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Effects of a major deletion in the SARS-CoV-2 genome on the severity of infection and the inflammatory response: an observational cohort study

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Summary

Background Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants with a 382-nucleotide deletion (Δ382) in the open reading frame 8 (ORF8) region of the genome have been detected in Singapore and other countries. We investigated the effect of this deletion on the clinical features of infection.

Methods We retrospectively identified patients who had been screened for the Δ382 variant and recruited to the PROTECT study—a prospective observational cohort study conducted at seven public hospitals in Singapore. We collected clinical, laboratory, and radiological data from patients’ electronic medical records and serial blood and respiratory samples taken during hospitalisation and after discharge. Individuals infected with the Δ382 variant were compared with those infected with wild-type SARS-CoV-2. Exact logistic regression was used to examine the association between the infection groups and the development of hypoxia requiring supplemental oxygen (an indicator of severe COVID-19, the primary endpoint). Follow-up for the study’s primary endpoint is completed.

Findings Between Jan 22 and March 21, 2020, 278 patients with PCR-confirmed SARS-CoV-2 infection were screened for the Δ382 deletion and 131 were enrolled onto the study, of whom 92 (70%) were infected with the wild-type virus, ten (8%) had a mix of wild-type and Δ382-virus variants, and 29 (22%) had only the Δ382 variant. Development of hypoxia requiring supplemental oxygen was less frequent in the Δ382 variant group (0 [0%] of 29 patients) than in the wild-type only group (26 [28%] of 92; absolute difference 28% [95% CI 14–28]). After adjusting for age and presence of comorbidities, infection with the Δ382 variant only was associated with lower odds of developing hypoxia requiring supplemental oxygen (adjusted odds ratio 0·07 [95% CI 0·00–0·48]) compared with infection with wild-type virus only.

Interpretation The Δ382 variant of SARS-CoV-2 seems to be associated with a milder infection. The observed clinical effects of deletions in ORF8 could have implications for the development of treatments and vaccines.

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Introduction Since the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), efforts have been made to map the genetic diversity of the virus and to identify variants with a selective advantage.1 The variations of interest include changes in immune targets, such as the spike glycoprotein; changes in primer-binding and probe-binding sites, which can reduce the sensitivity of diagnostic tests; and genetic variations that might affect transmissibility and virulence.2,4

A SARS-CoV-2 variant with a 382-nucleotide deletion (Δ382) was detected in a cluster of cases in Singapore that occurred in January and February, 2020.2 The deletion truncates open reading frame (ORF) 7b and removes the ORF8 transcription-regulatory sequence, eliminating ORF8 transcription. This variant was successfully transmitted early during the epidemic, but was not detected after March, 2020. An identical Δ382 variant was also detected in February, 2020, in a traveller who returned from Wuhan, China, to Taiwan, and other SARS-CoV-2 isolates with different deletions in ORF8 have been reported from cases in Bangladesh (345 nucleotides), Australia (138 nucleotides) and Spain (62 nucleotides).4,6

In severe acute respiratory syndrome coronavirus (SARS-CoV), the virus responsible for the 2002–03 SARS epidemic, a characteristic 29-nucleotide deletion (Δ29) in ORF8 occurred soon after its zoonotic transmission from civets to humans in 2002, and larger deletions of 82 nucleotides and 415 nucleotides in the same genomic region were also reported.7 The effects of these deletions on the course of the SARS epidemic is unknown. However, in-vitro studies have indicated that the Δ29 variant of SARS-CoV replicates less efficiently than the wild-type virus, and consequently this variant has...
Deletions in open reading frame 8 (ORF8) of severe acute respiratory syndrome coronavirus were commonly detected during the severe acute respiratory syndrome outbreak of 2002–03. These deletions reduced viral replication in vitro, and an attenuated severity of infection was hypothesised, although the effect of this deletion on clinical outcomes remains unknown. A 382-nucleotide deletion (Δ382) was detected in the genome of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from a cluster of infections in Singapore. A literature search was done through MEDLINE to July 27, 2020, using the keywords “coronavirus disease 2019”, “COVID-19”, “SARS-CoV-2”, “deletion”, and “ORF8”, with no language restrictions. An identical 382-nucleotide deletion in ORF8 was reported from in a traveller who returned from Wuhan to Taiwan in February, 2020. The clinical effect of this deletion was not described. Viruses with other deletions in the ORF8 region have also been described from Bangladesh (345 nucleotides), Australia (138 nucleotides) and Spain (62 nucleotides), but no accompanying clinical data are available.

