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Title

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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Abstract

Introduction: IFITM3, an innate immune protein linked to COVID-19 severity, has recently been identified as a novel γ-secretase modulator. Independent research has shown that IFITM3 may facilitate SARS-CoV-2 neurotropism in an ACE2-independent manner. In a previous study, we had detected perturbations in IFITM3 networks in both the CNS and peripheral immune cells donated by AD patients. The purpose of this study is to explore the transcriptomic evidence of the SARS-CoV-2 / IFITM3 / AD interplay, validating previous findings from our group.

Methods: Exploratory analyses involved meta-analysis of bulk and single cell RNA data for IFITM3 and FYN differential expression. For confirmatory analyses, we performed gene set enrichment analysis (GSEA) on an AD gene signature from AD Consensus transcriptomics; using the Enrichr platform, we scrutinized COVID-19 datasets for significant, overlapping enriched biological networks.

Results: Bulk RNA data analysis revealed that IFITM3 and FYN were differentially expressed in two CNS regions in AD: the temporal cortex (AD vs. Controls, adj.p-value=$1.3e^{-6}$) and the parahippocampal cortex (AD vs. controls, adj.p-value=0.012). Correspondingly, single cell RNA analysis of IFITM3 and FYN revealed that it was differentially expressed in neuronal cells donated from AD patients (astrocytes, microglia and oligodendrocyte precursor cells), when compared to controls.

Discussion: IFITM3 and by extent FYN were found as interactors within biological networks overlapping between AD and SARS-CoV-2 infection. SARS-CoV-2-mediated FYN/IFITM3 induction would mechanistically result in increased Tau fibrilization and Aβ oligomerization. FYN recruitment by viral processes results in abrogation of both fusion of IFITM3 vesicles with lysosomes; immunoevasion, by FYN-mediated impairment of autophagy would then serve to promote impaired detoxification from Aβ, while propagating Tau pathology in an IFITM3-independent manner.

MAIN MANUSCRIPT

Introduction

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. IFITM3's role in intercepting and shuttling viral particles to the lysosomes[1] was recently complemented by its discovery as a novel γ-secretase modulator that promotes Aβ production[2]. Considering the accumulating evidence on common pathways between COVID-19 and Alzheimer's disease (AD)[3], we aimed to examine whether FYN, a kinase regulating IFITM3's localization[4] is accordingly perturbed in both AD and COVID-19 transcriptomes. Current state of the art transcriptomic evidence suggest that FYN interacts with SARS-CoV-2 during the course of infection[5], and was found to be upregulated in a recent meta-analysis of SARS-CoV-2 expression datasets[6]. Expanding on our previous research on IFITM3 networks in AD[5], we propose a comprehensive model of AD pathogenesis where viral induction of the IFITM3/FYN endocytosis signal could account for increased Aβ oligomerization via γ-secretase activation[2]. Furthermore, SARS-CoV-2 induced FYN dysregulation / overactivation[5,6] would concomitantly and independently promote Tau fibrilization[7], abrogate autophagy[4], and prepare APP[8] for processing by the previously activated (via IFITM3[2]) γ-secretase complex.

In order to explore FYN and IFITM3's expression in AD, we meta-analyzed gene expression data from both bulk tissue and single cell RNA sequencing studies, aside from those previously examined[2]. Subsequently, we aimed to investigate the overlap between FYN/IFITM3's biological networks and SARS-CoV-2 infectomics. Finally, we provide data on FYN/IFITM3 networks that arose in our previous study, and

integrated them in a comprehensive model of AD and AD-like manifestations of NeuroCOVID-19's pathogenesis.

METHODS

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 and FYN expression data[9] (Supplementary Materials 1 – Extended Methods)

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[10,11] (Supplementary Materials 1 – Extended Methods).

Confirmatory gene set enrichment analyses (GSEA)

We also performed confirmatory GSEA on differential gene expression data available from Morabito et al[10] to detect IFITM3 in COVID-19 related datasets. GSEA was performed via the Enrichr platform[12] (Available from: https://maayanlab.cloud/Enrichr/) on available COVID-19 datasets. These datasets included human ex vivo samples, murine and human cell lines as well as infectomics on organoids. For all analyses, adjusted p-values <0.05 were considered statistically significant.

