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LETTER TO THE EDITOR

Cryo-EM structure of full-length α-synuclein amyloid fibril with Parkinson's disease familial A53T mutation

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Dear Editor,

α-Synuclein (α-Syn) forms amyloid fibrils accumulating in Lewy bodies (LB) and Lewy neuritis (LN), which is a common histological hallmark of Parkinson's disease (PD) and other synucleinopathies.[1] Moreover, α-syn amyloid fibrils spread in the patient's brain via cell-to-cell transmission, which accounts for the disease progression.[2] Several single amino-acid mutations of α-syn, including A53T, E46K, H50Q, G51D, A30P and A53E, have been identified from familial PD patients, which are causative to the early-onset pathology with different clinical symptoms.[3] Among them, A53T is the first hereditary mutation of α-syn discovered in Italian and Greek families with autosomal dominant and early-onset PD.[4] Till now, A53T represents the most commonly reported cases of familial PD involving more than 10 families of Greek, Korean, Swedish and Chinese origin.[3] A53T mutation promotes α-syn fibril formation in vitro, and exhibits exacerbated PD-like pathology both in cellular and animal models.[5,6] Previous structural studies on the wild-type (WT) α-syn fibril reveal that A53 is one of the key residues in the interface of α-syn protofilaments.[7–9] However, it remains unclear how the A53T mutation may alter the fibril structure and exacerbate α-syn pathology.

To investigate the structure of α-syn A53T mutant fibril, we prepared recombinant full-length N-terminally acetylated α-syn with A53T mutation (Supplementary information, Fig. S1). Ac-A53T α-syn formed a different fibril morphology from the Ac-WT fibril characterized by negative-staining transmission electron microscopy (TEM) (Supplementary information, Fig. S2). To validate the biological relevance of the recombinant fibril, we examined the pathology of Ac-A53T fibril by using a well-documented α-syn preformed fibril seeds (PFFs)-based neuronal propagation assay.[10] We treated rat primary cortical neurons with Ac-A53T and Ac-WT α-syn PFFs, respectively, and monitored the induction of pathological aggregation of endogenous α-syn by immunostaining of pS129 α-syn. The result showed that Ac-A53T PFFs induced significantly more pS129-positive α-syn aggregates than the Ac-WT PFFs (Fig. 1a, b and Supplementary information, Fig. S3). Consistently, lactate dehydrogenase (LDH) assay showed that Ac-A53T α-syn PFFs are more toxic to neurons than the Ac-WT PFFs (Fig. 1c). Together, these results indicate that the A53T mutation re-arranges the α-syn fibril structure causing increased neuronal pathology.

Next, we reconstructed the cryo-EM 3D density map of the Ac-A53T fibril at an overall resolution of 3.49 Å (Supplementary information, Fig. S4 and Table S1). The Ac-A53T fibril consists of two nearly identical protofilaments intertwining along an approximate twofold screw axis. The helical rise between α-syn subunits is 2.42 Å and the helical twist is 179.55° (Fig. 1d). Based on the density map, we were able to unambiguously build a near-atomic structure model for the Ac-A53T fibril (Fig. 1e). In the fibril structure, Ac-A53T α-syn subunit folds into a Greek key-like architecture composed of residues 37–99, which is similar to that in the Ac-WT and a recent H50Q α-syn fibril structure[11] (Fig. 1f). However, their interfaces between opposing α-syn subunits, that is the interface between the paring protofilaments, are different (Fig. 1f). In particular, the protofilamental interface of the Ac-WT α-syn fibril involves residues 50–57, which form a typical class I steric zipper[12] with a buried surface area of 683 $Å^2$ per layer and confer a high stability to the overall fibril structure (Fig. 1g). In contrast, the protofilamental interface of the Ac-A53T fibril is much smaller with a buried surface area of 83 $Å^2$ per layer only consisting of T59 and K60 with no obvious interactions (Fig. 1g). The protofilamental interface of H50Q fibril is similar to that of the Ac-A53T, while the overall fibril morphology varies with different fibril twists (Fig. 1f). N-acetylation is not observed in both the Ac-A53T and Ac-WT fibril structures.

Structural polymorphism is an important characteristic commonly existed in pathological fibrils formed by different amyloid proteins.[7,13] Different α-syn fibril polymorphs may lead to different pathological symptoms in a broad clinical spectrum of synucleinopathies. Indeed, α-syn fibrils extracted from multiple system atrophy (MSA) and PD patients are morphologically and pathologically different.[14] Thus, the structural basis underlying the assembly of different fibril polymorphs and how these polymorphs are induced by genetic or environmental factors are of great importance to deciphering the pathological mechanism of the diseases. PD familial α-syn mutation A53T enhances the propagation and cytotoxicity of α-syn fibril and is causative to early-onset PD. Cryo-EM structures show that A53 is in the center of the WT protofilamental interface (Fig. 1g). A53T mutation disrupts the interface, and thus drives the re-arrangement of the protofilamental orientation. The new interface formed in the Ac-A53T fibril is smaller and less stable than that in the Ac-WT fibril (Fig. 1f). Thus, the Ac-A53T protofilaments may be more prone to dissociate from the mature fibril, which facilitates the seeding-dependent propagation process. Intriguingly, recent study shows that another PD familial mutation E46K induces a totally different protofilament structure of α-syn,[15] indicating that different mutations contribute differently to the structure and pathology of α-syn.