The epidemiological investigation was implemented under the Infectious Diseases Act (Singapore). Written informed consent was obtained from participants who provided clinical data and biological samples (as part of the PROTECT study). Study protocols were approved by ethics committees of the National Healthcare Group (2012/00917) and SingHealth Centralised Institutional Review Board (2018/3045). Healthy donor samples were collected under study numbers 2017/2806 and NUS IRB 04-140. Work undertaken at the Duke–NUS Medical School ABSL3 laboratory was approved by the Singapore Ministry of Health.

The clinical data and biological sample collection

The electronic medical records of patients enrolled in the PROTECT study were reviewed and their data were entered onto a standardised collection form adapted from the International Severe Acute Respiratory and Emerging Infection Consortium’s case record form for emerging severe acute respiratory infections.9 Serial blood and respiratory samples were collected during hospitalisation and post-discharge.

Clinical management

All patients with COVID-19 were isolated in hospital with airborne transmission precautions, regardless of disease severity. Supportive therapy including supplemental oxygen and symptomatic treatment were administered as required. Patients with moderate to severe hypoxia (defined as requiring a fraction of inspired oxygen ≥40%) were transferred to the intensive care unit for high-flow oxygen via nasal cannula and invasive mechanical ventilation if required. De-isolation was contingent on

Research in context

Evidence before this study

Deletions in open reading frame 8 (ORF8) of severe acute respiratory syndrome coronavirus were commonly detected during the severe acute respiratory syndrome outbreak of 2002–03. These deletions reduced viral replication in vitro, and an attenuated severity of infection was hypothesised, although the effect of this deletion on clinical outcomes remains unknown. A 382-nucleotide deletion (Δ382) was detected in the genome of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from a cluster of infections in Singapore. A literature search was done through MEDLINE to July 27, 2020, using the keywords “coronavirus disease 2019”, “COVID-19”, “SARS-CoV-2”, “deletion”, and “ORF8”, with no language restrictions. An identical 382-nucleotide deletion in ORF8 was reported from in a traveller who returned from Wuhan to Taiwan in February, 2020. The clinical effect of this deletion was not described. Viruses with other deletions in the ORF8 region have also been described from Bangladesh (345 nucleotides), Australia (138 nucleotides) and Spain (62 nucleotides), but no accompanying clinical data are available.

The biological function of the ORF8 protein in SARS-CoV-2 remains unclear. A recent study suggested that ORF8 mediates immune evasion by downregulating MHC-I molecules.10 A previously reported interactome analysis that used affinity-purification mass spectrometry also identified 47 human proteins—mainly associated with glycoprotein metabolism—that interact with ORF8, of which 15 are known drug targets.31 In-vitro evidence has suggested that the deletion does not affect viral replicative fitness, and an analysis of subgenomic RNA has shown that transcription of the ORF6 and N genes, known SARS-CoV interferon antagonists, is altered in Δ382 variants as compared with wild-type SARS-CoV-2.32 In this study, we compared the clinical outcomes and immune responses of patients infected with wild-type and Δ382 SARS-CoV-2.

Methods

Study design and participants

We retrospectively identified individuals who had been screened for the Δ382 variant and recruited to the PROTECT study. PROTECT is a prospective observational cohort study done at seven public hospitals in Singapore (the National Centre for Infectious Diseases, Singapore General Hospital, National University Hospital, Ng Teng Fong General Hospital, Changi General Hospital, Alexandra Hospital, Khoo Teck Puat Hospital). The study aimed to recruit all individuals hospitalised at one of the participating hospitals with confirmed SARS-COV-2 infection for the purpose of clinical characterisation of COVID-19.
resolved symptoms and two consecutive nasopharyngeal swabs at least 24 h apart that were negative for SARS-CoV-2 on PCR.

