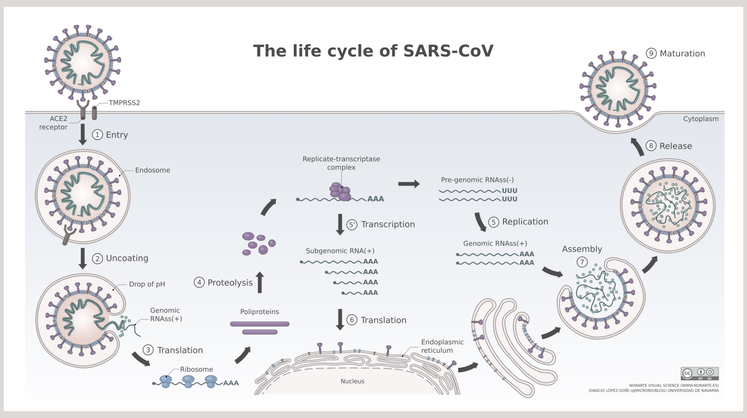
Missense variants in ACE2 affect SARS-CoV-2 Spike binding and could contribute to genetic risk in Covid-19

Stuart A. MacGowan[1], Michael I. Barton[2], Mikail Kutuzov[2], Omer Dushek[2], P. Anton van der Merwe[2], Geoffrey J. Barton[1].

1. Division of Computational Biology, School of Life Sciences, University of Dundee, Dundee, UK.
2. Sir William Dunn School of Pathology, University of Oxford, Oxford, UK.

Introduction

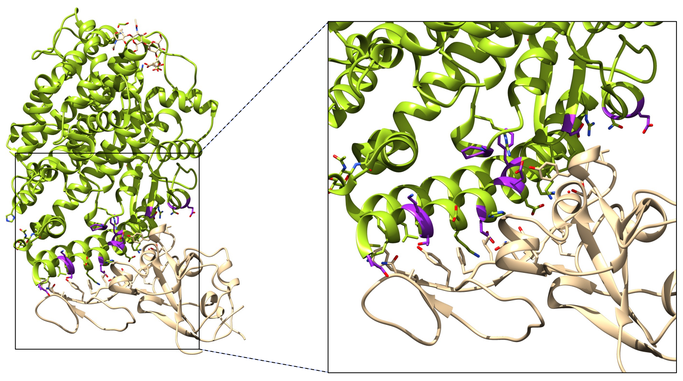
SARS-CoV-2 infection varies in severity from a mild illness to a fatal disease. As a key mediator of viral entry, ACE2 was one of the first candidate genes suspected to influence Covid-19 risk and susceptibility. The affinity of the virus Spike protein for host ACE2 influences the host-range of related coronaviruses and so we hypothesised that ACE2 alleles with altered binding could contribute to carriers' genetic risk. Over the last two years we have characterised ACE2 variants, initially with purely computational methods[1] and then by combining predictions with experimental data[2,3].



Vega Asensio - CC BY-SA 4.0, https://commons.wikimedia.org/

1. Population variants in ACE2

There are hundreds of rare coding variants in ACE2 reported in the gnomAD[4] database. Amongst the majority that are missense, some occur at the Spike binding site and are most likely to affect binding.



ACE2 (green) bound by SARS-CoV-2 Spike RBD. Key sites with missense variants in gnomAD are coloured magenta (PDB: 6vw1[5]).

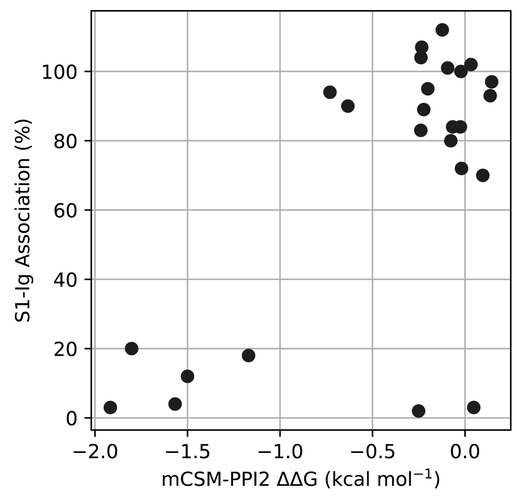
In April 2000, we predicted ΔΔG for all gnomAD variants and more with an algorithm called mCSM-PPI2[1,6] (A, below). Later, we measured the affinities of key variants with a set of accurate Surface Plasmon Resonance (SPR) experiments (C) and improved the predictions[2,3] (D). We found that there were ACE2 alleles with altered Spike binding.

ACE2 Variant	Dist.	(**A**) mCSM-PPI2 ΔΔG	(**B**) 2020 Insight	(**C**) SPR ΔΔG	(**D**) Refit ΔΔG	(**E**) 2022 Insight
S19P	2.6	−0.2	No effect	0.59	0.2	*High affinity*
K26R	6.0	0.0	No effect	0.26	0.4	*High affinity*
E37K	3.2	−1.2	*Resistance*	−1.33	−1.3	*Resistance*
G326E	5.5	1.0	*High affinity*	−0.65	*2.1	*Low affinity*
G352V	5.4	−1.1	*Resistance*	-	−1.2	*Resistance*
D355N	3.5	−1.3	*Resistance*	<−3.16	−1.5	*Near-total protection*

