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Multiple introductions of SARS-CoV-2 B.1.1.214 lineages from mainland Japan preceded the third wave of the COVID-19 epidemic in Hokkaido

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ABSTRACT

Background: The third wave of the COVID-19 epidemic in the island of Hokkaido, the second largest island in Japan, began abruptly in October 2020.

Methods: We conducted a phylodynamic analysis of the SARS-CoV-2 genome sequences obtained from tertiary medical centers in the Greater Tokyo Area and Sapporo, the largest city in the island of Hokkaido, and genome sequences published by GISAID, an international SARS-CoV-2 genome database. We also analyzed the statistics on the person-nights of travelers in the island of Hokkaido from the Greater Tokyo Area in 2019 versus 2020.

Results: At least eight sub-lineages belonging to the B.1.1.214 lineage were introduced to the island of Hokkaido from the island of Honshu, the mainland of Japan from late July to November 2020, during the governmental travel promotion program. Five of the eight sub-lineages originated from the Greater Tokyo Area. Comparison of the monthly ratios of the person-nights of travelers in the island of Hokkaido from the Greater Tokyo Area in 2019 and 2020 revealed that the highest value occurred in October 2020.

Conclusion: We contend that the Japanese governmental travel promotion program contributed to the introduction of the B.1.1.214 sub-lineages from the main island of Honshu to the island of Hokkaido, and drove the third wave in Hokkaido, even if we are unable to establish the causality.

1. Introduction

The third wave of the COVID-19 epidemic in the island of Hokkaido, which represents the second largest island in Japan, began abruptly in October 2020. The number of infections in the third wave was much larger than that in the first or the second wave, with more than 10,000 people becoming infected. However, event contributing to this third wave of COVID-19 in the island of Hokkaido, if any, has remained unclear.

Viral genomic analyses in the United Kingdom, which is separated from continental Europe by the English Channel, showed that cross-island travel contributes significantly to viral spread [1]. In that

Hokkaido is separated from the most populated main land of Japan named Honshu, which is home to Tokyo as the capital city of Japan, and that most (i.e., more than 85%, <https://www.mlit.go.jp/common/001193700.pdf>) of the visitors to the island of Hokkaido arrive by air and that there have virtually been few foreign visitors, if any, introduction of the virus from the island of Honshu region into the island of Hokkaido, triggering the third wave, is a distinct possibility [2]. The steady increase in the airline passenger traffic between Tokyo and Sapporo, the largest city in the island of Hokkaido, from April 2020 to October 2020 lends support to this hypothesis: The ratio of the number of passengers in 2020 to those in 2019 was about one-tenth in April 2020, but only about one-third in October 2020. (<https://www.anahd.co.jp/group/pr/>). An

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alternative hypothesis is that the third wave was caused by resurgence of pre-existing viral lineages in the island of Hokkaido.

Recently, a group from the United States conducted phylogenetic analysis, and demonstrated by phylogenetic analysis, that a large number of independent introductions of the alpha lineage into the US had occurred [3]. We hypothesized that comparison of the genomic sequences of the viral lineages isolated between the island of Honshu and the island of Hokkaido may enable to clarify if the third wave in the island of Hokkaido was brought about by the introduction and rapid transmission of incoming sub-lineages, or by a resurgence of pre-existing lineages. In the present study, we performed phylodynamic analysis of SARS-CoV-2 genome sequences isolated at several tertiary medical centers in Japan, including in the island of Hokkaido, together with the genomic data available from the international repository, the Global Initiative on Sharing All Influenza Data (GISAID) EpiCoV™ database [4], in an attempt to identify the trigger for the third wave of the COVID-19 epidemic in the island of Hokkaido.

In addition, because mass tourism has been shown to be a major factor in the spread of the disease [1], and because the Japanese government supported the recovery of industries that suffered economic distress as a result of the restrictive measures, including behavioral restrictions, imposed during the first wave by promoting domestic travel between July 22 and November 24, 2020 [5], we analyzed the tourist traveler trends and cell phone data between prefectures to infer the temporal relationship between changes in the mobility of the populace and the introduction of specific viral sub-lineages into the island of Hokkaido.

The governmental domestic tourism promotion program offered significant discounts on hotel rates. In addition, from October, coupons were issued under “cash-back” program [6]. Until September 30, 2020, any travel to Tokyo and that by residents of Tokyo were excluded from the promotion program because of concerns about the ongoing epidemic. On October 1, 2020 such exclusion was stopped. However, in October and November of 2020, the number of COVID-19 patients increased in the island of Hokkaido, and the travel promotion program was suspended on November 24, 2020.

2. Materials and methods

2.1. Ethics statement

The present study was conducted at Keio University Hospital, a tertiary-care medical institution in the Greater Tokyo Area (Tokyo, Japan), and Hokkaido University Hospital, a tertiary-care medical institution in Sapporo, the largest city in the island of Hokkaido. The present study protocol was approved by the ethics committee of the Keio University School of Medicine (approval number: 20200062). The use of an opt-out method was approved. The requirement for written informed consent was waived owing to it being an observational study the urgent need to collect COVID-19 data and safety.

