Shared Autoantibody Signature Is Not Identified in CSF During Long COVID

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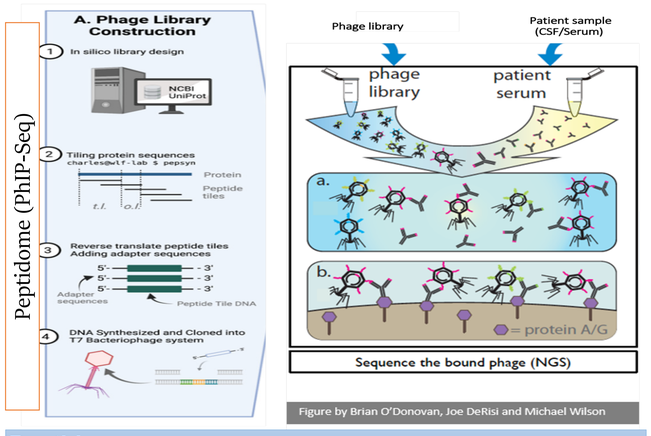
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BACKGROUND

A subset of people who recover from acute COVID-19 develop a range of emergent or persistent symptoms lasting months to years, collectively termed Long COVID (LC). We investigated whether autoimmune antibodies in the CNS associate with neurologic Long COVID symptoms (n-LC).

METHODS

Participants with LC (WHO criteria) and cognitive symptoms or headache enrolled in The COVID Mind Study (n-LC, n=39). Controls (n=25) were healthy pre-pandemic participants (n=22) or post-COVID without any LC symptoms (n=3). Cerebrospinal fluid (CSF) was collected for research. Mouse brain sections were immunostained with CSF from n-LC and controls at 1:4 dilution and visualized with anti-human IgG to screen for anti-neural autoantibodies. CSF IgG was incubated with a human proteome-wide phage display library (PhIP-Seq; Donovan et al., Brain Communication 2020) to identify candidate antibody targets enriched in n-LC compared to controls (enrichment threshold of sum rpk>100, fold change>10, z-score>3), with the minimum patient cutoff of two, to identify enriched peptides in the neuro-PASC patients compared to controls. We orthogonally validated candidate autoantigen by split-luciferase-binding assay (SLBA) and HEK293T cell overexpression.



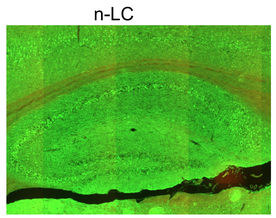
Peptidome:

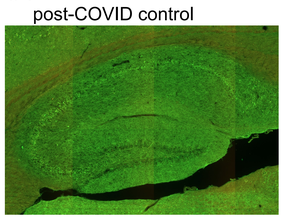
- Human proteome-wide coverage (including alternative splice forms)
- with 49 amino acid (AA) peptide length and 24AA resolution

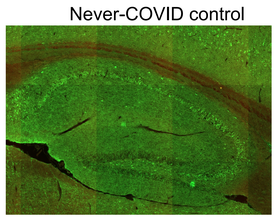
RESULTS

1. Mouse Anatomical Brain staining showed no differences in staining patterns between n-LC, healthy pre-pandemic controls , and post-COVID controls without any LC symptoms

14/39 n-LC samples showed positive staining on mouse brain sections, with moderate to strong cytoplasmic and nuclear staining, specifically in the thalamus, cortex, hippocampus, and brainstem. 15/24 never-COVID and 3/3 post-COVID controls displayed similar cytonuclear staining patterns in mostly all brain regions. The figure depicts the hippocampal region of mouse brain sections stained with control and n-LC CSF, all exhibiting similar staining patterns.







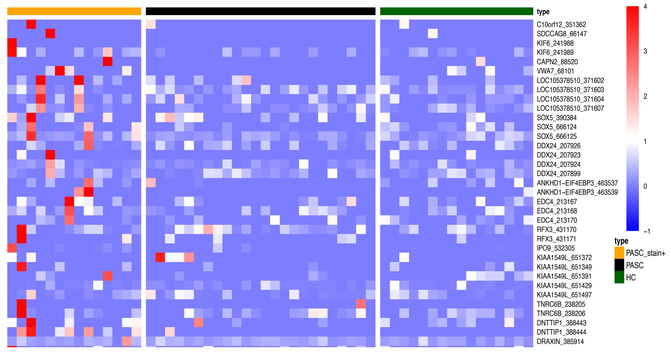
2. PhIP-seq comparison between positively stained n-LC cases vs controls yielded neuronal peptides as top hits

