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Genomic Sequencing of a SARS Coronavirus Isolate That Predated the Metropole Hotel Case Cluster in Hong Kong, *Stephen S.C. Chim,[1] Yu-Kwan Tong,[1] Emily C.W. Hung,[2] Rossa W.K. Chiu,[1] and Y.M. Dennis Lo[1]** (Departments of [1]Chemical Pathology and [2]Paediatrics, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong; * address correspondence to this author at: Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Room 38023, 1/F Clinical Sciences Bldg., 30-32 Ngan Shing St., Shatin, New Territories, Hong Kong SAR; fax 852-2194-6171, e-mail loym@cuhk.edu.hk)

The epidemic of severe acute respiratory syndrome (SARS) swept across the globe, with reported cases in more than 30 countries. As of July 11, 2003, the number of reported probable cases was 8437, with 813 deaths *(1)*. A novel coronavirus, SARS-CoV, was promptly implicated as the causative agent *(2–4)*. Macaques infected with SARS-CoV subsequently developed respiratory symptoms and pathology similar to SARS patients, thus fulfilling the Koch postulates *(5)*. Efforts in sequencing the viral genome promptly followed, and the genomic sequence revealed little homology to previously characterized strains of coronaviruses *(6, 7)*. The complete genomic sequences of several SARS-CoV isolates have since been made publicly available (www.ncbi.nlm.nih.gov).

Several sequence variations exist among isolates. In general, based on these sequence variations, the majority of the isolates can be segregated into two groups: isolates that were obtained from individuals who were epidemiologically linked to and those who were not linked to the Metropole Hotel in Hong Kong *(8–10)*. Ruan et al. *(10)* compared the genomic sequences of 14 SARS-CoV isolates and suggested that a haplotype comprising four nucleotide positions, namely, 9404, 17564, 22222, and 27827 [GenBank accession no. AY274119 *(7)*], clearly defined two distinct genotypes. Isolates that were epidemiologically linked to the Metropole Hotel cluster have the configuration T:T:T:T, as opposed to the sequence C:G:C:C seen in the unassociated strains. (Note: The usage of the DNA-based code for the designation of SARS-CoV haplotypes does not imply that this virus possesses a DNA genome.)

SARS was first reported in Guangdong Province, China, in November 2002 *(11)*. Isolates that demonstrated the C:G:C:C haplotype were epidemiologically traceable to the early part of the epidemic *(9)*. On the other hand, SARS was first reported in Hong Kong when a cluster of cases was noted among visitors to the Metropole Hotel. This case cluster comprised international travelers who subsequently brought SARS to other countries, including Vietnam, Canada, and Singapore *(11)*. Epidemiologic investigations revealed that the cases were traceable to a nephrologist from Guangdong Province, China, who checked into the hotel on February 21, 2003 *(8, 11)*. These data suggest that since the emergence of SARS-CoV in

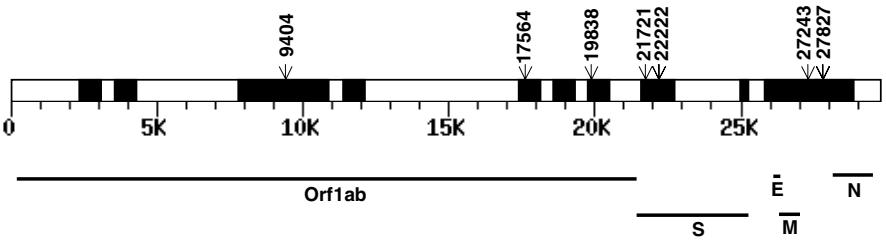

Fig. 1. Partial genomic sequence of SARS-CoV isolate CUHK-L2.

Regions of the CUHK-L2 isolate that were sequenced are indicated in *black*. *Arrows* indicate sites used for haplotype comparison. Open reading frames and nucleotide positions are shown in reference to the Tor2 sequence. *Orf1ab*, *S*, *E*, *M*, and *N* denote orf 1ab polyprotein, spike glycoprotein, envelope protein, membrane protein, and nucleocapsid protein, respectively.

southern China, at least two strains of the virus had emerged *(9)*.

We recently confirmed a case of SARS that presented in Hong Kong before the report of the case cluster at the Metropole Hotel. It would be of interest to determine whether this strain is related to either of the reported groups. This patient, designated A, presented to a hospital in Hong Kong on February 17, 2003, with a 2-day history of fever, chills, rigors, dry cough, and intense malaise. She resided in the US, but before symptom onset, she had been visiting her ailing mother in Guangzhou, China. Her mother died on February 12, 2003, of a cause unknown to the family. After admission, patient A deteriorated rapidly and required intensive care. Her chest radiograph revealed patchy infiltration, and serologic testing subsequently showed markedly increased antibody titers against SARS-CoV. Four household members and two healthcare workers later developed fever and respiratory symptoms.

In view of the distinct epidemiologic history, serum samples were retrieved from patient A. Viral RNA was extracted from 280 μL of the patient's serum with a QIAamp Viral RNA Mini Kit (Qiagen), according to the manufacturer's instructions, and eluted in 50 μL of RNase-free water; 11 μL of viral RNA was then reverse transcribed by Superscript III (Invitrogen) with reverse primers (PCR-R) targeting regions on the SARS-CoV genome that flank 20 selected polymorphic sites. Because polymorphisms seen in a single isolate could potentially be a result of culture or sequencing artifacts, we selected the target sites based on polymorphisms that were shared by at least two SARS-CoV isolates published in GenBank at the time of this study. The product was then amplified with use of 14 pairs of forward (PCR-F) and reverse (PCR-R) primers in a cDNA polymerase mixture (BD Clontech), with initial denaturation at 95 °C for 1 min and 35 cycles of denaturation at 95 °C for 0.5 min, annealing at 55 °C for 0.5 min, and extension at 68 °C for 1.5 min, and a final extension at 68 °C for 10 min. Seminested PCR was performed with the PCR-F and BSEQ-R series of primers with the same thermal profile. Primer sequences are available in the Data Supplement accompanying the online version of this Technical Brief at http://www.clinchem.org/content/vol50/issue1/. Multiple negative PCR controls were included in each amplification.

