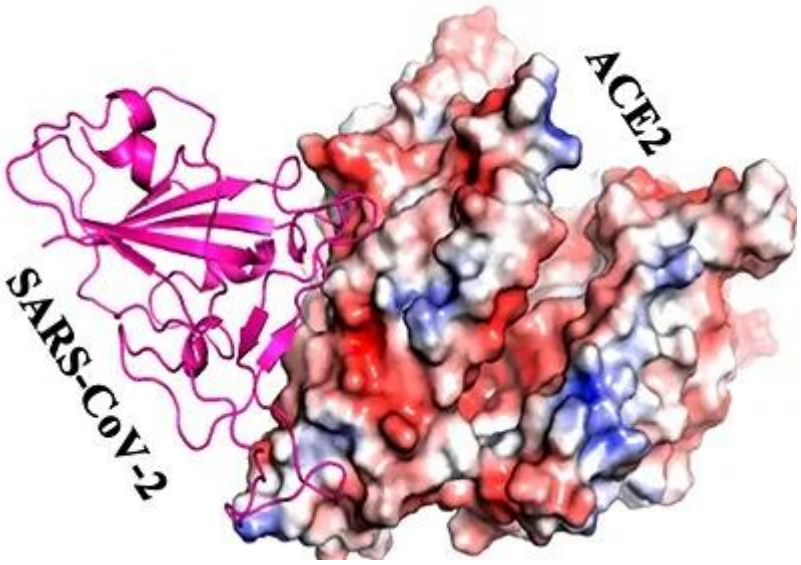
Prions are infectious proteins that can switch from non-aggregated state to self-templating highly ordered aggregates. They can self-propagate, leading to the misfolding of proteins. Aberrant protein folding underlies many neurodegenerative diseases. The authors from the United States performed an *in silico* study, using a prion-like amino acid composition (PLAAC) analysis, to investigate the presence of prion-like domains in the SARS-CoV-2 spike (S) protein and major differences in the prion-like domains of the S protein between emerging variants, including Omicron.

Similar to other beta-coronaviruses (β-CoVs), the genome of the SARS-CoV-2 virus encodes structural proteins required for the formation of infectious virions, including the spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins. The surface-located S protein plays a critical role in infection by mediating viral attachment to host cell-surface receptors and facilitating viral entry. The S protein consists of two large regions, the N-terminal S1 and C-terminal S2. S1 is composed of the N-terminal domain (NTD), the receptor binding domain (RBD) with a receptor binding motif (RBM), and two C-terminal domains, and has higher sequence variability than S2. The RBD in the S1 subunit is responsible for attachment to host cells. The membrane-embedded S2 region that is responsible for fusion, is more highly conserved compared to S1.

The RBD in the S1 subunit allows the virus to bind directly to the host ACE2 complex. ACE2 is the common receptor for SARS-CoV-2 and SARS-CoV, but SARS-CoV-2 has a higher binding affinity for ACE2 than SARS-CoV. In their previous study, the same research group identified, for the first time, prion-like domains within viral surface proteins involved in receptor binding and fusion with the host cell. Importantly, they discovered that prion-like domains enhance viral binding to the ACE2 receptor.



In 1982 Stanley Prusiner isolated a suspected infectious agent, a protein that he called a prion. He identified the gene behind the prion protein and determined its presence in healthy people and animals. Stanley Prusiner showed that the prion molecules are folded differently than the normal proteins and that the folding of the prion can be transferred to normal proteins.

In common nomenclature, the naturally folded form of the prion protein is referred to as PrPC, while the misfolded form is referred to as PrPSc (for “scrapie”).

Prions and prion-like regions in certain proteins may drive protein misfolding, associated with numerous neurodegenerative diseases, including Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, amyotrophic lateral sclerosis, and frontotemporal dementia. In addition, the prion-like domains can aggregate with heparin-binding proteins, amyloids, a-synuclein, tau, and other prion proteins.

The human prion diseases that arise from protein misfolding, are caused by the accumulation and aggregation of a misfolded “scrapie” isoform (PrPSc) of the native cellular prion protein (PrPc), whereby external PrPSc acts as an infectious agent to facilitate the misfolding of the same protein expressed in neurons. These diseases are characterized by transmissibility and progressive neurological impairments. Creutzfeldt-Jakob disease is the primary human prion disease, and it is always fatal. Fatal familial insomnia is a rare fatal

genetic disease caused by certain mutations in the prion protein.

In 2020, Tavassoly O et al. suggested that peptides derived from the S protein might cross-seed existing amyloidogenic human proteins to accelerate fibril formation and trigger neurodegeneration. (ACS Chem Neurosci. 2020, 11:3704-3706)
https://pubs.acs.org/doi/10.1021/acschemneuro.0c00676

About the study

One of the numerous applications of a statistical tool called Hidden Markov Modeling (HMM) involves evaluating compositional similarities between proteins and prion sequences. A prion-like amino-acid composition (PLAAC) analysis uses HMM technology to identify candidate prionogenic domains in proteins of viruses, prokaryotes, and eukaryotes. The log-likelihood ratio (LLR) generated by PLAAC estimates the likelihood that an investigated protein is prionogenic. (Cureus 15(2): e34872)
https://www.cureus.com/articles/129846-a-potential-role-of-the-spike-protein-in-neurodegenerative-diseases-a-narrative-review#!/

In the present study, researchers used PLAAC analysis to perform the first detailed evaluation of the prion-like domains in the S protein of SARS-CoV-2. They also investigated substantial differences in the prion-like domains of the S1 subunit between emerging SARS-CoV-2 variants and other human-pathogenic β-CoVs. The researchers pointed out that a high threshold of the PLAAC score was used for protein identification, and only proteins with a high probability of prionogenic properties were included in the analysis.