**Detection of Δ382 variant**

To detect the 382-nucleotide deletion in the SARS-CoV-2 genome, we used two specific PCR primers (forward 5'-TGTTAGAGTACAACTTCTTT-3'; reverse 5'-GGTAGTAGAATACCACCATCTTTGA-3') flanking the deleted region. For samples with high cycle threshold (Ct) values, hemi-nested PCR was done with a second forward primer (5'-TGTTTATAACACTTTGCTTCACA-3') and the same reverse primer as before. The PCR mixture contained the cDNA primers (10 µM each), 10× Pfu reaction buffer (Promega, Madison, WI, USA), Pfu DNA polymerase (Promega), and 10 mM dNTP mix (Thermo Scientific, Waltham, MA, USA). PCR was done in a thermal cycler (Applied Biosystems Veriti, Foster City, CA, USA) with the following conditions: 95°C for 2 min, followed by 35 cycles at 95°C for 1 min, 52°C for 30 sec, and 72°C for 1 min; and a final extension at 72°C for 10 min. Deletions in the PCR products were visualised with use of a QIAxcel DNA screening cartridge on QIAxcel Advanced capillary electrophoresis system (Qiagen, Hilden, Germany).

**Multiplex microbead-based immunoassay**

Levels of specific immune mediators in the first plasma samples collected from patients with COVID-19 during hospitalisation were quantified by multiplex microbead-based immunoassays. Plasma samples were treated with 1% Triton X-100 solvent-detergent (SD) mix for virus inactivation. Immune mediator levels were measured with the Luminex assay using the Cytokine/Chemokine/Growth Factor 45-plex Human ProcartaPlex Panel 1 (ThermoFisher Scientific; appendix p 1). Patient samples with a concentration out of measurement range were assigned the value of the logarithmic transformation of the limit of quantification. Data analysis was done with Bio-Plex Manager 6.1.1 software. TM4-MeV Suite (version 10.2) was used to compute hierarchical clustering and generate a heatmap of immune mediators, scaling concentrations to between 0 and 1 for visualisation. Biological processes and immune pathways were predicted from the significant immune mediators with Ingenuity Pathway Analysis (version 52912811). Protein–protein interaction networks of these immune mediators and previously reported host proteins targeted by SARS-CoV-2 ORF8 were predicted and illustrated with the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database version 11.0. All these interactions were derived from the STRING database see https://string-db.org.

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**Figure 1:** Capillary electrophoresis of the ORF8 gene showing differences across the duration of disease in four patients co-infected with wild-type and the Δ382 variant of severe acute respiratory syndrome coronavirus 2

Approximate band sizes were 880 bp for the wild-type virus and 500 bp for the Δ382 variant. Nuclease-free water was used as a non-template negative control for PCR1. This resultant reaction was used as the negative control for the nested reaction. Δ382=382-nucleotide deletion. M=100-bp marker.
from high-throughput lab experiments and previous knowledge in curated databases at a confidence threshold of 0·5.

**Epidemiological investigation**

As part of the COVID-19 outbreak investigation in Singapore, all patients with SARS-CoV-2 infection were interviewed to elucidate activity histories from 14 days preceding symptom onset until isolation in hospital, including recent travel history and possible contact with confirmed cases. The Singapore Ministry of Health initiated contact tracing to identify close contacts (prolonged time within 2 m of a person with confirmed COVID-19) and other contacts who had significant interactions with the infected person. Active case finding was done to detect additional COVID-19 cases among these contacts.

