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**REVIEW ARTICLE**

# COVID-19 and SARS-CoV-2: Molecular Genetics Perspectives

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**ABSTRACT**

Corona virus disease-19 (COVID-19) is a zoonotic viral disease caused by severe acute respiratory syndrome corona virus (SARS-CoV-2), which was first reported from Wuhan, Hubei province, in China in December 2019. The source of virus is believed to be from bats and the intermediate host is pangolins (ant eaters). The SARS-CoV-2 genome is fully sequenced and genome data are available now. Recent molecular studies on the three corona viruses- SARS-CoV, MERS-CoV(Middle East Respiratory Syndrome Corona virus), and SARS-CoV-2 can shed light on the mechanisms of COVID-19 infection, which could help the world to identify therapeutic target molecules, formulate control measures, and adopt appropriate preventive measures including development of vaccine(s).

**Key Words:** COVID-19, SARS-CoV-2, MERS-CoV, genome, infection, sequence.

## INTRODUCTION

Severe acute respiratory syndrome (SARS) was reported from China in 2002-2003 and the causative corona virus was named SARS corona virus (SARS-CoV). Later in 2011, a corona virus named MERS-CoV was reported to cause Middle East Respiratory Syndrome (MERS) in Saudi Arabia. A third time in the *late 2019,* the latest corona epidemic was reported from Wuhan, Hubei province, in China, from a sea food and live animal market, whence the disease was named corona virus disease-19 (COVID-19) by the World Health Organisation (WHO). Subsequently, WHO declared COVID-19 as a Public Health Emergency of International Concern (PHEIC) on 31st of January 2020 directing an international coordinated response across the globe.The International Virus Classification Commission named the causative virus of COVID-19 as SARS-CoV-2. of late, WHO declared COVID-19 as pandemic, which

means that the virus can spread across the globe rapidly so that it may affect people worldwide and cautioned countries to take measures to contain the spread of COVID-19 (Heymann and Shindo, 2020; Lu *et al*., 2020)

## Molecular genetics of SARS-CoV-2

Corona viruses are usual inhabitants in wild as well as domestic animals. The viruses become infective and cause diseases under two conditions: (1) when virulence (capacity of virus to produce disease) of viruses increases under suitable conditions such as changed climate; and/or (2), when the immunity of the host (animals/humans) decreases under conditions of stress. This article addresses the current knowledge on mechanistic processes at molecular level leading to increase in virulence of viruses and factors leading to immune deficits making humans more susceptible to the corona virus attack.

*All viruses*, in general, are obligatory intracellular, in the sense that a virus to survive it needs to be inside a living cell. Outside the living cell, viruses survive only for a short while. Entry of corona viruses into the living cell is through the mucosa of respiratory or digestive tract or eyes. That is how disinfecting hands (by frequent washing and use of sanitizer) and avoidance of hands touching mouth, nose and eyes help to prevent entry of viruses in to the body. Viruses possess either RNA or DNA as their genetic material. Unlike other cells, viruses neither contain a nucleus nor cell organelles like mitochondria or ribosomes. Instead, they use the host cellular machineries for their normal metabolic processes. For example, when corona virus infects human cells they use the human cellular organelles and ultimately kill the cells.

SARS-CoV-2 has a single stranded positive stranded RNA genome, which is of 26-32kb in size (Figure 1). The genome size of SARS-CoV and MERS-CoV is 27.9 kb and 30.1 kb, respectively. Among the four corona virus genera of $\alpha$, $\beta$, $\gamma$ and $\delta$, $\alpha$ (HCoV-229E and NL63) and $\beta$ (MERS-CoV, SARS-CoV, HCoV-OC43 and HCoV-HKU1) corona viruses infect humans. The efforts to identify the source of SARS-CoV-2 are in progress and maximum genetic identity of the SARS-CoV- 2 (99%) was with the corona virus in pangolins (ant eaters)(Zeng *et al*., 2020; Prompetchara *et al*., 2020).

The genome of corona virus encodes non-structural proteins (NSPs) that forms viral replicase transcriptase complex (that controls viral multiplication inside human cells) and four structural proteins, viz, spike (S), envelope (E), nucleocapsid (N) and membrane (M) proteins. NSPs coded by two-third of the viral genome, control replication of viruses (virus multiplication) and formation of proteins once they enter inside the human cells (viral transcription). The remaining one-third of the viral genome codes structural proteins, which has a pivotal role in the entry of viruses in to the cells.

