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Early detection of community acquired of SARS-CoV-2 lineage B.1.351 in Hong Kong

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ABSTRACT

Background: Prior to this report, variants of concern for SARS-CoV-2 were only detected from imported cases in Hong Kong.
Objective: Multiple cases of SARS-CoV-2 lineage B.1.351 have been identified in local community. We reported the phylogenetic relationship of these cases.
Study design: SARS-CoV-2 cases were screened for the key non-synonymous substitutions in spike protein by different assays. Preliminary positive cases were further tested by whole genome sequencing.
Results: From Dec 2020 to May 2021, 55 SARS-CoV-2 cases belonged to lineage B.1.351. Among them, eight genomes were clustered together, all of them were local cases with epidemiological link.
Conclusions: To track variants of SARS-CoV-2 and to allow early implementation of control measures, SARS-CoV-2 genomic surveillance must be consistently performed.

1. Introduction

The SARS-CoV-2 has been spreading around the world since 2019 and its variants harboring various mutations have been increasingly identified (henceforth: 'variant' stands for virus with one or more mutations that are different from Wuhan-Hu-1 reference sequence (GenBank accession no. MN908947.3) [1]. Several new variants that emerged in late 2020 had raised concerns and called variant of concern (VOC) as they share common features, notably a high genetic changes in the spike (S) protein [2–6] which is a key target for vaccine, virus entry and infectivity [7].

In Hong Kong, the Public Health Laboratory Services Branch (PHLSB) implemented intensive surveillance system to track the emergence of variants in Hong Kong since December 2020. Variants of SARS-CoV-2 B.1.1.7 (the PANGO lineage nomenclature system is used throughout the article) and B.1.351 were identified last year [8,9]. These two variants demonstrated high non-synonymous substitutions in the S protein when compared with other SARS-CoV-2 viruses detected in Hong Kong in 2020 [10]. Although SARS-CoV-2 variants were identified worldwide, they were only detected from imported cases in Hong Kong.

Between April 2021 and May 2021, 11 cases of the SARS-CoV-2 B.1.351 were identified in local community setting. Here, we report the early detection community acquired variants of SARS-CoV-2 cases in Hong Kong.

2. Methods

2.1. COVID-19 cases investigation

In Hong Kong, all COVID-19 confirmed cases were either diagnosed or confirmed by PHLSB. Contact tracing investigations were conducted by Communicable Disease Branch. Epidemiological information of confirmed cases were announced to the public through daily press conference. Epidemiologic information described in the manuscript were retrieved from public datasets [11–13].

2.2. SARS-CoV-2 variants screening

From December 2020, all SARS-CoV-2 cases were screened for the key non-synonymous substitutions in S gene by partial S gene sequencing and/or real-time RT-PCR assays. Majority worldwide circulating variants were screened out. These in-house developed protocols are available on request.

In brief, the RNA of the original specimens were put to RT-PCR. Sanger sequencing of the partial length covering 400–530 amino acid positions of S protein were performed using a pair of primers. Four different SNP real-time RT-PCRs were designed with probes targeting 452R, 484K, 484Q and 501Y. All of them were located in the S gene. These four PCRs could screen out variants including B.1.1.7, B.1.351, P.1, P.2, P.3, B.1.427, B.1.429, B.1.525, B.1.526, B.1.617.

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Table 1
Patients' characteristics of the local SARS-CoV-2 cases having 484K and 501Y and their affected individuals.

Patient	HK Case	Age / sex	Confirmed date	Date of onset	484K & 501Y mutations	Lineage
1	Case 11643	29 yrs / M	17 Apr 2021	asymptomatic	Yes	B.1.351
2	Case 11672	31 yrs / F	18 Apr 2021	asymptomatic	Yes	B.1.351
3	Case 11773	39 yrs / F	30 Apr 2021	23 Apr 2021	Yes	B.1.351
4	Case 11774	10 mos / F	30 Apr 2021	29 Apr 2021	Yes	B.1.351
5	Case 11792	36 yrs / F	5 May 2021	asymptomatic	Yes	NA
6	Case 11793	46 yrs / F	5 May 2021	asymptomatic	Yes	B.1.351
7	Case 11797	38 yrs / F	5 May 2021	asymptomatic	Yes	B.1.351
8	Case 11800	67 yrs / F	7 May 2021	5 May 2021	Yes	B.1.351
9	Case 11815	42 yrs / M	12 May 2021	asymptomatic	Yes	NA
10	Case 11816	40 yrs / F	13 May 2021	asymptomatic	NA	NA
11	Case 11825	4 mos / M	16 May 2021	asymptomatic	Yes	B.1.351

NA: Not applicable, results were not known due to low viral load of the samples.

2.3. Whole genome sequencing

Preliminary cases of significant SARS-CoV-2 variants were then put to whole genome sequencing.

