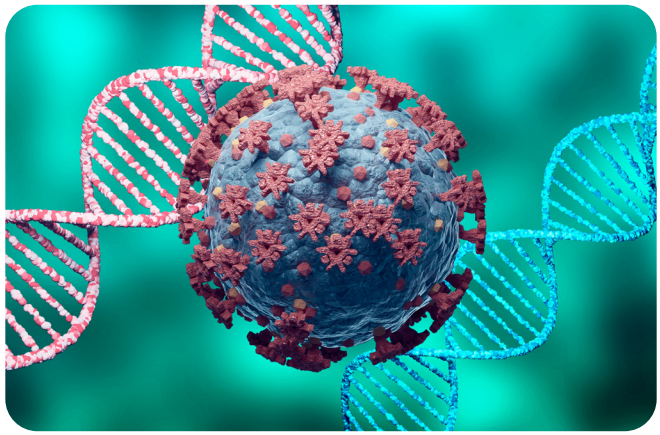
Learning from our ancestors: Can we be better prepared for the next pandemic?

Despite the vast array of epidemiological tools now available, the current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic swept across the globe at an unprecedented pace, emphasizing the vulnerability of human populations to novel viral pressures. Advances in genomic and proteomic technologies, combined with bioinformatics, have played a fundamental role in the global fight against COVID-19. For example, researchers have been able to investigate large numbers of genes and proteins in high throughput to answer fundamental questions relating to viral infection, host response, and more. These findings have not only facilitated the development of several vaccines against SARS-CoV-2, but also enabled the identification of protein-protein interactions that could be potentially targeted by antiviral drugs.

The SARS-CoV-2 pandemic is not the first coronavirus outbreak the world has experienced in the past 20 years – in 2002, SARS-CoV infected more than 8,000 people and killed over 800, and four years later MERS-CoV infected over 2,400 and killed more than 850 – and it likely won't be the last. However, it has opened a window of opportunity for countries to rethink the way they prepare for public health crises of the future.

In a recent study,[1] a team of international researchers used genomic data from modern human populations to better understand how humans have adapted to historical coronavirus outbreaks (Figure 1). "If we can identify ancient pandemics, then that knowledge could, and should, feed into our pandemic preparedness strategy in the future," explained Kirill Alexandrov, Professor of Synthetic Biology



at Queensland University of Technology in Australia and one of the study's lead authors. He is part of a team of researchers from the University of Arizona, the University of California San Francisco, and the University of Adelaide who collaborated on this project. "If we know what viruses have jumped previously, this can be a predictor of the future and so we can be more prepared in terms of diagnostics, therapeutics, and vaccines than we were with this pandemic." He added that genes with ancient viral histories might also aid researchers in their search for potential antiviral drugs.

Using evolutionary information to combat COVID-19

Modern human genomes contain evolutionary information tracing back tens of thousands of years, which could potentially provide key information about the viruses that have impacted our ancestors.

"All biological systems are constantly mutating. If these mutations are harmless, they are retained, and if they are harmful, they are eliminated by selection processes," explained Alexandrov. "However, when a population comes under the attack of a virus then those who, just by random chance, have mutations that are advantageous will survive better and pass these on to the next generation." Notably, throughout the evolutionary history of humans, this process of positive selection has frequently targeted virus-interacting proteins (VIPs), leading to the fixation of gene variants encoding VIPs at three times the rate observed for other classes of genes – a process called a selective sweep.[1]

With this in mind, the researchers examined genomic data from 26 human populations from five continental regions to identify possible signatures of adaptation to viruses, with a particular focus on VIPs. The team analyzed a total of 420 CoV-VIPs, including 332 identified through high-throughput mass spectrometry to interact with SARS-CoV-2. Interestingly, they observed a strong enrichment of sweep signals (selection that drives a beneficial variant to substantial frequencies in a population) across five East Asian populations, which was absent from the 21 non-East Asian populations that were tested. This suggests that an ancient coronavirus epidemic, or a virus interacting similarly with hosts, drove an adaptive response in ancestors of East Asians, according to the researchers.

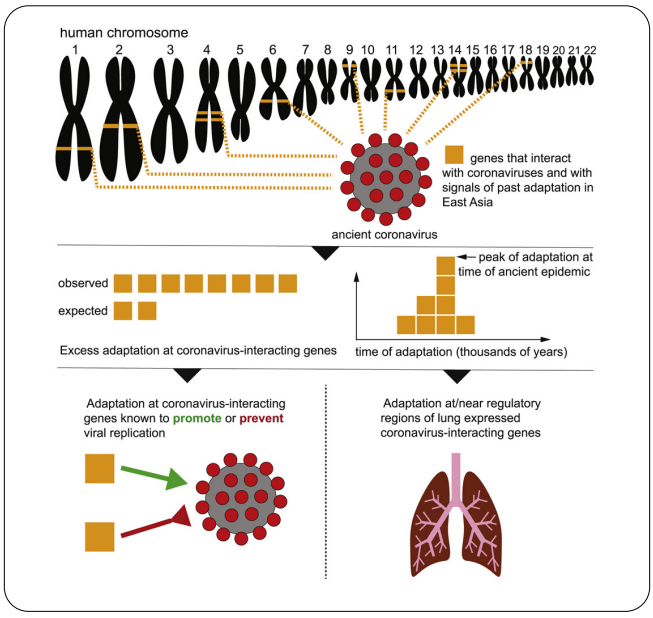


Figure 1: **Graphical abstract from 'An ancient viral epidemic involving host coronavirus interacting genes more than 20,000 years ago in East Asia'**. (Image credit: Souilmi Y, Lauterbur M, Tobler R, Huber C, Johar A, Moradi S et al.[1])