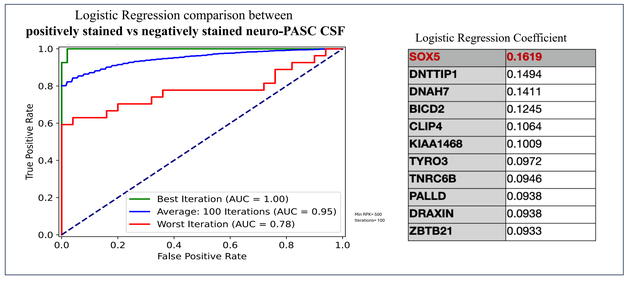
Analysis identified human peptides in n-LC CSFs with positive staining in mouse brain sections, compared to the negatively stained n-LCs and controls leading to the selection of SOX5 for further investigation due to its role in immune cells, memory B and TH17 cell differentiation, neuronal signaling, and enrichment in neuro-PASC cases.

3. Logistic regression analysis showed SOX5 as the most discriminatory peptide between Neuro-PASC samples that did or did not have mouse brain immunostaining with 100 iterations, and area under the curve of 0.95.

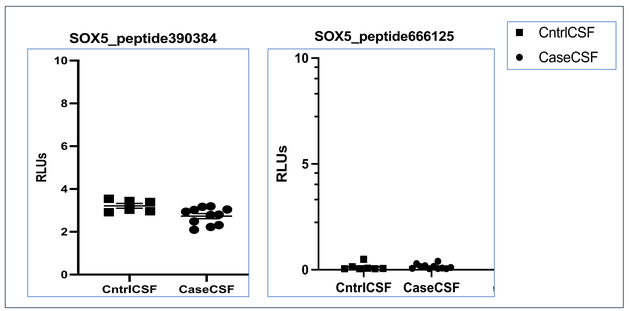
4. Orthogonal validation of the PhIP-seq hit Sox5 via the Split-Luciferase binding assay

Peptide tiling across SOX5 was inserted into a luciferase construct with a HiBiT tag. Translated peptides were measured for relative luciferase units, then incubated with neuro-PASC and control CSFs, followed by immunoprecipitation. However, the orthogonal validation of SOX5 IgG with SLBA did not yield any differences between neuro-PASC and control CSFs.

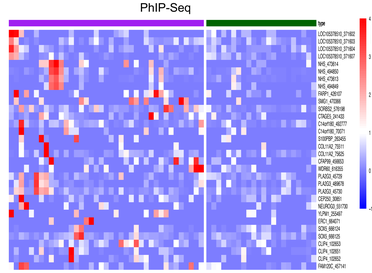




SOX5	0.1619
DNTTIP1	0.1494
DNAH7	0.1411
BICD2	0.1245
CLIP4	0.1064
KIAA1468	0.1009
TYRO3	0.0972
TNRC6B	0.0946
PALLD	0.0938
DRAXIN	0.0938
ZBTB21	0.0933



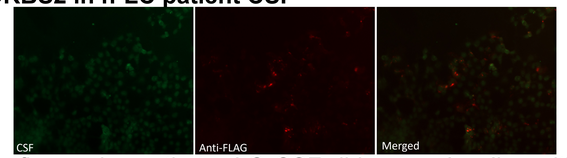
5. PhIP-seq and Logistic Regression comparison between all n-LC cases vs all never- COVID pre-pandemic controls



Logistic Regression

C14orf180	0.0925732279379750
SORBS2	**0.0919952639530330**
LOC105369276	0.0913657588294490
DDX17	0.0886052680157506 0
CD163L1	0.0865721813070614 0
UBE2E1	0.0807525834982180
SPATA31C1	0.0795897411459950
PPP1R13B	0.0786704852877743 0
C16orf86	0.0772623269260448 0
LMOD1	0.0764679455857929 0
TOX3	0.0748675459838561 0
POGK	0.0740726863849832 0
GPSM1	0.0730866934883586 0
QTRT1	0.0702799456744624 0
NEURL1B	0.0701190199866757
SOX5	0.0691337817623578 0

6. HEK293T overexpression assay did not validate SORBS2 in n-LC patient CSF



The figure shows that n-LC CSF did not co-localize with anti-FLAG SORBS2 in HEK293T cells overexpression assay.

CONCLUSIONS

- **Whole human proteome antigen discovery with CSF from individuals with and without n-LC did not identify a shared autoantigen in n-LC post-orthogonal validations.**
- Our current observations suggest that autoantibody signatures are against multiple cytoskeletal proteins, cell adhesion molecules, and ubiquitinases.
- There was no difference in the frequency and pattern of immunostaining on rodent brain slices between n-LC and controls.
- ***Our preliminary work did not identify a unified autoantibody signature in the CSF of patients with n-LC.***

ADDITIONAL KEY INFORMATION

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