

Influenza-like illness caused by the 2019 novel coronavirus (2019-nCoV) via the person-to-person transmission

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Abstract

The ongoing pandemic of the 2019 novel coronavirus disease (COVID-19) raises a global health crisis, which has resulted in 75,778 confirmed cases with 2130 deaths in China and beyond. Atypical symptom renders it challenging to earlier recognize the 2019-nCoV carrier with the potential ability of equivalent transmission. Therefore, it is needed to gain full spectrum of COVID-19. Here we report clustered COVID-19 cases of person-to-person transmission. The symptoms of typical pneumonia are shared by the two familial members, namely son (Patient 1) and father (Patient 2). Unexpectedly, an influenza-like illness (ILI) is also caused in Patient 3 having close contact with Patient 1 at personal dinner party. Combined with clinical and epidemiological study, chest computed tomography (CT) and molecular diagnosis demonstrate that all the three cases tested positive for COVID-19 with distinct symptoms by human-to-human transmission. To the best of knowledge, it closes in part (if not all), a missing gap of clinical repertoires of COVID-19 outbreaks and underlines the possibility that neglect of cryptic/asymptomatic/mild cold-like syndromes gives biased screen in the earlier stage of COVID-19 cases.

Key words: Influenza-like illness, 2019-nCoV, Person-to-person transmission

Introduction

Like the other two outbreaks of the 2003 SARS (severe acute respiratory syndrome) in China ^{1, 2} and the 2012 MERS (middle eastern respiratory syndrome) in Saudi Arabia ^{3, 4}, the 2019 novel coronavirus disease (COVID-19) is also a newly-emerging pandemic of global public concern ⁵. The causative agent of COVID-19 (provisionally termed 2019-CoV by WHO ⁶⁻⁸, and then renamed SARS-CoV-2 by the international Committee on Virus Taxonomy with great debate ⁹) is a new lineage within β -coronaviruses featuring with a single-stranded, positive RNA genome ^{6, 10}. In fact, COVID-19 is initially recognized as an un-explained pneumonia in Wuhan, China in the late of December of 2019 ^{11, 12}, and then determined to be a viral respiratory illness caused by a new pathogen 2019-nCoV ⁶⁻⁸. This agent is believed to be a zoonotic agent in that earlier cases of COVID-19 is found to display the history of close contact with “Huanan seafood wholesale wet market” in Wuhan City, China ^{6, 8}. Extensive analysis of genomic epidemiology further suggests that all the isolates of 2019-nCoV are nearly identical, and phylogenetically-related to three bat SARS-like CoVs of bat origins ^{6, 10, 13}. Among them, bat-SL-CoVZC45 (29,802bp, Acc. no.: [MG772933](#)) and bat-SL-CoVZXC21 (29,732bp, Acc. no.: [MG772934](#)) are sampled from *Rhinolophus sinicus*, in Zhoushan, Zhejiang, 2017 ¹³, and the β -CoV/bat/Yunnan/RaTG13/2013 (Acc. no.: [EPI_ISL_402131](#)) is collected from fecal swabs of *Rhinolophus affinis*, in Yunnan, 2013 ⁶.

Worrisomely, this deadly pathogen 2019-CoV exhibits an unprecedented ability of human-to-human transmission ¹⁴⁻¹⁶. In the past two months since its recognition of atypical pneumonia with unknown etiologic agent on Dec. 8 of 2019, over 75,205 confirmed COVID-19 cases with 2014 deaths have been accumulated worldwide (<https://www.gisaid.org/epiflu-applications/global-cases-covid-19>). Not surprisingly, China is the most devastating epidemic place of COVID-19, in that 74,188 cases tested positive for 2019-nCoV, of which 2006 deaths are recorded thus far. Apart from the mainland of China ^{7, 15}, Hongkong ¹⁷, and Taiwan ¹⁸, 2019-CoV has spread to 24 other countries (<https://www.cdc.gov/coronavirus/2019-nCoV/summary.html>), including USA ¹⁴, Thailand ¹⁹, Germany ²⁰, and etc. Along with the lockdown and strict regulation of multiple cities (esp. Wuhan), China, the increasing number of confirmed COVID-19 cases in China and beyond, prompted WHO to declare it as a global public emergency of international concerns. A number of clinical and epidemiological studies have described in part (if not all) the possible transmission of 2019-CoV and its resultant clinical features in patients with COVID-19 ^{11, 12, 21}. Indeed, the confirmed 2019-CoV case differs greatly, ranging from the severe patients with severe respiratory failures ^{3, 11}, the pneumonia of mild symptoms ^{14, 15}, to asymptomatic carriers ^{20, 21}. This renders the monitoring of COVID-19 clinically complicated (and/or challenging). Surprisingly, none of the

accumulated 1174 cases of 2019-nCoV infections dies in Zhejiang Province, in spite that it is geographically close to Hubei Province, a centering site of this COVID-19 outbreak. It hints that local prompt administrative response plays an important role in efficient monitoring, transparent tracking, and timely supportive therapies upon the onset of suspected cases detected.

Because that current situation of COVID-19 proceeds rapidly, our understanding the dissemination and spectrum of COVID-19 remains fragmentary. Here we report sporadic cases of COVID-19 in Lishui City, Zhejiang Province, China. To the best of knowledge, we describe influenza-like illness (ILI) is caused by 2019-nCoV via the person-person transmission. In addition to the serological investigation ²², recent analysis of single-cell transcriptomics elucidated the high abundance of cellular receptor ACE2 expression in intestine, suggesting an alternative route for intestinal route of 2019-nCoV entry/shedding in the digestive system. We believe that our results might close in part (if not all) a gap of clinical repertoire of COVID-19 pandemic. More importantly, this finding underscores the urgency and importance of additional concerns with cryptic/asymptomatic/mild cold-like syndromes in the earlier recognition of COVID-19 cases, decreasing the miss diagnosis rate by biased screen and even leading to favorable prognosis by its supportive therapy.

Methods

Ethics Statement

This was a retrospective study of COVID-19 conducted in Lishui People's Hospital, Zhejiang Province of China. According to the guideline of the National Health Commission, China, all the confirmed COVID-19 cases received the isolation and supportive therapy in the designated provisional hospital. All the clinical data collection (blood biochemical tests and chest computed tomography) and epidemiological surveys were conducted under the approval by Ethics Committee of Lishui People's Hospital (LLW-FO-401). Given the importance of clinical data sharing in public and academic societies, the routine written informed consent of patients was waived during this special period of ongoing COVID-19 epidemic in China and beyond.

Real-Time Quantitative PCR (qPCR)

The samples of nasal and throat swabs collected from the suspected patients were subjected to viral RNA extraction with QIAamp RNA Mini Kit (Qiagen, Hilden, Germany) as recommended by the manufacturer. In this multiplex RT-PCR kit (SMSJ-HX-321B, BioGerm, Shanghai, China), the three target genes of 2019-nCoV referred to ORF-1a/b, E, and N, respectively. These genes were detected with three different fluorescence dyes, namely FAM for ORF-1a/b, HEX/VIC for E, and CY5 for N. In general, the standard curve of amplification was supposed to be plotted in "S" form. The specimens whose cycle threshold (Ct) was less than 35, tested positive for 2019-nCoV on real-time fluorescent qPCR. Of note, the primers designed here exhibited appreciable specificity, in that no cross-reactions with the other four human CoVs (229E, HKU1, OC43, and NL63) were detected.

Phylogenetic Analysis

The nucleotide sequence of RdRp and spike protein sequence of 2019-nCoV isolates were retrieved from 37 the GISAID website (<https://gisaid.org/CoV2020>), and the equivalent sequences of SARS-CoV and bat SARS-like CoV were downloaded from the NCBI database (<http://www.ncbi.nlm.nih.gov>). Sequence alignment were conducted with Cluatal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo>). Maximum likelihood-based phylogenetic trees were generated with both MEGA software and TreeView.