2.2. Whole viral genome sequencing

The sequence data belonging to the B.1.1.214 lineage as per the definition coined by the Phylogenetic Assignment of Named Global Outbreak Lineages [7], were downloaded from the GISAID database together with the corresponding metadata. Some of the SARS-CoV-2 genomes were generated by our group and submitted to the GISAID database as follows.

SARS-CoV-2 genomes were obtained directly from nasopharyngeal swab or saliva specimens by next-generation sequencing using the Illumina technology, as previously reported [8]. Polymerase chain reaction-based amplification was performed using ARTIC nCoV-2019 primers version 3 in two multiplex reactions, according to the globally accepted nCoV-2019 sequencing protocol (<https://www.protocols.io/view/ncov-2019-sequencing-protocol-bbmuik6w>). The sequencing

library for amplicon sequencing was prepared using the Next Ultra II DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA). Paired-end sequencing was performed on the MiSeq platform (Illumina, San Diego, CA, USA). The bioinformatic pipeline used in this study, the mutation calling pipeline for amplicon-based sequencing of the SARS-CoV-2 viral genome, is available at <https://cmg.med.keio.ac.jp/sars-cov-2/>. All single-nucleotide substitutions, including non-synonymous and synonymous mutations, were annotated using ANNOVAR software and assessed using VarSifter (<https://research.nhgri.nih.gov/software/VarSifter/>).

2.3. Phylodynamic analysis

Phylodynamic analysis [9], including subsampling, alignment, phylogenetic inference, temporal dating of ancestral nodes, and discrete trait geographic reconstruction, as well as inference of the most likely transmission events, was performed using the Nextstrain pipeline suite [10], as previously described [11]. Briefly, we aligned sequences, subsampled sequences (see below), added sequences from the rest of the world for phylogenetic context based on genomic proximity, reconstructed the phylogeny using IQ-Tree version 2.1.2 [12]. A maximum likelihood analysis implemented using the program TreeTime version 0.8.1 was then used to infer a time-scaled phylogeny [13].

2.4. Temporal changes in the proportions of viral lineages

Temporal changes in the number of genomes of SARS-CoV-2 viral lineages as classified by the Pangolin nomenclature system [7] in the island of Hokkaido and in Japan, overall, were calculated from the metadata available from the GISAID database [4].

2.5. Extraction of sequences derived from the B.1.1.214 lineage

From the entire B.1.1.214 lineage dataset of 17,156 sequences and associated metadata available from GISAID as of July 9, 2021, a smaller set of 7,971 B.1.1.214 genomes were generated using a subsampling method implemented in the Augur software package [10]. We included the 590 samples of B.1.1.214 lineages from the island of Hokkaido (Supplementary Table). The sample PG-10684 with extremely increased number of mutations for collection date was discarded.

2.6. Analysis of inter-prefecture travel data

We used the data which are openly available from the Overnight Travel Statistics Survey [14] conducted by the Japan Tourism Agency of the Japanese government on monthly number of travelers to the island of Hokkaido from other prefectures who stayed in hotels targeted for tourists multiplied by the number of nights stayed (number of person-night travelers). Travelers were considered to be on a sightseeing trip when they stayed in a hotel in which more than 50% of the guests were on a sightseeing trip (on annual basis) according to governmental statistics.

The number of person-night travelers from the Greater Tokyo Area, including Tokyo, Kanagawa, Saitama, Chiba, Tochigi, Ibaraki, Gunma, and Yamanashi, to the island of Hokkaido were counted monthly. The ratios of the static for the hotel stay (i.e., number of person-night travelers) in 2020 versus those in 2019 were calculated month-on-month to clarify the possible influence of the COVID-19 pandemic and the governmental travel promotion.

Because Hyogo was later shown to be one of the originating locations of the introduction of B.1.1.214 to the island of Hokkaido (see Results section), the number of person-night travelers from Hyogo Prefecture to the island of Hokkaido was counted monthly. The ratios of the static for the hotel stay (i.e., number of person-night travelers) in 2020 versus those in 2019 were calculated month-on-month.

2.7. Analysis of mobile phone location data

As an indicator of the effectiveness of social distancing measures, we used mobility data provided by Google [15]. The data were downloaded from Google COVID-19 Community Mobility Reports (<https://www.google.com/covid19/mobility/>). These reports define the mobility changes for the following six categories, that is, Retail and recreation, Grocery and pharmacy, Parks, Transit stations, Workplaces, Residential, as compared to the baseline defined as the median mobility in January 2020 before the start of the global pandemic of COVID-19.

3. Results

3.1. Geographical and temporal transmission of B.1.1.214 lineages in Japan

Our previous work and other work on the genome sequence of SARS-CoV-2 obtained from Japan showed that most, if not all, of the lineages observed between June and November 2020 were the B.1.1.284 or B.1.1.214 lineages, both of which have been observed almost exclusively in Japan [11,16]. Further analysis of the metadata of B.1.1.284 and B.1.214 from Hokkaido alone and all Japan showed that the B.1.1.284 lineage was the most prevalent lineage during the late summer of 2020 and the B.1.1.214 lineage became more prevalent after October 2020 (Fig. 1).

