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FYN, SARS-CoV-2, and IFITM3 in the neurobiology of Alzheimer's disease: a regulatory feedback loop governing Tau and Aβ pathology

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Introduction

The Interferon-induced transmembrane protein 3 (IFITM3) belongs to a family of proteins that act as a second line of defense against enveloped viruses, including SARS-CoV-2. Athough IFTM3 is known to intercept and shuttle viral particles to the lysosomes[1], Hur et al[2] uncovered a novel role for IFITM3 as a γ-secretase modulator that promotes Aβ production[2], (i.e as a direct consequence of an innate immune response). Considering the accumulating evidence on common pathways between COVID-19 and Alzheimer's disease (AD)[3], we aimed to examine their currently unexplored converge on IFITM3. Bulk and single cell RNA expression studies of AD patients, as well as COVID-19 infectomics converge on IFITM3 and FYN, its regulating kinase, as differentially expressed genes common between AD and COVID-19. Expanding on Hur et al's findings[2] and our previous research on IFITM3 networks in AD[4], we propose a model where IFITM3 decoupling from FYN regulation can account for Aβ oligomerization and Tau fibrilization, while concomitantly abrogates autophagy, as a model of AD pathogenesis.

The purpose of this study was to validate IFITM3's potential role in AD by meta-analyzing gene expression data from both bulk tissue and single cell RNA sequencing studies, aside from those previously examined. Subsequently, we aimed to determine the overlap between IFITM3's biological networks and SARS-CoV-2 infectomics.

Methods

1. **Bulk RNA-seq data: The Accelerating Medicines Partnership Alzheimer's Disease Project (AMP-AD) consensus datasets**

We inquired the publicly available Alzheimer's disease consensus datasets (accessible via: http://swaruplab.bio.uci.edu:3838/bulkRNA/) for IFITM3 expression data[5] (See Supplementary Materials 1 – Expanded Methods for further details).

2. **Single cell RNA transcriptomics**

For single-cell expression studies, the scREAD database (Available from: https://bmbls.bmi.osumc.edu/scread/) was interrogated, to further characterize IFITM3' and FYN's expression in AD-donated tissue[6] (See Supplementary Materials 1 – Expanded Methods for further details).

3. **Confirmatory gene set enrichment analyses (GSEA)**

We also performed confirmatory GSEA on differential gene expression data available from Morabito et al[5] to detect IFITM3 in COVID-19 related datasets and viral infection induced gene signatures. GSEA was performed via the Enrichr platform[7] (Available from: https://maayanlab.cloud/Enrichr/) on the available COVID-19 datasets. For all analyses, adjusted p-values <0.05 were considered statistically significant.

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4. Ethical approval

Dataset used in this study were anonymized; The specific protocol for informed consent and institutional review board approval can be found in detail elsewhere[5,6].

Results

Analysis of bulk RNA data revealed that IFITM3 was differentially expressed in two regions in the discovery datasets: the temporal cortex (Mayo Clinic Study, AD vs. Controls, adj. p-value=1.3e^{-6}) and the parahippocampal cgyrus (MSSM study, AD vs. controls, adj. p-value=0.012); (Fig.1). In the validation datasets, IFITM3 was differentially expressed in Zhang et al. study[8] (AD vs. Controls, adj. p-value<2.2e^{-6}; Fig. 2). FYN was differentially expressed in the temporal cortex (Mayo Clinic Study, AD vs. Controls, adj. p-value=2.9e^{-5}), the prefrontal cortex (ROSMAP Study, AD vs. Controls, adj. p-value=0.004) and the (MSSM study, AD vs. controls, adj. p-value=2.9e^{-5}); Fig. 3. In the validation datasets, validation datasets, FYN was differentially expressed in two datasets, including Zhang et al's study[8] (AD vs. Controls, adj. p-value<2.2e^{-16}; Fig 4).