Re-analysis of RBD-ACE2 Complex

Cryo-EM structure of full length spiker protein was reported by Wrapp et al.²³. The X-ray structure of RBD of 2019-nCoV complexed with its cellular receptor ACE2 was resolved by Wang's research group²⁴. The detailed interface between S protein and its partner ACE2 was analyzed using the software of PyMol. The critical binding residues were highlighted, which are

implicated into the formation of hydrogen bonds in the context of 2019-nCoV interaction with its ACE2 receptor.

Results

Case Reports

We identified an unusual cluster of COVID-19 infections originating from Lishui City of Zhejiang Province, China. It involved three patients (Patient 1 to Patient 3, [Table 1](#)). A familial transmission of 2019-nCoV was recorded from Patient 1 to his father, termed Patient 2 ([Fig. 1](#)). To our surprise, Patient 3 presenting mild influenza-like illness ([Table 1](#)), was confirmed to be positive for COVID-19. Retrospectively, Patient 3 experienced a close contact with Patient 1 at a dinner party in Qingyuan County, on Jan 18, 2020 ([Fig. S1](#)). Of note, this timepoint was around a week before the onset of dry cough ([Table 1](#) and [Fig. 1](#)).

Familial Transmission of 2019-nCoV from Son to Father (Patient 1 to 2)

In brief, Patient 1 is a local resident of 45-year old in Qingyuan County, Lishui City. On Jan 18, 2020, he attended a dinner party ([Fig. 1](#)). Two days later, series of severe respiratory symptoms were developed in Patient 1, which referred to fever, dry cough, nasal congestion, etc. ([Table 1](#)). On Jan 23, 2020, three days after the onset of clinical presentation ([Fig. 1](#)), he was admitted to local hospital and received supportive therapy. Similar to those of earlier- reported cases of COVID-19 ^{11, 12, 21}, chest computed tomography (CT) revealed that a number of ground-glass opacities are distributed in two lungs (from upper lobe to lower lobe) of the patient 1 ([Figs 2A-C](#)). Indeed, these pathological changes of lungs are indicatives of viral pneumonia. As anticipated, on Jan 25, throat swabs obtained from Patient 1 tested positive for 2019-nCoV ([Fig. 1](#)), following the nucleic acid assays of real-time reverse-transcription polymerase chain reactions (RT-PCR). Meanwhile, medical department of local government hunted for all those suspected persons possessing the history of close contact with Patient 1. It returned two hits, namely his father of 72-year old (Patient 2) and a 46-year old man (Patient 3). Of being noteworthy, this confirmed COVID-19 patient 1 was immediately transferred to the provisional designated hospital for isolation and professional therapy. The condition of Patient 1 worsened seriously on Jan 28, 2020 and was greatly improved with stable vital signs on February 3, 2020. Following the supportive therapy as well as antiviral therapeutics, Patient 1 was recovered from illness, and discharged smoothly from hospital on Feb 12, 2020 ([Fig. 1](#)). As for the infection of Patient 1 with 2019-nCoV, we have no exact evidence supporting this is directly correlated with “animal-to-human intraspecies jump” or “person-to-person transmission”. While, it is of possibility that Patient 1 might be infected by 2019-nCoV during the journey of returning from Leqing County, to Qingyuan County on Jan 18, 2020. The reason lied in that he passed by Wenzhou City, a seriously affected area of COVID-19 epidemic we have recognized now. In fact, this city has already been closely monitored, whose traffic was under the strict regulation.

Different from that of Patient 1, the clinical onset of Patient 2 (a 72-year old man) was characterized with “chills, chest tightness, myalgia, etc.”, which was developed on the second day after his son was hospitalized ([Table 1](#)). Thus, he was also admitted immediately to local hospital of Qingyuan County on Jan 25. As expected, certain pathological damages of lungs by viral infection were illustrated with chest CT images of Patient 2 ([Fig. 2](#)). Not only did the single ground-glass alteration appear in the right lower lobe of lung ([Fig. 2A](#)), but also multi-focal ground-glass opacities dispersed from middle lobe to lower lobe of left lung ([Figs 2B-C](#)). In addition, similar scenarios were seen in the right lobe of lungs ([Fig. 2D](#)). On Jan 27, Patient 2 was also verified by real-time RT-PCR positive for COVID-19. A similar repertoire of COVID-19 progression (from severe to mild) replayed on the father of Patient 1, after he was transferred to the designated place of isolation ([Fig. 1](#)). Fortunately, Patient 2 was recovered well and allowed to leave hospital on Feb 19, 2020 ([Fig. 1](#)). Thus, we believed this constitutes a case of familial transmission of COVID-19 from son to father.

Influenza-Like Illness Caused by 2019-nCoV in Patient 3

Soon after recognition of Patient 1 as the confirmed COVID-19 case on Jan 25, a young man of 36-year old (designated Patient 3) was tracked and strictly requested to proceed the stay of home isolation on Jan 26 ([Fig. 1](#)). This was because that Patient 3 had ever been closely exposed to Patient 1, the confirmed 2019-nCoV carrier in Qingyuan County, on Jan 18, 2020. Epidemiological analysis showed that i) Patient 1 auto-drove to return his hometown (Qingyuan County) from Leqing County, close to Wenzhou City (a severely affected area by COVID-2019 according to current viewpoint) on Jan 18 ([Fig. S1](#)); ii) Patient 3 returned Lishui City from Qingyuan after the dinner party on the same day, and auto-drove from Lishui City to Wenzhou City on January 21, visited Qingyuan County on Jan 24, and arrived in his hometown Lishui City on Jan 26 ([Fig. S1](#)). During the journey of leaving for Lishui City on Jan 26, Patient 2 was reached by a phone call from the local administrative staff and formally informed to stay at home because of being a suspected case of COVID-19.

Though that Patient 3 was shocked by such unexpected disclosure at this very moment, he came to recognize his own statue of being discontinuously dry coughing, which was supposed to start on Jan 23, 2020 ([Fig. 1](#)). Unlike the father and son (Patient 2 & 1), this young person only gave a little bit mild symptom “dry cough”, a typical sign of flu-like syndrome ([Table 1](#)). Even during the whole period from home isolation (Jan 26, 2020), centralized isolation (Jan 27, 2020), to hospitalization (on Jan 29, 2020), none of the bellowed clinical symptoms was developed in this patient 3, including fever/chills, headache/dizziness, chest tightness, fatigue, etc. ([Table 1](#)). This was generally consistent with the almost normal parameters of blood

biochemical tests ([Table 2](#)). Chest CT imaging of Patient 3 elucidated the presence of an inflammatory focus in the middle lobe of right lung ([Figs 4A-B](#)), implying viral infection. In addition, the small pulmonary nodule was seen in left lower lobe of lung ([Fig. 4C](#)), and the pulmonary emphysema was visualized in left lung ([Fig. 4D](#)). On Jan 31, 2020, throat and nasal swabs from Patient 3 were subjected to the molecular diagnosis of real-time RT-PCR. Three specific genes 2019-nCoV targeted in this assay separately referred to ORF1a/b, E and N ([Fig. S3A](#)), as recommend by China CDC with minor improvement. As a result, the cycle threshold (Ct) was 30.09 for ORF1a/b ([Fig. S3B](#)), 30.17 for E ([Fig. S3B](#)), and 29.49 for N ([Fig. S3C](#)), respectively. Obviously, Patient 3 tested positive for COVID-19 on real-time RT-PCR assays. Together with clinical descriptions, genetic data allowed us to confidently recognize Patient 3 as a cryptic carrier of COVID-19 ([Fig. 1](#)). Moreover, the body temperature of this patient behaved at normal level, varying from 35.8°C to 36.8°C, five days post-hospitalization ([Fig. S2](#)). This posed the possibility that 2019-nCoV remains inactive in this patient during the 20 days after the onset of dry cough ([Fig. 1](#)). It is unusual, but not without any precedent, in that a rare case of asymptomatic COVID-19 carrier was recently reported in German ²⁰. Thanks to effective therapy, the lung inflammation of Patient 3 was largely relieved ([Figs 5A-B](#)). During 10 days of medical observation since the patient transfer on Feb 1, vital signs of Patient 3 remained stable. Thus, he was discharged smoothly from the designated hospital on Feb 11, 2020 ([Fig. 1](#)). Given that viral load of 2019-nCoV in upper respiratory tracts of asymptomatic case was recently found to be comparable to that in severe patient ²⁵, we believed that the potential transmission of mild COVID-19 cases with/without influenza-like illness cannot be neglected. It seemed a bias that we preferred on persons with severe syndrome in the former screen of COVID-19. Therefore, our discovery could benefit the improvement in screening practices of COVID-19, through extending from symptomatic individuals to asymptomatic persons with close contact of the confirmed 2019-nCoV case.