Data Availability Statement

All data used in this manuscript are available online via Mendeley Data (https://data.mendeley.com/datasets/5bypp2h5kj/1).

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 gyrus (MSSM study, AD vs. controls, adj. p-value=0.012); (Fig.1). In the validation datasets, IFITM3 was differentially expressed in Zhang et al's study[9] (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 parahippocampal gyrus(MSSM study, AD vs. controls, adj. p-value=$2.9e^{-5}$); Fig. 3. In the validation datasets, FYN was differentially expressed in two datasets, including Zhang et al's study[13] (AD vs. Controls, adj. p-value<$2.2e^{-16}$; Fig 4).

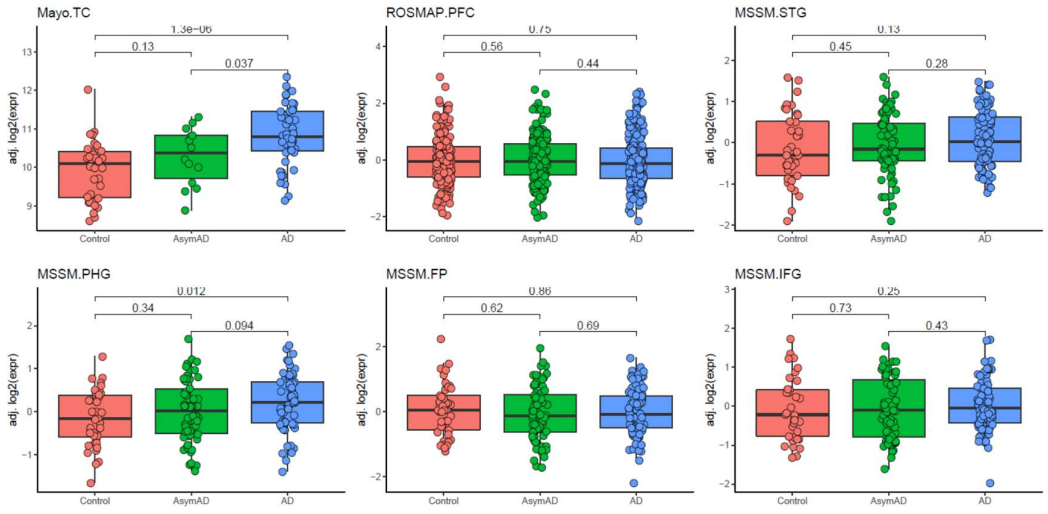

Figure 1. IFITM3 expression across the three comparison groups included in Morabito et al's study – Cognitively Normal Controls, Asymptomatic AD susceptible and AD patients. The numbers on horizontal brackets indicate adjusted p-values per comparison group. Mayo.TC: Mayo Clinic Brain Bank (Mayo) temporal 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).

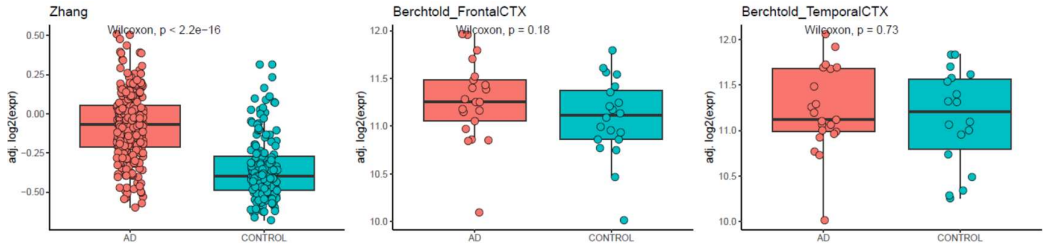

Figure 2. IFITM3 expression across the validation datasets included in Morabito et al's study – Comparisons were made between cognitively normal controls and late-onset AD patients. The numbers on horizontal brackets indicate adjusted p-values per comparison group.