3.2. Spread of the B.1.1.214 lineage in the island of Hokkaido

The evolution of the B.1.1.214 lineage in Japan is shown in Fig. 2. The data points denoted by the purple circles represent the sequences obtained in the island of Hokkaido. The B.1.1.214 lineage (C18167U (NSP14:P43L), G21518U (NSP16:R287I)) first appeared on June 1 in 2020 on the island of Honshu. This lineage accumulated mutations in a serial manner and three discrete haplotype groups were recognized. The first haplotype had C8917U (NSP4:F121F), which occurred around June 4, 2020 (95% confidence interval [CI], June 4 to June 18). The second one had C6433U (NSP3:T1238T), which occurred around June 27, 2020 (95% confidence interval [CI], June 21 to June 27). The third one had A6592G (NSP3:K1291K), G28541A (N: A90T) and G1738A (NSP2: V311V), which occurred around July 25, 2020 (95% confidence interval [CI], July 19 to August 3).

The group of C8917U branched out five more times. The first time was the C29679U (3'UTR) mutation, which occurred around July 2 (95% CI, June 11 to July 10). The second was C12049U (NSP7:N69N), which occurred around July 25(95% CI, July 22 to July 31). The viral

lineages with the C8917U > C29679U > C12049U haplotype were divided into to two subgroups: the subgroup that acquired the additional mutation G20560U (NSP15:V314F) around August 19 (95% CI, August 13 to August 23) and the other subgroup that acquired G23587U (S: Q675H) and C29353U (N:Y360Y) around August 18 (95% CI, August 8 to September 4). The latter subgroup acquired two additional mutations C17502U (NSP13:F422F) > C6380U (NSP3:L1221F) around September 29 (95% CI, September 22 to September 29).

The B.1.1.214 lineages entered the island of Hokkaido through at least eight discrete routes during the governmental travel promotion period of July 22, 2020 to November 24, 2020 (Fig. 3). For each haplotype detected in Hokkaido by the month of November, there was an ancestral genome detected earlier in the main island of Honshu (Fig. 4). Five of the eight routes derived from the Greater Tokyo Area (Supplementary material).

Two haplotypes propagated in the island of Hokkaido. The major one had the C8917U > C29679U > C12049U > G23587U, C29353U > C17502U > C6380U haplotype which was inferred to originate from Hyogo prefecture (Supplementary Figure (h)). The next largest cluster is presumed to be the influx from the Greater Tokyo Area (Supplementary Figure (c)). According to time view shown as the left panel of Supplementary Figure (c), the haplotype with C8917U was also observed in June and early July before the governmental travel promotion, we cannot deny the possibility that the influx from Honshu during this period was latent until November. However, according to divergence view shown as the right panel of the same figure, latent direct import from the Greater Tokyo Area to the island of Hokkaido is more likely.

3.3. Time-series analysis

The monthly numbers of travelers who travelled to the island of Hokkaido from other area and stayed in hotels targeted for tourists multiplied by the number of person-night travelers were available for all the prefectures in Japan from January 2019 to December 2020.

This statistic, the number of person-night travelers, for each month was compared between 2019 and 2020 (Fig. 5). The statistic for 2020 divided by that for 2019 represents the balance between the discouraging effect of the COVID-19 pandemic and counteracting effect of the governmental travel promotion. The monthly ratios of the static on tourists visiting in the island of Hokkaido from the Greater Tokyo Area in 2020 to those in 2019 revealed that the ratio was the highest in October 2020, when the national governmental travel promotion program between Tokyo and Hokkaido was launched.

The annual trends of the six indices of mobility obtained from the Google COVID-19 Community Mobility Reports in 2020 were analyzed