Clinical and Epidemiological Study

Patient 1 and his father, Patient 2, constituted a family cluster of severe pneumonia associated with COVID-19 (**Figs 2 and 3**). Although none of clinical presentations was shared by the two patients (**Table 1**), chest CT scans showed that multiple ground-glass opacities consistently occurred in their two lungs (**Figs 2 and 3**). Such pathological alterations of lungs were generally consistent with the presence of the deadly virus, 2019-nCoV, in the two patients. Because that the clinical onset (like fever) of Patient 1 on Jan 20, was four days earlier than that of his father (**Fig. 1**), it was reasonable to believe his son was infectious resource within this familial cluster of COVID-19. Patient 1 disclosed that he passed by Wenzhou City during the journey of returning hometown Qingyuan County from Leqing County on Jan 18 (**Fig. S1**). As we knew as of Feb 22, 2020, Wenzhou City was one of severely affected area of Zhejiang Province. This is because that 504 individuals were confirmed to be positive for COVID-19 in this city of Wenzhou alone, whereas the whole province of Zhejiang contained 1204 cases of 2019-nCoV infections. Thus, Patient 1 was anticipated to get infected on the way to home on Jan 18, 2020.

Unlike the father and son (Patients 1 & 2) with severe pneumonia, Patient 3 only gave influenza-like illness (ILI) with a hallmark of dry cough, but without any fever (**Table 1**). To the best of our knowledge, it is a first report of ILI case associated with 2019-nCoV infections in Zhejiang Province. Epidemiological surveys allowed us to conclude that this is due to the close contact with Patient 1 at the dinner party held in Qingyuan County, Jan 18, 2020 (**Fig. S1**). Patient 3 reported that the clinical onset of “dry cough” appeared on Jan 23, five days after this dinner party (**Fig. 1**). Despite that almost nothing was unusual in the blood biochemical tests (**Table 2**), a focus of inflammation of right lung was detected by chest CT imaginings (**Figs 4A-B**). This well explained why Patient 3 clinically presented with “dry cough”, rather than “high fever and chest tightness” (**Table 1**). Thanks to the recognition of Patient 1 to be positive for COVID-19, Patient 3 with a mild symptom was officially requested to stay at home, then admitted to the designated hospital, and finally diagnosed to as a COVID-19 case (**Figs 1 and S4**). The fact that the screening practice of 2019-nCoV gave a higher priority to those patients with severe acute respiratory syndrome suggested biased selection criteria used in an earlier stage of the COVID-19 outbreak. Presumably, this led to the neglect of certain asymptomatic COVID-19 cases. Given the carriage of high viral load in asymptomatic COVID-19 cases, its potential capability of human-to-human transmission posed a risky challenge to public health in China and beyond.

Phylogenetic Placement of 2019-nCoV

As of February 18, a total of 131 viral sequences of 2019-nCoV can be

accessed from the website of GISAID (<https://www.gisaid.org/>). Among them, three viral isolates from COVID-19 of Zhejiang Province were deposited, namely β -CoV/Zhejiang/WZ-02/2020 (Acc. No.: [EPI_ISL_404228](#)), β -CoV/Zhejiang/WZ-01/2020 (Acc. No.: [EPI_ISL_404227](#)), and β -CoV/Hangzhou/HZ-01/2020 (Acc. No.: [EPI_ISL_406970](#)). In fact, 107 isolates of 2019-nCoV were retrieved after the removal of 24 uncomplete/unqualified contigs. Using RNA-dependent RNA polymerase (RdRp), an evolutionary ruler, we generated a maximum likelihood-based phylogenetic tree that is consisted of two distinct lineages, termed subclade I & II ([Fig. 6A](#)). All the 2019-nCoV isolates are clustered in Subclade I, which is neighbored with Subclade II comprising SARS-CoV and bat SARS-like CoVs ([Fig. 6A](#)). The phylogeny of RdRp raises two possibilities for 2019-nCoV origin: i) 2019-nCoV is a cousin to SARS-CoV in parallel evolution; ii) 2019-nCoV is an evolutionary progenitor for SARS-CoV. Given that bat SARS-like CoV appeared as a bridge between SARS-CoV and 2019-nCoV in our phylogeny, we favored to believe the latter hypothesis ([Fig. 6A](#))²⁶. To some extent, the fact that β -CoV/bat/Yunnan/ RaTG13/2013 has 96% similarity to 2019-nCoV augmented our proposal and underscored its bat reservoir⁶. A similar scenario was also seen in the phylogenetic tree with spiker protein ([Fig. 6B](#)). Unlike that SARS-CoV is featuring with quasi-species, genetic heterogeneity is barely seen in the 2019-CoVs circulating in China and beyond thus far. It is of possibility that certain variants of 2019-CoV with low virulence appeared to adapt within human host in near future. Therefore, we anticipated that the causative of current COVID-19 epidemic is ancestral version, rather than a sister when compared with SARS-CoV, an etiological agent of the 2003 SARS outbreak^{1, 2, 27}.

Alternative 2019-nCoV Transmission in Intestine

Like those of SARS-CoV and MERS-CoV, the spread of 2019-nCoV proceeded primarily by respiratory droplets of the close contacts. The spiker glycoprotein, a type I viral fusion protein of 2019-nCoV is responsible for its efficient binding to the cellular ACE2 receptor²³, which is followed by the event of cellular and viral membrane fusion²⁶. In general agreement with our recent study of modeling²⁶, the x-ray crystal structure of 2019-nCoV RBD/ACE2 complex defined a fine binding interface ([Fig. S4A](#)), in which a number of contact residues are highlighted ([Figs S4B-C](#))²⁴. Thus, an efficient production of the ACE2 receptor is pre-requisite for active entry of 2019-nCoV into the infected host. As expected, high viral loads were recently detected in nasal and throat swabs from patients with COVID-19, because that the upper respiratory tract is attributed to a primary route of 2019-nCoV dissemination²⁵. Of particular being noteworthy, serological data suggested that certain anal swabs are positive for 2019-nCoV²², which is similar to a scenario seen with the stool specimen reported by Chen and coauthors¹¹. It was of possibility

that an alternative route of COVID-19 dissemination/shedding occurs in intestine. While it needed further mechanistic evidence.

To address this unanswered question, an approach of single-cell transcriptomics recently was exploited to systematically compare distinct expression profile of cellular receptor ACE2 in different tissues as well as various cell types ²⁸. Indeed, the expression of ACE2 is variable in different tissues and organs ^{28, 29}. In addition to the ACE2 receptor ²⁸, the transmembrane serine protease (TMPRSS) that is responsible for the cleavage of spiker protein into two functional domains (S1 and S2), was also determined to be produced appreciably in intestines ²⁹. This hints an alternative route of 2019-nCoV dissemination and or shedding in intestine, posing a risk of fecal-to-oral spread. Therefore, we speculated that dynamic expression of ACE2 and TMPRSS can be related to viral pneumonia of 2019-nCoV with different severity. Probably it is due to i) the limited number of susceptible ACE2-producing AT2 cells and/or ii) 2019-nCoV infection stage-correlated expression of ACE2 in certain AT2 cell types of lungs ^{28, 29}. Together, variable expression of ACE2 in patients determined different susceptibility and severity of respiratory symptoms, which can explain the existence of asymptomatic/mild 2019-nCoV carrier, like flu-like syndrome.

