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Peer Review

Review of: "The Contentious Origins of SARS-CoV-2: A Comprehensive Review of Current Knowledge"

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This paper is the latest in a series of reviews by R Domingo on this topic, dating back to 2020 and periodically updated since. It is clearly and concisely written and makes a cogent but not exhaustive attempt to summarize the state of the art concerning the origin of SARS-CoV-2. It has the overwhelming advantage of being brief, fair, optimistic, and forward-looking. Figure 3 is a very valuable resource for those who wish for a quick introduction to this topic, by subtopic, in reference to a short list of especially significant papers. Most reviews are encyclopedic, with the reference list being the most valuable part. This approach is refreshing. The review benefits from the author being largely an observer, rather than a direct participant in the ongoing back-and-forth war of words on this issue.

The paper has a finality about it, ending with several excellent recommendations. In the current geopolitical atmosphere, it is very unlikely they will come to pass.

I have a number of comments that are intended not so much as critique but to provide context and draw some omissions to the author's attention.

1. The review focuses on the published literature, with major papers in this area not formally published until late July of 2020. The two camps were well established by then. It misses the intense drama of early 2020 when scientists were communicating frequently, globally, and openly by phone, email, and informal blog posts. The virology blog moderated by Andrew Rambaut of Edinburgh was particularly important in the sharing of information in real time (1). The review misses this element of incredibly rapid pace and complete openness of that time. It is not so much a fault of the review as it is a missing link to how the origin controversy evolved.

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2. “Contentious” is a polite description of the passions stirred, the words written, and the unfortunate outright threats and harassment aimed at those defending the natural origin as a matter of fact and principle.

3. Full disclosure: I have been firmly grounded in the natural origin hypothesis since Feb 6, 2020. However, for the two weeks prior to that, I too was greatly fearful that the hand of man might be in it when I first viewed the protein sequence of the S protein on GenBank. As a longtime expert in viral Class I entry proteins, the RRAR motif immediately caught my attention. I feared that someone had inserted a furin cleavage site.

It was also reported that Shi Zhengli of the Wuhan Institute of Virology (WIV) spent long hours reassuring herself, and probably also her superiors in Beijing, that SARS-CoV-2 was not in her collection and something she had somehow overlooked. So the prospect of an engineered virus or lab leak was paramount as our first response to the novel sequence not found in any Sarbecovirus with which we were familiar.

Shi discarded the lab leak theory when she found only the related bat coronavirus RaTG13, collected in 2013, as closest to SARS-CoV-2. She made this sequence available on June 24, 2020. Instead of quieting the storm, it exacerbated it, since RaTG13 contained a run of 509 identical amino acids flanking either side of the PRAA insert. A US Senator actually suggested in public that a missile be used to “take out” the WIV.

With great urgency, I closely examined the nucleotide insert and a genomic alignment of RaTG13 and SARS-CoV-2. I found that the latter could not, in fact, be created from the former because there were too many synonymous wobble-base mutations different between the two. Also, the insert itself showed no evidence of human logic. I posted my analysis at midnight on Feb 6 as strongly disfavoring a lab origin of SARS-CoV-2 (2,3). I suspect others were doing so privately. The Holmes et al “Proximal Origin” paper from a global coalition comparing protein sequences I knew to be in process. It was posted on February 17 (4,5). With our analyses as background, Dr. Fauci and others closer to decision-makers were instrumental in preventing an outbreak of armed conflict with China.

Whether one remained rooted in the “lab leak hypothesis” or transitioned to the “natural origin hypothesis” depends on really one choice: to have the history of pandemic viruses (see comment 7 below) uppermost in your mind, or to focus on the virological and epidemiological data that continues to emerge concerning the initial spread of the virus within China.

4. The questions were, in my mind, actually settled by the data quite early, in a sequence of blog posts and public preprints through March of 2020, as well as a World Health Organization (WHO) report of Feb 24 that included work on the ground in China (6). Data drives science. What followed was politics with regard to an epidemic, to a degree I have not seen since AIDS was thought to be confined to the 4 H's – hemophiliacs, Haitians, homosexuals, and heroin addicts. Villification of the "other." Wrong then, wrong now. Our only enemy is the virus.

Our conclusions must be fact-driven. But we must remain open to any verifiable data that can change our conclusions at any time. Domingo is correct when he says that the pathway to the Huanan Market and what brought it there remains unknown. One fact can upend our conclusions. We must always remain humble before the reality that we do not know everything.

5. Another fact that is missing here is the Dec 2024 release, at long last, of an additional 56 sequences from the WIV collection by Dr. Shi (7). Her contention that they do not contain any sequences closer to SARS-CoV-2 than those already known has been confirmed by the Edinburgh group. I have yet to examine them directly.

6. Domingo asserts that the donor sequence for the furin site has not been found. This is not true. On May 2, 2020, I posted on virological.org and subsequently published, finding an alignment of a run of 10 out of 12 nucleotides in the insert, in a bat coronavirus S protein of strain HKU-9, isolated in 2006 (3). Since it represents a transposition of sequence from downstream in the S protein, out of frame, and codes not for PRRA but TSAG in HKU9, it has been disregarded as "improbable." But the alignment is unquestionable.

We have already seen SARS-CoV-2, while still circulating in China, transpose a non-coding splice acceptor RNA to insert a new coding splice acceptor hundreds of nucleotides to the end of the SR-rich region and in advance of the aggregation region of NP, creating a new open reading frame (8). Coronaviruses are unable to read our rulebook about what they can and cannot do with their genome. Mixed infection and recombination are both prolific and promiscuous (9), with natural selection the only arbiter of success or failure.