Results

The findings revealed prion-like domains in the S proteins of all β-CoVs analyzed. A more precise mapping of prion-like domains revealed a striking difference in their localization.

Importantly, the SARS-CoV-2 was the only virus with prion-like domains identified within the RBD of its S protein. The scientists hypothesized that the presence of prion-like domains in the RBD of the SARS-CoV-2 might explain why SARS-CoV-2 binds to ACE2 tighter than SARS-CoV, even though both viruses share the same host-cell receptor. The same researchers previously demonstrated that prion-like domains in the RBD of SARS-CoV-2 enhanced viral binding to ACE2 compared to SARS-CoV which lacks prion-like domains in its RBD structure. It suggests that prion-like domains are particularly associated with viral

adhesion and entry. However, their absence in the RBD of other β-CoVs shows that prion-like domains are beneficial, but not necessary, for receptor-mediated virion attachment to the host cell.

Further analysis showed that SARS-CoV-2 contains five substituted amino acids in the RBD compared to SARS-CoV: S460→Q474, T488→N481, N480→Q493, Y485→Q498, and T488→N501.

The subsequent analysis of the ACE2 protein revealed prion-like domains within the α1 helix of the ACE2 and a pattern in which five of the seven amino acids localized within prion-like domains of the viral RBD interact with the host cell ACE2. These specific amino acids (Q474, N481, Q493, Q498, and N501) enable the prionogenity of the SARS-CoV-2 RBD that directly interacts with ACE2.

The analysis of prion-like domains of the S1 subunit in the SARS-CoV-2 variants revealed a higher score for prionogenesis in the Delta variant than in the original Wuhan strain, whereas Omicron had a substantially lower score. Higher LLR scores indicate a greater possibility that the analyzed protein is a prion. Compared to the LLR score of the Wuhan strain, only the Delta variant has an elevated LLR score for the S protein (LLR was 6.025). Delta variant is known for its highest transmissibility, high mortality, and high risk of hospitalization. The Omicron LLR was the lowest among all SARS-CoV-2 variants (3.080). These findings are consistent with results from a recent article by Perez J-C, Moret-Chalmin C, and Luc Montagnier, a 2008 Nobel Prize winner, who discovered that the prion region was not present in the Bat RaTG13 which has sometimes been touted as the source of the Wuhan virus and that the prion region has completely disappeared in the Omicron variant. Since the prion region in the S protein of SARS-CoV-2 has a higher mutation density than the rest of the S protein, the authors found the complete disappearance of the prion region from the Omicron variant very strange. They raised the question of the origin of this prion region as “natural” or “chimeric” (man-made). Perez J-C, Moret-Chalmin C, Luc Montagnier. Emergence of a New Creutzfeldt-Jakob Disease: 26 Cases of the Human Version of Mad-Cow Disease, Days After a COVID-19 Injection. International Journal of Vaccine Theory, Practice, and Research 2023; 3(1), 727-770. https://doi.org/10.56098/ijvtpr.v3i1.66

Importantly, the prion-like domains, amyloid peptide-binding, and other domains in the SARS-CoV-2 S1 subunit of the spike proteins may play a role in systemic amyloidogenesis, which in turn supports systemic inflammation, CNS inflammation, neurodegeneration, neural cell atrophy and/or death. Recently, several case studies reported that SARSCoV-2 infection was associated with a new-onset or exacerbation of prion Creutzfeldt-Jakob

disease. Zhao Y, Jaber VR and Lukiw WJ (2022) SARS-CoV-2, long COVID, prion disease and neurodegeneration. Front. Neurosci. 16:1002770.
https://doi.org/10.3389/fnins.2022.1002770

In the abovementioned article, Perez J-C, Moret-Chalmin C, and Luc Montagnier presented 26 cases of Creuzfeldt-Jacob disease diagnosed in 2021 within on average 12 days after the Pfizer, Moderna, or AstraZeneca COVID-19 mRNA vaccination. Based on the progression and etiopathogenesis of these atypical and new cases of human prion disease, scientists concluded that this appears to be a completely new, rapidly evolving form of Creuzfeldt-Jacob disease.

Conclusion

This study has shown that different β-CoVs contain prion-like domains in the S proteins and that SARS-CoV-2 was the only β-CoV with prion-like domains identified within the receptor-binding domain of its S protein. Five of the seven amino acids localized within prion-like domains of the viral RBD interact with the host cell ACE2.

The authors concluded that further analyses of prion-like domains in SARS-CoV-2 spike protein may improve the understanding of COVID-19 and its pathophysiology, as well as the possible targets for developing therapies, such as antiprion compounds.

The article was published in Microorganisms.

Journal Reference

Tetz, G.; Tetz, V. Prion-like Domains in Spike Protein of SARS-CoV-2 Differ across Its Variants and Enable Changes in Affinity to ACE2. Microorganisms 2022, 10, 280. (Open Access). https://doi.org/10.3390/microorganisms10020280


UNLIMITED DOWNLOADS
VectorStock
ROYALTY FREE VECTOR GRAPHICS