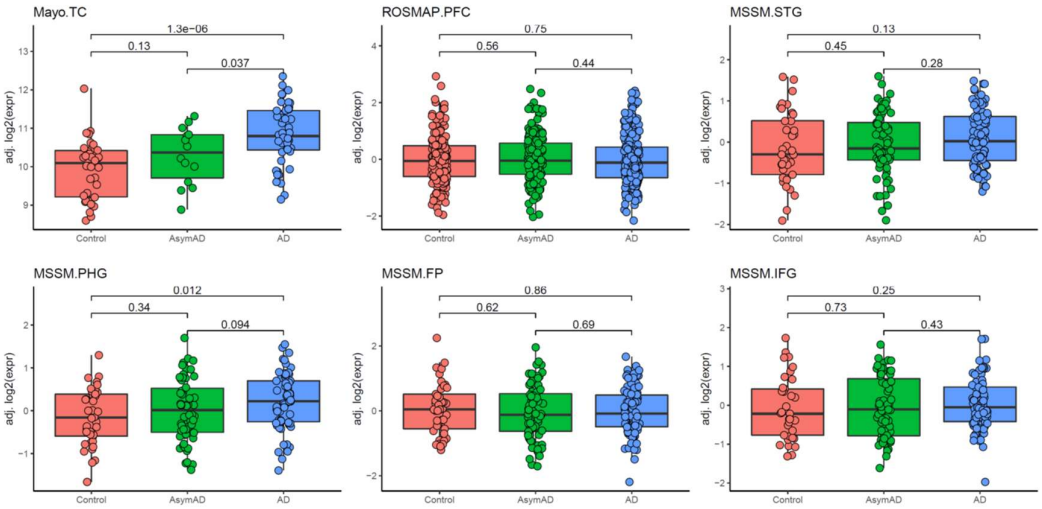

Figure 1. IFITM3 expression across the three comparison groups included in Morabito et al's study – Cognitively Normal Controls, Asymptomatic AD susceptible and AD patients. The numbers on horizontal brackets indicate adjusted p-values per comparison group. Mayo.TC: Mayo Clinic Brain Bank (Mayo) temporal cortex (TC); ROSMAP.PFC: Religious Orders Study and Memory and Aging Project (ROSMAP) prefrontal cortex (PFC); MSSM.PHG,IFG,FP: Mount Sinai School of Medicine (MSSM) para-hippocampal gyrus (PHG), inferior frontal gyrus (IFG), superior temporal gyrus (STG) and frontal pole (FP).

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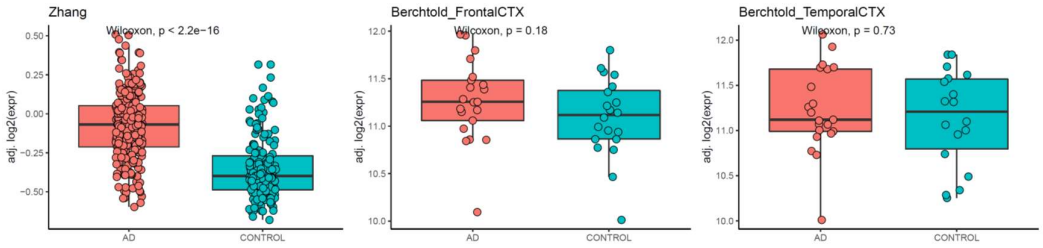

Figure 2. IFITM3 expression across the validation datasets included in Morabito et al's study – Comparisons were made between cognitively normal controls and late-onset AD patients. The numbers on horizontal brackets indicate adjusted p-values per comparison group.

