Genome composition and divergence of the novel coronavirus (2019-nCoV)
originating in China

Aiping Wu1*, Yousong Peng2*, Baoying Huang3*, Xiao Ding1*, Xianyue Wang1, Peihua Niu1, Jing Meng1, Zhaozhong Zhu2, Zheng Zhang2, Jiangyuan Wang1, Jie Sheng1, Lijun Quan4, Zanxian Xia5, Wenjie Tan3#, Genhong Cheng6#, Taijiao Jiang1#

1Center for Systems Medicine, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100005, Suzhou Institute of Systems Medicine, Suzhou, Jiangsu 215123, China;

2College of Biology, Hunan Provincial Key Laboratory of Medical Virology, Hunan University, Changsha, 410082, China;

3Key Laboratory of Medical Virology, National Health and Family Planning Commission, National Institute for Viral Disease Control and Prevention, China CDC, 102206, Beijing, China;

4School of Computer Science and Technology, Soochow University, Suzhou, China;

5Department of Cell Biology, School of Life Science, Central South University, Changsha 410013, China; Hunan Key Laboratory of Animal Models for Human Diseases, Hunan Key Laboratory of Medical Genetics & Center for Medical Genetics, School of Life Science, Central South University, Changsha 410013, China;

6Department of Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles, Los Angeles, USA;

* These authors contributed equally to this work.

# To whom correspondence should be addressed. Email: taijiao@ibms.pumc.edu.cn (T. J.) gcheng@mednet.ucla.edu (G. C.) and tanwj28@163.com (W.T.)
Summary

An in-depth annotation of the newly discovered coronavirus (2019-nCoV) genome has revealed differences between 2019-nCoV and severe acute respiratory syndrome (SARS) or SARS-like coronaviruses. A systematic comparison identified 380 amino acid substitutions between these coronaviruses, which may have caused functional and pathogenic divergence of 2019-nCoV.
Main Text

A novel coronavirus (CoV), named ‘2019 novel coronavirus’ or ‘2019-nCoV’ by the World Health Organization (WHO), is responsible for the recent pneumonia outbreak that started in late December 2019 in Wuhan City, Hubei Province, China (Huang et al., 2020; Zhou et al., 2020; Zhu et al., 2020). This outbreak is associated with a large seafood and animal market, and further investigations are ongoing to determine the origins of the infection. To date, thousands of human infections have been confirmed in China along with many exported cases across the globe (China CDC, 2020).

Coronaviruses mainly cause respiratory and gastrointestinal tract infections and are genetically classified into four major genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus* and *Deltacoronavirus* (Li, 2016). The former two genera primarily infect mammals, whereas the latter two predominantly infect birds (Tang et al., 2015).

Six kinds of human CoVs have been previously identified. These include HCoV-NL63 and HCoV-229E that belong to the *Alphacoronavirus* genus, and HCoV-OC43, HCoV-HKU1, severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome coronavirus (MERS-CoV) that belong to the *Betacoronavirus* genus (Tang et al., 2015). Coronaviruses did not attract worldwide attention until the 2003 SARS pandemic followed by the 2012 MERS and most recently 2019-nCoV outbreaks (China CDC, 2020; Song et al., 2019). SARS-CoV and MERS-CoV are considered to be highly pathogenic (Cui et al., 2019), and it is very likely that both SARS-CoV and MERS-CoV were transmitted from bats to palm civets (Guan et al., 2003) or dromedary camels (Drosten et al., 2014), respectively, and finally
to humans (Cui et al., 2019).