Discussion

As of Feb 23, 2020, totally 17 confirmed 2019-nCoV cases with no deaths were accumulated in Lishui City, Zhejiang Province. 14 of 17 patients have been discharged smoothly from the designated provisional hospital. This is in part (if not all) attributed to the rapid and efficient recognition of asymptomatic COVID-19 cases in the incubation time by local government. It seemed likely that Malayan pangolins act as one of intermediate host for 2019-nCoV circulation. This is because that a number of pangolin coronaviruses are identified to share 90% similarity to that of 2019-nCoV³⁰. Obviously, the event of genetic re-arrangement is necessitated prior to the onset of 2019-nCoV spread.

The data we reported here, represented a first example that influenza-like illness is caused by the person-to-person transmission of 2019-nCoV. Also, this cluster case of COVID-19 involved a familial transmission of severe viral pneumonia from son to father. Our findings provided additional insights into full spectrum of COVID-19 in various severity. As an infectious resource in this COVID-19 case, Patient 1 disclosed that he had no history of close contact with “Huanan Seafood Wholesale Wet Market”. Thus, it ruled out the possibility of animal-to-human dissemination”. By contrast, Patient 1 might get infected with 2019-nCoV on the way to his hometown, because that he passed by Wenzhou City with 504 confirmed COVID-19 cases ([Fig. S1](#)). Unfortunately, Patient 2, also presented severe pneumonia, which is analogous to that of his son, Patient 1. This was a severe familial transmission of COVID-19. On the contrary, Patient 3 only gave the mild symptom “dry cough” of ILI after his contact with Patient 1. This suggested the diversity of clinical spectrum with those recipients of 2019-nCoV donated from a severe patient. Meanwhile, we are concerned with whether certain mild case of COVID-19 can spread to those contacts, giving severe syndromes. Given that ACE-2 also functioned as a cellular receptor for 2019-CoV, we speculated that different syndromes might be determined by various level of ACE2 expression in susceptible cell types as well as cell counts within the infected host. Because that the analysis of single-cell transcriptomics elucidated higher level of ACE2 expression in intestine than that of lungs, the paradigm organ infected with 2019-nCoV, we concluded that an alternative pathway for 2019-nCoV might exist in intestine. Similar scenarios have ever been seen in other two related coronaviruses, SARS-CoV³¹ and MERS-CoV³¹. Very recently, this prediction was supported by the positive detection of 2019-nCoV in anal swabs²².

In conclusion, we described a first example that influenza-like illness is caused by the 2019-nCoV transmission from a patient with heavy pneumonia. It was probable that the cryptic carrier of 2019-nCoV is more risky challenge than those severe cases of COVID-19. Thereafter, this underlined the

importance of additional concerns with cryptic/asymptomatic/mild cold-like syndromes in the earlier recognition of COVID-19 cases, decreasing the miss diagnosis rate by biased screen.

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Declaration of interest statement

The authors declare that they have no competing interests.

Author contributions

YF and WW designed and supervised this project; YF, HZ, WW, NW, WL, and CQ performed experiments; YF, WW, HZ, XJ, CS and JW analyzed the data and prepared the figures; YF, HZ, and WW drafted this manuscript.

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Table 1 The relationship and clinical symptoms of COVID-19 patients

Description	Patient 1	Contact	
		Patient 2	Patient 3
Relationship	Son of Patient 2	Father of Patient 1	NA
Age	45	72	36
Gender	Male	Male	Male
Symptoms and signs			
Cough	+ (dry)	--	+ (dry)
Fever	+	--	--
Generalized weakness	--	+	--
Nasal congestion	+	--	--
Headache	+	--	--
Dizziness	+	--	--
Fatigue	--	--	--
Chills	--	+	--
Chest tightness	--	+	--
Tachypnea	--	+	--
Arthralgia	--	+	--
Myalgia	--	+	--

The plus symbol “+” denotes the presence of certain symptom, whereas the minus symbol “--” refers to the absence of relevant clinical symptoms.

Table 2 Blood biochemical index of patient 3 with influenza-like illness

Biochemical index	Units	Reference range	Patient 3
White blood cell counts	10^9 /L	3.5~9.5	3.2 ↓
Lymphocyte relative value	NA	0.200~0.500	0.423
Monocyte relative value	NA	0.030~0.100	0.110 ↑
Neutrophil relative value	NA	0.400~0.750	0.461
Eosinophils relative value	NA	0.004~0.080	0.006
Basicyte relative value	--	0.000~0.100	0.000
Lymphocyte counts	10^9 /L	1.10~3.20	1.35
Monocyte counts	10^9 /L	0.10~0.60	0.35
Neutrophil counts	10^9 /L	1.80~6.30	1.48 ↓
Eosinophils counts	10^9 /L	0.02~0.52	0.02
Basicyte counts	10^9 /L	0.00~0.06	0.00
Red blood cell counts	10^{12} /L	4.30~5.80	6.17
Hemoglobin	g/L	130~175	179 ↑
Hematocrit	NA	0.400~0.500	0.53 ↑
Mean corpuscular volume	fL	82.0~100.0	86.5
Mean corpuscular hemoglobin content	pg	27.0~34.0	29.0
Mean corpuscular hemoglobin concentration	g/L	316~354	335
Red blood cell volume distribution width	%	11.5~15.5	10.9 ↓
Platelet counts	10^9 /L	125~350	172
Platelet specific volume	NA	0.108~0.282	0.220
Mean platelet volume	f1	7.4~12.5	12.8 ↑
Hypersensitive C-reactive protein	mg/L	0.0~10.0	4.0

Figure legends

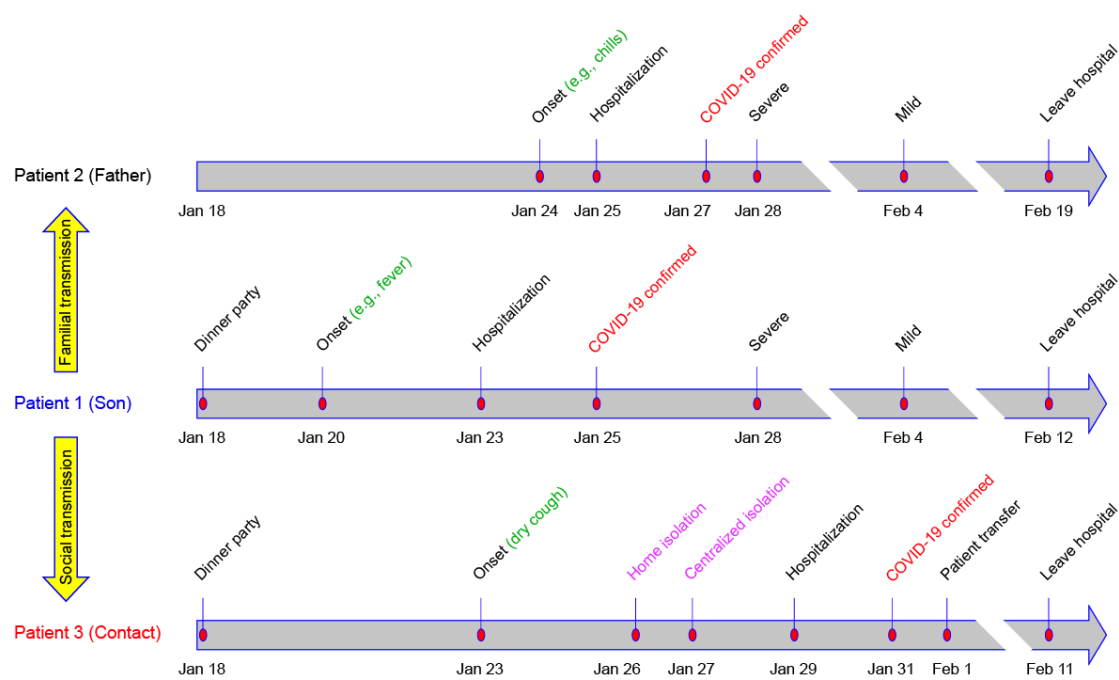


Fig. 1 Epidemiological analysis of three COVID-19 patients with the close contact history suggests the human-to-human transmission of 2019-nCoV (SARS-CoV-2)

