# The Pneumonia outbreak: High isoleucine and high valine plus glycine contents are features of the proteins of COVID-19 virus

Yulin Wan[1], Sirui Yan[1], Ying Zhang[2], Shanshan An[1], Kaiqiao Yang[1], Huan Xu[3], Tao Gan[4], Weiguo Cao[5], Qiuyun Liu[1,*]

[1]*State Key Laboratory of Biocontrol, Biomedical Center, Guangdong Provincial Key Laboratory of Improved Variety Reproduction in Aquatic Economic Animals, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China.*

[2]*Guangzhou Center for Disease Control and Prevention, Guangzhou 510440, China.*

[3]*School of Electronic Information and Automation, Guilin University of Aerospace Technology, Guilin 541004, Guangxi, China.*

[4]*Department of Biotechnology, School of Basic Medicine, Gannan Medical University, Ganzhou 34100, China.*

[5]*Department of Genetics and Biochemistry, Clemson University, Clemson, SC 29634, USA.*

Yulin Wan and Sirui Yan made equal contributions to this work.

*Corresponding author: Qiuyun Liu, Ph.D, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China. Tel: +86 (020)84110296; E-mail: lsslqy@mail.sysu.edu.cn

**Abstract**

The current pneumonia epidemic could evolve into a pandemic on a global scale if not effectively contained. The COVID-19 possesses a 61-amino acid open reading frame resembling SARS-CoV virulence factor - ORF6 peptide. The isoleucine content is 15.9% in ORF6 of SARS-CoV versus 16.4% of that in COVID-19. Given the proton affinity in the carbonyl oxygen in isoleucine, augmented proton traffic can enhance proton-ion antiport and prompt cell swelling. Calorie restriction has been confirmed in animal studies to extend lifespan, and its underlying mechanism is not fully known. As the content of essential amino acids in the open reading frame of COVID-19 reaches 57.4%, a starch/vitamin diet served for short period of time does not give rise to essential amino acids and halts virion production, which could be adopted as prophylactic approach of many viral infections. Plant-based diet or fasting/boiled rice water can also minimize the intake of essential amino acids or all amino acids respectively. Furthermore, several proteins of COVID-19 possess high valine plus glycine content which is implicated in heart disease, justifying the aforementioned approaches.

**Keywords**

COVID-19; Proton affinity; Calcium oxalate; Isoleucine, Valine; Glycine; Prophylactic approach

## Introduction

The current pneumonia outbreak could develop into a pandemic on a global scale if not effectively controlled. The World Health Organization named the virus as COVID-19. Based on available data, the infection rate is lower in children and teenagers than in adults. According to news reports on autopsies of the deceased patients, the lungs are very sticky which may be the cause of respiratory difficulties.

## Results and discussion

The COVID-19 has a 61-amino acid open reading frame resembling SARS-CoV virulence factor - the 63-residue ORF6 protein. The basic amino acid content is slightly higher in ORF6 of SARS-CoV with 11.1% versus 9.8% in its counterpart of Wuhan strain nCoV-2019 (WIV02) (Fig. 1) (Zhou et al., 2020, Corman et al., 2020, Huang et al., 2020, Wang et al., 2020), whereas the isoleucine content is relatively close (15.9% in ORF6 of SARS-CoV versus 16.4% of that in COVID-19 with identical number of isoleucine residues in the two open reading frames (orf)). However, the isoleucine residues are more packed in a shorter segment in SARS-CoV ORF6 protein, allowing more robust proton relay as isoleucine displays proton affinity (Gu et al., 1994; He et al., 2017). High fever and metabolic acidosis are two of the major symptoms in severe patients with COVID-19 infections, and are related to hydrogen bonding and proton traffic. The positively charged basic amino acids attract anions such as $Cl^-$, enhancing the formation of strong acids. Augmented proton traffic promotes proton-ion antiport and cell swelling. The intake of boiled red meat without higher protein structures confers slight cancer risks, and the β-amyloid plaques and neurofibrillary tangles correlate to the onset of Alzheimer's disease, suggesting that protein primary structures are implicated in disease etiology. The lower rate of COVID-19 in children can be at least partly explained by the less robust Krebs cycle in young kids generating less protons, consequently yielding less proton stress. Women also have less strong energy metabolism than men. The human transmissibility and the long latency period of the Wuhan strains may drive the spread of the virus to other regions of world, underscoring the urgency for preventive measures. As the percentage of essential amino acids in the