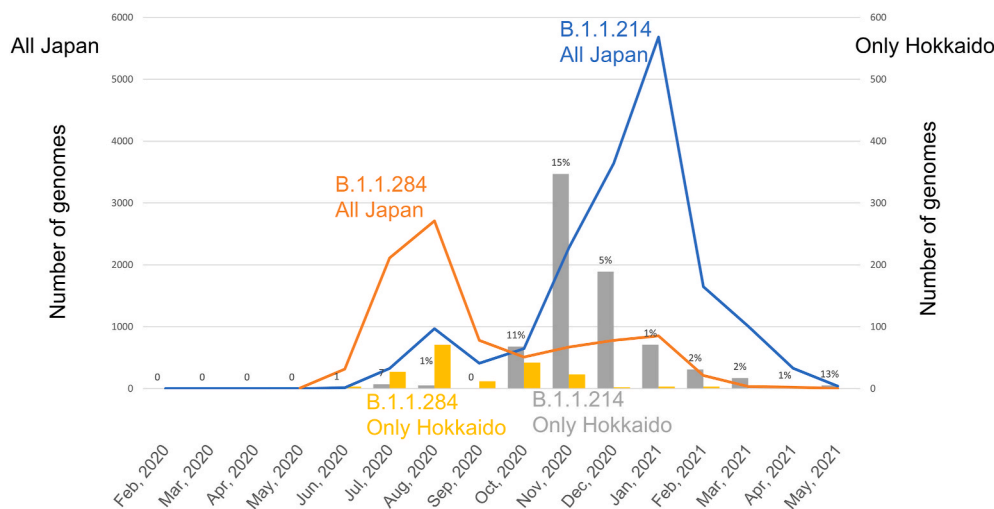


Fig. 1. Monthly number of genomes of SARS-CoV-2 sub-lineages B.1.1.284 and B.1.1.214 in Hokkaido and All Japan during 2020. Bar graphs show monthly number of COVID-19 genomes in Hokkaido. Yellow and grey bars indicate B.1.1.284 and B.1.1.214, respectively. Line graphs show monthly number of COVID-19 genomes in Japan including Hokkaido. Orange and blue lines indicate B.1.1.284 and B.1.1.214, respectively. Note B.1.1.214 started to take over B.1.1.284 in October both in Japan. B.1.1.214 had not been observed until October 2020 in Hokkaido. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

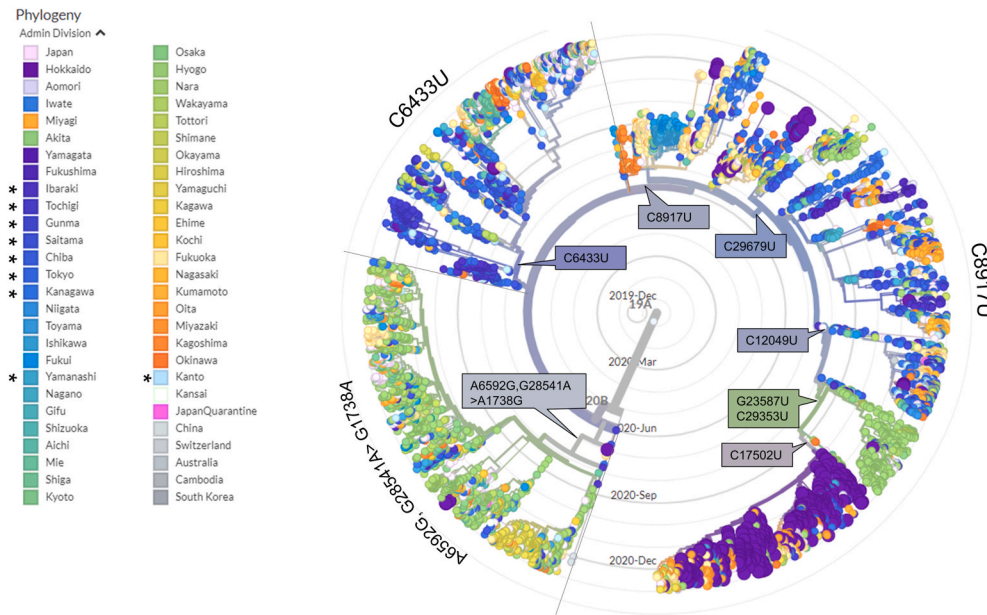


Fig. 2. Phylogenetic tree of the B.1.1.214 lineages observed in Hokkaido in 2020. All the B.1.1.214 lineages isolated in Hokkaido are displayed together with relatively similar B.1.1.214 sequences observed in the non-Hokkaido regions of Japan. The tree is displayed in a radial layout and the branch lengths correspond to the time (i.e., number of mutations in comparison with the original SARS-Cov-2 genomic sequence determined in Wuhan, China). The circles correspond to individual samples. Mutation characterizing haplotype groups are shown at the corner of the branches. The circles (i.e., samples) are colored according to the prefectures from which the samples were collected. Circles representing cases for which the sampling location was not known are colored pink. Samples collected from Hokkaido are indicated by the large purple circles. Supplementary Figures (a), (b), (c), (e) and (f) are derived from the Greater Tokyo Area. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

