

NERVTAG paper: SARS-CoV-2 variants

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Summary of paper

- Reason for bringing to NERVTAG
 - To alert the group to a potential gap in understanding of the implications of the evolution of SARS-CoV-2 virus.
- Key conclusions of the paper (and level of confidence in these)
 - Mutations in SARS-CoV-2 are occurring that may have implications for virus behaviour, diagnosis, serology, vaccines and treatments. Mutation / recombination is highly likely, but the impacts are uncertain.
- What are the key questions to be considered by NERVTAG / SAGE / DHSC?
 - Are we currently adequately prepared to understand and act upon important changes in the virus as they occur?
- Recommendations or proposed next steps (if any)
 - A coordinated functional biology research programme should be initiated so that genetic changes in SARS-CoV-2 detected by COG and wider groups can be translated into knowledge on the implications for disease epidemiology and control.

SARS-CoV-2 genetic variation: Early evidence and implications.

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The genetic material of coronaviruses and SARS-CoV-2 is RNA and in the positive sense orientation. Coronavirus RNA synthesis is complex and involves the replication of the nearly 30 kb genome (the largest RNA genomes so far discovered) and the synthesis of a nested set of subgenomic mRNAs.

During the synthesis of viral RNA two main errors can occur that result in virus genome variation, these are recombination and mutation. Examples of both types of genetic change have occurred already in SARS-CoV-2:

Recombination can have several effects. Parts of the genome can be deleted, or new sequences incorporated producing a chimeric virus. New sequence can be acquired from the same type of coronavirus or a different source of RNA altogether. This is thought to account for the zoonotic origin of these viruses.

1. The current SARS-CoV-2 virus is thought to have been generated by a recombination event(s) between other animal coronaviruses. One consequence of recombination is the presence of a furin protease cleavage site exists at the S1/S2 junction in the spike glycoprotein gene. The SARS-CoV-2 spike glycoprotein gene is very similar in sequence to the SARS-CoV spike glycoprotein apart from the presence of this furin cleavage site. This furin cleavage site may have been acquired through recombination with host RNA. This sequence seem to enhance infectivity in human lung cells (Hoffmann et al. 2020). Other RNA viruses are known to undergo this type of genetic change (Gischke et al. 2020). Avian influenza viruses acquire similar furin cleavage sites in haemagglutinin and this generates highly pathogenic avian influenza strains (Sun et al. 2016). Whether the high virulence or transmissibility of SARS-CoV-2 is partly determined by this furin cleavage site is not yet known. Human coronavirus OC43 is thought to have acquired a hemagglutinin-esterase gene from recombination with influenza virus or a common ancestor (Zhang et al. 1992).

2. Several deletions have already been identified in the SARS-CoV-2 genome either in cell culture and/or in the analysis of the viral genome/RNA in patients. For example, work published in preprint (Davidson et al 2020; Liu et al 2020) has shown deletion of the S protein furin cleavage site in virus populations in cell culture. Similar deletions have been found in patients (unpublished). Studies in animal models show viruses with the deletion are attenuated (Lau et al. 2020). Deletions have also been found in the envelope gene and further towards the 3' end of the genome. These tend to exist in mixed virus populations within an individual.

Point mutations can have several effects. These can either be neutral (no effect), deleterious (decreases viral load) or advantageous (increases viral load and transmission). Coronaviruses

encode enzymatic functions that result in error correction. Therefore, their error rate may be up to ~10 times lower than other RNA viruses. Nonetheless point mutations are observed:

1. Analysis of the spike gene sequence by teams from the University of Sheffield and the USA, and published on a preprint server, have identified a coding (D614G) variant that predominates in virus populations (Korber et al. 2020). However, no functional biology is associated with the work that measures whether this mutation causes an increase in transmissibility of the virus.
2. Published work in April by groups in Italy (Pachetti et al. 2020), based on analysis of 220 sequences (from December 2019 to March 2020) have suggested that European, North American and Asian strain variants might co-exist.
3. A preprint released this week from a Japanese research group show that the SARS-CoV-2 ORF3b protein acts as a more potent interferon antagonist than the SARS-CoV homologue (Konno et al. 2020). This protein is only 22 amino acids long due to a point mutation that generated a premature STOP codon in orf3b. Artificially generated longer SARS-CoV-2 ORF3b proteins were even more efficient in vitro interferon antagonists. Moreover, two viruses in Ecuador carrying point mutations that resulted in longer orf3b proteins were associated with severe infection or death.

Conclusion:

As SARS-CoV-2 continues to circulate in humans we might expect it to accumulate genetic change, and some variants might be selected for that confer advantage. The drivers of evolution might include further adaptation to the human host to increase transmissibility. In addition, increasing immunity in the population might select for antigenic variants, and widespread use of antiviral agents might select for drug resistance. During the last influenza pandemic in 2009, three waves of virus circulation in the UK saw accumulation of genetic changes that affected virus phenotype (Elderfield et al. 2014).

Understanding the significance of the genetic changes in SARS-CoV-2 viruses as they arise is important.