The virus enters in to the human cells through the receptors located on the surface of the human cells. The cell surface receptors on human cells are unique for each virus and this determines the susceptibility of various organs in the human body for viral attack. The genome sequence analysis using spike protein determining entry of receptor-binding domain (RBD) shows that for both SARs-CoV and SARs-CoV-2, the predominant cell surface receptor is angiotensin-converting enzyme 2 (ACE-2), where as, for MERS-CoV the predominant receptor is dipeptidyl peptidase 4 (DPP4, also known as CD26). The basic reproductive number ($R_0$), the average number of individual getting infected from an infected individual, in COVID-19 ranges from 2.2 to 2.6, with a doubling time of 6.4 days. Continued transmission is predicted if $R_0$ is greater than 1. The $R_0$ value of SARS-COV and MERS-COV was less than 1 and 1.4 - 2.5, respectively. The higher $R_0$ of SARS-COV-2 implies more contagious nature of the virus and the potential of SARS-CoV-2 as a pandemic, as we see now [5]. An interdisciplinary research into the molecular mechanisms and better understanding of the receptor binding and viral entry mechanisms will help to understand how SARS-CoV-2 virus causes disease conditions in humans and how we can prevent the viral entry.

**Molecular genetics of COVID-19**

As mentioned earlier, the structural envelope spike glycoprotein (S) is the significant determinant of viral entry in to the human cells. ACE-2 receptors are ubiquitous in a wide range of wild and domestic animal species except in rats and mice. In the lungs, the ACE-2 receptors are predominantly seen in a specific subset of cells in the lung alveoli- the alveolar type 2 cells. The immune cells in the lung- monocytes and macrophages also express ACE-2 receptors, but only a few cells have ACE-2. Recent studies show additional mechanisms of viral entry into susceptible individuals. The S protein binds to the ACE-2 and TMPRSS-2 receptors in case of SARS-COV-2, to the ACE-2 and CD209L (L-SIGN, a C-type lectin) in case of SARS-CoV and, to the DPP4 in MERS-CoV. Other mechanisms identified for the corona virus entry in to the human cells are through S protein activation mediated either through proteolytic cleavage of S protein or through furin-mediated activation. Moreover, the affinity of SARS-CoV-2 to ACE-2 is also different- SARS-CoV-2 has 10-20 fold more affinity to ACE-2 compared to SARS-CoV, another reason for high virulence of SARS-CoV-2. After receptor binding, viral envelope protein fuses with plasma membrane (the outer membrane of human cells) enabling the entry of virus in to the cells. After viral entry in to the cell, viral genome is released in to the cytoplasm of the cell, starts multiplying (replication) and produce structural and non-structural proteins (translation). The new viruses thus formed fuses with the plasma membrane of the cells and virus infection of the cell is complete resulting in disease condition-COVID-19, in infected individual (Ahmed *et al*., 2020).

The SARS-CoV infection reported in 2003 was successfully contained, thanks to the concerted efforts of scientists, public and policy makers in making aware the whole population regarding the threats of a pandemic. After the SARS outbreak in 2003, there were only a few follow-on disease reports mainly due to accidental out breaks in research laboratories. After 2003, there had been no new reports of SARS-CoV and hence SARS-CoV was considered eliminated from human populations. The origin of SARS-CoV, MERS-CoV and SARS-CoV-2 is common bats; and the intermediate hosts harboring the viruses are palm civets; camels and pangolins, respectively. SARS-CoV causing contagious pneumonia like infection in healthcare personals originated from the live animal market in Guangdong province in China (Wu *et al*., 2020).

The mean incubation period of SARS-COV-2 is 6.4 days within a range of 2.1 to 11.1 days and the primary mode of infection is human-to-human transmission through close contact. The SARS-COV-2 gets entry in to the body through exhaled air or through cough or sneeze. The international travel of infected people mainly caused spread of the disease across the nations. SARS-CoV-2 has a different epidemiology and it behaves like other corona viruses that cause common cold. They predominantly replicate at the upper respiratory tract initially without causing any abrupt severe symptoms. Hence, infected individuals carry significant viral load at the upper respiratory tract while they carry on usual day-to-day activities, allowing others to get infected.