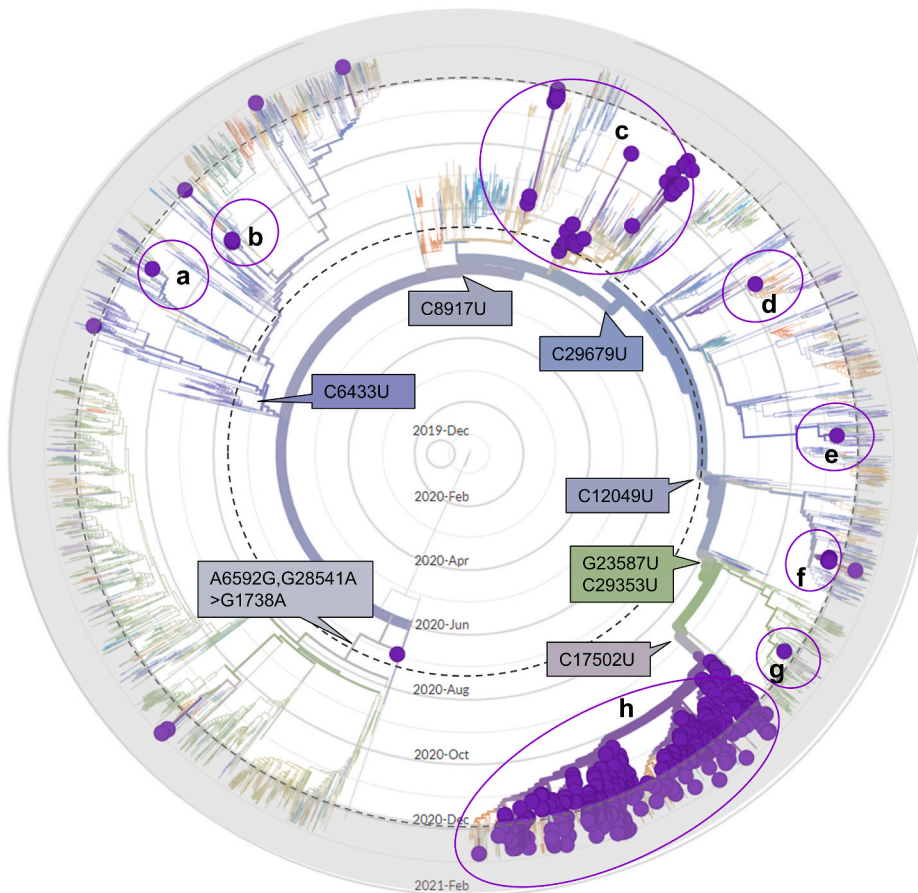


Fig. 3. Phylogenetic tree of the B.1.1.214 lineages focus on Hokkaido in 2020. The dashed lines indicate the duration of the Go To campaign. (a) to (h) are enlarged views of a to h in the overall subtree. The top row is the mutations along the path from the root of the phylogenetic tree. The frame is the diverged point just before diverging into the Hokkaido sub-lineage.

(Fig. 6). The trend for “Parks” reflected simple seasonal changes, that for “Grocery and pharmacy” remained relatively stable,” the trends for “Workplaces” and “Residential” showed an inverse relationship (high for “Workplaces” and low for “Residential” on weekdays, data not

shown), and the trends for “Retail and recreation” and “Transit stations” were similar: they increased in parallel three times during the governmental travel promotion in the summer to fall of 2020. The first increasing phase was correlated with the summer vacation. In many

