Pandemic analysis of infection and death correlated with genomic open reading frame 10 mutation in severe acute respiratory syndrome coronavirus 2 victims

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Abstract

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues the pandemic spread of the coronavirus disease 2019 (COVID-19), over 60 million people confirmed infected and at least 1.8 million dead. One of the most known features of this RNA virus is its easiness to be mutated. In late 2020, almost no region of this SARS-CoV-2 genome can be found completely conserved within the original Wuhan coronavirus. Any information of the SARS-CoV-2 variants emerged through as time being will be evaluated for diagnosis, treatment, and prevention of COVID-19.

Methods: We extracted more than two million data of SARS-CoV-2 infected patients from the open COVID-19 dashboard. The sequences of the 38-amino acid putative open reading frame 10 (Orf10) protein within infected patients were gathered output through from National Center for Biotechnology Information and the mutation rates in each position were analyzed and presented in each month of 2020. The mutation rates of A8 and V30 within Orf10 are displayed in selected counties: United States, India, German, and Japan.

Results: The numbers of COVID-19 patients are correlated to the death numbers, but not with the death rates (stable and <3%). The amino acid positions locating at A8(F/G/L), I13, and V30(L) within the Orf10 sequence stay the highest mutation rate; N5, N25, and N36 rank at the lowest one. A8F expressed highly dominant in Japan (over 80%) and German (around 40%) coming to the end of 2020, but no significant finding in other countries.

Conclusion: The results demonstrate via mutation analysis of Orf10 can be further combined with advanced tools such as molecular simulation, artificial intelligence, and biosensors that can practically revealed for protein interactions and thus to imply the authentic Orf10 function of SARS-CoV-2 in the future.

Keywords: Big data analysis; Coronavirus disease 2019; Open reading frame 10; Protein mutation analysis; Severe acute respiratory syndrome coronavirus 2

1. INTRODUCTION

Emerging the first victim of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) reported at the end of 2019, the spread of coronavirus disease 2019 (COVID-19) and the death numbers due to SARS-CoV-2 are continuing to make history.[1] At least three strategies applied to decline the broadly expanded pandemic transmission are ongoing as expected: (1) design in time of precise detection methods to confirm the infection of SARS-CoV-2 (diagnostic aspect);[2–4] (2) discovery of curable therapies for patients with less side effects after recovery (treatment aspect);[5–7] (3) develop potentially effective vaccine for prevention of SARS-CoV-2 infection (prevention aspect).[8–10] Whatever these strategies are applied for clinic precisely, the fundamental understanding about the genomic function of SARS-CoV-2 is indeed required.[11–14]

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Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Journal of Chinese Medical Association. (2021) 84: 478-484.

Received February 18, 2021; accepted March 11, 2021.

doi: 10.1097/JCMA.0000000000000542.

SARS-CoV-2 belongs to a new strain of RNA beta-coronavirus with similar but distinct to 2003 SARS and the later Middle East respiratory syndrome coronavirus (MERS-CoV).[15–17] To understand more detail about how viral proteins help invading into the human body and the following transmission among human population, and to the design for good and prolonged detection methods and even vaccines, genomic analysis on this virus mutations is one of the most essential tasks to do.[18–21] The invasion of SARS-like pathogens into host is generally needed the interaction of the viral Spike protein and the specific receptor protein angiotensin I converting enzyme 2 on host cell surface membrane.[15,16] The mutations within some parts of the viral genome such as the Spike protein can be the major portion to be transmitted fast, and which causes for various prognosis outcome after recovering (or failure to survive). Take the Spike protein mutations as example, some positions were found to be the major cause for boosting the transmission[21] and some mutants were found to help virus escaping from vaccine killing.[22] Within the genome of SARS-CoV-2, a novel open reading frame 10 (Orf10) with relatively unique properties comparing to other SARS-like viruses, for example, SARS, MERS, and any other coronavirus that do not have such gene segment.[23–26] More recently, reinfected by the second strain of SARS-CoV-2 was found and noticed in certain mutation sites, including Orf10.[27–30] Herein, we gathered the whole genomic information of SARS-CoV-2 from more than two million patients all over the world through the database from National Center for Biotechnology Information (NCBI). The relationship between infected populations and the death numbers/death rate was statistically presented. The genomic mutation status of Orf10 in SARS-CoV-2 from selected countries, for example, the United States, Germany, Japan, and India during 8 months (from April to November) of 2020 was further analyzed and compared. Together the whole outcome here, with the simulation of molecular structures and monitoring works such as biosensing or detection will apply in proceeding information for understanding the five molecular mechanisms of transmitting spread pandemic crisis and bow the chance to end viral threat.