small orf of COVID-19 reaches 57.4%, a starch/vitamin diet served to the potentially exposed human subpopulation for short period of time does not give rise to essential amino acids such as isoleucine and lysine and reduces the viral load, which could be adopted as prophylactic approach of COVID-19 (Gan et al., 2018; Wan et al., 2019; He et al., 2018; Wahl et al., 2018). Peptides consisting of essential amino acids are often stressful (Tang et al., 2017). Plant-based diet, fasting/boiled rice water or sugar water/vitamins can be also used to minimize the intake of essential amino acids or all amino acids. During fasting or consuming sugar water, occasional limited co-intake of protein-containing nutrients may relieve some stress generated by abnormal ion traffic via ionic bonds or secondary chemical bonding with proteins, peptides or amino acids. Some of these are tried and tested ancient antiviral remedies which ensured human survival and can be explained by modern science. Appearance of essential amino acids may be the consequence of natural selection against viral and perhaps some other diseases as flu-stricken individuals tend to lose appetite, particularly appetite toward meat which is high in essential amino acids. Fasting is also used in some individuals in western society to extend lifespan, and it accounts for the prolonged lifespan of Americans during the Great Depression, known as calorie restriction. These food regimens should be tested in clinical trials, and individuals need to be instructed by a physician.

Valine and glycine content of the orfs of COVID-19 and SARS-CoV are shown in Table 2 and Table 3. Since valine and glycine harbor affinity to divalent cations such as calcium, and isoleucine possesses proton affinity (Qi et al., 2018; Wan et al., 2019; Xing et al., 2017; Wang et al., 2017), valine or glycine compete with isoleucine and alleviate fever and other symptoms. However, high content of valine or/and glycine are implicated in heart disease and other disorders by the formation of insoluble and rigid calcium oxalate which are stressful to the body (Qi et al., 2018; Wan et al., 2019), which accounts for the higher mortality rates in these patients concomitant with COVID-19 infections. Patients with renal stones, constipation, heart disease, Alzheimer's disease, Prion disease, *etc.* need to be cautious of COVID-19 as insoluble calcium salts may be present in their bodies which can be compounded by viral

infections. The higher mortality rates in Wuhan and Hubei Province than elsewhere can be attributed to the low temperature in winter without heating, driving robust energy metabolism to produce an excess of oxalate which leads to the formation of calcium oxalate (Zhou et al., 2017; Shi et al., 2017). The low food intake mentioned above could minimize the generation of oxalate via the reduced energy metabolism. Injection of physiological saline (divalent cation-free NaCl) may help dissolve insoluble and rigid calcium oxalate via the action of $Cl^-$. RNA interference can be adopted to decrease the production of oxalate. During the course of adopting fasting/rice water or sugar water regimens, amino acids with hydrogen bonding capabilities such as serine can be supplemented since these protein building blocks gather protons and help form acids which solubilize insoluble calcium oxalate. Individuals with rheumatic diseases or sensitive to humidity should not take these amino acid supplements with hydrogen bonding properties. Collected viral strains can be divided into two major types designated L and less aggressive S, representing leucine and serine polymorphisms respectively (Tang et al., 2017; Tang et al., 2020). Lysine preferentially attracts small anions such as $Cl^-$ and helps solubilize calcium oxalate, and the beneficial or adverse effects of lysine supplement need to be studied for severe COVID-19 patients since modest lysine intake modifies the proteome and enhances the synthesis of lysine-rich proteins. Due to the allergic response of arginine intake and potential calcium affinity of guanidyl group, arginine supplement is not recommended. High-purity NaCl solution or white vinegar can be smeared on the patients' chest, since white vinegar contains acetic acid and NaCl, and acetic acid inhibits the generation of oxalate (Zhang et al., 2020). All these regimens or treatments should be subjected to clinical trials before antiviral application.

