News Release

Title

Actin-binding protein filamin-A drives tau aggregation and contributes to progressive supranuclear palsy pathology

Key Points

- **Co-localization of filamin-A with tau pathology and genetic variations on *filamin-A* gene were identified in patients with progressive supranuclear palsy (PSP).**
- **Cellular experiments showed filamin-A promotes tau aggregation through actin filaments.**
- **Human filamin-A transgenic mice display tau aggregation in the brains.**

Summary

A group of researchers, headed by Prof. Masahisa Katsuno, Dr. Kentaro Sahashi and Dr. Koyo Tsujikawa (first author), Department of Neurology, Nagoya University Graduate School of Medicine have collaborated with Prof. Naomichi Matsumoto and Dr. Kohei Hamanaka, Yokohama City University Graduate School of Medicine; Adjunct Prof. Mari Yoshida, Institute for Medical Science of Aging, Aichi Medical University; Prof. Takaki Miyata, Anatomy and Cell Biology, Nagoya University Graduate School of Medicine; Prof. Takeshi Ikeuchi, Brain Research Institute, Niigata University; Prof. Takeshi Iwatsubo; Japanese Longitudinal Biomarker Study in PSP and CBD (JALPAC) Consortium; Japanese Alzheimer's Disease Neuroimaging Initiative (J-ADNI) and other researchers, and revealed that actin-binding protein filamin-A drives tau aggregation and contributes to progressive supranuclear palsy pathology. This work was supported by the Strategic Research Program for Brain Sciences from the Japan Agency for Medical Research and Development (AMED).

PSP is a pathologically defined tauopathy presenting with various neurological and cognitive impairment. The mechanism that drives tau aggregation in PSP has not been clearly explained, and thus there is no effective treatment for PSP.

This study showed that filamin-A is abundant and colocalized with tau in the brains with PSP. In addition, some patients with PSP were found to harbor genetic variations in the *filamin-A* gene. The cellular experiments showed filamin-A induces tau aggregation through interaction with actin filaments. The newly generated transgenic mice carrying human *filamin-A* gene showed neural and glial tau aggregation like the neuropathology of PSP. These results indicate that filamin-A is a driver for tau aggregation and a potential therapeutic target for PSP.

This work was published online in *Science Advances*.

Research Background

PSP is a neurodegenerative disorder characterized by tau aggregation in neurons and glial cells. Patients with PSP usually die in 5-10 years after onset, and their serious physical and mental disabilities increase caregiver burden. In the autopsied brains, tufted astrocytes are the

pathologically diagnostic hallmark for PSP. The clinically classical form of PSP is referred to as Richardson's syndrome, presenting with vertical supranuclear gaze palsy and postural instability. Recently, accumulated data from the autopsied brains have demonstrated that the clinical phenotype of PSP includes a broad spectrum ranging from abnormal movement-predominant types (e.g. PSP-parkinsonism) to abnormal behavioral-predominant types (e.g., PSP with frontotemporal dementia). This suggests that the number of the patients is higher than the previously estimated rare of 18 cases per 100,000. However, there is no effective treatment for PSP because its pathogenesis largely remains elusive. While amyloid-ß lies upstream of tau pathology in Alzheimer's disease, the mechanism that drives tau aggregation in PSP has not been clearly explained, and genetic animal models that fully phenocopy PSP are not available.

Research Results

To investigate molecular mechanisms underlying tau aggregation in PSP, we performed LC-MS/MS analysis and identified filamin-A abundance in the sarkosyl-insoluble fractions of the brains from PSP subjects. The sarkosyl-insoluble filamin-A protein levels were higher in PSP than in normal controls and other neurodegenerative disorders, including corticobasal degeneration, Alzheimer's disease, Parkinson's disease and dementia with Lewy bodies. The pathological investigation showed that filamin-A was co-localized with the aggregated tau in the brains with PSP (Figure 1), suggesting the protein interaction between filamin-A and tau. In addition, we identified the duplication of *filamin-A* gene in monozygotic twin concordant for tau pathology of PSP. Of 312 cases with sporadic PSP, 12 cases were found to carry rare variants (e.g. p.Ser2523Asn) of *filamin-A* gene. An association study using the dataset of 499 normal controls demonstrated that the rare variants of *filamin-A* gene can contribute to the risk of PSP with the odds ratio of 3.91.

Filamin-A is known to regulate neuronal migration during the development of the forebrain, whereas there is only limited data how filamin-A is related to tau-mediated neurodegeneration. Here, we performed the cellular experiments and showed that filamin-A enhances phosphorylation, protein stability and sarkosyl-insolubility of tau. In this context, the protein interaction between tau and filamin-A was confirmed by a co-immunoprecipitation. We also found that filamin-A augmented the interaction of ubiquitin and chaperone proteins. In addition, rat primary astrocytes co-expressing tau and filamin-A showed that hyper-phosphorylated tau aggregates were localized within the proximal portions of processes and cell bodies, reminiscent of tufted astrocyte in PSP.