The genome of coronaviruses, whose size ranges between approximately 26,000 and 32,000 bases, includes a variable number (from 6 to 11) of open reading frames (ORFs) (Song et al., 2019). The first ORF representing approximately 67% of the entire genome encodes 16 non-structural proteins (nsps), while the remaining ORFs encode accessory proteins and structural proteins (Cui et al., 2019). The four major structural proteins are the spike surface glycoprotein (S), small envelope protein (E), matrix protein (M) and nucleocapsid protein (N). The spike surface glycoprotein plays an essential role in binding to receptors on the host cell and determines host tropism (Li, 2016; Zhu et al., 2018). The spike proteins of SARS-CoV and MERS-CoV bind to different host receptors via different receptor-binding domains (RBD). SARS-CoV uses angiotensin-converting enzyme 2 (ACE2) as one of the main receptors (Ge et al., 2013), with CD209L as an alternative receptor (Jeffers et al., 2004), whereas MERS-CoV uses dipeptidyl peptidase 4 (DPP4, also known as CD26) as the primary receptor. Initial analysis suggested that 2019-nCoV has a close evolutionary association with the SARS-like bat coronaviruses (Zhou et al., 2020). Here, based on the first determined three genomes of the novel coronavirus (2019-nCoV) namely Wuhan/IVDC-HB-01/2019 (EPI_ISL_402119) (HB01), Wuhan/IVDC-HB-04/2019 (EPI_ISL_402120) (HB04) and Wuhan/IVDC-HB-05/2019 (EPI_ISL_402121) (HB05), an in-depth genome annotation of this virus was performed with a comparison to related coronaviruses, including 1008 human SARS-CoV, 338 bat SARS-like CoV and 3131 human MERS-CoV, whose genomes were published before
Comparison of genomes of these three strains showed they are almost identical with only five nucleotide differences in the genome of ~29.8kb nucleotides (Fig. S1). The 2019-nCoV genome was annotated to possess 14 ORFs encoding 27 proteins (Fig. 1A and Table S1a & S1b). The orf1ab and orf1a genes located at the 5’-terminus of the genome respectively encode the pp1ab and pp1a proteins that comprise 15 nsps from nsp1 to nsp10, and from nsp12 to nsp16 (Fig. 1A and Table S1b). The 3’-terminus of the genome contains 4 structural proteins (S, E, M and N) and 8 accessory proteins (3a, 3b, p6, 7a, 7b, 8b, 9b and orf14). At the amino acid level, the 2019-nCoV is quite similar to that of SARS-CoV, but there are some notable differences. For example, the 8a protein is present in SARS-CoV and absent in 2019-nCoV; the 3b protein is 154 amino acids in SARS-CoV but shorter in 2019-nCoV with only 22 amino acids (Table S1a). Further studies are needed to characterize how these differences affect the functionality and pathogenesis of 2019-nCoV.

As shown in a phylogenetic tree based on whole genomes (Fig. 1B and Fig. S2) with the Molecular Evolutionary Genetics Analysis (MEGA) (version 7.0), the 2019-nCoV is in the same betacoronavirus clade as human-CoV, MERS-CoV, bat-CoV, SARS-like bat CoV and SARS-CoV. The phylogenetic tree falls into two clades. The betacoronavirus genus constitutes one clade, while the alphacoronavirus, gammat coronavirus and deltacoronavirus genera constitute the other clade. The 2019-nCoV is parallel to the SARS-like bat CoVs, while the SARS-CoVs are
descended from the SARS-like bat CoVs, indicating that 2019-nCoV is closer to the SARS-like bat CoVs than the SARS-CoVs in terms of the whole genome sequence. Tables S1c and S1d also show that the genome of 2019-nCoV has the highest similarity with that of a SARS-like bat CoV (MG772933). In comparison, 2019-nCoV is distant from and less related to the MERS-CoVs. In terms of the encoded proteins of pp1ab, pp1a, envelope, matrix, accessory protein 7a and nucleocapsid genes, phylogenetic analyses showed that the 2019-nCoV is closest to the SARS-like bat CoVs (Fig. 1C and Table S1d). Regarding the spike gene, the 2019-nCoV is closest to the bat CoVs, while the 3a and 8b accessory genes are both closest to the SARS-CoVs. Although phylogenetic analyses for the whole genome and individual genes clearly show that the 2019-nCoV is most closely related to SARS-like bat viruses (Figs. 1B & 1C), we did not find a single strain of a SARS-like bat virus that harbors all proteins with the most similarity to counterparts of the 2019-nCoV (Figs. 1B & 1C). Given the close relationship between 2019-nCoV and SARS-CoVs or SARS-like bat CoVs (Figs. 1B and 1C), an examination of the amino acid substitutions in different proteins could shed light into how 2019-nCoV differs structurally and functionally from SARS-CoVs. In total, there were 380 amino acid substitutions between the amino acid sequences of 2019-nCoV (HB01) and the corresponding consensus sequences of SARS and SARS-like viruses (Fig. 2, Tables S1e and S1f). No amino acid substitutions occurred in nonstructural protein 7 (nsp7), nsp13, envelope, matrix, or accessory proteins p6 and 8b (Table S1f). 102 and 61 amino acid substitutions are located in nsp3 and nsp2, respectively. In addition, 27 amino acid substitutions were
found in the spike protein with a length of 1282 amino acids, including 6 substitutions in the receptor-binding domain (RBD) at amino acid region 357-528 and 6 substitutions in the underpinning subdomain (SD) at amino acid region 569-655. Moreover, four substitutions (Q560L, S570A, F572T and S575A) in the C-terminal of the receptor-binding subunit S1 domain (Fig. 2) are situated in two peptides previously reported to be antigens for SARS-CoV (Guo et al., 2004).