The false negatives occurred in nucleic acid diagnosis of COVID-19 can be attributed to the viscosity generated from insolubility of calcium oxalate and secondary chemical bonding between calcium and glycine and between calcium and valine, which also leads to delayed progression of the viral disease in some patients. The intake of NaCl might decrease viscosity and release viruses from the mucus. Alternatively, PCR buffers with lower pH or modified salt composition may reduce viscosity, improve

performance of the PCR amplifications and minimize false negatives in nucleic acid diagnosis. Lung stickiness could be the cause of respiratory difficulties (Figure 1). (Gu et al., 1994; Wan et al., 2019) The divalency of the calcium ion also enhances viscosity. For instance, hydrogen bonding is critical for the stickiness of boiled starch, whereas ionic and secondary chemical bonding between amino acids and calcium is adopted to make Tofu. Towels or paper towels soaked with NaCl solution can be placed on the chest of patients to give adequate time for the salt to be absorbed and penetrate into the lungs. In traditional medicine, individuals with constipation problem can drink lightly salty water to reduce stickiness of the feces itself or stickiness between feces and the colon, which could take effect in about half an hour. Salt use can reduce viscosity of fried food to iron pan during cooking. NaCl addition can also render Tofu--a Chinese food more tender by potentially minimizing secondary chemical bonding of amino acid residues to calcium. Therefore, chloride as a strong anion can disrupt hydrogen bonding or secondary chemical bonding to other cations. The high mortality rates in seniors can be attributed to the buildup of oxalate via the shunt of the normal Krebs cycle in people over 60 years old with attenuated respiratory chain activities. Due to the presence of insoluble calcium oxalate, cancer patients and young people with constipation problems should be very cautious about the COVID-19 virus.(An et al., 2020; Wan et al., 2019) Individuals with regular diarrhea might develop less severe symptoms as hydrogen bonding capacity allows the buildup of proton pool and acids, which are capable of solubilizing insoluble and rigid salts.

## Conclusions

The high contents of particular amino acids in COVID-19 virus can have profound impact on the human hosts. The high percentage of essential amino acids in this virus can be harnessed to develop prophylactic food regimens such as carbohydrate-based diet, plant-based diet and fasting/boiled rice water, minimizing virion production to give the host a grace period to fight off the existing pathogens. Combining food regimens and western medicine may yield unexpected outcomes.

**Conflict of interest statement**

The authors declare no conflict of interests.

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**Author Contributions**

QL contributed to the conception and design of the work. All authors contributed to the analysis and interpretation of data for the work. QL, YW and SY drafted the manuscript with input from all authors.

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Table 1 The amino acid content of ORF6 protein of SARS-CoV (GenBank accession no.: AY323977.2) versus its counterpart of COVID-19 (GenBank accession no.: MN908947.3).

| Amino Acid | SARS-CoV | | COVID-19 | |
|---|---|---|---|---|
| | No. of residues | Content % | No. of residues | Content % |
| Ala(A) | 2 | 3.2 | 1 | 1.6 |
| Arg(R) | 3 | 4.8 | 1 | 1.6 |
| Asn(N) | 2 | 3.2 | 4 | 6.6 |
| Asp(D) | 5 | 7.9 | 4 | 6.6 |
| Cys(C) | 0 | 0.0 | 0 | 0.0 |
| Gln(Q) | 2 | 3.2 | 3 | 4.9 |
| Glu(E) | 5 | 7.9 | 5 | 8.2 |
| Gly(G) | 0 | 0.0 | 0 | 0.0 |
| His(H) | 1 | 1.6 | 1 | 1.6 |
| Ile(I) | 10 | 15.9 | 10 | 16.4 |
| Leu(L) | 7 | 11.1 | 8 | 13.1 |
| Lys(K) | 3 | 4.8 | 4 | 6.6 |
| Met(M) | 3 | 4.8 | 3 | 4.9 |
| Phe(F) | 4 | 6.3 | 3 | 4.9 |
| Pro(P) | 3 | 4.8 | 1 | 1.6 |
| Ser(S) | 3 | 4.8 | 4 | 6.6 |
| Thr(T) | 3 | 4.8 | 3 | 4.9 |
| Trp(W) | 1 | 1.6 | 1 | 1.6 |
| Tyr(Y) | 2 | 3.2 | 2 | 3.3 |
| Val(V) | 4 | 6.3 | 3 | 4.9 |
| Basic Amino Acids(R,K,H) | 7 | 11.1 | 6 | 9.8 |
| Acidic Amino Acids(D,E) | 10 | 15.9 | 9 | 14.8 |
| Essential Amino Acids(K,W,F,M,T,I,L,V) | 35 | 55.6 | 35 | 57.4 |
| Leu/Phe(L,F) | 11 | 17.5 | 11 | 18.0 |
| Total | 63 | | 61 | |