2. METHODS

2.1. Confirmed and death number gathering

The COVID-19 patient data including numbers of the confirmed and death patients SARS-CoV-2 infected patients and the death were from the official website: COVID-19 dashboard (https://covid-19.nchc.org.tw) built by Regents of the National Center for High-performance Computing (https://www.nchc.org.tw/), Taiwan. These data were originally gathered from the Center for Systems Science and Engineering, Johns Hopkins University (https://github.com/CSSEGISandData/COVID-19) and Taiwan Centers for Disease Control (https://www.cdc.gov.tw/). The death rate is calculated by the monthly confirm number divide monthly death number.

2.2.Sequence resource and mutation of open reading frame 10 through each month

All the Orf10 protein sequence data of the patients were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/sars-cov-2/) and COVID-19 Host Genetics Initiative (https://www.covid19hg.org/partners/), and compared in each position in amino acid. The mutation rates within each month were statistically calculated through counting the one in certain position with the original wild type or mutated from the original strain found from world-first case in Wuhan.

3. RESULTS

3.1. Pandemic of coronavirus disease 2019 update and infection–death relationship

Calculating the statistical data extracted from the open sources such as NCBI; we found the total infected numbers more than 60 million in the world with over 1.5 million death at the beginning of the first case happened in Wuhan, China (November 2019) till the end of November 2020. These include almost 15 million in the United States (almost 300 000 death), nearly 10 million in India (over 150 000 death), near 1 million in Germany (over 15 000 death), almost 150 000 in Japan (over 2000 death), and surprisingly <1000 in Taiwan (seven deaths) (Table 1). Within these SARS-CoV-2 infected patients, the general world death rate is <3% (constantly around 2.35%) and varies with different countries like 2% in the United States, 1.46% in India, 1.53% in Germany, 1.44% in Japan, and 1.12% in Taiwan (Table 1). The transmission power of this SARS-CoV-2 is extremely strong, while the causing-death ability of it is relatively weak. And this could be the major reason why COVID-19 is still ongoing influence our daily life now, since the numbers in SARS-CoV-2-infected and death patients are both still increasing.

To find out whether there are correlations between the death numbers or the death rate and the infected numbers during the pandemic of COVID-19, these data from the whole world (Fig. 1A) or from different countries (Figs. 1C and 2; and death rates in Fig. 1B) were put together and compered in each month (from April to November 2020). Basically, the pattern (if there exists) can be significantly observed after June to the end of 2020, for example, both infected numbers and death numbers are indeed closely correlated (Fig. 1A in the world; Fig. 2 in selected countries), while the death rate is not influenced by (at least not correlated to) the infected in selected countries (Fig. 1B). Before June, there is a death rate peak in selected countries as high as 15% in Japan (Fig. 1B), probably due to the limitation of hospital care within very short time to handle large amounts of COVID-19 patients at the beginning of this pandemic. This peak soon dropped down to <3% after June and stably stayed around 2% to the end of 2020. In most of the selected countries except India, there was another wave starting at September and reached to a peak at the end of November, and the trend of death numbers raised also (Fig. 2). The timing of the

Table 1

Statistical data of SARS-CoV-2 patients from the whole world and selected countries

Rank	Country	Case/million	Confirmed	Recover (recover rate)	Death (death rate)
	World	7826	60 856 294	~39 000 000 (64.09%)	1 429 689 (2.35%)
1	United States	39 196	12 912 415	~12 637 000 (98.87%)	262 832 (2.04%)
2	India	6811	9 266 705	~8 680 000 (93.67%)	135 223 (1.46%)
12	Germany	11 977	995 879	~666 000 (66.88%)	15 210 (1.53%)
48	Japan	1094	137 735	~115 000 (83.49%)	1983 (1.44%)
164	Taiwan	26	625	555 (88.8)	7 (1.12%)

SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

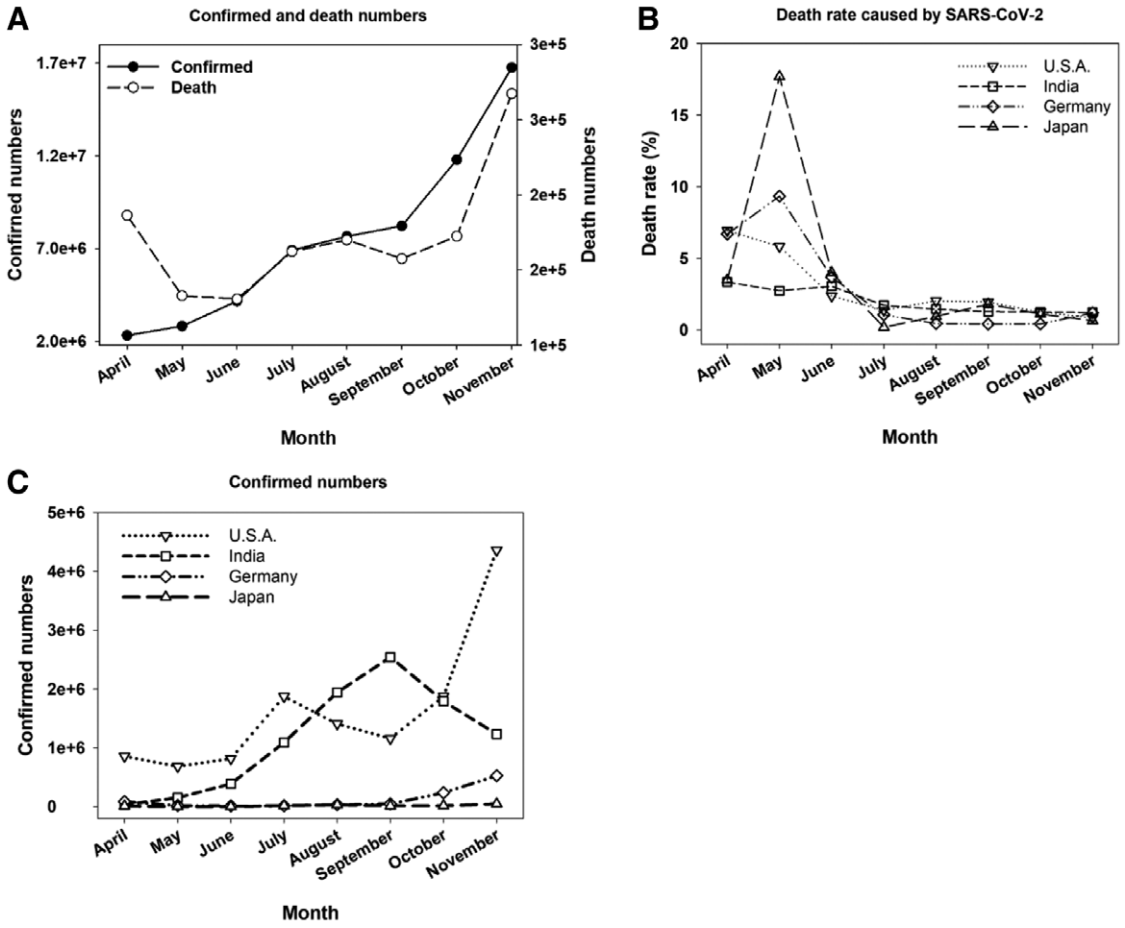

Fig. 1 Relationship between numbers of SARS-CoV-2 infected patients and the death numbers due to COVID-19 and the death rate. A, The confirmed numbers though time were plotted with the death number in the whole world. B and C, The death rate (B) and the death numbers in selected countries, United States (inverted triangle), India (rectangle), Germany (square), and Japan (triangle). COVID-19 = coronavirus disease 2019; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

latest wave near the end of 2020 may be matched to the seasons of Thanksgiving Day which is popular for European countries and the United States; but not in India (as counted both infected numbers and death rate decrease since September, Fig. 2B). Thus, the lockdown culture activities will be an efficient way to decrease the chance of transmitting SARS-CoV-2 in citizens and therefore to seize the transmission of COVID-19, although it is almost impossible.

3.2. Mutations within open reading frame 10 of severe acute respiratory syndrome coronavirus 2 infected patients

We will ask if the role of mutations in Orf10 is correlated to infected patient status? A putative protein consists 38-amino acids of Orf10 function is not clearly understand as well at present. According to the sequence alignment within various strains of coronavirus, Orf10 is unique and only exists within SARS-CoV-2 genome. It is debatable by scientist that Orf10 itself is a hard evidence for the hypothesis of artificial man-made.[17] As described previously, more data input on Orf10 genomic mutation from the SARS-CoV-2 patients are necessary. We therefore analyze the mutation rates of Orf10 genomes from different patients in different countries at protein level through the whole year 2020 in each month (Fig. 3A). The statistical data implied that almost every amino acid was changed at least 8 months comparing the original case found in Wuhan (Fig. 3B), except for three positions (N5, N25, and N36) which are relatively conserved (Fig. 3A). Furthermore, the mutation rates of three positions reached to 100% mutants in all infected populations: A8, I13, and V30 (Fig. 3A). We plotted the time course of the mutation rates of these six amino acids as shown in Fig. 3C to express the dynamic change of genomic mutations.

3.3. Open reading frame 10 A8 and V30 mutations in different countries

As the data shown in Fig. 3C, the regions of A8, I13, and V30 are the hottest spots to be mutated. We further traced the time course of Orf10 mutations found in four selected countries: United States, Japan, Germany, and India (A8 and V30 in Fig. 4A and Fig. 4B, respectively). In Germany, A8 is the major amino acid at the eighth position of Orf10 at April as shown in dash line with inverted filled triangle in Fig. 4A with no mutation which is identical to the first Wuhan case in China. Before July, the mutation rate of A8 is around 20-30%, but the rate dramatically increased between July and August (jumping up from nearly from 30% to >60%), and stably increased up to 80% on November. As in other countries such as the United States (solid line with filled circle in Fig. 4A) and India (dash line with empty triangle in Fig. 4A), the increasing rate of A8 mutations is relatively smooth and slow (from 60% to >80%; Fig. 4A). While in Japan, the rate jumped up from 40% to >60% within 1 month (April to May; dash line with empty circle in Fig. 4A).