Next, we performed *in utero* electroporation to overexpress various types of filamin-A in the murine forebrains. As a results, the protein levels of tau and neuronal migration defect were induced by wild-type filamin-A, but not by p.Ala39Gly mutant filamin-A that abrogates the binding ability to actin filament. The primary cortical neurons showed that the wild-type filamin-A caused hyper-phosphorylation of tau with actin accumulation, which was compromised by the treatment with an actin depolymerizer cytochalasin D. These results

indicate that filamin-A increases tau levels through direct interaction mediated by actin filament (Figure 2).

To induce overexpression of filamin-A in the adult murine brains, we employed an adeno-associated virus (AAV) vector-mediated approach and designed AAV expressing the truncated versions of filamin-A that contains essential domains for the interaction with tau (AAV-ΔFLNA). We performed stereotaxic injections of AAV-ΔFLNA into the frontal cortex of 2-month-old wild-type mice, and found increased murine tau. Similarly, we evaluated the effect of AAV-ΔFLNA on human tau protein in transgenic mice that harbor human tau in the absence of murine tau and found the increased protein levels of human tau in the ΔFLNA-transduced neurons.

We further generated transgenic mice expressing full-length human filamin-A (FLNA-Tg). The brains of the 5-month-old FLNA-Tg mice showed augmented sarkosyl-insoluble tau and co-localization of filamin-A with tau in the neurons and glial cells, similar to the brains with PSP. Finally, primary cortical neurons from the FLNA-Tg mice showed tau phosphorylation and defective neurite outgrowth, which were rescued by lentiviral short hairpin RNA-mediated knockdown of filamin-A.

This study underscores the potential of filamin-A as a driver for tau aggregation and suggests that filamin-A would be a determinant factor in tau pathology in the brain with PSP. Strategies aimed at inhibiting filamin-A-driven tau aggregations could stand as a disease-modifying therapy to combat devastating neurodegeneration observed in PSP.

Research Summary and Future Perspective

We are engaged in developing a novel assay for drug screening to cure PSP, in collaboration with a research group of Graduate School of Pharmaceutical Sciences, Nagoya University and with a domestic pharmaceutical company. We plan to modify FLNA-Tg mice and generate iPS-derived cells from patients with PSP for more elucidation of the pathogenesis and drug discovery.

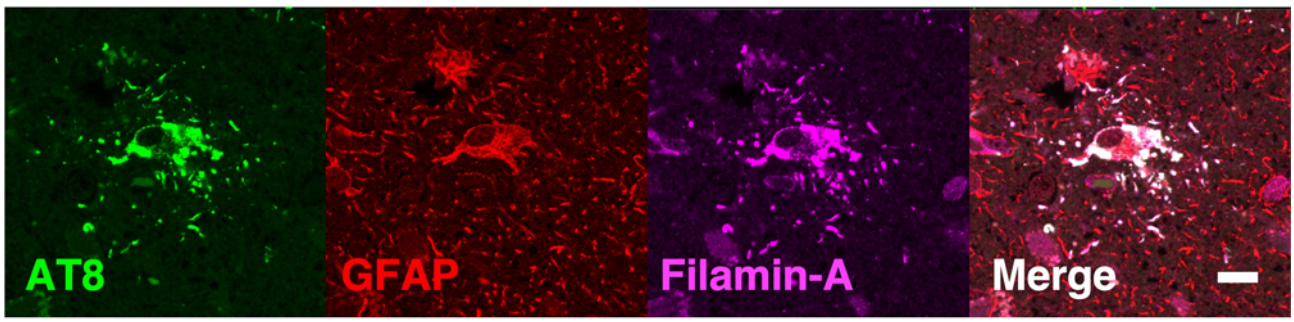

Figure 1. Immunofluorescence for tufted astrocyte in the subcortical gray matter of sporadic PSP. AT8 hyper-phosphorylated tau (green) is co-localized with filamin-A (magenta). Anti-GFAP antibody (red) was used to identify astrocytes.