The pandemic spread of SARS-CoV -2 is mainly due to its transmission through asymptomatic carriers (persons who do not show any symptoms while they carry virus). In a study among 277 COVID-19 confirmed individuals in Wuhan, 200 of them had never visited Wuhan market and were not in close contact with severely ill patients. Conversely, SARS-CoV mainly spread through persons who were severely ill, and thus the control of infection was relatively easy compared to SARS-CoV-2. Moreover, mathematical disease modeling of SARS-CoV shows transmission rate of 5% during asymptomatic period. Interestingly, SARS-CoV-2 also has affinity towards lower respiratory tract causing all the three common clinical symptoms of corona virus infection- common cold, mild pneumonia and acute severe pneumonia with acute respiratory distress syndrome (ARDS). Thus, although the case fatality rate of SARS-CoV-2 is low (2.8% compared to SARS-CoV (9.14%)and MERS-CoV (34.4%), SARS-CoV-2 is more disruptive than SARS-CoVand MERS-CoV due to its chances of rapid spread and the ability to produce life threatening respiratory syndrome in affected individuals (Rabi *et al*., 2020).

The Scientific and Technical Advisory Group for Infectious Hazards (STAG-IH), under WHO regularly assess the risk of COVID-19 in various countries. The current understanding on COVID-19 is that SARS-CoV-2 causes mild to

severe attacks in infected individuals producing life threatening ailments to the old and people with other illnesses such as diabetes and pulmonary disease pointing that immune-competent individuals have a lower risk of severe symptoms.

In a study among 752 COVID-19 patients in China, fever (86-90%), cough (49.1-51%), fatigue (25.2-27.1%), discharge of sputum (20- 23.1%) and headache (9.8-11.1%) were the common symptoms. The mortality rate also varied in different regions: 4.6% in Wuhan, 1.9% in Beijing, and 0.9% in Shanghai. In the same study the researchers also compared the results in 14,117 normal controls. There exists phenomenon of lymphocyte depletion (PLD)-a compensatory mechanism in patients with low immunity such as in HIV patients or in those under chemotherapy. The age related increased susceptibility to COVID-19 is explained through differential lymphocyte levels. As the age increased, the natural killer (NK) cells were elevated and the CD4, CD8 and B-lymphocytes decreased suggesting an age-dependent decline of B and T cell immune function. This clinical case study also revealed the reference ranges of individuals at high risk of COVID-19 infection- CD3+, CD4+ and CD8+ lymphocytes less than 900, 500 and 300 cells/mm, respectively. Study also recommended screening for lymphocyte subsets as soon as individuals are diagnosed as COVID-19 positive in order to assess the severity of viral attack (Zeng*et al*., 2020). Quantitative real time polymerase chain reaction (qRT-PCR) helps clinicians to assay lymphocyte subsets and assess severity of illness.

When an individual comes in close contact with SARS-CoV-2 source (virus carriers like bats or pangolins or with infected individuals) the human cells respond to the entry of viruses through antigen processing cells (APCs) called major histocompatibility complex (MHC) or human leucocytic antigens (HLAs) and virus-specific cytotoxic T lymphocytes (CTLs). The polymorphisms (gene sequence differences) in HLA genes explain individual differences in susceptibility of SARS-CoV-2 in different populations. Gene polymorphisms in mannose-binding lectin (MBL) also cause differences in corona virus susceptibility among different individuals and populations. The innate immune cells identify viral entry in to the cells by recognizing pathogen associated molecular patterns (PAMPS). For COVID-19, TLR3 and TLR7, the RNA receptors in the endosomes, and RIG-I/MDA5, RNA sensor in the cytosol, recognize either positive stranded RNA of SARS-CoV-2 or intermediary double stranded RNA generated from SARS-CoV-2 at its replication stage. Subsequently virus enters in the nucleus of the infected cell; and this is called nuclear translocation. This results in the induction of type 1 interferon and other pro-inflammatory cytokines, thus sparking the first line of defense against viral attack in the cells. This type 1 interferon mediated response trigger JAK-STAT pathway through interferon AR (IFNAR) and phosphorylate JAK1 and STAT1 using JAK1 and TYK2 kinases. Activated JAK-STAT form complex with IRF 9 and this activated complex stimulate expression of interferon-stimulated genes (ISGs) with the help of interferon stimulated response element (ISRE) (Li *et al*., 2020). If this interferon mediated response is successful, viral replication and dissemination is suppressed at an early stage; it means that the human host cells won the battle and the individual escaped from viral attack.

It was found that SARS-CoV and MERS-CoV mount suppression of type 1 interferon response leading to severe clinical manifestations. For example, SARS-CoV degrade MAVS and TRAF3/6-the RNA sensor adaptor molecules while, MERS-CoV repress modification of histones; both these potentially inhibit type 1 interferon response (Li *et al*., 2020). Many COVID-19 therapeutic experimental protocols under trial target this interferon mediated disease defense pathway where timing of intervention is found to be the most important factor in breaking the chain of disease progression.