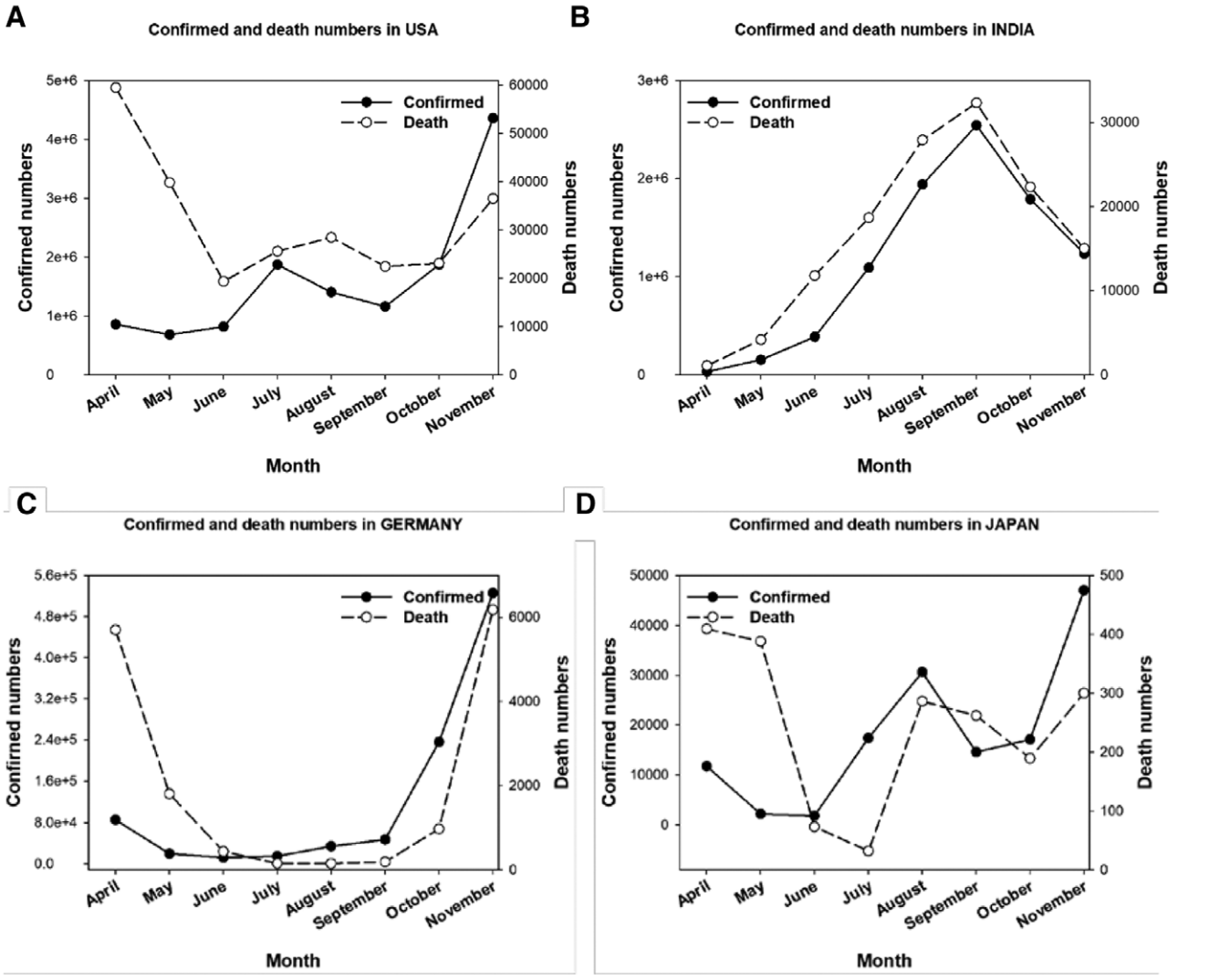

Fig. 2 Relationship between numbers of SARS-CoV-2 infected patients and the death numbers due to COVID-19 in selected countries. The confirmed numbers though time were plotted with the death number in four selected countries: United States (A), India (B), Germany (C), and Japan (D) though time were plotted with the death number within selected countries. COVID-19 = coronavirus disease 2019; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

Observing Japan at April (like A8 in Germany), the V30 region of Orf10 is the major amino acid at the 30th position, and the mutation rate of V30L jumped up from 0% to 60% within 1 month from April to May (dash line with open circle in Fig. 4B). In fact, such increasing trend also was observed in Germany (from 10% to 50% within 2 months from April to June; dash line with inverted filled triangle in Fig. 4B). The increasing trend of V30L mutation rate is smooth and slow in the United States and India (from 10% to >40%; solid line with filled circle and dash line with open triangle mark in Fig. 4B). Unknown expressed the unique trends in mutations of Orf10 especially in Germany and Japan (Fig. 4A and B) could influence the ability of SARS-CoV-2 to invade or not, whereas it is worthy for further analyzing the detailed mutations in Orf10.