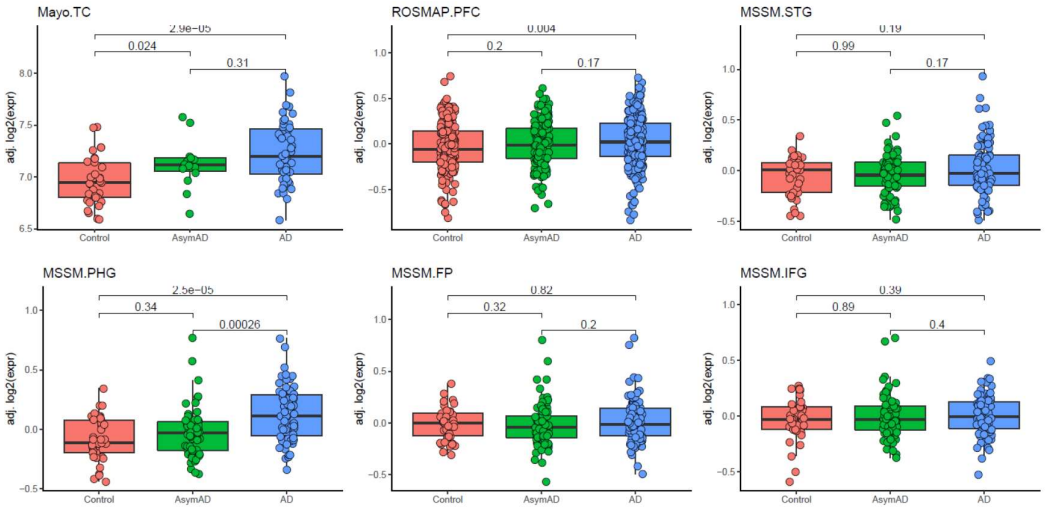

Figure 3. FYN expression across the three comparison groups included in Morabito et al's study – Cognitively Normal Controls, Asymptomatic AD susceptible and AD patients. The numbers on horizontal brackets indicate adjusted p-values per comparison group. Mayo.TC: Mayo Clinic Brain Bank (Mayo) temporal 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).

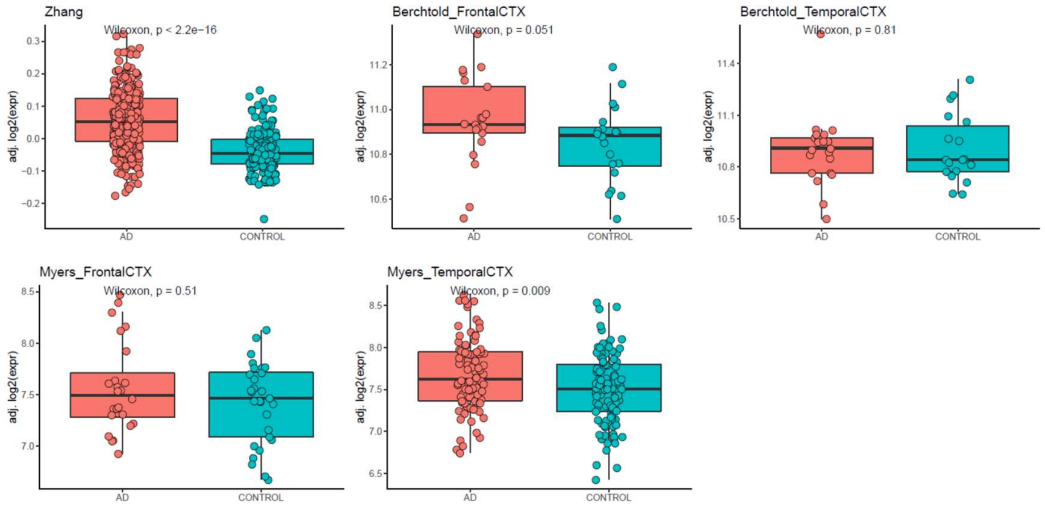

Figure 4. FYN expression across the validation datasets included in Morabito et al's study – Comparisons were made between cognitively normal controls and late-onset AD patients. The numbers on horizontal brackets indicate adjusted p-values per comparison group.

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 FYN and IFITM3 biological networks were significantly enriched in several COVID-19 datasets containing SARS-CoV-2 upregulated genes (Supplementary Materials 3; adjusted p-value<0.05).