| Δ382 variant only (n=29) | Mixed Δ382 variant and wild-type (n=10) | Wild-type only (n=92) | p value* | p value† |
|--------------------------|----------------------------------------|-----------------------|----------|----------|
| **Patient characteristics** |                                        |                       |          |          |
| Median age, years        | 37 (27–53)                             | 46 (39–56)            | 47 (35–61) | 0·041    | 0·018    |
| Age group, years         |                                        |                       |          |          |
| <45                      | 19 (66%)                               | 5 (50%)               | 43 (47%) | 0·019    | 0·17     |
| 45–64                    | 9 (31%)                                | 5 (50%)               | 39 (42%) | 0·90     | 0·17     |
| ≥65                      | 1 (3%)                                 | 0 (0%)                | 10 (11%) | 0·61     | 0·52     |
| Sex                      |                                        |                       |          |          |
| Female                   | 10 (34%)                               | 3 (30%)               | 39 (42%) | 0·66     | 0·52     |
| Male                     | 19 (66%)                               | 7 (70%)               | 53 (58%) | 0·74     | 0·26     |
| Chinese ethnicity        | 22 (76%)                               | 7 (70%)               | 67 (73%) | 0·92     | 0·81     |
| Charlson Comorbidity Index | 0 (0–0)                              | 0 (0–0)               | 0 (0–0)  | 0·64     | 0·51     |
| Diabetes                 | 1 (3%)                                 | 0 (0%)                | 11 (12%) | 0·22     | 0·29     |
| **Baseline symptoms and findings** |                                        |                       |          |          |
| Duration of symptoms, days | 6 (3–9)                               | 6 (5–9)               | 4 (2–7)  | 0·061    | 0·036    |
| Fever                    | 17 (59%)                               | 1 (10%)               | 72 (78%) | 0·054    | 0·05     |
| Cough                    | 20 (69%)                               | 7 (70%)               | 62 (67%) | 0·98     | 1·00     |
| Dyspnoea                 | 1 (3%)                                 | 2 (20%)               | 15 (16%) | 0·18     | 0·13     |
| Sore throat               | 15 (52%)                               | 5 (50%)               | 37 (40%) | 0·50     | 0·29     |
| Rhinorrhea               | 10 (34%)                               | 2 (20%)               | 21 (23%) | 0·42     | 0·22     |
| Heart rate (beats per min) | 85 (74–97)                        | 80 (77–108)           | 92 (83–100) | 0·26 | 0·78     |
| Systolic blood pressure, mm Hg | 136 (122–145)       | 131 (128–139)         | 133 (120–146) | 0·77 | 0·49     |
| Respiratory rate, breaths per min | 18 (18–18) | 18 (16–20) | 18 (17–19) | 0·92 | 0·68 |
| Oxygen saturation, %     | 98 (97–99)                             | 98 (96–99)            | 98 (96–98) | 0·45 | 0·06     |
| Temperature, °C          | 37·1 (36·6–37·7)                      | 37·8 (37·8–38·5)      | 37·7 (37·2–38·3) | 0·0064 | 0·0013   |
| Neutrophils, ×10⁹/L      | 2·6 (2·0–3·3)                          | 2·6 (2·3–4·4)         | 3·1 (2·1–4·1) | 0·50 | 0·24     |
| Lymphocytes, ×10⁹/L      | 1·3 (0·9–1·9)                          | 1·2 (0·8–2·0)         | 1·1 (0·8–1·5) | 0·22 | 0·079    |
| Platelet, ×10⁹/L         | 193 (173–245)                          | 206 (159–275)         | 190 (147–241) | 0·40 | 0·21     |
| C-reactive protein concentration, mg/L | 5·6 (2·1–10·7); n=25       | 13·7 (11·7–18·0); n=7 | 11·6 (3·0–4·7); n=86 | 0·018 | 0·023 |
| Lactate dehydrogenase concentration, U/L | 388 (341–509); n=27 | 375 (352–474); n=10 | 463 (368–616); n=86 | 0·10 | 0·041 |
| Alanine aminotransferase concentration, U/L | 26 (15–36); n=22 | 36 (24–84); n=7 | 29 (19–50); n=74 | 0·29 | 0·31 |
| Creatinine concentration, μmol | 64 (51–79); n=24 | 86 (71–93); n=8 | 68·5 (53–83); n=80 | 0·10 | 0·35 |
| Ct value of first nasopharyngeal SARS-CoV-2 PCR | 29·2 (24·8–34·2) | 26·2 (24·0–29·5) | 25·6 (21·6–30·6) | 0·11 | 0·040 |
| **Outcomes**              |                                        |                       |          |          |
| Pneumonia                | 15 (52%)                               | 5 (50%)               | 47 (51%) | 1·00     | 1·00     |
| Hypoxia requiring supplemental oxygen | 0 (0%) | 3 (30%) | 26 (28%) | 0·0050 | 0·0013 |
| Intensive care unit admission | 0 (0%) | 3 (30%) | 15 (16%) | 0·025 | 0·021 |
| Invasive mechanical ventilation | 0 (0%) | 3 (30%) | 10 (11%) | 0·020 | 0·12 |
| Death                    | 0 (0%)                                 | 0 (0%)                | 2 (2%)   | 0·65     | 1·00     |