3.4. Various mutations at A8 of severe acute respiratory syndrome coronavirus 2 open reading frame 10 in different countries

During mutation analysis, the patterns in different regions of Orf10 were found to be variable through time. For example, the amino acid A8 can be switched into F, G, or L (Fig. 5), but V30 can only be mutated into L (V30L, Fig. 4B). The details of A8 mutation status in different countries are shown in Fig. 5A–D. There's no significant pattern of A8 mutations in the United States (Fig. 5A) and India (Fig. 5B), but the position of A8F in Germany (Fig. 5C) and in Japan (Fig. 5D) seem to be the dominant mutant populations. Thus, the A8F (dash line with open circle) together with A8G (dash line with inverted triangle) are the major increase in A8 from July to August (Fig. 5C), and it might be correlated to the transmission of the wave of pandemic in Germany (Fig. 2C). On the other hand, the A8F is also expressed significant cause in Japan from July to August, and then from July to November (Fig. 5D), and the A8F again implied the tight correlation in both transmission and death numbers in Japan, 11 especially at the end of November (Fig. 2D).

4. DISCUSSION

A positive correlation between infected numbers and death numbers was found (the more people conformed to be SARS-CoV-2-infected, the more people died) after June 2020 (Fig. 1A). Such correlation still exists within different countries also after June

A

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	M	G	Y	I	N	V	F	A	F	P	F	T	I	Y	S	L	L	L	C
Jan.	0	0	0	0	0	0	0	0	0	0.001	0	0	0	0	0	0.042	0.0061	0.031	0
Feb.	0	0.0001	0	0.0005	0.003	0.01	0.04	0.05	0.005	0.008	0.001	0.02	0.018	0.043	0.054	0.081	0.014	0.072	0.001
Mar.	0	0.003	0	0.007	0.0081	0.05	0.089	0.08	0.012	0.014	0.032	0.056	0.023	0.14	0.081	0.15	0.018	0.14	0.024
Apr.	0	0.004	0.0001	0.015	0.012	0.064	0.15	0.64	0.045	0.024	0.041	0.091	0.14	0.21	0.13	0.18	0.024	0.18	0.081
May	0	0.009	0.0003	0.032	0.025	0.12	0.16	0.68	0.089	0.18	0.12	0.18	0.21	0.28	0.18	0.25	0.081	0.24	0.17
Jun.	0	0.01	0.0005	0.054	0.031	0.24	0.34	0.74	0.18	0.28	0.15	0.24	0.27	0.38	0.27	0.31	0.17	0.28	0.24
Jul.	0	0.045	0.07	0.057	0.052	0.32	0.42	0.88	0.31	0.37	0.24	0.28	0.35	0.41	0.29	0.39	0.24	0.31	0.28
Aug.	0	0.12	0.08	0.102	0.064	0.41	0.49	0.89	0.35	0.42	0.35	0.34	0.72	0.45	0.35	0.42	0.31	0.38	0.34
Sep.	0	0.34	0.12	0.26	0.071	0.43	0.54	0.92	0.39	0.51	0.42	0.51	0.81	0.46	0.43	0.48	0.35	0.47	0.38
Oct.	0	0.52	0.25	0.51	0.09	0.45	0.61	0.93	0.42	0.58	0.52	0.62	0.91	0.54	0.51	0.57	0.41	0.51	0.41
Nov.	0	0.67	0.34	0.63	0.13	0.51	0.64	0.94	0.51	0.62	0.67	0.72	0.94	0.61	0.59	0.67	0.52	0.71	0.59