For whole genome sequencing, extracted RNA was reverse transcribed to cDNA using LunaScript® RT SuperMix Kit followed by PCR amplification using the ARTIC network nCoV-2019 version 3 primer set. DNA libraries were prepared using the Illumina Nextera XT kit (Illumina, San Diego, CA, USA). Sequencing was performed on the MiSeq (Illumina) to generate paired-end 151-bp reads. The Illumina reads were aligned to the reference Wuhan-Hu-1 SARS-CoV-2 genome (MN908947.3) using BWA [14]. The iVar v1.3.1 was used to trim primer sequences from the aligned reads. Variant calling and consensus sequence generation were performed using SAMtools version v 1.12 and iVar v1.3.1 [15]. The consensus sequences generated in the present study have been deposited into GISAID (Appendix A).

Multiple sequence alignment was performed using MAFFT [16]. The maximum-likelihood whole genome phylogenetic tree was constructed using IQ-TREE v 2.1.2 using a GTR substitution model and the -czb option with 1000 bootstrap replicates [17].

3. Results

The first local SARS-CoV-2 case having 484K and 501Y was reported on 17 Apr 2021. The case was a 29-year-old male (Patient 1) who arrived in Hong Kong from Dubai on 19 March 2021 (Table 1). SARS-CoV-2 tests were negative throughout the quarantine period from 19 March 2021 to 8 April 2021. He stayed at his friend's home starting from 8 April 2021. He planned to return to Dubai and performed SARS-CoV-2 test at a community center on 15 April 2021. The test results were SARS-CoV-2 positive and possessed 484K and 501Y. The day after, his friend (Patient 2) was also tested positive for SARS-CoV-2 484K and 501Y.

On 30 Apr 2021, another two local SARS-CoV-2 484K and 501Y cases were reported. One of the cases was a 39-year-old woman (Patient 3) who worked as a domestic helper in Tung Chung. The 10-month-old baby (Patient 4) whom was taken care of by the helper, was also positive for SARS-CoV-2 484K and 501Y.

In view of the presence of local SARS-CoV-2 484K and 501Y cases, the government requested all foreign domestic helpers to undergo compulsory SARS-CoV-2 RT-PCR testing [18]. On 5 May 2021, a local SARS-CoV-2 484K and 501Y case was reported, the case was a 36-year-old woman (Patient 5), who was the employer of Patient 3. On the same day, two foreign domestic helpers (Patient 6, Patient 7) were tested positive for SARS-CoV-2 484K and 501Y.

From 7 May 2021 to 16 May 2021, four local SARS-CoV-2 484K and 501Y cases were reported (Patient 8–11). They were close contacts of local SARS-CoV-2 484K and 501Y cases, namely, the mother (Patient 8) and brother (Patient 9) of Patient 2; Patient 10 was a household contact of Patient 9, Patient 11 was the son of Patient 9.

Except three cases due to low viral load in the samples, all local SARS-CoV-2 484K and 501Y cases were put to whole genome sequencing. Only one virus from each case was analyzed. All local SARS-CoV-2 484K and 501Y cases belonged to B.1.351 lineage.

In order to determine the relatedness of the local SARS-CoV-2 B.1.351 cases, phylogenetic analysis was performed for all B.1.351 genome sequences detected by PHLSB. From 27 Dec 2020 to 24 May 2021, 74 cases had E484K and N501Y mutations. Whole-genome sequences were determined for 67 cases with sufficient reads for analysis. Among them, 12 belonged to P.3 lineage, 55 belonged to B.1.351 lineage. Eight local SARS-CoV-2 B.1.351 genomes were successfully sequenced, all of them formed a well-supported monophyletic cluster and marked by the presence of the SNP C17127T that was not seen in other B.1.351 sequenced imported cases (Fig. 1). These eight genomes were separated from one another by 0–2 single-nucleotide polymorphisms (SNP).

Contact tracing investigations found that all local SARS-CoV-2 B.1.351 cases were epidemiology linked. Patient 1 and Patient 2 attended gathering with Patient 9 and Patient 10 on April 13 in Chai Wan. Subsequently, Patient 9 and Patient 10 attended another gathering with Patient 3, Patient 6 and Patient 7 on April 18 in Sham Shui Po.

4. Discussion

Our data showed that during the six-month period of surveillance, we were capable of identifying the first local SARS-CoV-2 B.1.351 case and its transmission chain. By routinely applying SNP real-time RT-PCRs and partial S gene sequencing on SARS-CoV-2 positive samples, early detection of this VOC was achieved. Based on the patients' epidemiological information, Patient 1 was most likely the index case of this transmission chain. The virus was transmitted to other individuals through social gatherings. Subsequently, affected individuals further transmitted the virus to others.