Data are median (IQR) or n (%). p values are from Kruskal-Wallis test (for continuous variables) or χ² test (for categorical variables). Δ382=382-nucleotide deletion. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2. Ct=cycle threshold. *Δ382 variant only group versus wild-type only group versus mixed Δ382 variant and wild-type group. †Δ382 variant only group versus wild-type only group.

Table 1: Demographic and clinical characteristics of patients infected with Δ382 variant SARS-CoV-2, wild-type SARS-CoV-2, or both.
Outcomes
The primary endpoint for this study was the proportion of patients who developed severe COVID-19, defined by hypoxia requiring supplemental oxygen. Secondary outcomes were the concentrations of immune mediators in plasma samples. All other clinical findings and viral PCR Ct values were exploratory outcomes.

Statistical analysis
Data processing and analysis were done in the R statistical language (version 3.3.1) and Stata version 15. We compared continuous variables with Mann-Whitney U or Kruskal-Wallis tests, and categorical variables with Fisher’s exact test or χ² test as appropriate. Exact logistic regression was used to examine the association between the SARS-CoV-2 infection group and the development of hypoxia (the primary outcome). The following covariates were considered for inclusion in the multivariable exact logistic regression model: age group (<45 years, 45–64 years, or ≥65 years), sex, Charlson Comorbidity Index group (0 or ≥1) and infection group (wild-type, Δ382 variant, or mixed wild-type and Δ382 variant).

For the cytokine analysis, we compared logarithmically transformed concentrations between patients with Δ382 variant and those wild-type virus infections by use of an unpaired t-test. Plots were generated with GraphPad Prism software (version 8).

All statistical tests were two-sided, and p values less than 0·05 were considered to indicate statistical significance. Adjustment for multiple testing was not done.

Role of the funding source
The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. LFPN, GJDS, and BEY had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
Of the 432 individuals diagnosed in Singapore with PCR-confirmed SARS-CoV-2 infection between Jan 22 and March 21, 2020, 276 (64%) had residual samples available for PCR analysis (appendix pp 2–4)." SARS-CoV-2 was detected in 251 (91%) of these individuals, among which the Δ382 variant was detected in 44 (18%). 131 (52%) of the 251 individuals screened for the Δ382 variant had been enrolled in the PROTECT study and had clinical data available for further analysis (appendix pp 5–6). Among them, 92 (70%) were infected with the wild-type virus only and 39 (30%) with the Δ382 variant (29 [74%] of whom had the Δ382 variant only and ten [26%] of whom had co-infection with the wild-type virus). Serial respiratory samples were available for four individuals, and capillary electrophoresis of PCR products showed the Δ382 variant replacing wild-type virus as infection progressed into the second week from symptom onset (figure 1).

Infection groups were similar by sex and comorbidities (table 1). Comparing the Δ382-variant only group with the wild-type only group, those infected with the Δ382 variant were younger overall, with only one (3%) patient aged 65 years or older, in contrast to ten (11%) in the wild-type only group. Patients with Δ382-variant infection presented later after symptom onset, with a lower median temperature, and with less systemic inflammation according to baseline laboratory investigations than the wild-type only group (table 1). SARS-CoV-2 PCR Ct value from the first respiratory sample was lower from wild-type versus Δ382 infections though this difference was not apparent after adjusting for day of sample collection (appendix p 7).

Clinical outcomes were considerably better in patients infected with the Δ382-variant than with the wild-type virus. Although rates of pneumonia visualised on chest radiograph were similar across all three infection groups, fewer patients required supplemental oxygen in the Δ382-variant only group (0 [0%] of 29) than in the Δ382-variant and wild-type co-infection group (three [30%] of ten) and the wild-type only group (26 [28%] of 92; absolute difference 28% [95% CI 14–28]; p=0·0050 [χ² test]; table 1). After adjustment for age group and presence of comorbidities, patients infected with the Δ382-variant had lower odds of developing hypoxia (adjusted odds ratio 0·07 [95% CI 0·00–0·48]; table 2) compared with those infected with wild-type virus.