	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
	R	M	N	S	R	N	Y	I	A	Q	V	D	V	V	N	F	N	L	T
Jan.	0	0.005	0.008	0.009	0.01	0	0	0.01	0.007	0.007	0.008	0	0.004	0.005	0.002	0.001	0.002	0.001	0
Feb.	0.009	0.017	0.012	0.014	0.051	0.004	0.01	0.06	0.04	0.02	0.01	0.08	0.03	0.021	0.051	0.01	0.03	0.021	0
Mar.	0.015	0.08	0.081	0.102	0.12	0.014	0.09	0.09	0.08	0.07	0.07	0.11	0.1	0.11	0.11	0.051	0.021	0.051	0.003
Apr.	0.11	0.14	0.21	0.15	0.21	0.021	0.15	0.13	0.14	0.13	0.11	0.18	0.12	0.18	0.13	0.094	0.06	0.083	0.015
May	0.19	0.21	0.29	0.31	0.24	0.058	0.19	0.16	0.21	0.18	0.17	0.24	0.21	0.21	0.18	0.14	0.12	0.14	0.034
Jun.	0.24	0.27	0.34	0.41	0.31	0.12	0.24	0.21	0.25	0.28	0.3	0.31	0.25	0.25	0.21	0.19	0.18	0.18	0.057
Jul.	0.29	0.34	0.38	0.48	0.35	0.16	0.31	0.28	0.31	0.32	0.34	0.4	0.31	0.31	0.25	0.21	0.2	0.24	0.18
Aug.	0.34	0.39	0.42	0.52	0.41	0.19	0.41	0.31	0.41	0.47	0.49	0.46	0.35	0.38	0.3	0.28	0.24	0.28	0.24
Sep.	0.41	0.43	0.51	0.61	0.47	0.24	0.46	0.35	0.45	0.52	0.57	0.58	0.41	0.41	0.34	0.31	0.28	0.34	0.31
Oct.	0.51	0.51	0.64	0.68	0.51	0.29	0.5	0.39	0.49	0.551	0.61	0.63	0.46	0.48	0.41	0.35	0.31	0.38	0.35
Nov.	0.72	0.62	0.71	0.72	0.63	0.38	0.53	0.41	0.53	0.62	0.73	0.71	0.51	0.53	0.44	0.41	0.35	0.43	0.42

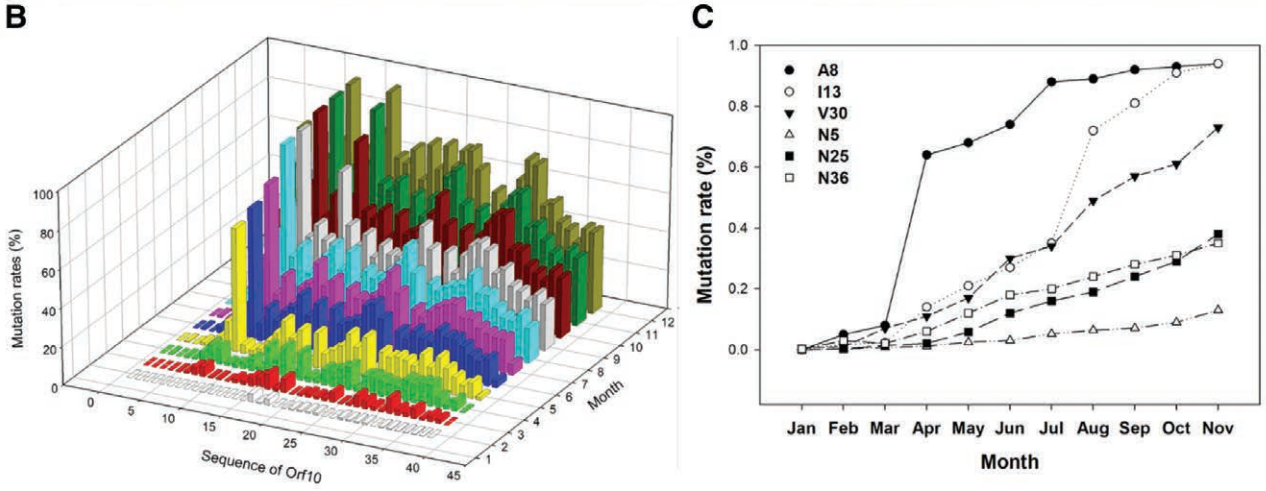

Fig. 3 Mutations in sequences of putative Orf10 protein from SARS-CoV-2 infected patients. A, Within the period from January to November (left column), the whole 38 proposed sequence of Orf10 is listed on the top part or A (with number above from 1 to 38). The mutation rates of each amino acid significantly increased through time except N5, N25, N36 of which rate is below 0.4. Nearly complete mutated in A8 (above 0.6 at April) and I13 (over 0.7 around August) was noticed (0.94), and the V30 is the third highest in mutation rate (0.73). B, The 3D bar graph represents the status of Orf10 mutations through different months. C, The mutation status of six positions (A8, I113, V30, N5, N25, and N36) is shown through each month. Orf10 = open reading frame 10; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

(Fig. 2). This means the transmission–death relationship in numbers may be general in world, not restricted in specific regions. However, the death rate due to COVID-19 remains constantly during these periods in different countries selected (Fig. 1B). The information from Figs. 1 and 2 (before and after June) implies that cluster infection (eg, during the holiday gathering, increasing the contact and thus getting the chance to be infected) and no effective guaranty strategy at the starting the pandemic time period may play the critical roles in the spread of transmission (compared to the situation in Taiwan its experience).[31]