Implications:

1. **Diagnostics.** Deletions and mutations can affect the accuracy of diagnostics. For nucleic acid based tests, deletions have been observed in the envelope gene at sites that match to WHO primer sets. We recommend that surveillance of genomes such as by COG UK continue, to ensure compatibility of primer/probe sequences.
2. **Contact Tracing using genomic epidemiology.** The lower error rate may make immediate contact tracing problematic (if based on point mutations). We recommend that this should be taken into consideration for outbreak assessments.
3. **Vaccinology.** Changes in S might affect the efficacy of vaccines that are being developed using the original S sequence described from the Wuhan epicentre in late 2019. We recommend studies that assess the consequence of genetic change in the S protein on ability of antibodies to recognize and neutralize virus. Changes in S protein can be introduced into

the plasmids used to generate pseudotyped viruses so that the effects on antigenicity can be checked. Neutralization assays using sera from vaccine recipients or recovered individuals can measure ability of sera to inhibit replication of contemporary viruses and monitor for changes that might affect vaccine efficacy. Large changes could even necessitate vaccine update. Neutralization assays of virus variants with mutations in S by monoclonal antibodies being considered for therapeutics, or convalescent plasma should also be carried out to confirm these therapeutics are still valid.

4. **Virus transmission and pathogenicity**. Deletions/mutations can affect transmission/disease associated with coronaviruses. In 10% of cats infected with feline enteric coronavirus (FeCV), mutations occur in the viral genome, many targeted to the furin cleavage site in the spike glycoprotein that lead to white blood cells becoming infected and cause a new disease, Feline infectious peritonitis virus (FIPV) (Licitra et al. 2013).

5. **Treatments**: Changes in the polymerase or protease genes might alter susceptibility to current or developed antiviral drugs.

Recommendations

We recommend that research funding be invested to understand the full implications of SARS-CoV-2 genetics changes in controlled studies. This will require analysis of the phenotype of viral variants in good model systems including primary cells and animal models. Investing in a UK shared reverse genetic system that allows individual mutations to be generated within a stable genetic background will allow a more robust analysis of each change. We recommend laboratories engage in surveillance of COG UK data via GISAID to ensure the spike glycoprotein is not changing significantly.

References:

Davidson et al. 2020. Characterisation of the transcriptome and proteome of SARS-CoV-2 using direct RNA sequencing and tandem mass spectrometry reveals evidence for a cell passage induced in-frame deletion in the spike glycoprotein that removes the furin-like cleavage site. bioRxiv 2020.03.22.002204; doi: <https://doi.org/10.1101/2020.03.22.002204>.

Elderfield et al. 2014. Accumulation of Human-Adapting Mutations during Circulation of A(H1N1)pdm09 Influenza Virus in Humans in the United Kingdom. *Journal of Virology* 88:13269–13283.

Gischke et al. 2020. Insertion of Basic Amino Acids in the Hemagglutinin Cleavage Site of H4N2 Avian Influenza Virus (AIV)-Reduced Virus Fitness in Chickens is Restored by Reassortment with Highly Pathogenic H5N1 AIV. *Int J Mol Sci.* 2020 Mar 28;21(7). pii: E2353. doi: 10.3390/ijms21072353.

Hoffmann M et al. A Multibasic Cleavage Site in the Spike Protein of SARS-CoV-2 Is Essential for Infection of Human Lung Cells. *Mol Cell.* 2020 Apr 28. pii: S1097-2765(20)30264-1. doi: 10.1016/j.molcel.2020.04.022.

Korber et al. 2020. Spike mutation pipeline reveals the emergence of a more transmissible form of SARS-CoV-2. bioRxiv preprint doi: <https://doi.org/10.1101/2020.04.29.069054>.

Konno et al. 2020. SARS-CoV-2 ORF3b is a potent interferon antagonist whose activity is further increased by a naturally occurring elongation variant. <https://www.biorxiv.org/content/10.1101/2020.05.11.088179v1>.

Lau SY et al. Attenuated SARS-CoV-2 variants with deletions at the S1/S2 junction. *Emerg Microbes Infect.* 2020 Dec;9(1):837-842. doi: 10.1080/22221751.2020.1756700.

Licitra et al. 2013. Mutation in spike protein cleavage site and pathogenesis of feline coronavirus. *Emerg Infect Dis.* 19(7):1066-73. doi: 10.3201/eid1907.121094.

Liu et al. 2020. Identification of a common deletion in the spike protein of SARS-CoV-2. bioRxiv 2020.03.31.015941; doi: <https://doi.org/10.1101/2020.03.31.015941>.

Pachetti et al. 2020. Emerging SARS-CoV-2 mutation hot spots include a novel RNA-dependent-RNA polymerase variant. *Journal of Translational Medicine.* <https://doi.org/10.1186/s12967-020-02344-6>.

Sun et al. 2016. A novel eight amino acid insertion contributes to the hemagglutinin cleavability and the virulence of a highly pathogenic avian influenza A (H7N3) virus in mice. *Virology.* 488:120-128.

Zhang et al. 1992. The hemagglutinin/esterase gene of human coronavirus strain OC43: Phylogenetic relationships to bovine and murine coronaviruses and influenza C virus. *Virology.* 186: 318–323.