Using an Ancestral Recombination Graph (ARG)-based method, known as Relate, the team also identified a specific set of 42 CoV-VIPs that exhibited an adaptive response that they believe emerged around 900 generations (~25,000 years) ago. "It looks like there was an Asian pandemic caused by something that looks very similar to the modern SARS-CoV-2 coronavirus that left significant changes in multiple CoV-VIP genes," explained Alexandrov. Further analysis revealed that selection for these 42 CoV-VIPs was highest around 25,000 years ago, likely when the population was first infected, before gradually waning as the population adapted to the viral pressure, or the virus lost its ability to cause disease. Of note, selection leveled off in frequency around 5,000 years ago. "The significance of this is that it tells us something about how ancient pandemics occurred, how long they lasted, and what impact they had on the population," said Alexandrov. "It also paints a very concerning picture because if this virus was there for hundreds of years last time, even with our modern tools and technologies, it probably means that we aren't near the end of the current pandemic."

Validation of direct physical interactions

In the next stage of their analysis, the researchers sought to validate the direct interactions between the selected CoV-VIPs and SARS-CoV-2 using an AlphaLISA® Protein-Protein Interaction (PPI) assay (Figure 2), which had previously been used for rapid analysis of the intra-viral PPI network of Zika virus.[2] Initially, these interactions were identified by high-throughput mass spectrometry; however, the researchers note that high throughput mass spectrometry can sometimes identify indirect interactions in a larger protein complex or false positives altogether, so further validation was still required. Commenting on their findings, Alexandrov said: "We found that over 70% of those interactions could be validated experimentally in the lab with the *in vitro* synthesized proteins using the AlphaLISA platform. This is a very high validation rate which provides strong, but still circumstantial evidence that those proteins were involved in interaction with an ancient coronavirus."

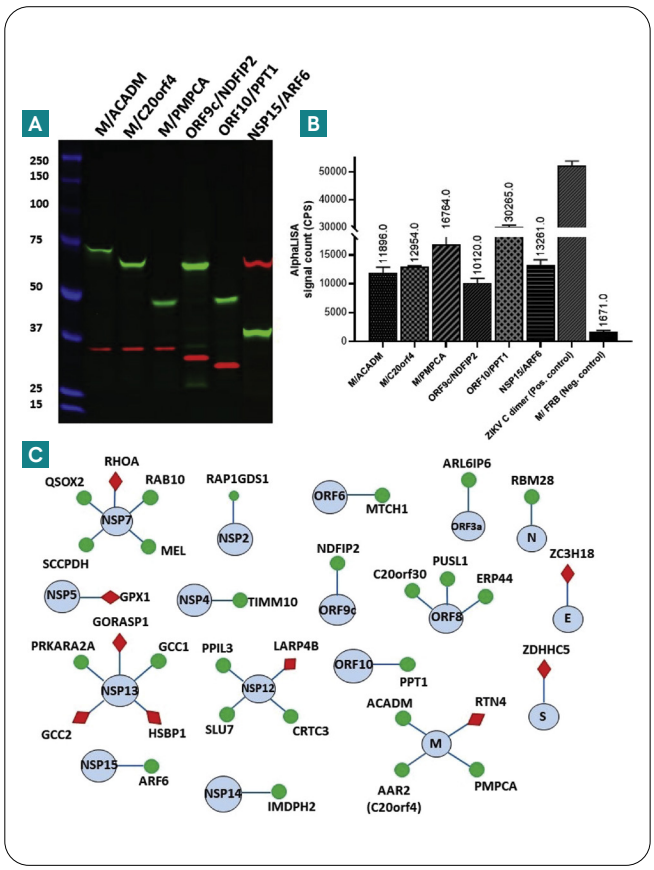


Figure 2: **Validation of selected CoV-VIPs/SARS-CoV-2 protein interactions using cell-free expressed proteins.** **(A)** A representative image of SDS-PAGE gel loaded with *in vitro* translation reactions co-expressing human VIPs/SARS-CoV-2 proteins in Leishmania tarentolae (LTE) system. Human proteins were tagged with EGFP at N terminus, and the viral proteins were tagged with mCherry at C terminus. The protein bands were visualized by fluorescence scanning; viral proteins: M,ORF9c, ORF10, and NSP5; human proteins: ACADM, C20orf4, PMPCA, NDFIP2, PPT1, and ARF6. **(B)** A plot of representative signals of AlphaLISA interaction assay for VIP/viral protein pairs shown in (A). Zika virus self-dimerizing C-protein tagged with Cherry and EGFP was used as positive interaction control. As the negative control, we used FKBP-rapamycin-binding (FRB) domain. **(C)** Graphic summary of the VIPs/SARS-CoV-2 interaction analysis: the confirmed interactions are shown with green circle, whereas interactions that could not be conformed using this assay are depicted as red diamond. (Image credit: Souilmi Y, Lauterbur M, Tobler R, Huber C, Johar A, Moradi S et al.[1])