Due to the very limited knowledge on this novel virus, we are unable to give reasonable explanations for the significant number of amino acids substitutions between the 2019-nCoV and SARS/SARS-like CoVs. For example, no amino acid substitutions were present in the receptor-binding motifs that directly interact with human receptor ACE2 protein in SARS-CoV (Ge et al., 2013), but 6 mutations occurred in the other region of the receptor-binding domain. Whether these differences could affect the host tropism and transmission property of the 2019-nCoV compared to SARS-CoV is worthy of future investigation.
Acknowledgments

This work has been supported by the National Key Plan for Scientific Research and Development of China (2016YFD0500301, 2016YFC1200200), CAMS Initiative for Innovative Medicine (CAMS-I2M, 2016-I2M-1-005), the National Natural Science Foundation of China (U1603126), the Central Public-Interest Scientific Institution Basal Research Fund (2016ZX310195, 2017PT31026 and 2018PT31016), and NIH R01AI069120.
Reference


Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu,


Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W.,
Lu, R., et al. (2020). A Novel Coronavirus from Patients with Pneumonia in China,
2019. The New England journal of medicine. Published online 2020/01/25 DOI:
10.1056/NEJMoA2001017.

(2018). Predicting the receptor-binding domain usage of the coronavirus based on
kmer frequency on spike protein. Infection, genetics and evolution: journal of
molecular epidemiology and evolutionary genetics in infectious diseases. 61, 183-184.
Published online 2018/04/07 DOI: 10.1016/j.meegid.2018.03.028.

Figure legends

Figure 1. (A) Schematic diagram of the genome organization and the encoded
proteins of pp1ab and pp1a for the IVDC-HB-01/2019 (HB01) strain. The largest
gene, namely the orf1ab, encodes the pp1ab protein that contains 15 nsps (nsp1-nsp10
and nsp12-nsp16). The pp1a protein encoded by the orf1a gene also contains 10 nsps
(nsp1-nsp10). Structural proteins are encoded by the four structural genes, including
spike (S), envelope (E), membrane (M), and nucleocapsid (N) genes. The accessory
genes are distributed among the structural genes. The protein-encoding genes of the
genome of 2019-nCoV were predicted by the online servers of GeneMarkS
(http://exon.gatech.edu/GeneMark/genemarks.cgi) and ORFfinder
relationship based on the whole genome for the HB01 strain and other coronaviruses.
All viral strains were classified by the genus and the type, which are presented on the left and right schematic phylogenetic trees, respectively. The four genera of the coronaviruses, including alphacoronavirus (red), betacoronavirus (blue), gammacoronavirus (green) and deltacoronavirus (violet) are blocked in the left phylogenetic tree. The MERS coronavirus (brown), the SARS-like bat coronavirus (violet), human SARS coronavirus (light blue) and the HB01 strain (red) are highlighted by lines of different colors in the right phylogenetic tree. (C) Schematic phylogenetic trees of individual genes for the HB01 strain. The coronavirus species were colored in the same way as (B). The amount of the strains in the phylogenetic clade is denoted by the area of the circles.

Figure 2. Amino acid substitutions of 2019-nCoV against SARS and SARS-like viruses. All 27 proteins encoded by 2019-nCoV have been aligned against SARS-CoVs and SARS-like bat CoVs using the FFT-NS-2 algorithm in MAFFT (version v7.407) (The number of aligned proteins were listed in Table S1e). An amino acid substitution was defined as an absolutely conserved site in the group of SARS/SARS-like CoVs while different with that of 2019-nCoV. In total, 380 amino acid substitutions in have been identified between the amino acid sequences of 2019-nCoV (HB01) and the corresponding consensus sequences of SARS/SARS-like CoVs.