The source of infection for Patient 1 was not known. There were three possible sources of infection. First, he was infected from Dubai before his travel to Hong Kong. However, SARS-CoV-2 tests were negative during the 3-week period of quarantine. In addition, the viral load for the specimen collected on 15 Apr 2021 was high (Ct 17.11) which indicated a recent infection. Second, he was infected by a silent carrier of SARS-CoV-2 484K and 501Y variant from community after quarantine. It was also unlikely since local SARS-CoV-2 484K and 501Y cases have not been reported before 15 April 2021. Lastly, he was infected during quarantine period. The local SARS-CoV-2 B.1.351 variant cases were most closely related to the other two imported cases, they were incoming travelers whom also stayed at the same designated quarantine

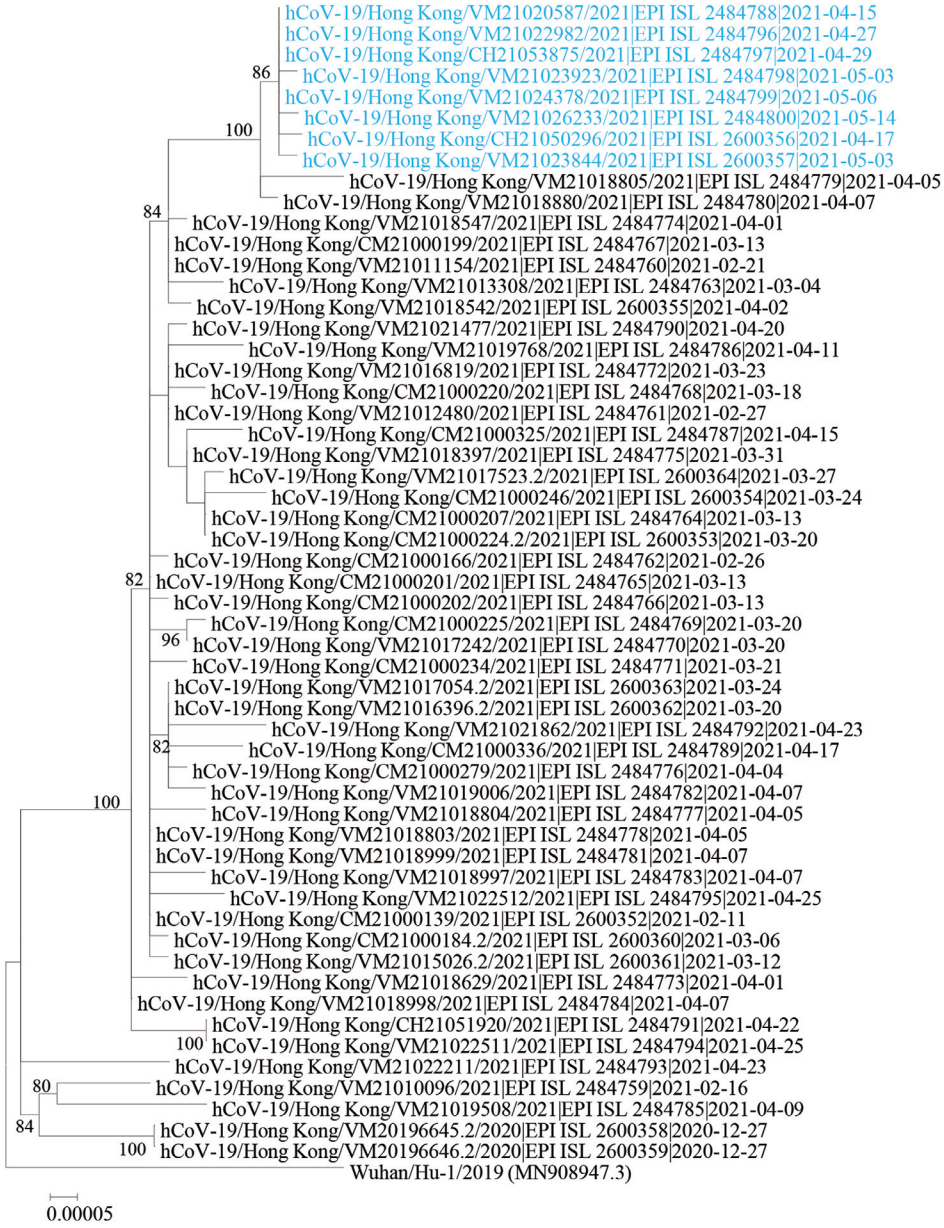

Fig.1. Phylogenetic analysis of 55 genomes of SARS-CoV-2 B1.351 detected in Hong Kong from Dec 2020 to May 2021. Cases highlighted in blue were local cases. The Wuhan-Hu-1 strain was included as reference sequence (GenBank accession no. MN908947).

hotel which Patient 1 had been staying. The local cluster and the two closely related cases had their own unique mutations: G2305T, T5941G, C13011T, C17127T, G24932T and T25333C, except ambiguous bases were called at position 5941 in Patient 2 and Patient 8 and at position 24,932 in Patient 11 due to low read depth.

Successful COVID-19 control could only be achieved by widespread testing, contacts tracing and cases isolation. At the time of writing this report (June 2021), local SARS-CoV-2 B.1.351 cases have not been detected since the last case reported on 16 May 2021. It is expected that variant of different types will be encountered in future. To track variants, control measures and SARS-CoV-2 genomic surveillance must be regularly performed to provide early warning on potential origins of cases so that effective control measures can be implemented on time.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jcvp.2021.100029.

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