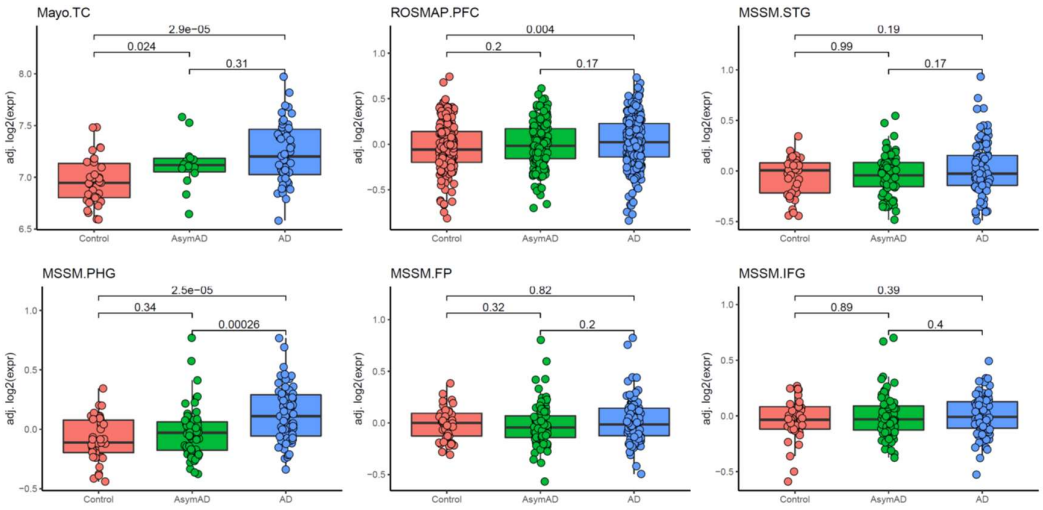

Figure 3. FYN expression across the three comparison groups included in Morabito et al's study – Cognitively Normal Controls, Asymptomatic AD susceptible and AD patients. The numbers on horizontal brackets indicate adjusted p-values per comparison group. Mayo.TC: Mayo Clinic Brain Bank (Mayo) temporal cortex (TC); ROSMAP.PFC: Religious Orders Study and Memory and Aging Project (ROSMAP) prefrontal cortex (PFC); MSSM.PFHG,IFG,FP: Mount Sinai School of Medicine (MSSM) para-hippocampal gyrus (PHG), inferior frontal gyrus (IFG), superior temporal gyrus (STG) and frontal pole (FP).

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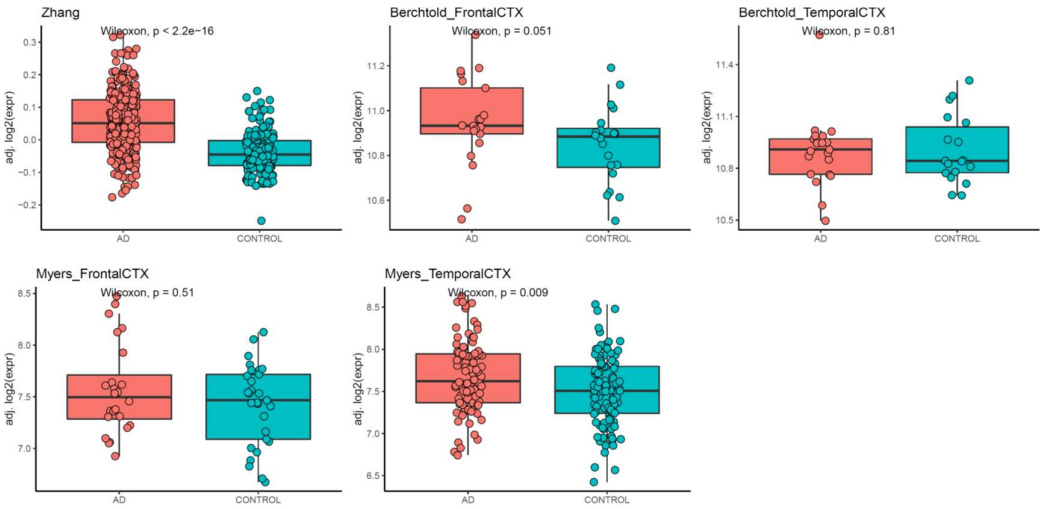

Figure 4. FYN expression across the validation datasets included in Morabito et al's study – Comparisons were made between cognitively normal controls and late-onset AD patients. The numbers on horizontal brackets indicate adjusted p-values per comparison group.

Correspondingly, cross-dataset comparisons of the scREAD database revealed that both IFITM3 and FYN were differentially expressed in neuronal cells donated from AD patients (astrocytes, microglia and oligodendrocyte precursor cells), when compared to controls (Supplementary Materials 2; adj. p-value<0.05).

Confirmatory GSEA indicated that IFITM3 networks were significantly enriched in several COVID-19 datasets containing SARS-CoV-2 upregulated genes (Supplementary Materials 3**;** adjusted p-value<0.05). These datasets included human ex vivo samples, murine and human cell lines as well as infectomics on organoids. Notably, FYN, a 59 kDa member of the Src family of tyrosine kinases that encodes a kinase that controls the cellular fate of IFITM3 vesicles and controls Aβ toxicity in a tau-dependent manner[9], was also an interactor of SARS-CoV-2 infection-related gene networks.