As for Patient 1 (the son of Patient 2 in Qingyuan County of Zhejiang Province, [Fig. S1](#)), the onset of COVID-19 is featuring with numbers of symptoms (esp., fever, [Table 1](#)) on Jan 18, 2020. He was in-hospitalized in the provincial designated hospital on Jan 23, and then diagnosed as the confirmed case of 2019-nCoV infection. Unlike the scenario of Patient 1, his father (Patient 2) exhibits the flu-like symptoms (like chills and generalized weakness) on Jan 24, went to hospital on the next day, and then identified as a confirmed case of COVID-19 on Jan 25, 2020.

Patient 3 who attended a dinner party along with Patient 1 in Qingyuan County of Zhejiang Province, on Jan 18 ([Fig. S1](#)), exclusively displayed dry cough, a representative sign of influenza-like illness from Jan 23 to Jan 29 ([Table 1](#)). Upon on patient 1 was verified to be positive for 2019-nCoV, the patient 3 was regarded as a suspected case, and immediately informed to stay at home on Jan 26, 2020. On Jan 27, he was transferred to the place of centralized isolation. Because of limited relief of dry cough, Patient 3 was hospitalized in Lishui People’s Hospital on Jan 29, confirmed as a COVID-19 case on Jan 31, and then transferred to a designated hospital of this city on Feb 1, 2020.

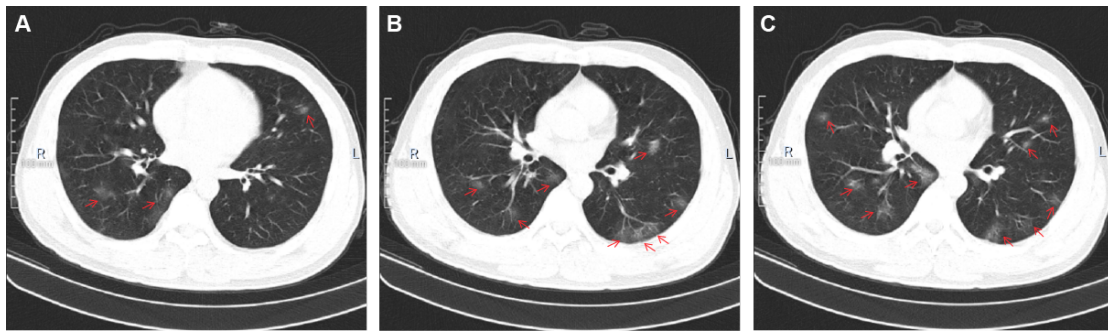


Fig. 2 Representatives of Chest computed tomography (CT) of Patient 1 with COVID-19

A. CT scans of lungs suggest that the pathological alteration of ground-glass opacity occurs in the left upper lobe (and right bottom lobe) of lung from Patient 1

B. Chest CT scan reveals that multi-focal ground-glass opacities appear in the two lungs (from middle lobe to lower lobe) of Patient 1

C. Distribution of multi-focal ground-glass opacities (from upper lobe to lower lobe) of two lungs from Patient 1

Chest CT scans with multiple sections were routinely performed. ground-glass opacity is showed with a red arrow.

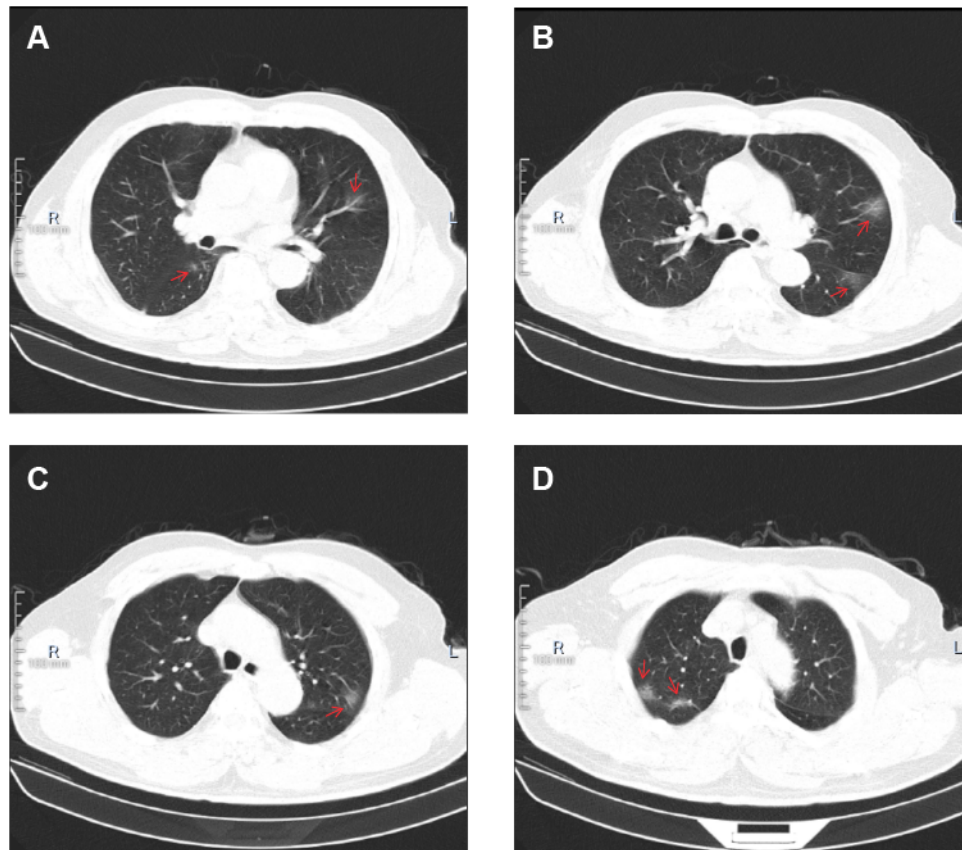


Fig. 3 Typical images of chest CT of lungs from Patient 2, the patient 1's father

A. A ground-glass alteration in the left middle lobe (and right lower lobe) of lung of the patient 2

B-C. Chest CT scan informed us multi-focal ground-glass opacities in left lung (from middle lobe to lower lobe) of the patient 2

D. Detection of other multi-focal ground-glass opacities by chest CT in the right lobe of lung from the patient 2

The chest CT scan with multiple sections was routinely performed, and the ground-glass opacity is highlighted with the red arrow.

