1 system was attributable to a partial deletion in the primer binding region in ORF1a gene. However, the µTAS Wako g1 system was able to correctly confirm the samples as SARS-CoV-2 positive by detecting the S gene, although it failed to detect the ORF1a gene because of partial base deletion in the primer binding region. The partial base deletion in the ORF1a gene of Omicron BA.5.2.1 seen in this study is considered to occur at a frequency of 1.0% (2,906/ 303,412) for Sample 1 and 0.1% (432/303,412) for Sample 2 on the analogy of the deletion of amino acids encoded at the deletion regions (Figure 2). This suggests the importance of setting target genes in multiple regions in the molecular diagnostic system of SARS-CoV-2 [9]. The discrepancy in detected genes in NAAT targeting multiple genes may be caused by two factors: false-positive due to non-specific reaction to the target gene and failure to detect the target gene due to mutation or deletion in the viral gene [6,10,11]. Because the S gene Cq values of 33.1 and 31.4 are relatively low, and because the S gene carrying L452R mutation was detected in the samples used in this study, it was considered that the discrepancy in the detected genes was more likely to have been caused by a failure to detect the ORF1a gene due to mutation or deletion in the viral gene rather than by false-positive of the S gene. Therefore, WGS of the virus was performed and it revealed a deletion of 15 bases at positions from 508 and a deletion of 9 bases at positions from 11,288 in the ORF1a gene region in SARS-CoV-2 genome in Sample 1 and a deletion of 9 bases at positions from 514 and a deletion of 10 bases at positions from 11,288 in the ORF1a gene region in SARS-CoV-2 genome in Sample 2. Of these, the deletion of 15 bases at positions from 508 in Sample 1 and the deletion of 9 bases at positions from 514 in Sample 2 overlap with the primer binding region for detecting the ORF1a gene. This indicated that the OGTF was considered to have been caused by ORF1a gene target failure due to a mismatch primer base sequence caused by a partial deletion of ORF1a gene in the SARS-CoV-2 genome.
In conclusion, this study identified the presence of a partial deletion in the primer binding region in ORF1a gene, which caused Omicron BA.5.2.1 to exhibit OGTF on µTAS Wako g1 system. It was considered desirable to implement a multi-target detection in the molecular diagnostic system of SARS-CoV-2 to address mutations or deletions in the viral gene.

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Ethical Considerations:
This study was approved by the Ethics Committee of Kindai University, School of Medicine (approval number: R04-183).

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Declaration of Interest:
Yamamoto Y and Kawabata T are employees of FUJIFILM Wako Pure Chemical Corporation.

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