

## COVID-19: Structural predictions of viral success

Since the beginning of the 21st century, three coronaviruses have crossed the species barrier and caused serious human disease: severe acute respiratory syndrome coronavirus (SARS-CoV) in November 2002,<sup>1,2</sup> Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012<sup>3,4</sup> and SARS-CoV-2 in 2019.<sup>5,6</sup> SARS-CoV-2,<sup>7</sup> initially called 2019-nCoV, is the aetiological agent of COVID-19, a highly contagious infectious illness that was first reported in December 2019 in Wuhan, China and subsequently spread globally.<sup>8</sup> As of May 24, 2020, COVID-19 has caused >5 370 000 infections and >343 000 deaths worldwide.<sup>9</sup>

Unfortunately, nearly 20 years after the SARS outbreak, and despite many attempts for vaccines and therapeutic agents directed against SARS and MERS, no approved prophylactics or therapeutics exist. As a result, the management of COVID-19 largely relies on supportive care<sup>10,11</sup> and on hopes surrounding compounds that appeared promising against previous coronaviruses.<sup>12,13</sup> This lost opportunity, in itself, offers a valuable lesson for current and future outbreaks, and the need for new experimental rationales to accelerate discovery.

The cellular entry of coronaviruses is fairly conserved across members of the *Coronaviridae* family and is mediated by the transmembrane spike (S) glycoprotein,<sup>14</sup> a homotrimer<sup>15,16</sup> that is often heavily glycosylated<sup>17</sup> and protrudes from the viral surface. Each of the three monomers of the spike glycoprotein consists of two functional subunits, S1, involved in membrane attachment, and S2, required for membrane fusion.<sup>15,18</sup> In many coronaviruses, the spike glycoprotein is cleaved at the S1/S2 interface by host cell proteases.<sup>19</sup> Within the S1 domain, the receptor binding domain (RBD) attaches to the cellular receptor, which in the case of both SARS-CoV and SARS-CoV-2 is the angiotensin-converting enzyme 2 (ACE2).<sup>19-21</sup> Another cleavage site, S2', is located within S2.<sup>17,19</sup> The spike glycoproteins of SARS-CoV and SARS-CoV-2 share 76% identity at the amino acid level,<sup>22,23</sup> although biophysical assays indicate that SARS-CoV-2 binds their common receptor, ACE2, with a 10-20-fold higher affinity than SARS-CoV.<sup>14</sup>

As we contemplate the dynamics of COVID-19 and the development of prophylactic and therapeutic interventions, one of the key considerations is the emergence and potential relevance of viral mutations. In the short time since the pandemic started, several missense mutations have been observed in various SARS-CoV-2 isolates.<sup>24</sup> One of these, the 23403A>G variant, substitutes the aspartic acid at position 614 of the viral spike glycoprotein with glycine (D614G), and is frequently documented in European countries but rarely observed in China.<sup>25</sup>

In the current issue of the *IJCP*, Becerra-Flores and Cardozo interrogate the impact of this mutation on pathogenicity and offer a structural correlate for their findings.<sup>26</sup> Their analysis includes confirmed COVID-19 cases and deaths as reported by the European CDC during the first week of April 2020 and examines the viral spike genomic sequences deposited in the GISAID database over that period, correlating the prevalence of the D614G mutation with fatality rates in the same regions. The authors then use cryo-electron microscopy data and *in silico* mutagenesis of this key residue to predict conformational preferences of the two variants of the spike protein.

The analysis indicates that viruses isolated from European patients predominantly expressed a glycine at position 614 of the spike glycoprotein, while a high percentage of the isolates collected from Far East patients favoured aspartic acid at the same position. The proportion of viral isolates having a glycine at this position significantly correlated with higher average and median case fatality rates across geographic areas. Interestingly, their data also imply a rationale for divergence in the behaviour of the disease between the East and West coasts of the United States, based upon the provenance of the viral "founders" in these regions, from the European and Asian variants, respectively.


Surprisingly, the authors' molecular modelling indicates that the presence of a glycine at position 614 *diminishes* binding to the cellular receptor when replacing the aspartic acid at that residue, mainly by reducing the spike protein's occupancy of the "up" or liganded state, when it is most amenable to receptor interaction. While seemingly counterintuitive, this finding opens at least two fascinating scenarios. As the authors hypothesise, a spike glycoprotein that harbours glycine at this position might be better protected from immune recognition, elicit the production of harmful antibodies, flood the host with ineffective antibodies or some combination of all three. A delay in immune recognition may impact viral transmission by delaying symptomatic presentation or allowing unfettered infection without effective immune response. An aberrant response, suited to the viral conformation at large but not the infective conformation, could equally allow for an increased—but poorly targeted—inflammatory cascade. The possibility of a harmful immune response is particularly thought provoking, as antibody-dependent enhancement, the phenomenon by which antibodies facilitate viral entry into host cells that do not necessarily have viral receptors,<sup>27,28</sup> has been reported for many viruses, including coronaviruses,<sup>27,29</sup> dengue virus,<sup>30,31</sup> feline infectious peritonitis virus,<sup>32</sup> Ebola virus<sup>33</sup> and HIV.<sup>34</sup> Another possibility, not mutually exclusive, is that the D614G mutation creates or exposes a novel cleavage site in the spike glycoprotein.

Delving into these molecular mechanisms with confirmatory in vitro studies will hopefully reap the benefits of decades of scientific strides while simultaneously highlighting deficiencies in key areas that can guide our approach to the current pandemic. One of the immediate questions involves the impact of this and other mutations on vaccine efficiency and the potential need to develop multiple candidate vaccines that cover a range of epitopes and their variants. In all likelihood, there is a lengthy and tortuous road ahead, but characterising significant variants will allow us to better understand many elusive aspects of this virus' success—the latent/incubation period, immune evasion and hyper-response, variable receptor binding, replication dynamics and organ-specific pathogenesis—and discover host vulnerabilities that mutations such as D614G seem to exploit.

The D614G mutation appears to become more common as the pandemic unfolds.<sup>35</sup> That this phenomenon is simply the result of a founder effect is possible but unlikely, and rather may be explained by this variant's selective advantage allowing more efficient spread. Whether this advantage is conferred by infectivity, immune evasion or pathogenicity—or some combination of these—is yet to be understood. Interestingly, this mutation is now known to travel simultaneously with other mutations, including one that affects the RNA-dependent RNA polymerase, with implications for proofreading, replication efficiency (and thus, viral titre) and the emergence of drug-resistant viral phenotypes.<sup>36</sup>

Addressing these molecular questions relies heavily on widespread efforts to assemble accurate and comprehensive data on population infection rates and mortality and frequent sampling of the genotypes of circulating isolates on a global basis. So far, this feat has been challenging and continued deficiencies will translate into missed singular opportunities to link molecular findings with population-level consequences, ultimately leaving us less prepared to address both this and future pandemics.

The valuable and timely experimental strategy used by Becerra-Flores and Cardozo serves as an important analytic model that should be employed routinely to understand the “molecular strategy” of this virus in the context of the evolving pandemic. This approach will also prove to be an indispensable instrument if also employed routinely at the onset of future outbreaks, which are all but guaranteed in the coming years, given the only recently appreciated ease of global spread of viruses in the modern world. In summary, this set of tools allows us to perform active surveillance, monitor the emergence of deleterious mutations prior to their widespread distribution, and use informed in silico and structural data to make informed decisions guiding molecular research and epidemic preparedness.

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