Subsequent to antigen presentation, another line of defense against SARS-CoV-2 infection is the B cell and T cell mediated humoral and cellular immunity. In severe acute viral infections, there is a typical pattern of immunoglobulin G and M (IgG and IgM) profile, where IgM is present only for a few weeks; IgG lasts longer. IgG is S and M specific in SARS-CoV infection. T cell proliferation, delayed type hypersensitivity (DTH) response and production of interferon-γ (IFN-γ) persist for years in recovered individuals. The plasma of the recovered individuals may contain an abundance of immune molecules; this is the basis of convalescent serum therapy, also

called donated plasma therapy. As we can imagine, the action of convalescent serum would be transient, and may require repeated infusions for getting a prolonged effect (Li *et al*., 2020).

Acute respiratory distress syndrome is the major cause of death in SARS-CoV-2 mediated through cytokine storm (that is, uncontrolled release of pro-inflammatory cytokines such as interferons and interleukins). The cytokine storm induced progression of disease severity is common for SARS, MERS and COVID-19. In general, the cytokine flux initiates virus-induced sepsis and lung inflammation causing shock, organ failure, respiratory failure and potentially, death. However, some of the interferons have protective effect against SARS-CoV and MERS-CoV (for example IFN-$\alpha$ and IFN-$\beta$), which are strong therapeutic target molecules as SARS-CoV-2 specific drugs.

For diagnosis, the first clue is obtained from the epidemiological history and clinical manifestations. The auxiliary diagnostic methods include detection of viral nucleic acid (the single stranded SARS-CoV-2 RNA, either through qRT-PCR or nucleic acid sequencing), CT scan, immunoglobulin assay (point-of-care testing, POCT) of IgM/IgG, enzyme-linked immunosorbent assay (ELISA) and the blood culture. For qRT-PCR diagnosis of SARS-CoV-2, the Chinese Center for Disease Control and Prevention (China CDC) suggests using specific primers and probes in the open reading frame 1ab (ORF1ab) and N gene regions. Once both targets are positive, they suggest, the COVID-19 is confirmed (Nguyen *et al*., 2020). The qRT-PCR can be done using saliva samples, which makes sample collection easy and non-invasive. CT scan images show bilateral pulmonary parenchymal ground glass and consolidative pulmonary opacity making CT scan a valuable aid for clinicians for differential diagnosis.

## Vaccines against COVID-19

Since the immune response is identical in SARS, MERS and COVID-19, there exists a possibility of designing cross-reactive immunization against all the three human corona viruses – SARS-CoV, MERS-CoV and SARS-CoV-2. Deoxyribo nucleic acid (DNA) vaccine, viral vector vaccine, subunit vaccine, vaccine using virus like particles, live attenuated virus vaccine and formaldehyde/gamma irradiated whole virus vaccine are most promising COVID-19 vaccines. Full-length S protein or S1 that contains receptor-binding domain (RBD) is considered a good antigen, as explained in the previous sections. This vaccine can target viral attachment to the host cell preventing early infection (Ahmed *et al*., 2020).

## Treatment against COVID-19

Developing treatment protocols mainly target repurposing of current antiviral drugs to intervene viral entry or disease progression pathways. Long-term memory of immune cells in corona-infected individuals may also help devising therapeutic strategy against COVID-19- including the one described earlier, the convalescent serum therapy or donated plasma therapy (Li *et al*., 2020).

## Conclusions and future strategies

In a pandemic like COVID-19, control of spread of the disease and developing therapeutic strategy are of primary concerns. Identifying the molecular mechanisms operating at cellular level may help to develop vaccines and formulate control measures. This may also help to find therapeutic target molecules and effective drugs against the disease. Extensive viral detection and characterization of SARS-CoV-2 in different regions of the world may aid to identify genetic differences among the virus and formulate vaccines and antiviral drugs.

The big challenges we are facing such as in developing vaccine for SARS-CoV-2, or treating COVID-19 patients, identifying causes of increasing virulence of SARS-CoV-2, and understanding mechanisms of reduced immunity among animals and humans against COVID-19, cannot be solved by a single discipline alone. Emphatically, insight

into the holistic/stand alone approaches of the ayurvedic and several other indigenous systems have promising role in the future transdisciplinary consortium to address the impending issues across the globe, including those arising from climate change.