The DNA of each amplicon was sequenced by the dideoxy dye terminator method on an automated DNA sequencer (3100 Genetic Analyzer; Applied Biosystems) based on capillary electrophoresis. The PCR-F, ASEQ-F, BSEQ-F, ASEQ-R, and BSEQ-R series of oligonucleotides were used as sequencing primers. Sequences were edited and aligned, and comparisons were made with the SeqScape software (Applied Biosystems). Regions that revealed nucleotide substitutions were confirmed by resequencing with a combination of different primer sets to ensure the quality of the sequencing data. On the whole, the sequencing covered one-third of the virus genome (Fig. 1) and was deposited at GenBank (accession nos. AY443086 to AY443095).

In contrast to the SARS-CoV isolates sequenced to date, the viral genomic sequence obtained from patient A, CUHK-L2, revealed a haplotype configuration of T:G:C:C (Table 1), which represents a combination of the two genotypes that typify the isolates associated and those not associated with the Metropole Hotel *(10)*. This is particularly interesting in view of the epidemiologic history of patient A. She had a history of travel to southern China, whereas her presentation clearly predated the Metropole Hotel cluster. The CUHK-L2 sequence represents the third SARS-CoV genotype directly traceable to southern China early in the course of the SARS epidemic, with a transitory sequence that bridges the two major genotypes reported earlier.

We then compared the CUHK-L2 sequence with the complete genomic sequences of other SARS-CoV isolates. We note that in addition to the four-nucleotide haplotype, the nucleotide sequences at three additional positions, 19838, 21721, and 27243 (Table 1), also distinctly segregated the isolates associated with the Metropole Hotel from the unassociated strains with the exception of CUHK-L2 and CUHK-W1 (GenBank accession no. AY278554). Although the isolates that are or are not linked to the Metropole Hotel showed a sequence of A:G:C or G:A:T, respectively, both CUHK-L2 and CUHK-W1 had a configuration that is a combination of the two haplotypes, A:A:C. CUHK-W1, as reported previously *(9)*, was isolated from a patient who presented at approximately the same time as a major hospital outbreak in Hong Kong *(12)*. The latter hospital outbreak was epidemiologically linked to the hotel cluster. However, similar to CUHK-L2, the CUHK-W1 isolate was obtained from a patient who had no connection with the Metropole Hotel but had been traveling in southern China before symptom onset *(9)*.

When we used the combined haplotype consisting of all seven nucleotides (Table 1), the segregation between the genotypes linked or not linked to the Metropole Hotel became less distinctive. CUHK-L2 shares three and

Table 1. Haplotype comparison of SARS CoV strains from Hong Kong and two transitory genotypes.

Nucleotide position[a]	Isolate (GenBank accession no.)										Amino acid
	Linked to Metropole Hotel				Transitory		Not linked to Metropole Hotel				
	Tor2 (AY274119)	Urbani (AY278741)	SIN2500 (AY283794)	CUHK-Su10 (AY282752)	CUHK-L2 (AY443086–AY443095)	CUHK-W1 (AY278554)	BJ01 (AY278488)	BJ02 (AY278487)	BJ03 (AY278490)	GZ01 (AY278489)	
9404[b]	T	T	T	T	T	C	C	C	C	C	T, valine; C, alanine
17564[b]	T	T	T	T	G	G	G	G	G	G	T, aspartic acid; G, glutamic acid
19838	A	A	A	A	A	A	G	G	G	G	Silent
21721	G	G	G	G	A	A	A	A	A	A	G, glycine; A, aspartate
22222[b]	T	T	T	T	C	C	C	C	C	C	T, isoleucine; C, threonine
27243	C	C	C	C	C	C	T	T	T	T	C, proline; T, leucine
27827[b]	T	T	T	T	C	C	C	C	C	C	Noncoding

[a] Nucleotide positions are stated in reference to the Tor2 sequence.
[b] Part of the four-nucleotide haplotype reported by Ruan et al. *(10)*.

CUHK-W1 shares two common nucleotide sequences with the hotel-linked isolates, whereas the remaining four nucleotides for CUHK-L2 and five nucleotides for CUHK-W1 are in common with the isolates not linked to the Metropole Hotel. Epidemiologically, CUHK-L2 and CUHK-W1 were obtained from patients who presented before or around the time of the report of the hotel cluster. Thus, there is both temporal and molecular evidence to suggest that CUHK-L2 and CUHK-W1 may represent two transitory strains of SARS-CoV that bridge the evolution between the earlier SARS-CoV strains and those that are linked to the Metropole Hotel.

These data confirm that during the early part of the epidemic, the SARS-CoV was undergoing gradual evolution. However, at present, it is uncertain whether these cumulative changes contributed to the infectivity and propagation efficiency of the SARS-CoV and, thus, the development of the SARS epidemic. It remains to be seen whether this evolutionary transition of the SARS-CoV may have implications on a possible future reemergence of SARS.

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