Plasma samples were available for 97 patients: 64 (66%) patients infected with wild-type virus, 25 (26%) with the Δ382-variant, and eight (8%) with mixed wild-type and Δ382-variant infection (figure 2). Higher concentrations of IFN-γ and lower concentrations of the

### Table 1: Cytokine concentrations and clinical outcomes

| Age, years | OR (95% CI) | p value | Adjusted OR (95% CI) | p value |
|------------|-------------|---------|---------------------|---------|
| <45        | 1 (ref)     | 1 (ref) |                     |         |
| 45-64      | 1·15 (0·73–1·85) | 0·21 | 1·00 1·85 (0·76–5·04) | 0·39 |
| ≥65        | 1·35 (0·86–2·12) | 0·18 | 1·00 2·05 (0·94–4·49) | 0·08 |

Sex

|                | OR (95% CI) | p value | Adjusted OR (95% CI) | p value |
|----------------|-------------|---------|---------------------|---------|
| Female         | 1 (ref)     | 1 (ref) |                     |         |
| Male           | 1·51 (0·58–4·17) | 0·49 | 1 (ref)             |         |

Charlson Comorbidity Index

|                | OR (95% CI) | p value | Adjusted OR (95% CI) | p value |
|----------------|-------------|---------|---------------------|---------|
| 0              | 1 (ref)     | 1 (ref) |                     |         |
| ≥1             | 1·00 (2·67–24·110) | 0·0001 | 6·36 (1·76–25·68) | 0·0030 |

Infection

|                | OR (95% CI) | p value | Adjusted OR (95% CI) | p value |
|----------------|-------------|---------|---------------------|---------|
| Wild-type only | 1 (ref)     | 1 (ref) |                     |         |
| Δ382 variant   | 0·07 (0·00–0·40) | 0·0008 | 0·07 (0·00–0·48) | 0·0035 |

Δ382=382-nucleotide deletion. Odds ratio. *Adjusted for age, Charlson Comorbidity Index, and infection group.

As the conditional maximum likelihood estimate is unbounded (ie, infinite), the median unbiased estimate (ie, regression estimate that places the observed sufficient statistic at the median of the conditional distribution) is computed.

Table 2: Exact logistic regression analysis of candidate predictors for hypoxia requiring supplemental oxygen
Heatmap of immune mediator levels in plasma samples of patients infected with either wild-type (n=64), Δ382 variant (n=25), or mixed wild-type and Δ382 variant severe acute respiratory syndrome coronavirus 2 (n=8; indicated by asterisks in figure) during the first collection timepoint upon hospital admission (median 8 days from symptom onset). Each colour represents the relative concentration of a particular analyte (blue=low concentration; red=high concentration). Δ382=382-nucleotide deletion.

Figure 2: Concentrations of 45 immune mediators quantified using a 45-plex microbead-based immunoassay

Table: Concentrations of 45 immune mediators quantified using a 45-plex microbead-based immunoassay

| Immune Mediators | Wild-type | Δ382 |
|------------------|-----------|------|
| IFN-γ            |           |      |
| TNF-α            |           |      |
| IL-2              |           |      |
| IL-5              |           |      |
| RANTES            |           |      |
| VEGF-A           |           |      |
| MIP-1β           |           |      |
| MCP-1            |           |      |
| IL-10            |           |      |
| HGF              |           |      |
| LIF              |           |      |
| PIGF-1           |           |      |
| IFN-γ            |           |      |
| IP-10            |           |      |
| HGF              |           |      |
| LIF              |           |      |
| VEGF-A           |           |      |
| RANTES           |           |      |

Discussion

The Δ382 variant of SARS-CoV-2, which emerged in Wuhan early in the pandemic and was exported to Singapore and Taiwan, was transmitted as a co-infection with the wild-type virus, and became the dominant virus in the second week of illness. The Δ382 variant causes...
clinically significant illness, including pneumonia, but infections tended to be milder compared with those caused by the wild-type virus, with less pronounced cytokine release during the acute phase of infection. The observed attenuated clinical features further suggest that ORF8 is a possible target for therapeutic intervention and for the development of a SARS-CoV-2 controlled human infection model.