In summary, we report a near-atomic structure of the Ac-A53T α-syn fibril. The structure reveals that A53T mutation has no significant disturbance on the fold of α-syn; instead, it changes the paring geometry of the protofilaments, and thus forms a different morphology of mature fibril. The protofilamental interface of the mutant fibril is less stable than that of the WT, which alters the fibril dynamics and may facilitate fibril dissociation and self-propagation. Our work suggests that the way of protofilament

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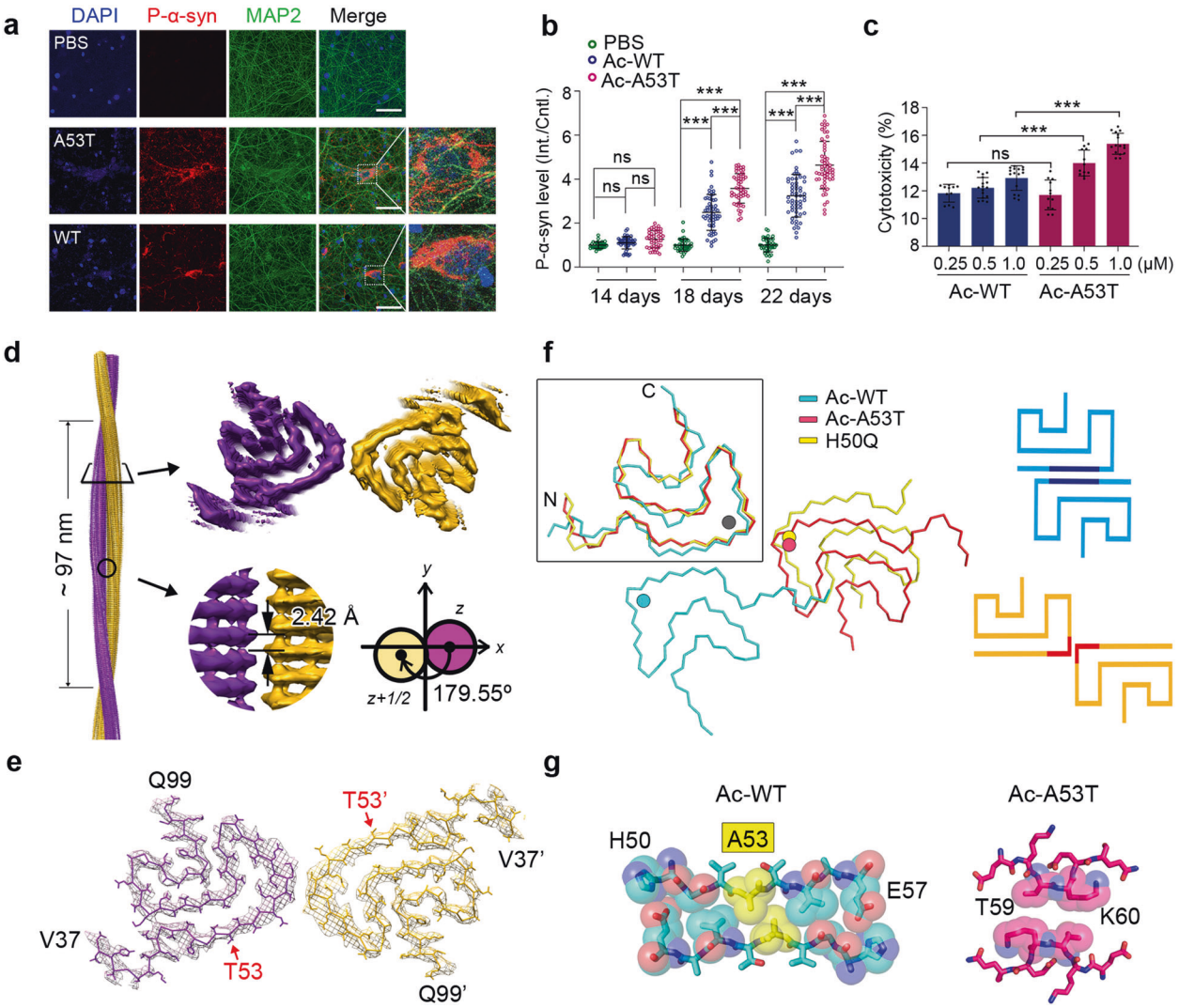