Table 2 Amino acid composition of COVID-19 proteins (GenBank accession no.: MN908947.3).

| | COVID-19 (aa %) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ORF1ab | S | ORF3a | E | M | ORF6 | ORF7a | N |
| I | 5.2 | 6.0 | 7.6 | 4.0 | 9.0 | 16.4 | 6.6 | 3.3 |
| V | 8.5 | 7.6 | 9.1 | 17.3 | 5.4 | 4.9 | 6.6 | 1.9 |
| G | 6.0 | 6.4 | 5.1 | 1.3 | 6.3 | 0.0 | 3.3 | 10.3 |
| V+G | 14.5 | 14.1 | 14.2 | 18.7 | 11.7 | 4.9 | 9.9 | 12.2 |
| Basic amino acids | 11.4 | 9.4 | 9.1 | 6.7 | 11.7 | 9.8 | 12.4 | 15.3 |
| Acidic amino acids | 11.0 | 8.6 | 8.7 | 4.0 | 5.9 | 14.8 | 8.3 | 8.6 |

Table 3 Amino acid composition of SARS-CoV proteins (GenBank accession no.: AY323977.2).

| | SARS-CoV (aa %) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ORF1ab | S | ORF3a | E | M | ORF6 | ORF7a | N |
| I | 4.9 | 6.1 | 8.0 | 4.0 | 8.1 | 15.9 | 5.7 | 2.6 |
| V | 8.2 | 7.3 | 7.3 | 18.4 | 7.2 | 6.4 | 5.7 | 2.6 |
| G | 5.9 | 6.2 | 5.1 | 2.6 | 6.8 | 0.0 | 3.3 | 10.7 |
| V+G | 14.1 | 13.5 | 12.4 | 21.1 | 14.0 | 6.4 | 9.0 | 13.3 |
| Basic amino acids | 11.8 | 9.1 | 8.8 | 5.3 | 10.9 | 11.1 | 13.1 | 15.4 |
| Acidic amino acids | 10.5 | 9.3 | 8.0 | 5.3 | 5.9 | 14.3 | 8.2 | 8.5 |

## Long carbonyl bond length

Weakened bond

## Carbonyl oxygen of glycine/valine residues

Web-like structure          Secondary chemical bonding

## Insoluble calcium oxalate
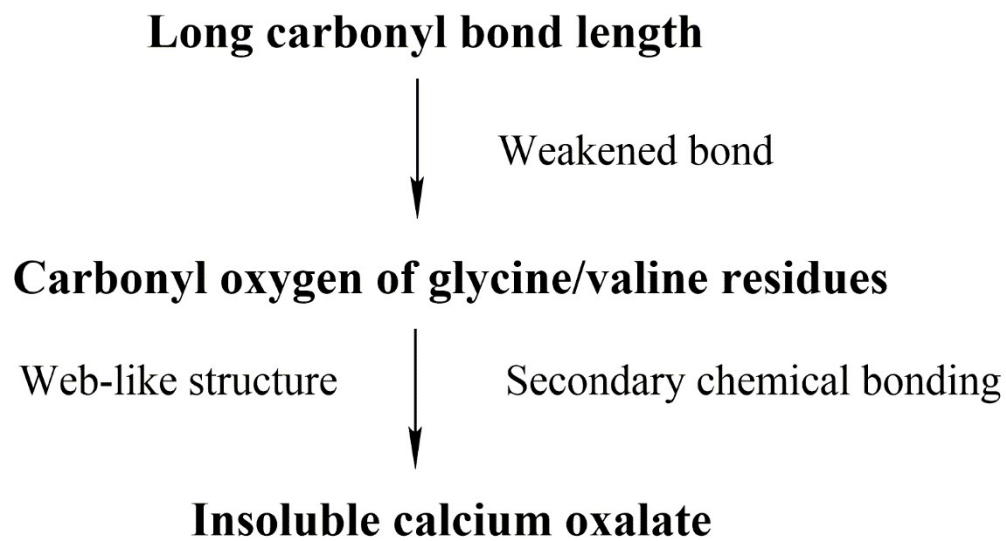
Figure 1. Lung stickiness can be explained by the extensive secondary chemical bonding between calcium and glycine and between calcium and valine residues.