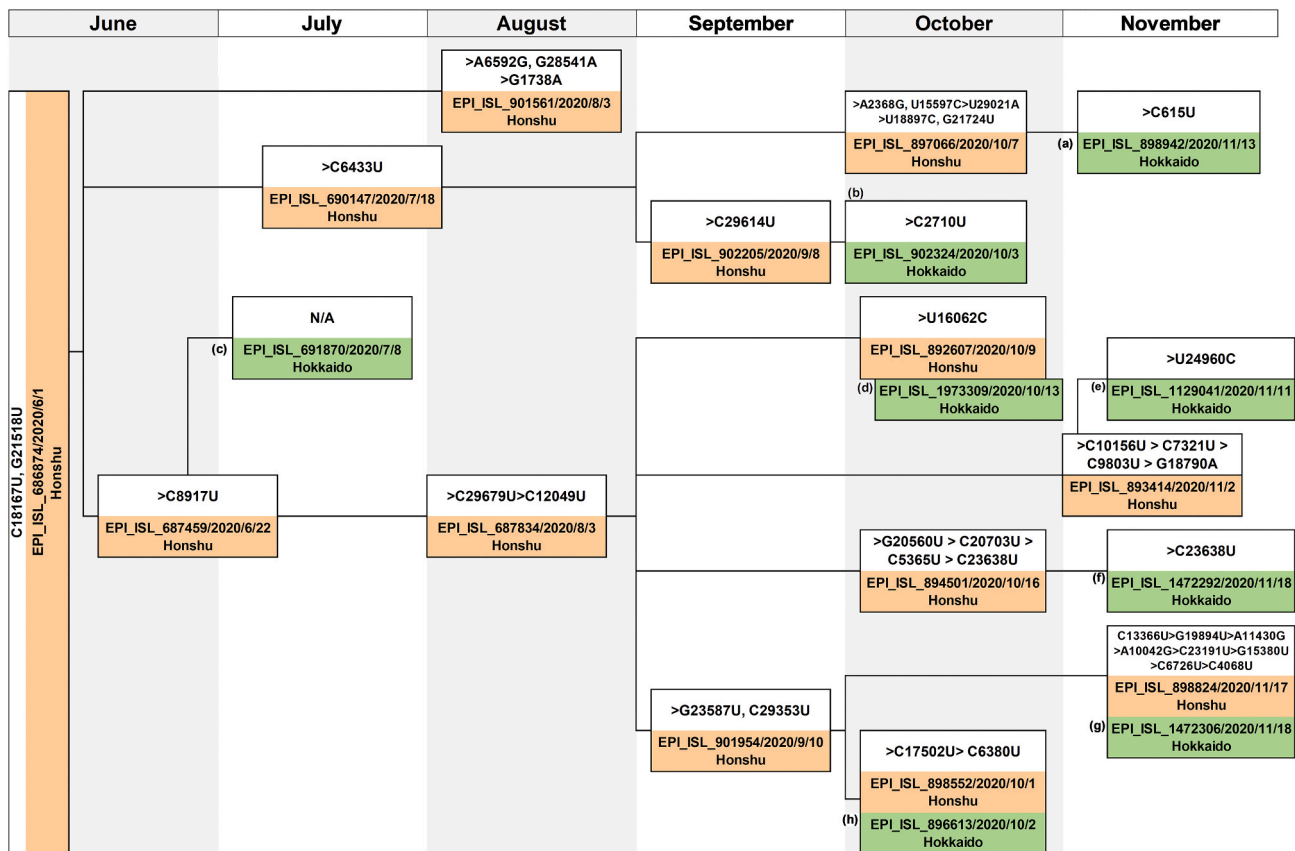


Fig. 4. Chronological accumulation of mutations in the B.1.1.214 sub-lineages. The date of first appearance of new sub-lineages with additional mutations are shown. Sub-lineages detected in the island of Honshu are colored orange, whereas those detected in the island of Hokkaido are colored green. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

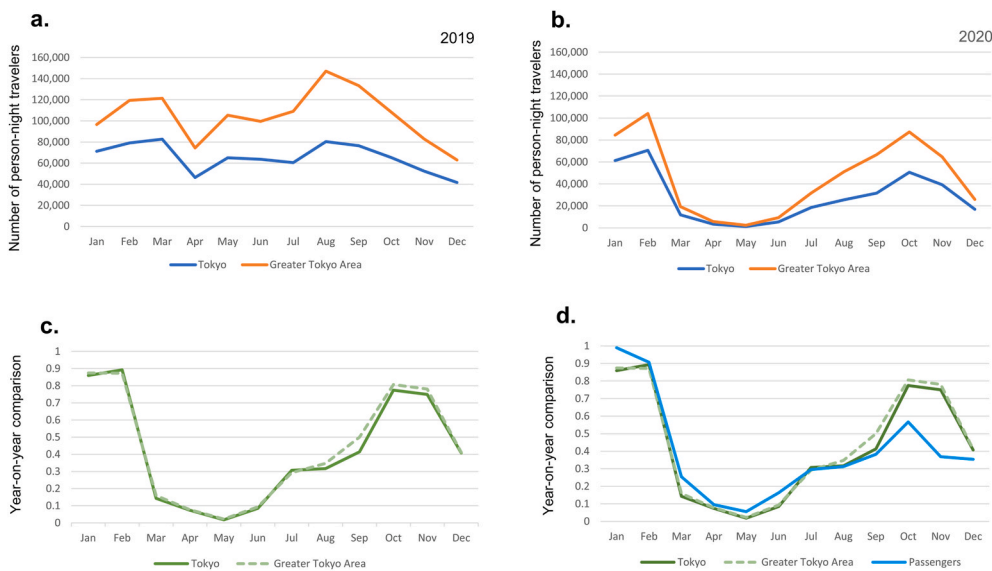


Fig. 5. Trends in monthly number of travelers to Hokkaido from the Greater Tokyo Area who stayed in hotels targeted for tourists multiplied by the number of nights stayed (the number of person-night travelers) in 2019 and 2020. The number of person-night travelers (a, b) Data indicate the trends in 2019 (a) and 2020 (b). The blue lines and orange lines correspond to the statistic of residents of Tokyo and the Greater Tokyo Area including Tokyo, respectively. The trend in Tokyo and that in other Greater Tokyo Area are essentially the same. (a) In 2019, there were two peaks: One in the winter skiing season in February and March, and the other in August. The statistic was the maximal in August and decreased steadily thereafter. (b) In 2020, there was a marked decrease, almost down to zero, during the initial phase of the COVID-19 epidemic until June 2020. From July to September, the statistic increased gradually and there was a steep rise in October. Hence, the peak was delayed by two months, from August to October. (c, d) Each monthly statistic of 2020 was divided

by the statistic for the corresponding month of 2019 to adjust for seasonal changes in Hokkaido visitors. The peak was in October. Note that the ratio of the statistic approached 0.8, indicating that sightseeing in October was near the yearly standard before the pandemic. The 2020 statistic for the monthly passenger traffic on the Tokyo-Sapporo flight on one of the two major domestic airlines, All Nippon Airways, was compared with the corresponding 2019 statistic. Note that the 2020 versus 2019 ratio of the statistic for hotel stay (number of guests multiplied by the length of stay) exceeded that for the number airline passengers. This discrepancy may reflect an increase in the length of stay. The discrepancy was exaggerated in October, when the governmental travel promotion between Tokyo and Hokkaido was launched. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