The existence of Orf10 within the whole genome of SARS-CoV-2 represents itself as a great mystery awaited to be further verified in many aspects.[17,24–26] We would ask first whether Orf10 will be essential for the biologic effect of SARS-CoV-2, such as the transmission or replication efficacy of SARS-CoV-2?[24] Second, can Orf10 of SARS-CoV-2 be encoded into a real protein or Orf10 be just an RNA segment for nothing?[23] Some authors believe (a theoretical analysis of the putative ORF10 protein in SARS-CoV-2),[25,26] but some disagree.[24] Third, Orf10 can be translated into a protein with the function what it will interact with its own target(s) inside host (human or any living organisms it can co-survives) cells?[18,32] Fourth, how is the stability of this genomic sequence and how is the genetic variance status of Orf10 after many runs of transmission (in this article survey)? Fifth, can Orf10 or any mutant of Orf10 contribute to the transmission and/or the lethal death of SARS-CoV-2?

The mutation analysis on Orf10 here demonstrates that most regions of the expanded 30-amino acid putative protein can be mutated during yearly pandemic (Fig. 3). Our survey to demonstrate that the hot spots (A8, I13, and V30; mutated with their function to be determined) and cold spots (N5, N25, and N36; conserved, whether essential remains further explored) within Orf10 are found. However, it is unverified about the consequence of these Orf10 variants to the viral

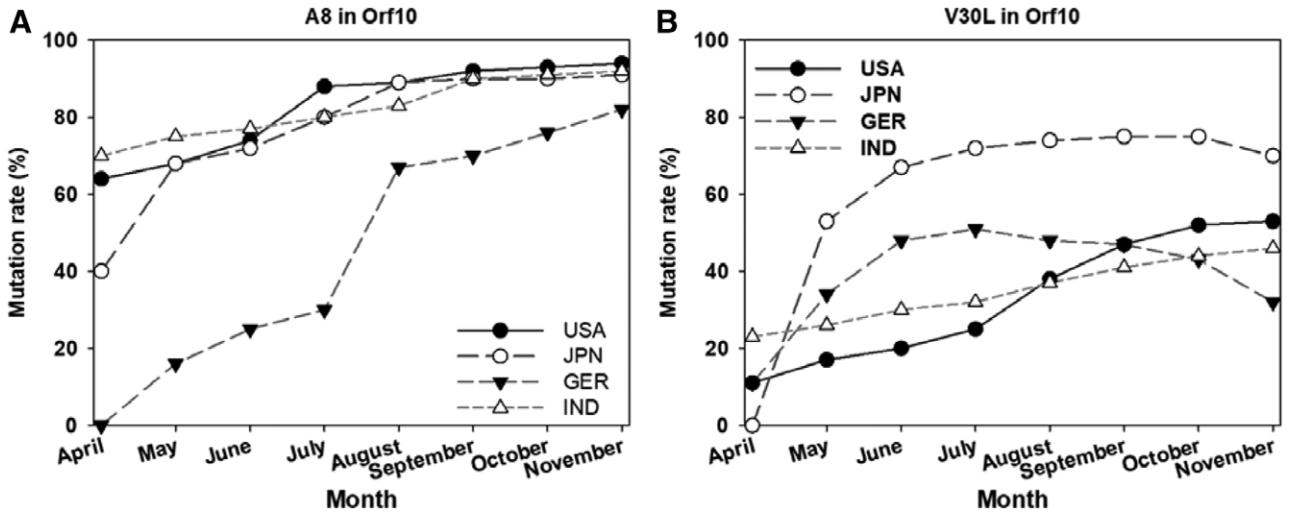

Fig. 4 Mutations at the A8 and V30 of Orf10 within SARS-CoV-2 infected patients in different countries. In four selective countries, United States (solid line with filled circle), India (dash line with open triangle), Germany (dash line with inverted filled triangle), and Japan (dash line with open circle), of which data extracted from April to November. A8 mutation in (A), and V30L shown in (B). Orf10 = open reading frame 10; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