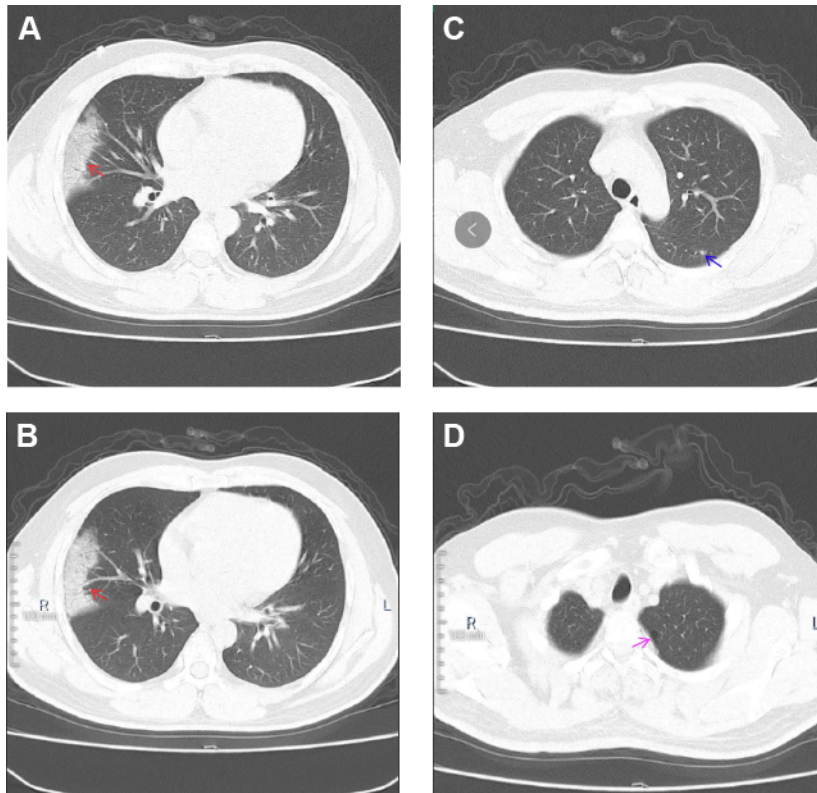


Fig. 4 Chest CT of Patient 3 with influenza-like illness

Chest scans with multiple sections were routinely performed.

A-B. Chest CT of Patient 3 reveals the inflammatory focus of middle lobe of right lung

The inflammatory focus is indicated with a red arrow.

C. A representative CT image for the small pulmonary nodule in left lower lobe of lung from Patient 3

The small pulmonary nodule is shown with a blue arrow.

D. Chest CT suggests the presence of pulmonary emphysema in left lung of Patient 3

The vesicular emphysema is highlighted with a magenta arrow.

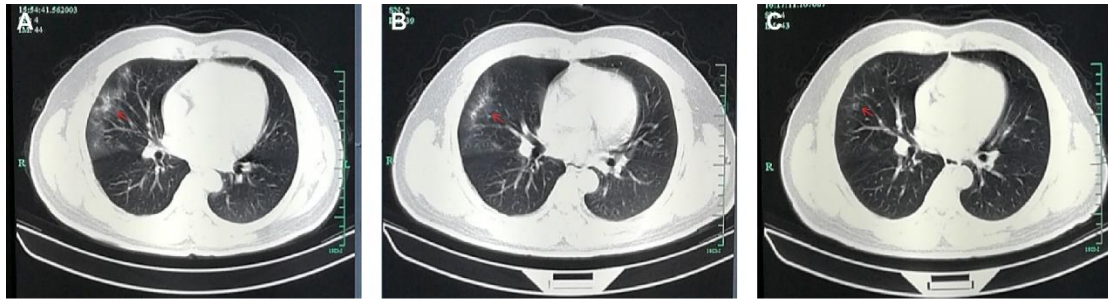


Fig. 5 Recovery of lung inflammation by active therapy in Patient 3 with COVID-19

A. Chest CT-based visualization of lung inflammation in Patient 3 with COVID-

B. Partial relief of lung inflammation in the intermediate stage of active therapy

C. In the late stage of active therapy, the COVID-19 patient 3 recovered largely

As for Patient 3 on different days post-hospitalization, chest CT scans were routinely conducted, giving numbers of images at various sections. The focus of inflammation was indicated with the red arrow.

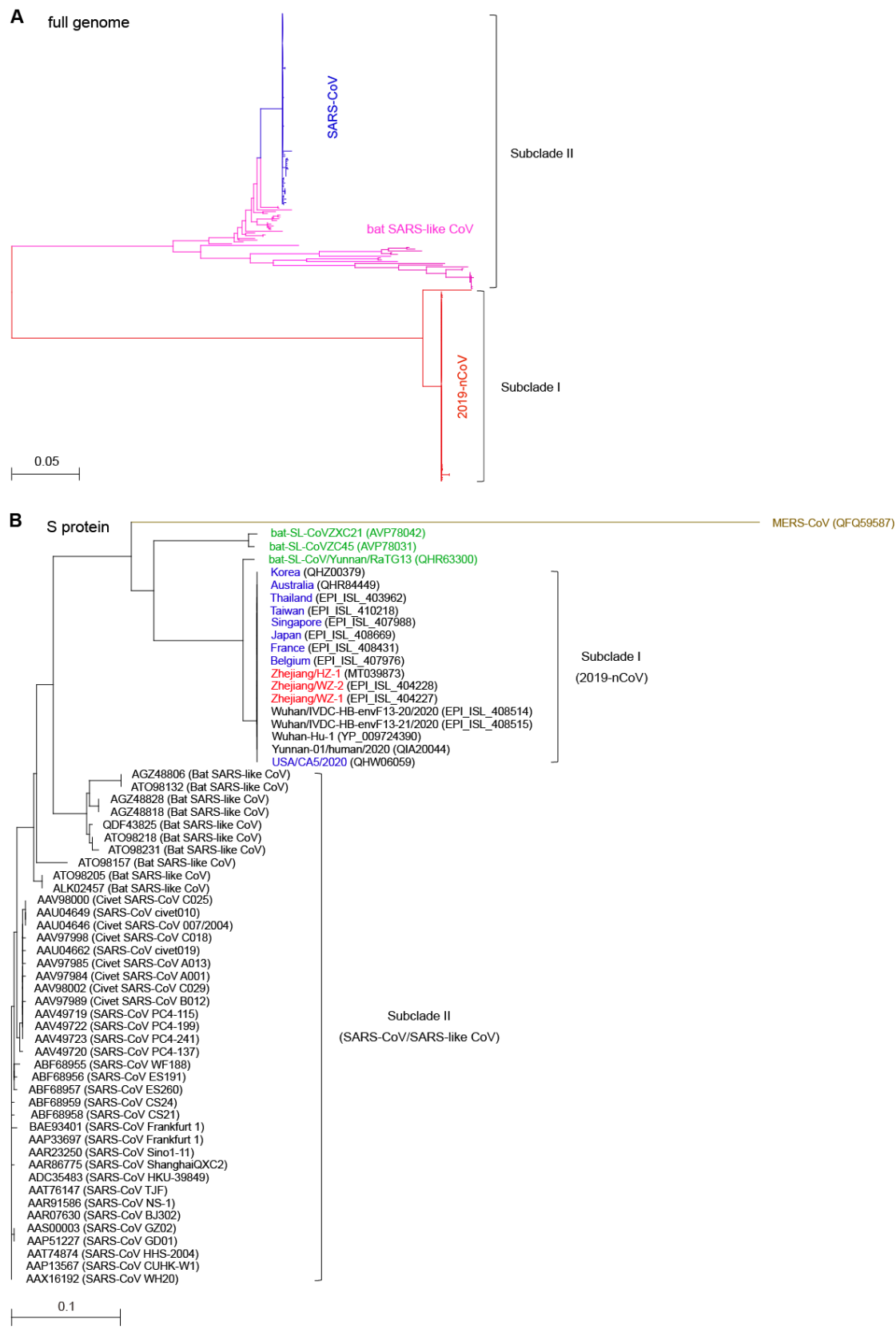


Fig. 6 Use of phylogenetic analyses to infer the evolutionary placement of the causative of COVID-19 pandemic, 2019-nCoVs

A. RdRp-based phylogeny of 2019-nCoV isolates

As of Feb 18, 2020, 107 2019-nCoV with complete genomes were retrieved

by excluding 24 uncomplete contigs from the total 131 viral sequences deposited into from the database of GISAID (<https://www.gisaid.org/>). Along with the related SARS-CoVs and bat SARS-like CoVs, the RdRp-encoding genes of the afore-mentioned COVID-19 viruses were applied to generate the generate phylogenetic tree. The maximum likelihood-based phylogeny is given with the MEGA software.