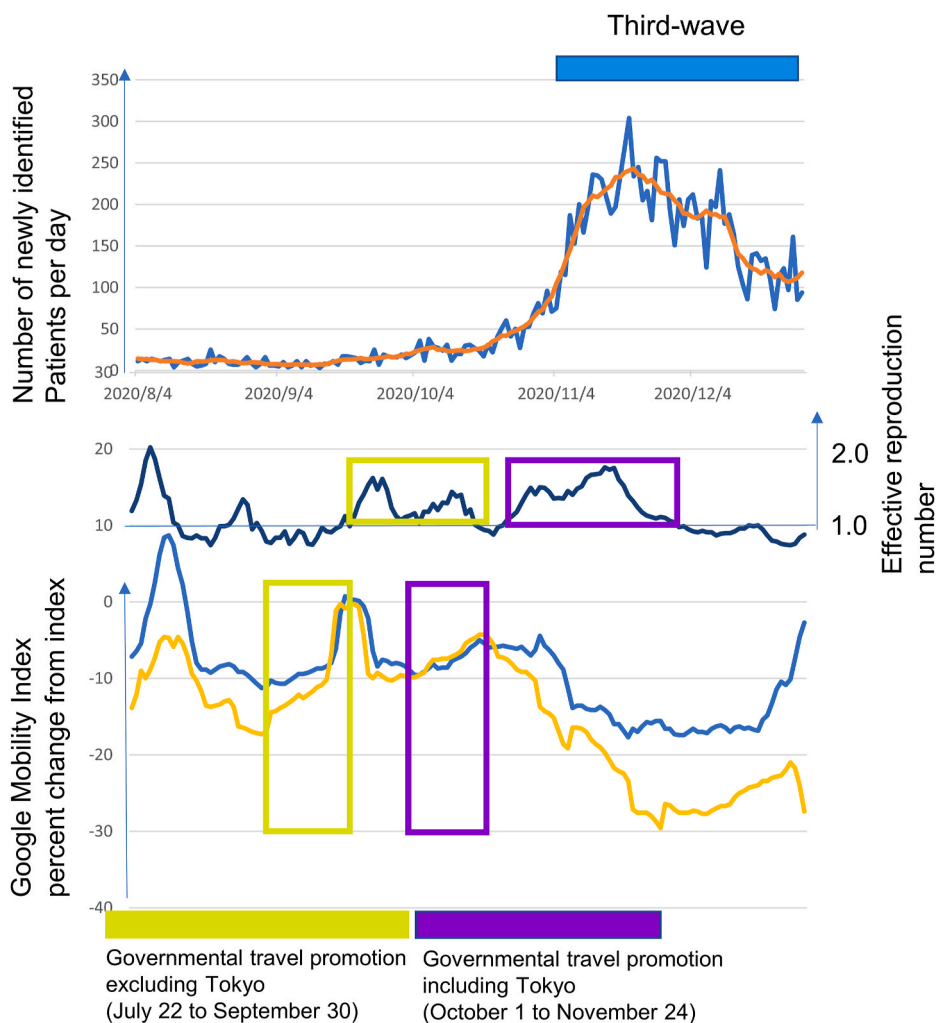


Fig. 6. The temporal relationship between the COVID-19 epidemic and human mobility in Hokkaido from August 1 to December 31, 2020. **Top:** Number of newly identified COVID-19-positive cases per day and the sliding average. **Middle:** Effective reproduction number. The light green squares and purple squares represent periods during which the effective reproduction number was above 1.0 and the epidemic was expanding. **Bottom:** Google mobility index. Percent change from the baseline of the index for “Retail and recreation” (blue) and that for “Transit stations” (dark yellow). The light green square corresponds to the first phase during which both the indices for “Retail and recreation” and “Transit stations” were increasing. Note the increasing phases depicted in light green and purple led two periods with effective reproduction numbers of over 1.0, depicted in light green and purple, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

schools in the island of Hokkaido, summer vacation started on August 8 and ended on August 18. The second increasing phase occurred during the 4-day holiday from September 19 to September 22. This holiday predated the start of the governmental travel promotion from Tokyo and coupons campaign.

The monthly ratios of the static on tourists visiting in the island of Hokkaido from Hyogo prefecture in 2020 to those in 2019 revealed that the ratio increased steadily from May to November 2020 (data not shown).

4. Discussion

Here, we document the results of a phylodynamic analysis of the B.1.1.214 lineages of SARS-CoV-2 in Japan for the period from June 2020 to December 2020, to better define the situation in the island of Hokkaido, which is a unique region in that it represents the second largest island of Japan that is separated from the main island (Honshu) by the sea, so that most visitors to the island of Hokkaido arrive by air. We inferred that the B.1.1.214 lineages entered the island of Hokkaido independently from other parts of Japan on multiple occasions. Specifically, the B.1.1.214 lineages entered the island of Hokkaido through at least eight discrete routes between October 1 and November 30, 2020. Five of them derived from the Greater Tokyo Area. Most of the patients who were infected after October 1, 2020, were infected with the B.1.1.214 lineage. One sub-lineage, which significantly propagated in Hokkaido was introduced from Hyogo prefecture far away from Tokyo.