Fig. 1 The cryo-EM structure and pathology of Ac-A53T α-syn fibril. a Confocal microscopic imaging of rat primary cortical neurons treated with 250 nM Ac-A53T or Ac-WT α-syn PFFs at 8 days in vitro (DIV) for 22 days. The fixed neurons were immunostained for DAPI (blue), pS129 α-syn (P-α-syn, red) and microtubule-associated protein 2 (MAP2) (green). Scale bar: 50 μm. **b** Quantitative analysis of the mean gray value of pS129 α-syn from different neuronal samples indicated. > 6 images were randomly taken for each sample. > 6 individual samples were analyzed for each time point. ns, not significant; ***$P < 0.001$ for one-way ANOVA followed by Tukey HSD post-hoc test. **c** The cytotoxicity of Ac-WT and Ac-A53T α-syn PFFs to primary neurons at 8 DIV for 23 days measured by LDH assay. ns not significant; ***$P < 0.001$ for one-way ANOVA followed by Tukey HSD post-hoc test. **d** Cryo-EM 3D reconstruction density map of the Ac-A53T α-syn fibril. Pitch length, helical rise, and twist angle are marked. A cross-section view is shown. The two protofilaments are colored in yellow and purple, respectively. **e** One layer of the Ac-A53T fibril structure model in the density map. T53 is highlighted in red. **f** Overlay of one layer of the Ac-WT (6A6B), Ac-A53T and H50Q (6PES) fibril structures. The structures are aligned based on one subunit of α-syn. The solid circles, colored same as the individual structures, indicate the relative positions of the α-syn subunits in the different structures. Cartoon on the right shows the interfaces between the Greek-key-like subunits in the WT (blue) and mutant (orange) structures. **g** The protofilament interfaces of Ac-WT (cyan) and Ac-A53T (pink) are shown in sticks. Residues involved in the inter-protofilamental interactions are indicated in spheres. A53 in the Ac-WT structure is highlighted in yellow.

bundling is a key factor defining the pathology of α-syn amyloid fibril.

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AUTHOR CONTRIBUTIONS

C.L., K.Z and D.L. designed the project. K.Z and Z.L prepared the fibril samples. Y.S., K.Z and X.S. performed the Cryo-EM experiments and determined the structure of fibrils. S.H. and H.L performed the cellular assays. J.G. and Y.Z performed the mass spectrum-based experiment. All of the authors are involved in analyzing the data and contributed to manuscript discussion and editing. D.L. and C.L. wrote the manuscript.

ADDITIONAL INFORMATION

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Yunpeng Sun[1,2], Shouqiao Hou[1,2], Kun Zhao[1,2], Houfang Long[1,2], Zhenying Liu[1,2], Jing Gao[1,2], Yaoyang Zhang[1,2], Xiao-Dong Su[3], Dan Li[4,5] and Cong Liu[1,2]

[1]Interdisciplinary Research Center on Biology and Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 201210, China; [2]University of Chinese Academy of Sciences, Beijing 100049, China; [3]State Key Laboratory of Protein and Plant Gene Research, and Biomedical Pioneering Innovation Center (BIOPIC), School of Life Sciences, Peking University, Beijing 100871, China; [4]Bio-X-Renji Hospital Research Center, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200240, China and [5]Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders, Ministry of Education, Shanghai Jiao Tong University, Shanghai 200030, China

These authors contributed equally: Yunpeng Sun, Shouqiao Hou, Kun Zhao

Correspondence: Dan Li (lidan2017@sjtu.edu.cn) or Cong Liu (liulab@sioc.ac.cn)

REFERENCES

1. Goedert, M., Spillantini, M. G., Del Tredici, K. & Braak, H. *Nat. Rev. Neurol.* **9**, 13–24 (2013).
2. Luk, K. C. et al. *Science* **338**, 949–953 (2012).
3. Rosborough, K., Patel, N. & Kalia, L. V. *Curr. Neurol. Neurosci. Rep.* **17**, 31 (2017).
4. Polymeropoulos, M. H. et al. *Science* **276**, 2045–2047 (1997).
5. Giasson, B. I. et al. *Neuron* **34**, 521–533 (2002).
6. Narhi, L. et al. *J. Biol. Chem.* **274**, 9843–9846 (1999).
7. Li, Y. et al. *Cell Res.* **28**, 897–903 (2018).
8. Li, B. et al. *Nat. Commun.* **9**, 3609 (2018).
9. Guerrero-Ferreira, R. et al. *eLife* **7**, e36402 (2018).
10. Volpicelli-Daley, L. A., Luk, K. C. & Lee, V. M. *Nat. Protoc.* **9**, 2135–2146 (2014).
11. Boyer, D. R. et al. *Nat. Struct. Mol. Biol.* **26**, 1044–1052 (2019).
12. Sawaya, M. R. et al. *Nature* **447**, 453–457 (2007).
13. Guerrero-Ferreira, R. et al. *eLife* **8**, e48907 (2019).
14. Peng, C. et al. *Nature* **557**, 558–563 (2018).
15. Zhao, K. et al. *bioRxiv* https://www.biorxiv.org/content/10.1101/870758v2 (2019).