It seems likely that Subclade II comprises SARS-CoV colored blue, and bat SARS-like CoV colored magenta. Whereas, 2019-nCoV colored red is exclusively clustered in Subclade I.

RdRp denotes RNA-dependent RNA polymerase.

B. Phylogenetic tree of the S protein from 2019-nCoV isolates

Maximum likelihood-based phylogenic tree of S protein from 2019-nCoV isolate is given, in which the outgroup refers to MERS-CoV (Acc. no.: [QFQ59587](#)). Three Zhejiang isolates are colored red, and the isolates from the cases exported abroad are indicated in blue. A similar scenario is seen in the S protein-based phylogeny, because that it also displays Subclade I (2019-nCoV) together with Subclade II (SARS-CoV and Bat SARS-like CoV).

Supplemental Materials for

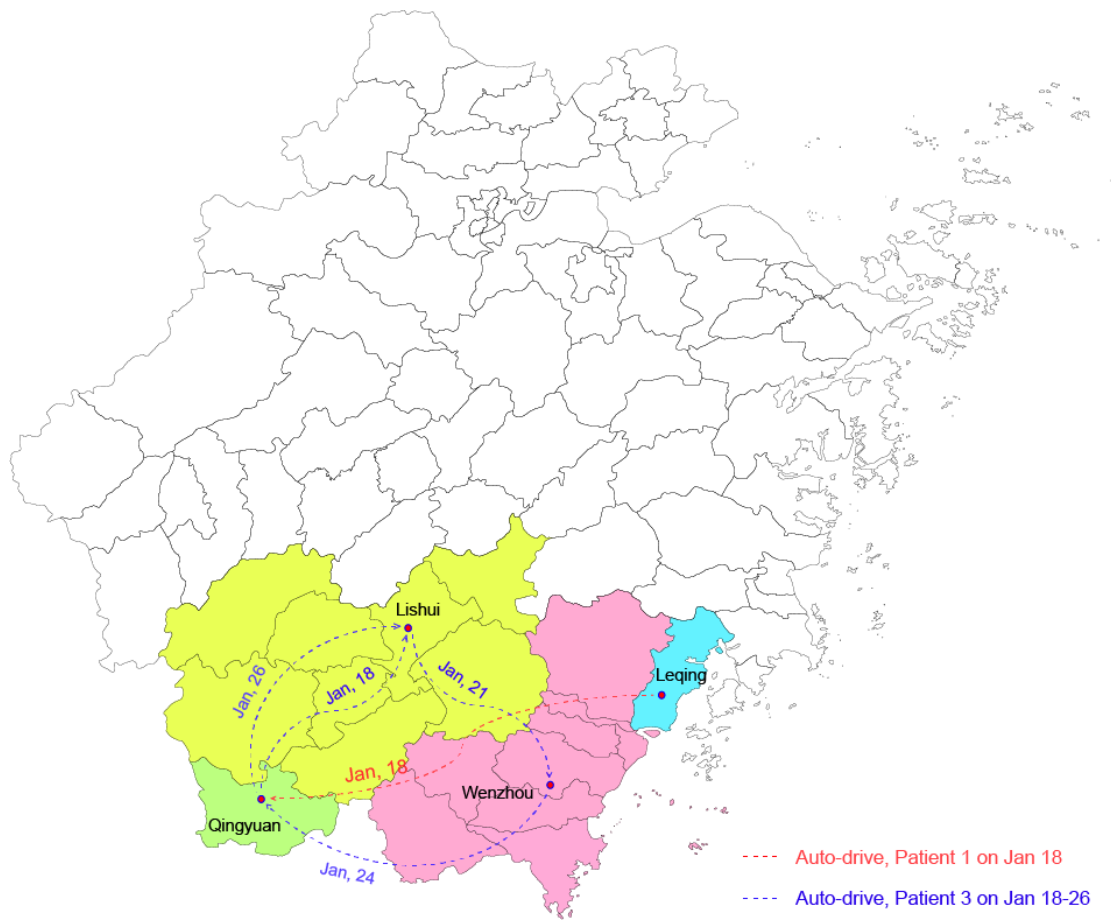


Fig. S1 Tracking the movement of Patient 1 and the contact Patient 3, prior to the onset of COVID-19 symptoms

On Jan 18, 2020, Patient 1 returned from Leqing County to hometown Qingyuan County for a dinner party. Of note, Patient 3 was also attended this party.

On Jan 18, 2020, Patient 3 auto-drove back home in Lishui City, visited Wenzhou City, on Jan 21 of 2020, passed by Qingyuan County on Jan 24 of 2020, and finally returned Lishui City on Jan 26 of 2020. The movement tracks of the two COVID-19 patients were recorded with arrowed/dashed lines (red for patient 1, and blue for patient 3).

The map of Zhejiang province is given with Adobe Illustrator, and the cities/counties where patients visited are colored.

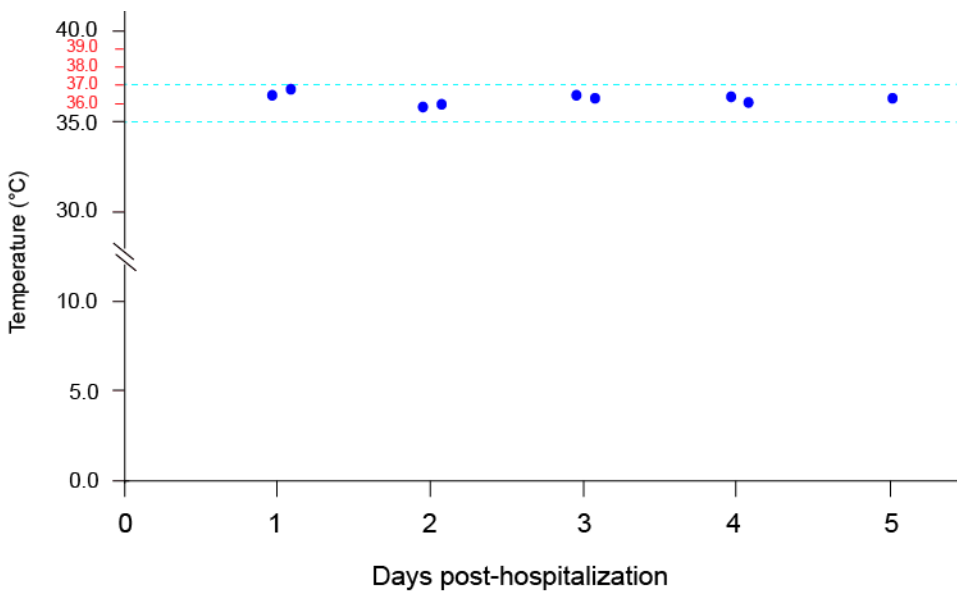


Fig. S2 Patient 3 has no fever in the 5 days post-hospitalization
The body temperature remains at the normal level (varying from 35.8°C to 36.8°C)