**Outside range of interpolation*

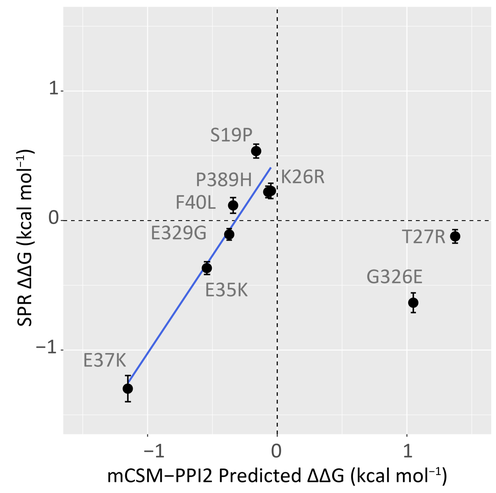
2. Three benchmarks

Before our SPR experiments, we tested mCSM-PPI2 with data on SARS-CoV. Low affinity predictions seemed reliable, but we saw hints that high affinity predictions were less so. This benchmark enabled our 2020 insights (see B, bottom left).



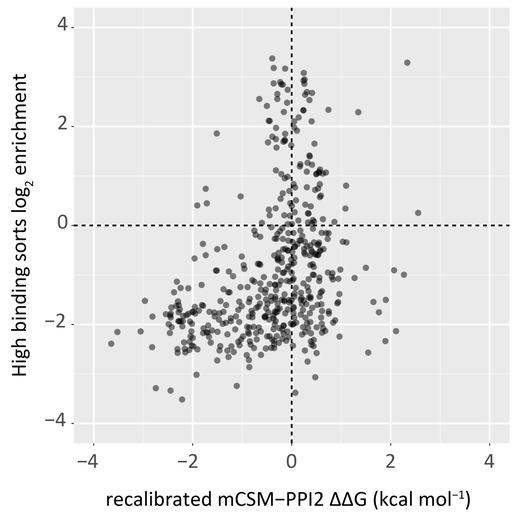
An excerpt of our first benchmark with SARS-CoV mutant data[1,7]

SPR experiments showed we were right about inhibitory alleles, but high affinity predictions were unreliable. Also, an offset masked the high affinities of two more common alleles. We recalibrated mCSM-PPI2 with the well-modelled variants (D, bottom left).



Experimental ΔΔG G from SPR vs. mCSM-PPI2 predicted ΔΔG

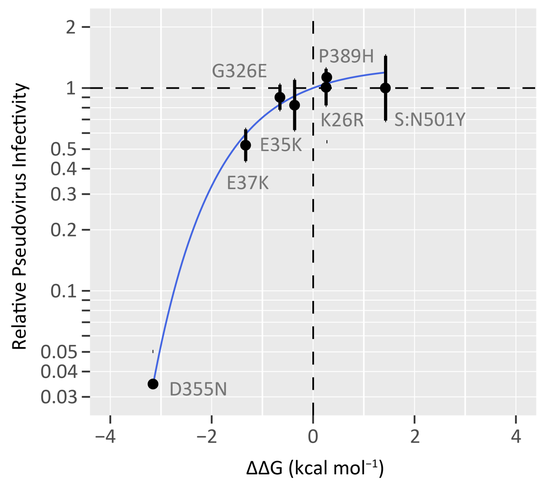
These conclusions were re-iterated by comparing predictions to binding data from a deep mutagenesis screen (DMS)[8]. This clarifies the effects of ACE2 mutations.



Binding data from deep mutagenesis[8] vs re-calibrated ΔΔG

3. Affinity vs. Infectivity

When we compared our affinity data to published pseudovirus infectivities[9], we found a stronger correlation with our SPR experiments than any other ACE2 mutant binding dataset. *This confirmed that inhibitory variants reduce infectivity*.



Relative pseudovirus infectivity[9] vs experimental SPR affinity

High affinity variants have normal infectivity here, but this doesn't rule out a biological effect. We argue that higher affinity could facilitate entry into cells with lower ACE2 surface abundance. This idea is consistent with work in other viruses[10] and the improved fitness of later SARS-CoV-2 strains[2].

This informed our 2022 insights (see E, bottom left)

What have we learned?

- There are rare ACE2 alleles that are likely to confer complete resistance to SARS-CoV-2
- Two relatively common alleles, S19P and K26R, enhance Spike binding, but their biological effect is less clear
- Predictions gave the first insights into the effects of ACE2 variants on Spike binding and now help to expand and interpret experimental data
- The next generation of affinity predictors should focus on improving affinity enhancing predictions

Acknowledgements

Thanks to Jim Procter, Johannes Pettmann, Anna Huhn, and the Dundee Research Computing team.

This work was funded by awards from the BBSRC (BB/J019364/1 and BB/R014752/1) and Wellcome Trust (101651/Z/13/Z and 207537/Z/17/Z828).

References

1. MacGowan and Barton. bioRxiv (2020)
2. Barton, MacGowan, Kutuzov, et al. Elife (2021)
3. MacGowan, Barton, Kutuzov, et al. PLoS CB (2022)
4. Karczewski, Francioli, Tiao, et al. Nature (2020)
5. Shang, Ye, Shi, et al. Nature (2020).
6. Rodrigues, Myung, Pires, et al. NAR (2019)
7. Li., Zhang, Sui, et al. EMBO J (2005)
8. Chan, Dorosky, Sharma, et al. Science (2020)
9. Shukla, Roelle, Suzart, et al. PLoS Pathog (2021)
10. Hasegawa, Hu, Nakamura, et al. J Virol (2007)