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Previews

Making Sense of Mutation: What D614G Means for the COVID-19 Pandemic Remains Unclear

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In this issue of *Cell*, Korber et al. found that a SARS-CoV-2 variant in the spike protein D614G rapidly became dominant around the world. Although clinical and *in vitro* data suggest that D614G changes the virus phenotype, the impact of the mutation on transmission, disease, and vaccine and therapeutic development are largely unknown.

Introduction

After the emergence of SARS-CoV-2 in China in late 2019, and the rapid expansion of the COVID-19 pandemic in 2020, questions about viral evolution have come tumbling after. Did SARS-CoV-2 evolve to become better adapted to humans? More infectious or transmissible? More deadly? Virus mutations can rise in frequency due to natural selection, random genetic drift, or features of recent epidemiology. Because these forces can work in tandem, it's often hard to differentiate when a virus mutation becomes common through fitness or by chance. It is even harder to determine if a single mutation will change the outcome of an infection, or a pandemic.

The new study by Korber et al. (2020) in this issue of *Cell* sits at the heart of this debate. They present compelling data that an amino acid change in the virus's spike protein, D614G, emerged early during the pandemic, and viruses containing G614 are now dominant in many places around the world. The crucial questions are whether this is the result of natural selection and what it means for the COVID-19 pandemic. For viruses like SARS-CoV-2, transmission really is everything—if they don't get into another host their lineage ends. Korber et al. (2020) hypothesized that the rapid spread of G614 was because it is more infectious than D614. In support of their hypothesis, the authors provided evidence that clinical samples from G614 infections have a higher levels of viral RNA and produced

higher titers in pseudoviruses from *in vitro* experiments, results that now seem to be corroborated by others (e.g., Hu et al., 2020; Lorenzo-Redondo et al., 2020; Ozono et al., 2020; Wagner et al., 2020).

Still, these data do not prove that G614 is more infectious or transmissible than viruses containing D614. And because of that, many questions remain on the potential impacts, if any, that D614G has on the COVID-19 pandemic.

Will D614G Make Outbreaks Harder to Control?

To answer this question, we must first explore how G614 became the dominant genotype and what impacts it could have on transmission. As an alternative hypothesis to the one described above, the increase in the frequency of G614 could be explained by chance and the epidemiology of the pandemic. In February, the area with the most COVID-19 cases shifted from China to Europe, and then in March on to the United States. As this and other work shows, the great majority of SARS-CoV-2 lineages in the United States arrived from Europe, which is unsurprising considering the amounts of travel between the continents. Whether lineages become established in a region is a function not only of transmission but also of the number of times they are introduced. There is good evidence that for SARS-CoV-2, a minority of infections are responsible for the majority of transmission (Endo et al., 2020). Therefore, although most introductions go extinct,

those that make it make it big (Lloyd-Smith et al., 2005). Over the period that G614 became the global majority variant, the number of introductions from China where D614 was still dominant were declining, whereas those from Europe climbed. This alone might explain the apparent success of G614.

Even if viruses containing G614 got “lucky” in escaping China, the variant could still provide a transmission boost. The clinical and *in vitro* data provided by Korber et al. (2020) certainly make this a plausible scenario. However, higher detection of SARS-CoV-2 RNA in oral and nasal swabs might not be a direct reflection of transmission potential. In addition, much transmission likely happens in the presymptomatic stage, and we don't know how these differences during the symptomatic phase compare.

The pseudovirus assays used in this study can demonstrate the ability to infect a cell in culture, and the results are important, but it's not clear what it means for the ability to productively transmit to a new host. These assays don't account for the effect of other viral or host proteins and the parade of biochemical host-pathogen interactions that must occur to support infection and transmission. Therefore, as prior experience with the 2013–2016 Ebola epidemic suggests (Marzi et al., 2018), it's impossible to conclude that a single mutation alone would have a major impact in a large, diverse human population based on *in vitro* infectivity and fitness data.



If G614 truly is more transmissible in equivalently mixing populations, then yes, the virus will be harder to control. But we cannot definitively answer this question at the moment.

Will D614G Make Infections More Severe?

So far there is no evidence that infection with SARS-CoV-2 containing the G614 variant will lead to more severe disease. By examining clinical data from 999 COVID-19 cases diagnosed in the United Kingdom, [Korber et al. \(2020\)](#) found that patients infected with viruses containing G614 had higher levels of virus RNA, but they did not find a difference in hospitalization outcomes. These clinical observations are supported by two independent studies: 175 COVID-19 patients from Seattle, WA ([Wagner et al., 2020](#)) and 88 COVID-19 patients from Chicago, IL ([Lorenzo-Redondo et al., 2020](#)) in the United States. Viral load and disease severity are not always correlated, particularly when viral RNA is used to estimate virus titer. The current evidence suggests that D614G is less important for COVID-19 than other risk factors, such as age or comorbidities.

Will D614G Impact Therapeutic and Vaccine Designs?

Although the D614G mutation is located in the virus's external spike protein that receives a lot of attention from the human immune system, and thus *could* have an influence on the ability of SARS-CoV-2 to evade vaccine-induced immunity, we think that it's unlikely for these reasons. D614G is not in the receptor-binding domain (RBD) of the spike protein, but in the interface between the individual spike protomers that stabilize its mature trimeric form on the virion surface through hydrogen bonding. [Korber et al. \(2020\)](#) propose that this could result in the loss of between-protomer hydrogen bonds, modulate interactions between spike pro-

tomers, or change glycosylation patterns. Although any of these changes could alter infectivity, it is less likely that it would drastically alter the immunogenicity of RBD epitopes thought to be important for antibody neutralization. Furthermore, [Korber et al. \(2020\)](#) and others ([Hu et al., 2020](#); [Ozono et al., 2020](#)) found that the antibodies generated from natural infection with viruses containing D614 or G614 could cross-neutralize, suggesting that the locus is not critical for antibody-mediated immunity. The D614G mutation is therefore unlikely to have a major impact on the efficacy of vaccines currently in the pipeline, some of which exclusively target the RBD.

Because the specific effect of D614G on spike function in entry and fusion is unknown, the impact of this mutation on therapeutic entry inhibitors is unknown. There is no current evidence that it would interfere with therapeutic strategies such as monoclonal antibodies designed to disrupt spike binding with ACE2 or drugs that modulate downstream processes such as endosomal acidification. However, until we better understand the role of D614G during natural SARS-CoV-2 infection, the mutation should be taken into consideration for any vaccine or therapeutic design.

Conclusions

Although there has already been much breathless commentary on what this mutation means for the COVID-19 pandemic, the global expansion of G614 whether through natural selection or chance means that this variant now *is* the pandemic. As a result, its properties matter. It is clear from the *in vitro* and clinical data that G614 has a distinct phenotype, but whether this is the result of bonafide adaptation to human ACE2, whether it increases transmissibility, or will have a notable effect is not clear. The work by [Korber et al. \(2020\)](#) provides an early base for more extensive epidemiological, *in vivo* experimental,

and diverse clinical investigations to fill in the many critical gaps in how D614G impacts the pandemic.

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