Finally, we include significantly enriched FYN signatures from entorhinal cortex neurons containing neurofibrillary tangles and hippocampal cortex neurons from our previous study[5] for comparisons (Supplementary Materials 4_{a-d})

DISCUSSION

Viral induction of FYN / IFITM3 endocytosis signal as the trigger for tau aggregation and Aβ oligomerization

Previous studies in AD have pinpointed innate immunity, endocytosis and lipid processing gene networks as major contributors to the neurobiology of AD[10]. In this study, we confirm, localize and expand this concept via multi-omics on FYN /IFITM3, as regulators of all aforementioned processes. The premise explored by Hur et al[2] is now expanded by FYN dysregulation, as a driving force behind tau pathology in an Aβ independent manner[4], complementing Aβ dependent, Tau-independent mechanisms[14]. FYN, however, has been shown has been shown to direct Tau pathology in both Aβ dependent and independent manners[4]. While the importance of IFITM3's dysregulation in AD cerebral tissue was only recently confirmed[2], we consider here for the first time FYN's concomitant dysregulation within a functional context: that of an endocytosis signal, induced by viral infection[4,15]. In this setting, the concomitant priming of the γ-secretase[2] and APP[8] would correspond to increased production of Aβ oligomers. Considering their antiviral proteins, Aβ overproduction coupled with a viral endocytosis signal would represent an efficient innate antiviral immune response[16], immediately challenging invading pathogens in the extracellular milieu.

SARS-CoV-2-related neurocognitive deficits and their importance in AD pathobiology

COVID-19 has put tremendous pressure in researchers worldwide in order to mount an efficient response to the threat it represents. Increased awareness and intensive research efforts yielded increasing and evolving understanding of the its spectrum, one that includes both phenotypic and genomic overlap with neurodegenerative disease[5,17]. Among the more albeit easily underdiagnosed manifestations are neurocognitive symptoms, including memory defects, even among those patients recovered from mild disease[18].

A study reporting on 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[19]. These findings indicate that SARS-CoV-2 neurotropism can affect sites not only related to AD pathology, but spread via anatomically connected sites. Notably, olfactory dysfunction as an early symptom of AD has been previously attributed to the

dysfunction of prohibitins[20], proteins targeted by SARS-CoV-2 during the establishment of cellular latency[21].

The overlap between SARS-CoV-2 neuroinvasion and AD features, would inadvertedly shift the focus back to whether the novel coronavirus can provide arguments in favor of the antimicrobial protection hypothesis. The answer to this question, lies within the IFITM3 / FYN regulatory network.

FYN, IFITM3 and SARS-CoV-2 induced tauopathy: its importance in AD pathogenesis

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[22]. The importance of IFITM3's antiviral functions[1] become even more pronounced when considering its role as a gamma secretase[2]: a FYN-phosphorylated IFITM3 remains membrane bound, enhancing rather than restricting SARS-CoV-2's cellular entry[23]. At the same time, IFITM3 remains available to interact with the γ-secretase complex[2], while FYN activation independently promotes both Tau fibrillization and Aβ oligomerization (**Figure 5**).