Discussion

In one of the largest AD GWAS to date[8], immunity, pathogenic SNPs indicated that immunity, endocytosis and lipid processing contributed to the neurobiology of AD. In this study, we confirm and localize this concept via multi-omics in IFITM3, as regulator of all three processes. The premise explored by Hur et al[2] is expanded by FYN dysregulation, as a driving force behind tau pathology in an Aβ independent manner[9], complementing Aβ dependent, tau-independent mechanisms[10]. Hence, IFITM3 and FYN perturbations may complimentarily cover the entire spectrum of AD pathology; FYN, however, as we have shown in our study, may independently drive Tau pathology in both Aβ dependent and independent manners, and hence may interact with but not depend on IFITM3 pathways.

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SARS-CoV-2's relevancy in AD pathobiology

Transcriptomics presented both here and in our original study concur on a global dysregulation of IFITM3 expression in AD. The range and level of evidence is compelling: Bulk and single cell RNA expression studies across different tissues, research groups and even technologies.

In our previously published work, IFITM3 signatures belonged to the significantly enriched "Response to type I interferon" biological network in entorhinal cortex neurons containing neurofibrillary tangles, and innate immunity pathways across all other datasets[4]. Larger scale studies confirmed that immunity, along with lipid metabolism and endocytosis may play an important role in at least late onset AD[5,8]. By design, IFITM3 regulates all three, while competing with invading viruses for the control of vesicular trafficking and lipid metabolism[1].

FYN, however, regulates IFITM3's trafficking and ubiquitination[9], and via Aβ oligomer-PrP^c interactions furthermore regulates tau fibrillization, NDMAR-dependent excitoxicity and neuronal spine moprhology[10].

SARS-CoV-2 infection experiments with brain organoids have recently revealed that neuroinvasion is followed by altered distribution of hyperphosphorylated Tau[11], indicating that aside from IFITM3, FYN may also be inescapably implicated in the neuronal defense response.

SARS-CoV-2 and IFITM3 and the neurobiology of Alzheimer's disease: Targeting FYN

SARS-CoV-2 has unique benefits in studying IFITM3 role in the neurobiology of AD. The recent identification of IFITM3 SNVs associated with severe COVID-19 and mortality all but assure that IFITM3 will inevitably interact with an invading SARS-CoV-2 as a post-entry form of defense.

Conversely, an unbiased GSEA on differentially expressed genes associated with AD consistently revealed significantly enriched IFITM3 and FYN biological networks, associated with COVID-19 infectomics on human ex vivo samples, human and murine cell lines and organoids. This finding indicates that an external stimulus, i.e. viral infection, would mechanistically induce Aβ production (via IFITM3). FYN upregulation could reflect a compensatory regulatory mechanism, considering that both Aβ and Tau induce is expression[9].

As such, Aβ-accumulation would stimulate expression of the nonreceptor tyrosine kinase FYN; in turn, FYN mediated phosphorylation of IFITM3 on Tyrosine 20 (Tyr-20) would mark it for ubiquitination, restricting Aβ production. Interestingly, Tyr-20 phosphorylation however, does not modify its antiviral properties; this latter finding indicates that IFITM3 induction – FYN phosphorylation may represent a feedback loop restricting buffering Aβ levels in infection[9]. Therefore, IFITM3 overexpression and any accumulation of Aβ oligomers would furthermore activate FYN and induce both tau aggregation and NMDAR-dependent perturbations such as excitoxicity[9,10].

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Notably, FYN may be recruited in viral processes such as viral RNA replication, with its inhibition conferring mechanistic antiviral effects[12]. In a FYN-virus interaction scenario, FYN restriction of IFITM3-mediated Aβ production would be abrogated, and consequently, Aβ levels and oligomerization would remain unchecked. Recruitment of Src (FYN/LYN) family kinases in viral processes however may subvert autophagy in favor of viral egress[12], and therefore abrogate a clearance mechanism for tau fibrils.

Interestingly, FYN inhibitors repurposed as a COVID-19 treatments[13], have been also investigated as disease modifying treatments in AD[14], supporting the convergence of common pathways between SARS-CoV-2 and AD in IFITM3's regulatory network.