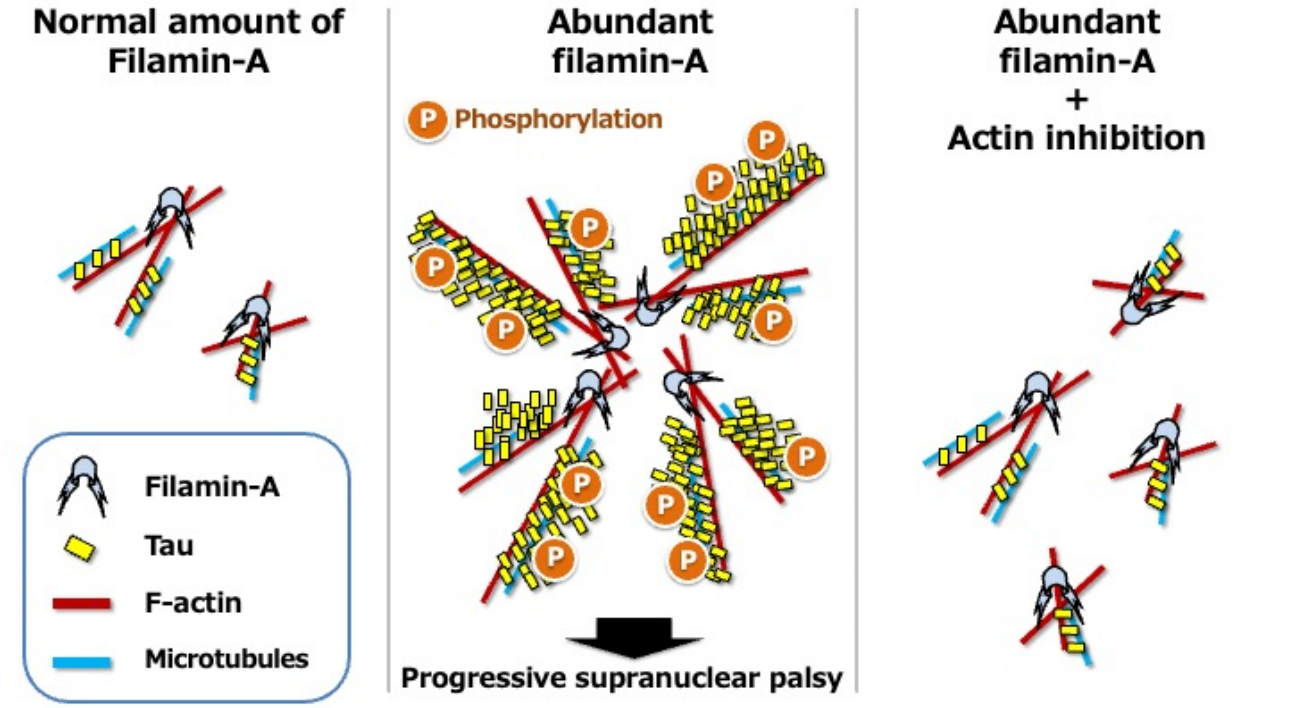

Figure 2. Graphical abstract. Filamin-A increases protein aggregation of microtubule-associated protein tau through direct interaction mediated by actin filament (F-actin).

Publication

Journal: Science Advances

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Authors: Koyo Tsujikawa[1-3], Kohei Hamanaka[4], Yuichi Riku[1,5], Yuki Hattori[6], Norikazu Hara[7], Yohei Iguchi[1], Shinsuke Ishigaki[1,8], Atsushi Hashizume[1,9], Satoko Miyatake[4,10], Satomi Mitsuhashi[4,11], Yu Miyazaki[1], Mayumi Kataoka[1], Li Jiayi[1], Keizo Yasui[2], Satoshi Kuru[3], Haruki Koike[1], Kenta Kobayashi[12], Naruhiko Sahara[13], Norio Ozaki[14], Mari Yoshida[5], Akiyoshi Kakita[15], Yuko Saito[16], Yasushi Iwasaki[5], Akinori Miyashita[7], Takeshi Iwatsubo[16], Japanese Alzheimer's Disease Neuroimaging Initiative (J-ADNI)[a], Takeshi Ikeuchi[7], Japanese Longitudinal Biomarker Study in PSP and CBD (JALPAC) Consortium[b], Takaki Miyata[6], Gen Sobue[8], Naomichi Matsumoto[4], Kentaro Sahashi[1], Masahisa Katsuno[1,9]

Affiliations:

[1] Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya, Japan

[2] Department of Neurology, Japanese Red Cross Aichi Medical Center Nagoya Daini Hospital, Nagoya, Japan

[3] Department of Neurology, National Hospital Organization Suzuka National Hospital, Suzuka, Japan

[4] Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan

[5] Department of Neuropathology, Institute for Medical Science of Aging, Aichi Medical University, Nagakute, Japan
[6] Department of Anatomy and Cell Biology, Nagoya University Graduate School of Medicine, Nagoya, Japan
[7] Department of Molecular Genetics, Brain Research Institute, Niigata University, Niigata, Japan
[8] Research Division of Dementia and Neurodegenerative Disease, Nagoya University Graduate School of Medicine, Nagoya, Japan
[9] Department of Clinical Research Education, Nagoya University Graduate School of Medicine, Nagoya, Japan
[10] Clinical Genetics Department, Yokohama City University Hospital, Yokohama, Japan.
[11] Department of Genomic Function and Diversity, Medical Research Institute Tokyo Medical and Dental University, Tokyo, Japan
[12] Section of Viral Vector Development, National Institute for Physiological Sciences, Okazaki, Japan
[13] Department of Functional Brain Imaging, National Institutes for Quantum and Radiological Science and Technology, Chiba, Japan
[14] Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya, Japan
[15] Department of Pathology, Brain Research Institute, Niigata University, Niigata, Japan
[16] Department of Neurology and Neuropathology (The Brain Bank for Aging Research), Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology, Tokyo, Japan
[17] Department of Neuropathology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
DOI：10.1126/sciadv.abm5029

Japanese ver.
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