We observed that patients infected with the Δ382 variant had lower concentrations of proinflammatory cytokines, chemokines, and growth factors that are strongly associated with severe COVID-19. Notably, patients with pneumonia from the Δ382 variant had higher concentrations of SDF-1α, which is usually downregulated during hypoxia. These findings corroborated our clinical observations that patients infected with the Δ382 variant had better clinical outcomes, as shown by the lower proportion of patients in the Δ382 variant group who had hypoxia requiring supplemental oxygen. The in-vitro replication kinetics of the Δ382 variant are similar to those of wild-type SARS-CoV-2, consistent with the viral loads observed in these patients, indicating that the ORF8 deletion does not reduce replicative fitness. This finding is contrary to the reduced replication observed in SARS-CoV viruses with an ORF8 deletion.

Further analysis of immune mediator profiles in patients with mild symptoms revealed that patients infected with Δ382 variants had more effective T-cell responses and platelet regulation during the early phase of the infection. T-cell responses are severely impaired in patients with SARS-CoV-2 infection. Lymphopenia and functional exhaustion of T cells correlate with disease severity in COVID-19. The more robust production of IFN-γ during the early phase of the infection, which was observed in patients infected with the Δ382 variant, could promote and maintain the effector functions of T cells, which might mediate rapid and effective antibody responses against SARS-CoV-2.

SARS-CoV-2 ORF8 targets host proteins in endoplasmic reticulum quality control, extracellular matrix organisation, and glycosaminoglycan synthesis. Our STRING analysis showed that host proteins in endoplasmic reticulum trafficking are interacting partners pivotal to multiple pathways in T-cell-mediated immunity and platelet regulation. This finding is consistent with an interaction between the viral protein encoded by ORF8 and host MHC-I leading to downregulation of cytotoxic CD8 T-lymphocyte-mediated antiviral activity. Given the important roles of ORF8 in mediating SARS-CoV-2 immune evasion, inhibition of its function could be investigated as a potential therapeutic strategy.

The repeated emergence of SARS-CoV-2 viruses with a deletion in ORF8 suggests this region is important for viral adaptation to humans. Studies have reported that ORF8 is strongly immunogenic, and that antibodies are produced against ORF8 early during SARS-CoV-2 infection. Significant CD4-positive and CD8-positive T-cell responses to ORF8 have also been described in patients who recover from COVID-19.

Figure 3: Chain of transmission between cases as established by epidemiological investigations

Δ382=382-nucleotide deletion. *Possible epidemiological links were identified when individuals with COVID-19 had a common physical location or timing but direct interaction could not be clearly established; possible epidemiological links would also reflect transmissions that have arisen from close contact between a case and a few possible sources in the same cluster.
exhibit altered virus transmissibility, but belong to early outbreak sequences for which no significant difference in transmissibility is observed.25

Our study has a number of limitations. First, respiratory samples for SARS-CoV-2 PCR were collected as part of routine clinical care. We did not have samples from early in the course of the illness for many patients, and some patients’ samples were not available. Second, although every effort was made to corroborate epidemiological data, the data are subject to recall bias and linkages might have been missed or incorrectly inferred. Third, we adjusted for known major determinants of severe COVID-19 in the multivariable model, but there might have been unmeasured confounders that could explain some of the differences in clinical outcomes. The presence of transmission clusters can also amplify bias, and it is possible that recruited patients were not representative of their infection groups. Fourth, some mixed-wt-type and A382 variant infections are suspected from epidemiological linkage but were undetected by PCR. This could reflect limitations of this assay for detecting mixed infection, or virological sampling only later in the course of infection. Finally, blood samples were collected as early as possible in the course of the illness, but inevitably they were not available on the same day post-infection or symptom onset.

In summary, ORF8 is a hotspot for genetic variation in coronaviruses. The clinical effect of deletions in this region appears to be a milder infection with less systemic release of proinflammatory cytokines. Further study of these variants could improve our understanding of SARS-CoV-2 virology and pathogenesis and could have implications for the development of treatments and vaccines.

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