To further clarify that an ancient viral epidemic caused the strong burst of selection observed, the team tested whether the 42 selected CoV-VIPs were enriched with antiviral or proviral effects relative to other CoV-VIPs. Analysis revealed that 50% of the selected CoV-VIPs had high-confidence for anti- or proviral effects compared to 29% for all 420 CoV-VIP, supporting their hypothesis that the selective pressure observed was likely due to a viral epidemic. The group also showed that the inferred underlying causal mutations were situated near to regulatory variants active in lungs and other tissues negatively affected by COVID-19, including blood and arteries, adipose tissue, and the digestive tract, indicating that the tissues impacted in the ancient pandemic in East Asia match those affected by SARS-CoV-2.

Overall, the study suggests that an ancient coronavirus, or a closely related pathogen, triggered an epidemic in East Asia around 25,000 years ago which involved host coronavirus interacting genes.

Utilizing evolutionary genetic information

Alexandrov notes that a limitation of their approach is that they can identify statistical associations but not causal links. “It is an interesting question as to whether we can analyze different populations, and in particular their genetic background, and see whether a population is better adapted to a pandemic. However, I think that societal factors and medical interventions probably play a much greater role – accessibility to healthcare, medicine, living conditions, densities, and society probably override genetic adaptations,” he said.

The team’s analysis did identify several candidate genes that might aid current efforts to find a treatment for patients with COVID-19. The authors note that four of these genes – *SMAD3, IMPH2, PPIB, and PRX1* – are targets of eleven drugs currently used or investigated in clinical trials to mitigate COVID-19 symptoms, while an additional five are targeted by multiple drugs to treat a variety of non-coronavirus diseases. “It remains to be established whether the genes identified in this study might help drug-repurposing efforts and provide a basis for future drug and therapeutic development,” said Alexandrov.

Collaborative approach

This study is a prime example of collaboration that is interdisciplinary, international, and multi-institutional, and notably conducted by a group of researchers who have never met faceto- face. “One of the remarkable and positive outcomes of the pandemic is that we have become better at working across national and institutional boundaries,” said Alexandrov. “The world got a lot flatter, and in this instance, two fields emerged that were not really talking to each other. We had a synthetic biology and

protein engineering background and were interested in analyzing large datasets of protein interactions, while other groups were interested in mining genomic data to see what happened with ancestors of individuals in the distant past. The pandemic created the question that only a combination of these skills and interests could have answered."

The next focus for Alexandrov and his collaborators will be focused around broad-spectrum antivirals and coronavirus evolution. "This work will be driven by our bioinformatics colleagues. We want to look at their interaction with the host and explore what makes coronaviruses cross the species barrier," he said. "We also want to look at protein-protein interaction networks in humans and bats to see how different it is and what has changed as viruses evolved."

Alexandrov concluded that an important offshoot of their research is the ability to identify viruses that have caused epidemics in the distant past and may do so in the future. "This, in principle, enables us to compile a list of potentially dangerous viruses and then develop diagnostics, vaccines, and drugs for the event of their return," he said.

References

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2. Varasteh Moradi, S., Gagoski, D., Mureev, S., Walden, P., McMahon, K.A., Parton, R.G., Johnston, W.A., and Alexandrov, K. (2020). Mapping interactions among cell-free expressed Zika virus proteins. *J. Proteome Res.* 19, 1522–1532.

Professor Kirill Alexandrov

Professor Kirill Alexandrov obtained his master's degree in Invertebrate Zoology at the Leningrad State University, Russia in 1989 and completed his PhD in Cell Biology at EMBL Heidelberg, Germany in 1995. He continued on to postgraduate work at the Department of Physical Biochemistry at the Max- Planck Institute in Dortmund, Germany, and remained with the Institute for 13 years, becoming a group leader in 1999. He joined the Institute for Molecular Bioscience and the Australian Institute for Bioengineering and Biotechnology of the University of Queensland, Australia in 2008 as an Australian Research Council Future Fellow. He co-founded the German biotechnology company JenaBioscience GmbH in 1998 and the UK/ Australian Synthetic Biology company Molecular Warehouse Ltd in 2015. In 2018 he joined Queensland University of Technology as a CSIROQUT Inaugural Professor of Synthetic Biology. His group is interested in protein engineering of artificial sensing and signal transduction, two-way connectivity between biology and electronics, as well as diagnostics.

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