As a final note, it is worth considering the scarce yet emerging evidence of SARS-CoV-2's neuroinvasive potential. Specifically, a 3-month follow up of patients recovering from COVID-19 uncovered microstructural alterations in the entorhinal cortex, associated with hyposmia, whereas memory loss was associated with hippocampal cortex remodeling[15]. These findings indicate that SARS-CoV-2 neurotropism can induce both phenotypic and radiological AD-like features, albeit within interactions indicated by the heterogeneity of the host's biology.

In a comprehensive model of AD pathogenesis supported by our data, interferon induction of IFITM3 in the setting of neuroinflammation would also implicate FYN, as a regulator of its localization and degradation. IFITM3-APP Aβ oligomerization would furthermore serve as both a feedback signal via PrP^c, maintaining FYN in an active state and promoting Tau pathology. The latter step, rather than Aβ accumulation, was shown to be the primary neurotoxic stimulus in the scenario of SARS-CoV-2 neuroinvasion[10], rather than IFITM3 mediated Aβ oligomerization / accumulation. Persistent neuroinflammation, viral latency in case of defective endosome – lysosome fusion in a vulnerable area (i.e. the entorhinal cortex in transcribial neuroinvasion) would then account for a switch from tau pathology to Aβ oligomer / Interferon signal spreading through neuronal projections to afferent sites, such as the hippocampi, as seen in SARS-CoV-2- related olfactory and memory impairment[15] (Figure 5).

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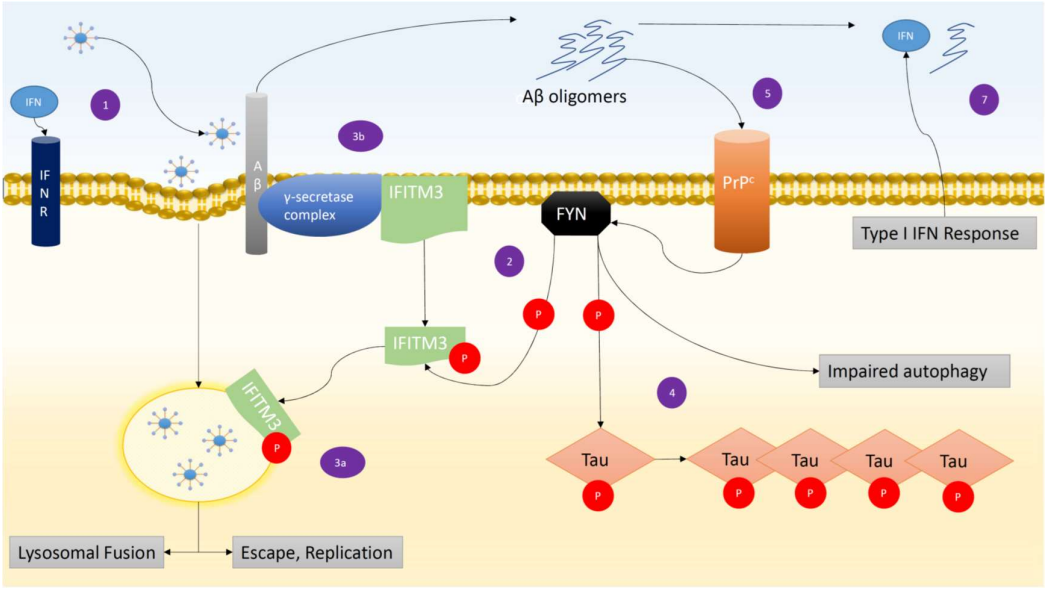

Figure 5. A comprehensive model of FYN regulation of AD pathogenesis in the setting of infection. (1) Interferon or viral infection-mediated induction of IFITM3 in the setting of neuroinflammation implicates FYN, as a regulator of its localization and degradation. FYN-mediated Tyr^{20} phosphorylation (2) governs the localization of IFITM3 in either the membrane (3b) or the endosomes (3a), as well as its potential ubiquitination. IFITM3-enhanced γ-secretase oligomerization of Aβ (3b) would furthermore serve as both a feedback signal via PrP^{c} (5), maintaining FYN in an active state and promoting Tau pathology (4). The latter step, rather than Aβ accumulation, has been shown to be the primary neurotoxic stimulus in the scenario of SARS-CoV-2 neuroinvasion, rather than IFITM3 mediated Aβ oligomerization / accumulation. Persistent neuroinflammation, viral latency in case of defective endosome – lysosome fusion in a vulnerable area (i.e. the entorhinal cortex in transcribial neuroinvasion) would then account for a switch from tau pathology to Aβ oligomer / Interferon "signal" spreading through neuronal projections to afferent sites, such as the hippocampi, as seen in SARS-CoV-2- related olfactory and memory impairment (6).