Our analysis of the travel data from the Greater Tokyo Area to the

island of Hokkaido published by the Japan Tourism Agency lent support to the notion that the governmental travel promotion succeeded in promoting tourist travel to the island of Hokkaido, especially in October 2020 (Fig. 5). Among the six mobility indices in the island of Hokkaido obtained from the Google COVID-19 Community Mobility Reports in 2020, “Retail and recreation” and “Transit stations” reflected tourism, because they were high during the summer vacations, the 4-day holiday in September, and 3-day holiday in October (Fig. 6). It is noteworthy that the increase in mobility during the summer vacation or the 4-day holiday in September did not contribute to resurgence of the pre-existing lineage B.1.1.284 lineages, despite reports that increase in recreational activities, including restaurant and bar visits, can lead to super-spreading of the virus [17]. In other words, measures to maintain social distancing were relatively effective until September 2020, that is, right before the program for travel promotion from Tokyo to the island of Hokkaido and coupons campaign were launched on October 1, 2020. Although the causality is difficult to establish, it is not unreasonable to suggest the governmental travel promotion contributed to the introduction of the B.1.1.214 lineages.

In view of the propagation of the C8917U > C29679U C12049U > G23587U, C29353U > C17502U > C6380U haplotype in the island of Hokkaido in November and December, the introduction from Hyogo prefecture, which is in the island of Honshu is outside of the Greater Tokyo Area but contributed significantly to the third wave of COVID-19 in the island of Hokkaido. Because governmental travel promotion program started in late July 2020, and there was no change in the incentives for travel promotion from Hyogo prefecture to the island of

Hokkaido in October 2020, the contribution of travel promotion program is less apparent for this haplotype.

The present study had several limitations. First, it is possible that more sub-lineages of B.1.1.214 might have entered the island of Hokkaido, since sequencing was conducted only for limited proportions of PCR-positive cases. Second, there is the theoretical possibility of the emergence of haplotype groups after entry of the sub-lineages into the island of Hokkaido. However, the relative complexity of the haplotypes observed in the island of Hokkaido makes such a possibility unlikely. Third, precise inferences about the geographical origins of samples are strongly dependent on the comprehensiveness of the Japanese dataset. Transmission origins cannot be inferred for areas for which genomic data are not available.

Some events including the Marathon race of the Olympic Games 2021 are to be held in the island of Hokkaido in August 2021 [18]. Potentially, many visitors from the island of Honshu and from other regions are expected to visit the island of Hokkaido, mingle, and then return to their respective home countries. The lessons learned from the resurgence of COVID-19 caused by the B.1.1.214 sub-lineages in the island of Hokkaido in October 2020 should be borne in mind when planning relaxation of social distancing measures for the event. It is prudent that the local government has decided to hold all events at Sapporo Dome in the island of Hokkaido without spectators, and has asked people to refrain from watching along the marathon course.

In retrospect, increase in the mobility of the people seems to have preceded the resurgence in the of number of infections in the island of Hokkaido in October 2020. Real-time monitoring of the mobility data may benefit governmental stakeholders to decide the best time to discontinue the travel promotion program [19,20]. Considering that Hokkaido is an isolated island mainly connected to the Honshu region by air, pre-boarding antigen screening or PCR screening of passengers for COVID-19 may be helpful as a preventive measure [19].

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Author contributions

Conceived and designed the study: TS, KK. Designed and/or performed experiments: TS, KA, TT, MY, KK. Analyzed patients' data: KA, MS, SN, KK, SK. Analyzed and interpreted data: TS, KA, TT, MY, MS, SK, TT, KK. Wrote the manuscript: TS, KA, TT, MY, HS, MS, SN, KK, SK, TT, KK. All authors read and approved the final manuscript.

CRediT authorship contribution statement

Takako Shimura: Conceived and designed the study, Designed and/or performed experiments, Analyzed patients' data, Analyzed and interpreted data, Wrote the manuscript. **Kodai Abe:** Designed and/or performed experiments, Analyzed patients' data, Analyzed and interpreted data, Wrote the manuscript. **Toshiki Takenouchi:** Designed and/or performed experiments, Analyzed and interpreted data, Wrote the manuscript. **Mamiko Yamada:** Designed and/or performed experiments, Analyzed and interpreted data, Wrote the manuscript. **Hisato Suzuki:** Wrote the manuscript. **Makoto Suematsu:** Analyzed patients' data, Analyzed and interpreted data, Wrote the manuscript. **Sho Nakakubo:** Analyzed patients' data, Wrote the manuscript. **Keisuke Kamada:** Analyzed patients' data, Wrote the manuscript. **Satoshi Konno:** Analyzed patients' data, Analyzed and interpreted data, Wrote the manuscript. **Takanori Teshima:** Analyzed and interpreted data, Wrote the manuscript. **Kenjiro Kosaki:** Conceived and designed the study, Designed and/or performed experiments, Analyzed and interpreted data, Wrote the manuscript.

Declaration of competing interest

The authors declare no competing interests. Funding sources had no role in the design and conduct of the study, collection, management, analysis and interpretation of the data; and preparation, review, or approval of the manuscript.

Acknowledgments

We downloaded the full nucleotide sequences of the SARS-CoV-2 genomes from the GISAID database (<https://www.gisaid.org/>). Sequence data were obtained from GISAID and tables listing all accession numbers of the sequences and contributors are available as supplementary information.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tmaid.2021.102210>.

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