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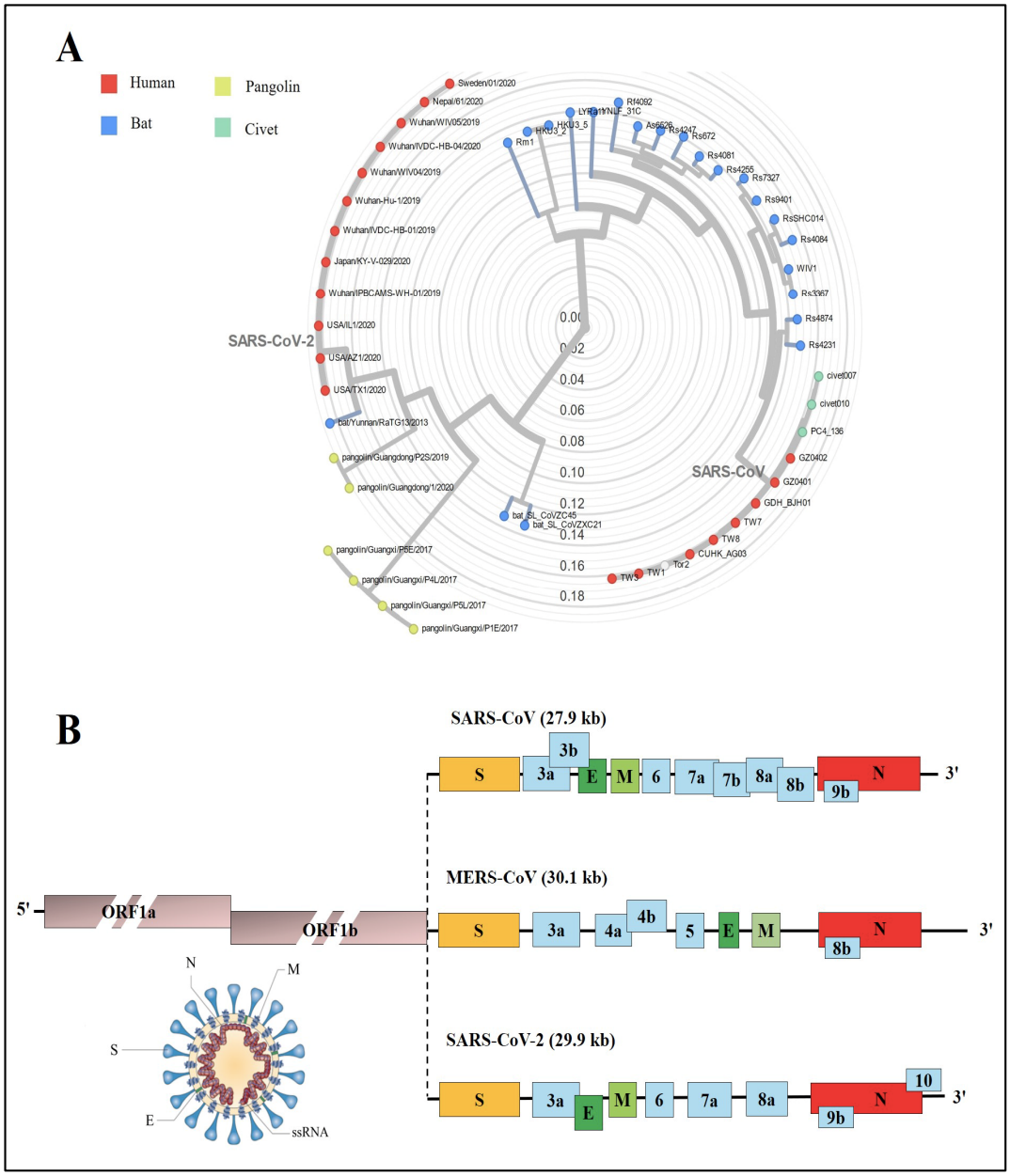


**Figure 1. Molecular phylogeny and structure of recent corona viruses. A) The relationship between the SARS-CoV and SARS-CoV-2 identified from different sources. Note that the SARS-CoV-2 was more closely related to corona virus isolated from bats and pangolins (ant eaters). B) The structure of corona virus and its genome organisation. ssRNA-single strand RNA; M, N, S and E-the viral structural proteins; ORF-open reading frame; the total genome size of SARS-CoV, MERS-CoV and SARS-CoV-2 is also given (adapted from Li *et al*., 2020).**