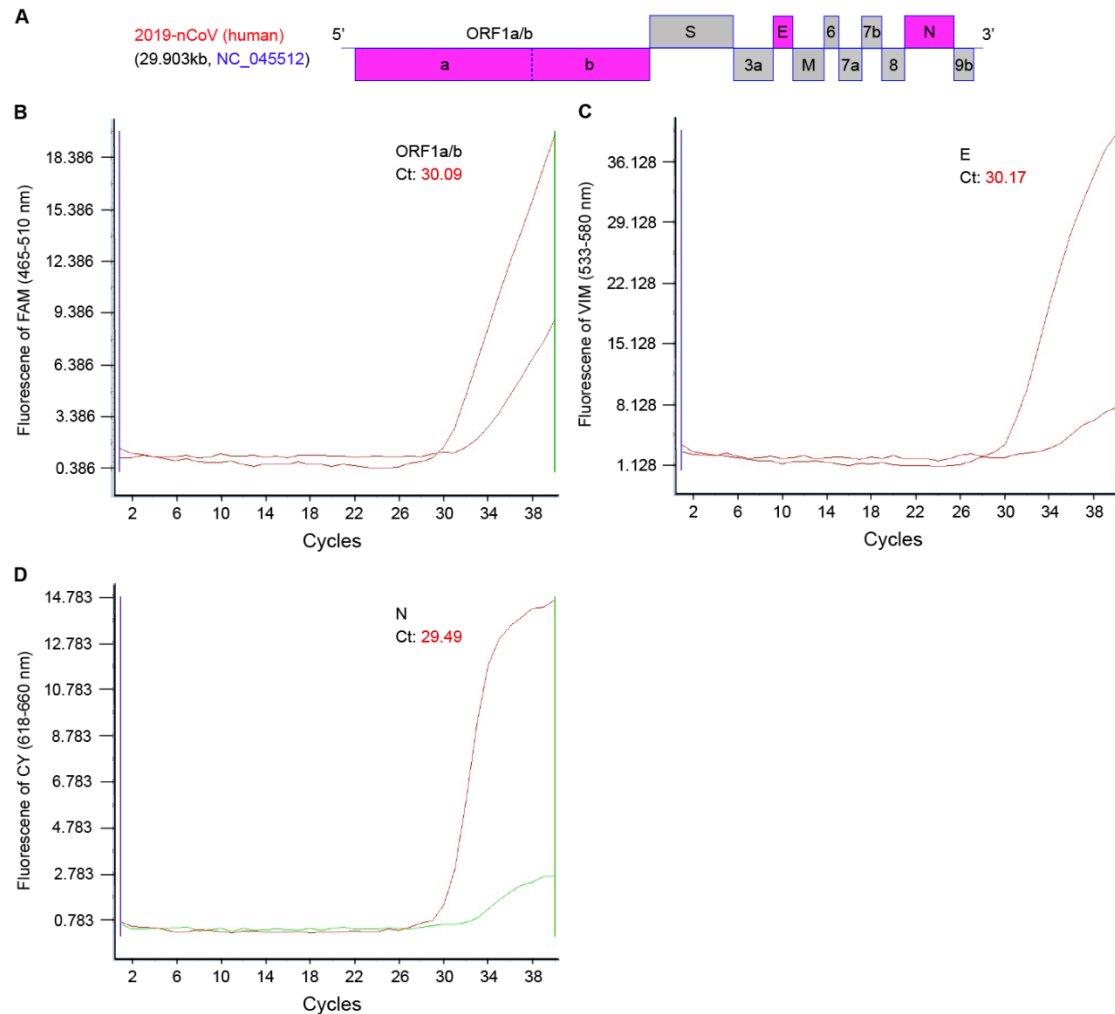


Fig. S3 Real-time quantitative PCR detection of the patient 3 with influenza-like illness

A. Cartoon scheme for 2019-nCoV (renamed as SARS-CoV-2) genome
The three target genes (highlighted in magenta), refer to ORF1a/b, E, and N, respectively.

B. Real-time qPCR detection (FAM) for the ORF1a/b-specific locus of 2019-nCoV (SARS-CoV-2)

C. qPCR detection (VIM) of 2019-nCoV (SARS-CoV-2) with specific primer against E protein-encoding gene

D. Real-time qPCR detection (CY) for the nucleocapsid (N) protein-specific locus of 2019-nCoV (SARS-CoV-2)

The values of Ct (cycle threshold) are 30.09 for ORF1a/b, 30.17 for E, and 29.49 for N, respectively.

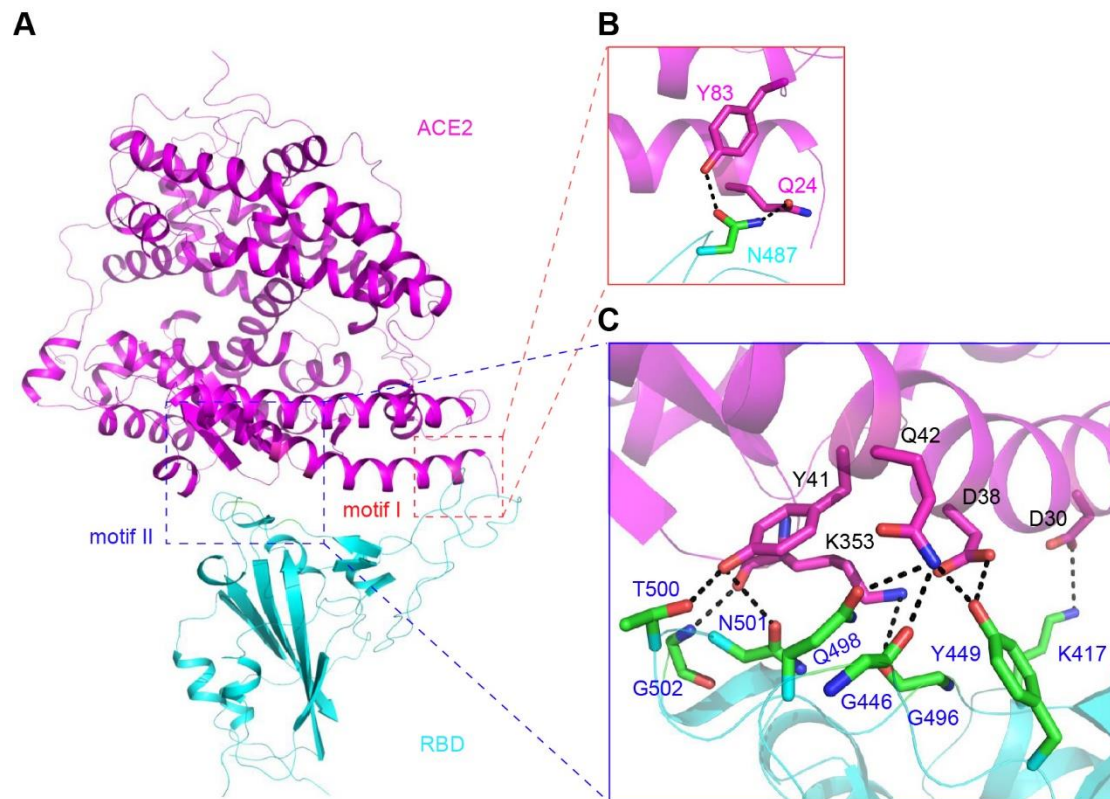


Fig. S4 Structural insights into the binding of 2019-nCoV to cellular receptor ACE2

A. Ribbon representation of complex structure of 2019-nCoV RBD and the N-terminal peptidase domain of ACE2 receptor

Presumably, two motifs (motif 1 & 2) contributed to the maintenance of the stable interface between the cellular receptor ACE2 and RBD of 2019-nCoV spiker protein. Motif 1 is squared with blue dashed line, and motif 2 is squared with red dashed line. ACE2 is colored magenta, and RBD of 2019-nCoV is indicated in cyan.

B. Structural snapshot of motif 1 with contact residues labeled

N487 of RBD is suggested to interact with the two residues (Q24 and Y83) of cellular receptor ACE2.

C. Enlarged view of motif 2 of the RBD-ACE2 interface

Five residues of ACE2 (D30, D38, Y41, Q42 & K353) are contact residues interacting with the eight residues of RBD of 2019-nCoV surface protein (K417, G446, Y449, G496, Q498, T500, N501, and G502).

Structural re-analysis was conducted on the basis of the PDB information of RBD/ACE2 reported by Lan *et al.* ¹.

Supplemental references

1. Lan, J. *et al.* Crystal structure of the 2019-nCoV spike receptor-binding domain bound with the ACE2 receptor. *bioRxiv* (2020).