Overall, SARS-CoV-2 as a model virus has potentially uncovered a link between AD and innate immunity, namely the IFITM3 / FYN connection. The findings of the study suggest that a novel IFITM3-FYN interaction, is implicated in the pathogenesis of AD and is furtherly associated with COVID-19 infectomics.

References

1. Iadecola, C., Anrather, J. & Kamel, H. Effects of COVID-19 on the Nervous System. *Cell* **183**, 16-27 e11, doi:10.1016/j.cell.2020.08.028 (2020).
2. Hur, J. Y. *et al.* The innate immunity protein IFITM3 modulates gamma-secretase in Alzheimer's disease. *Nature* **586**, 735-740, doi:10.1038/s41586-020-2681-2 (2020).

3. Rahman, M. A., Islam, K., Rahman, S. & Alamin, M. Neurobiochemical Cross-talk Between COVID-19 and Alzheimer's Disease. *Mol Neurobiol*, doi:10.1007/s12035-020-02177-w (2020).
4. Vavougios, G. D. *et al.* Double hit viral parasitism, polymicrobial CNS residency and perturbed proteostasis in Alzheimer's disease: A data driven, in silico analysis of gene expression data. *Mol Immunol* **127**, 124-135, doi:10.1016/j.molimm.2020.08.021 (2020).
5. Morabito, S., Miyoshi, E., Michael, N. & Swarup, V. Integrative genomics approach identifies conserved transcriptomic networks in Alzheimer's disease. *Hum Mol Gen* **29**, 2899-2919, doi:10.1093/hmg/ddaa182 (2020).
6. Jiang, J., Wang, C., Qi, R., Fu, H. & Ma, Q. scREAD: A Single-Cell RNA-Seq Database for Alzheimer's Disease. *iScience* **23**, 101769, doi:10.1016/j.isci.2020.101769 (2020).
7. Kuleshov, M. V. *et al.* Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res* **44**, W90-97, doi:10.1093/nar/gkw377 (2016).
8. Zhang, B. *et al.* Integrated systems approach identifies genetic nodes and networks in late-onset Alzheimer's disease. *Cell* **153**, 707-720, doi:10.1016/j.cell.2013.03.030 (2013).
9. Briner, A., Gotz, J. & Polanco, J. C. Fyn Kinase Controls Tau Aggregation In Vivo. *Cell Rep* **32**, 108045, doi:10.1016/j.celrep.2020.108045 (2020).
10. Um, J. W. & Strittmatter, S. M. Amyloid-beta induced signaling by cellular prion protein and Fyn kinase in Alzheimer disease. *Prion* **7**, 37-41, doi:10.4161/pri.22212 (2013).
11. Ramani, A. et al. SARS-CoV-2 targets neurons of 3D human brain organoids. *EMBO J* **39**, e106230, doi:10.15252/embj.2020106230 (2020).
12. Kumar, R., Agrawal, T., Khan, N. A., Nakayama, Y. & Medigeshi, G. R. Identification and characterization of the role of c-terminal Src kinase in dengue virus replication. *Sci Rep* **6**, 30490, doi:10.1038/srep30490 (2016).
13. Weisberg, E. *et al.* Repurposing of Kinase Inhibitors for Treatment of COVID-19. *Pharm Res* **37**, 167, doi:10.1007/s11095-020-02851-7 (2020).
14. Li, M.Y., Naik, T.S., Siu, L.Y.L. et al. Lyn kinase regulates egress of flaviviruses in autophagosome-derived organelles. *Nat Commun* **11**, 5189 (2020). https://doi.org/10.1038/s41467-020-19028-w
15. Lu, Y. *et al.* Cerebral Micro-Structural Changes in COVID-19 Patients - An MRI-based 3-month Follow-up Study. *EClinicalMedicine* **25**, 100484, doi:10.1016/j.eclinm.2020.100484 (2020).