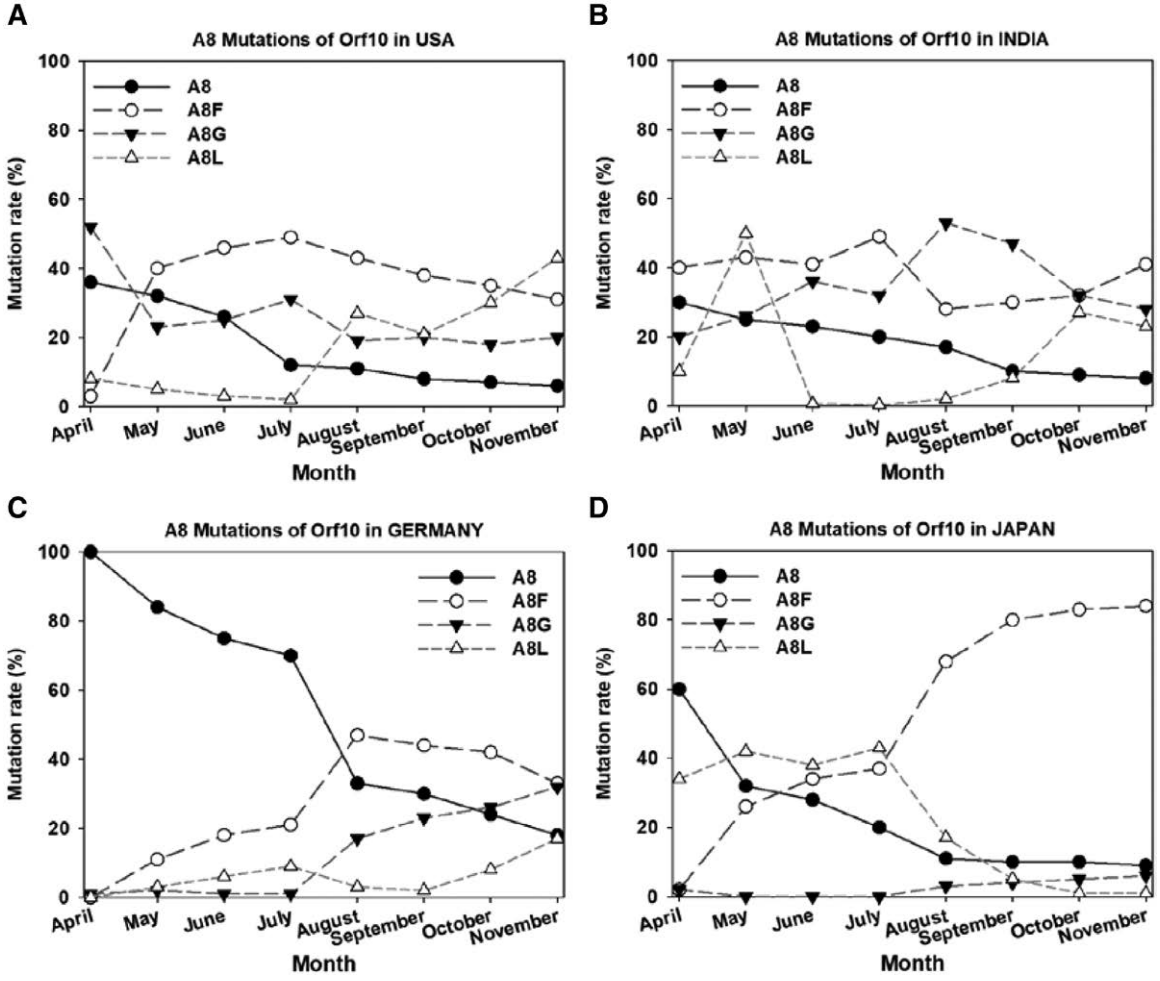

Fig. 5 Detail mutation status of A8 in Orf10 of SARS-CoV-2 patients from four selected countries. The dynamic changes of amino acid at the eighth position of SARS-CoV-2 virus from the original A (A8) into F, G, and L are shown in different symbols: filled circle, empty circle, filled inverted triangle, and empty triangle, respectively, in different countries, United States (A), India (B), Germany (C), and Japan (D). Orf10 = open reading frame 10; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

transmission, reinfection (eg, V30L was found in one case of a reinfected patient)[30] or even death-causing in the patients. From the structure and function viewpoints, the details about Orf10s (including variants) interact with some target proteins can be assayed through a kind of biosensing based on the fluorescence resonance energy transfer.[33,34] As for the possible target(s) of Orf10, ZYG11B (Protein zyg-11 homolog B) within the Cullin 2 (CUL2)-RING ubiquitin E3 ligase complex which could be one of the candidates for being important in the quality control of protein N-myristoylation.[18] As reported by computer simulation analysis, the 1-β chain of hemoglobin with which porphyrin inside can be bound, captured and thus attacked by Orf10 and associated Orfs; and then exert the following inhibition of human heme metabolism.[31] Although heme-Orf complex hypothesis is challenged and should be further confirmed,[35,36] there are no other defined Orf10 targets in addition to ZYG11B and hemoglobin to be found at present. Available resources (eg, ORF10 Google Drive Data at https://prokoplab.com/orf10/) combined with several advanced tools with big data analysis, artificial intelligence, and protein structure simulation including molecular docking[37] should support advance clarification of Orf10 function in processes.

ACKNOWLEDGMENTS

This work was supported by the Ministry of Science and Technology of Taiwan (MOST 105-2320-B-075-002, 108-2745-8-075-001-) and Taipei Veterans General Hospitals.

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