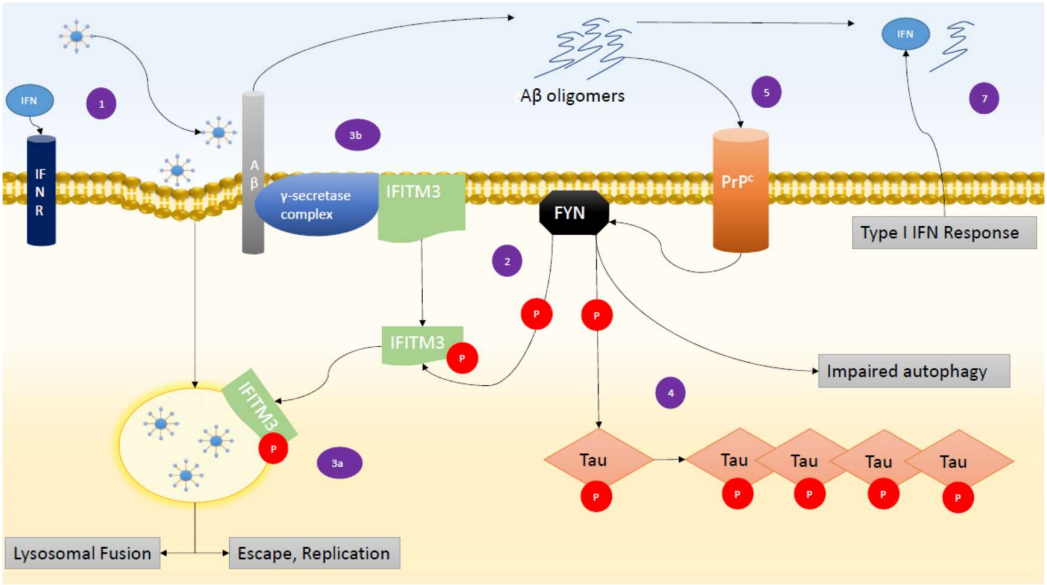

Figure 5. A comprehensive model of FYN regulation of AD pathogenesis in the setting of infection. (1) Interferon / viral infection-mediated induction of IFITM3 in the setting of neuroinflammation implicates FYN, as a regulator of its localization and degradation in the setting of an endocytosis signal. 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. 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).

FYN overexpression secondary to SARS-CoV-2 infection has been reported by several studies [5,6]. Furthermore, it was independently confirmed in this study by an unbiased GSEA on differentially expressed genes associated with AD; This analysis revealed significantly enriched FYN and IFITM3 biological networks, associated with COVID-19 infectomics on human ex vivo samples, human and murine cell lines as well as organoids (Supplementary Materials 3).

Within the setting of this model, the primary event of SARS-CoV-2 induced neurodegeneration would be the induction of tau pathology in neurons. SARS-CoV-2 infection experiments with brain organoids have recently revealed that neuroinvasion is followed by altered neuronal distribution of hyperphosphorylated Tau[24]. Even in non-neuronal cells, systems biology approaches have identified the induction of the tau kinase pathway and impaired autophagy following SARS-CoV-2 neuroinvasion[25].

It is important to consider that aside from host entry, FYN has been found to be actively recruited in intracellular viral processes such as viral RNA replication[26], while subvert autophagy in favor of viral lifecycles[27]. In this setting, Tau fibril toxicity would function as a restricting mechanism for viral parasitism, killing the host and limiting productive infection. Considering that his proposed mechanism is linked to Aβ production, both would serve as an innate immune response: active viral parasitism would inescapably be limited by accumulating tau cytotoxicity, whereas simultaneous and continuous Aβ oligomerization would both prepare an antiviral milieu, and promote inflammation. Translating this model of SARS-CoV-2 neurodegeneration in the setting of the antimicrobial hypothesis of AD pathogenesis would posit major tau fibrilization and Aβ oligomerization are part of an innate immune response, with FYN / IFITM3 functioning as both a mediator and sensor of viral entry. In this sense, tau pathology would predominate the site of primary infection (i.e. olfactory cortex) whereas Aβ oligomers would feedforward neuroinflammation via afferent projections, i.e. from the olfactory cortex to the hippocampi. The latter scenario is in line with significantly enriched FYN/IFITM3 networks uncovered in our previous work[9] (Supplementary Materials $4_{a\text{-}d}$). This scenario could explain the COVID-19 neurocognitive manifestations, as well as olfactory impairments, as well as their associated radiological features[19] .

Our current work hypothesis should also be considered within its limitations. Currently, no study has evaluated the longitudinal development of pathologically proven AD following exposure to COVID-19. Furthermore, we are aware of only one study examining the mechanistic development of tauopathy in SARS-CoV-2 infected neurons[24], and only one study on that included neuroimaging follow-up in post-COVID-19 recoverees presenting with memory impairments[19]. To date, no study combines both a clinical and a basic science concept that can verify our model in a uniform manner. Another important limitation is that other AD-related genes are also implicated in COVID-19 infection, such as APOE[28] and ACE2[29]. While this implication further bolsters SARS-CoV-2's potential role in AD pathogenesis, genetic variability

and gene interactions between these genes and the FYN/IFITM3 switch should be studied in detail, in order to elucidate their mechanistic effects.

Our findings support FYN dysregulation in AD and suggest novel roles for FYN/IFITM3 in SARS-CoV-2 neuroinvasion. FYN dysregulation, as an independent mediator of tau pathology expands on recent findings by completing the spectrum of AD pathology. Further studies are needed to explore the FYN/IFITM3 regulatory loop proposed here in both AD and COVID-19's neurocognitive manifestations, particularly considering that this pathway overlap is already druggable by tyrosine kinase inhibitors used both in AD and COVID-19[30].

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