7. There is no mention that the furin site in SARS-CoV-2 is a true example of "molecular mimicry", in that the peptide sequence RRARSVASS duplicates exactly a furin cleavage site found in a human ion channel protein ENaC (10). The exact mimicry assures that the S protein is cleaved with an efficiency equal to that of an essential human protein. The peptide sequence is highly conserved in a variety of mammals, from rats to humans, a conservation that extends back tens of millions of years to as far as 160 million years.

As such, it could have an incredible effect on the effective host range of the recombinant SARS-CoV-2, greatly facilitating its passage from southern Asia into the Huanan market, potentially infecting more than one animal species there.

This point has been buried because it became tangled up in the origin war, when Harrison and Sachs (11) linked it to a strong insinuation that Shi and her US collaborator Charles Baric “copied” the human ENaC gene and inserted it into the precursor of SARS-CoV-2 in a “gain of function” experiment sometime in 2018-2019.

In his testimony before the US Congress in June of 2024 (12), Dr. Robert Garry, a highly visible proponent of the natural origin hypothesis, stated that the insinuation of Harrison and Sachs was “unsound”. The alignment of the nucleotide sequence of human ENaC from chromosome 12 with the SARS-CoV-2 insert is shown below, as I had provided it to Dr. Garry in 2022.

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HS ENAC CCGCCTCACG/GGGCCCGTCGAG\CCCGTACCGTGTCCAGCTTG SARSCoV2 cagactaatt/*ctcctcggcggg*\cacgtagtgtagctagtcaa

There is no way one can copy the ENaC sequence to create the 12 nucleotide insert of the virus. Quite simply, carried away with their zeal, Harrison and Sachs forgot to check the alignment of the DNA sequence. Case closed.

However, the concept of “molecular mimicry” as applied to the host range of SARS-CoV-2 is quite valid. The serendipitous and accidental molecular mimicry of a conserved mammalian furin cleavage site would have powerful consequences in spillover from bats to mammals. It should not be a casualty of the war of words.

8. To be fair, no account of the origin question is complete without noting that the “lab origin hypothesis” is firmly anchored in the history of Chinese secrecy concerning severe epidemics within their borders, which has several times either started pandemics or left those outside China unprepared to deal with them. In halting pandemics, days matter, days often lost due to failure to be transparent. Often, critical information was not released for years or had to be learned through intelligence services.

In 1957, H2N2 influenza began in Guizhou province and heavily involved China before being reported from Singapore in mid-February of 1957. Even then, the extent was not fully reported. A small outbreak hit the US in June, giving false promise of little effect. In the week of 12 October, it slammed into US children after their return to school. Only 9-day shutdowns of schools and workplaces quelled the spread.

I was 12 at the time. There was no word from China to prepare us for what transpired through March. A similar history is found for the 1968 Hong Kong flu, with news of substantial antecedent flu epidemics in the Chinese interior well before spillover into Hong Kong.

The case of the so-called 1977 “Russian flu” was the worst. The outbreak actually began in a cluster of towns in Northwest China, before spreading to Russia and the rest of the world. The virus itself was identical to a 1950 genotype of influenza, and the only way that kind of stability could be achieved was in a lab freezer. It is widely assumed that the Chinese had thawed out a 1950 sample, attempted to make a vaccine, and started a pandemic instead (13). Notably, administrations of both parties have been made familiar with these facts and done nothing about them. This virus would become the seasonal flu from 1977 through 2008. All of the H1N1 influenza deaths during that period constitute cases of negligent homicide. There is some angst to bring China to task.

The 2009 pandemic flu that still circulates emerged in Perote, Mexico, and was relatively avirulent, with a death in only 1 of 10,000 cases. While it is considered to have arisen as a central Mexican recombinant of Asian, North American, and European ancestors (14), two gene segments were new to Mexico in the pandemic strain. One of these, PB1, encodes a regulatory protein PB1 frame 2 (PB1f2). In the pandemic strain, PB1f2 has a very unusual genotype that contains three STOP codons in place of the Trp wild-type (15), inactivating the protein. The only places that Triple STOP an artificial construct are in Peter Palese’s lab with the purpose to attenuate the viral pathology (16), demonstrated in mice. The intent was to develop means of creating a vaccine. The mild pathogenesis of the 2009 flu may have been due to the defective PB1f2, consistent with it being an accidental release of a vaccine attempt in imitation of the Palese protocol, as in 1977.

Mexico was not even aware at the time of the genotype of Mexican influenza strains in swine. But the Chinese were active in 2009 in Mexico, acquiring massive pig farms in Mexico, including Carroll Farms in Perote, Mexico, as part of acquiring the Smithfield conglomerate in the US. They discontinued the acquisition in the wake of the 2009 pandemic but later acquired Carroll Farms and Smithfield as wholly owned subsidiaries of a massive global Chinese agribusiness in 2014.

It would be in the interests of the Chinese to develop a vaccine that would protect the production of nearly a million swine a year from Carroll Farms after the acquisition.

In other words, where there is smoke, there is fire, and this remains a solid premise when investigating outbreaks of severe respiratory disease emanating from China.

The “lab leak hypothesis” is fueled in part by some not being able to clear the smell of smoke from pandemics past from their senses. For that, I cannot blame them.

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Note

This discourse is dedicated to the memory of my former wife, Betty Jean Burton Grier Gallaher, RN, who died from COVID acquired in treating hospital patients in late 2020. Like many others, she knew the danger but went in anyway and gave her life in service to humankind.

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Declarations

Potential competing interests: No potential competing interests to declare.