Molecular Neurobiology (2019) 56:621–631
https://doi.org/10.1007/s12035-018-1102-0

First-in-Rat Study of Human Alzheimer's Disease Tau Propagation

Tomas Smolek[1,2] · Santosh Jadhav[1,2] · Veronika Brezovakova[1] · Veronika Cubinkova[1,2] · Bernadeta Valachova[1] · Petr Novak[1,3] · Norbert Zilka[1,2]

Received: 24 November 2017 / Accepted: 1 May 2018 / Published online: 16 May 2018
© Springer Science+Business Media, LLC, part of Springer Nature 2018

Abstract

One of the key features of misfolded tau in human neurodegenerative disorders is its propagation from one brain area into many others. In the last decade, in vivo tau spreading has been replicated in several mouse transgenic models expressing mutated human tau as well as in normal non-transgenic mice. In this study, we demonstrate for the first time that insoluble tau isolated from human AD brain induces full-blown neurofibrillary pathology in a sporadic rat model of tauopathy expressing non-mutated truncated tau protein. By using specific monoclonal antibodies, we were able to monitor the spreading of tau isolated from human brain directly in the rat hippocampus. We found that exogenous human AD tau was able to spread from the area of injection and induce tau pathology. Interestingly, solubilisation of insoluble AD tau completely abolished the capability of tau protein to induce and spread of neurofibrillary pathology in the rat brain. Our results show that exogenous tau is able to induce and drive neurofibrillary pathology in rat model for human tauopathy in a similar way as it was described in various mouse transgenic models. Rat tau spreading model has many advantages over mouse and other organisms including size and complexity, and thus is highly suitable for identification of pathogenic mechanism of tau spreading.

Keywords Tau spreading · Alzheimer's disease · Tau strains · Neurofibrillary tangles · Prion-like spreading

Introduction

Progressive accumulation of pathologically modified tau protein in the central nervous system is a defining feature of Alzheimer's disease (AD), Pick's disease, progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), and other human tauopathies [1]. In AD, the tau pathology follows a distinct spatiotemporal pattern, manifesting initially in the entorhinal cortex, followed by hippocampal and cortical regions [2]. The distribution and progression of tau pathology correlates well with the cognitive decline in AD patients [2, 3]. This progression of tau pathology is mediated trans-synaptically by retrograde or anterograde transmission of misfolded tau through anatomically connected regions in the brain [4, 5].

Spreading of tau pathology by injection of brain extract from animal models [6] or AD brain [7] or other tauopathies [7, 8] has been reported. In addition, misfolded tau protein from different tau maladies induces various tau lesions idiosyncratic for the specific human tauopathies [8], suggesting the existence of diverse pathological tau species or 'tauons' in tauopathies, as it was proposed by Michal Novak in 1994 [9]. Furthermore, various tau forms manifesting either as oligomers or fibrils have been shown to induce neurofibrillary pathology in mouse models [10–15]. Moreover, we and others have showed that the 3-repeat and 4-repeat tau isoforms differ in their seeding propensity and require the presence of a proper partner or substrate (e.g., as in these cases, the additional transgenic expression of tau, whether wild type, or altered) to drive disease progression [8, 16]. All this evidence suggests that tau protein itself serves as a pathogenic entity that induces and spreads tau pathology from one neuron to another, following a predictable sequence of transmission.

Although the abovementioned studies support the induction of tau pathology in transgenic mice models [6, 11], these

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12035-018-1102-0) contains supplementary material, which is available to authorized users.

✉ Norbert Zilka
norbert.zilka@savba.sk

[1] Institute of Neuroimmunology, Slovak Academy of Sciences, Centre of Excellence for Alzheimer's Disease and Related Disorders, Dubravska 9, 845 10 Bratislava, Slovak Republic

[2] Axon Neuroscience R&D Services SE, Dvořákovo nábrežie 10, Bratislava, Slovak Republic

[3] Axon Neuroscience CRM Services SE, Dvořákovo nábrežie 10, Bratislava, Slovak Republic

models predominantly express tau protein with a mutation at P301S or P301L [11, 14, 15, 17], which is observed exclusively in FTDP-17, but not in AD. On the other hand, it has been demonstrated that intracerebral inoculation of tau fibrils purified from AD brains resulted in the formation of tau pathology in non-transgenic mice [18]. However, it is important to note, that the amount of disease tau used for injection in the abovementioned study was at about 10 times higher than the concentration of tau used for transgenic mice. To sum up these findings, a high amount of tau, of either exogenous or endogenous origin, seems to be essential for induction and spread of tau pathology in mouse models.

It is not entirely clear whether the ability to induce and drive neurodegeneration by intracerebral injection of diseased tau can be reproduced only in experimental mouse models, or whether it is rather a general feature of diseased tau. In order to address this issue, we employed a rat model of tauopathy expressing human truncated tau that recapitulates AD neurofibrillary degeneration mainly in the brainstem. Interestingly, no neurofibrillary lesions were observed in the hippocampus this model, despite high transgene expression [19]. Here, we demonstrate that misfolded tau isolated from AD brain induced and propagated tau pathology in the hippocampus of said rat model. Tau pathology was able to spread in similar way as it was described previously in mouse models, although in the absence of mutated tau. We found that tau neurofibrillary tangles contain both tau isolated form human brain, and human transgenic and endogenous rat tau. By transforming insoluble tau into a soluble form, we have completely abolished its pathogenic activity. Our results support the idea that the seeding potency of tau is rather general feature tauopathies and can be recapitulated in various rodent species.

Materials and Methods

Human Brain Samples

In this study, frozen human brain tissue samples from parietal cortex of patients clinically diagnosed as having AD and one healthy control brain were used (see Table 1). The neuropathological examination confirmed the presence of numerous NFTs and amyloid plaques in selected brains. All brain samples were procured from respective brain banks in compliance with their material transfer agreements.

Table 1 Details of brain samples used in this study

Case no.	Braak stage	Age	Sex	PM delay (h)
AD1	5	87	F	4
AD2	6	85	M	29
AD3	5	64	M	28
AD4	6	85	M	29
AD5	5	82	F	12
Non-AD	1	82	M	8

Isolation of Sarkosyl-Insoluble Tau

Sarkosyl-insoluble tau was extracted according to published protocols [20, 21]. Cortices from control and AD brains were homogenised in buffer containing 20 mM Tris, 0.8 M NaCl, 1 mM EGTA, 1 mM EDTA, and 10% sucrose supplemented with protease inhibitors (Complete, EDTA free, Roche Diagnostics, USA) and phosphatase inhibitors (1 mM sodium orthovanadate, 20 mM sodium fluoride). After centrifugation at 20,000×*g* for 20 min the supernatant (S1) was collected and small fraction was saved as total protein extract; 40% *w*/*v* of *N*-lauroylsarcosine (sarkosyl) in water was added to a final concentration of 1% and mixed by stirring for 1 h at room temperature. The sample was then centrifuged at 100,000×*g* for 1 h at 25 °C using Beckmann TLA-100 (Beckmann instrument Inc., California, USA). Pellets (P2) were washed and resuspended in PBS to 1/50 volume of S1 fraction and sonicated briefly; 10 μg *w*/*v* of P2 fraction corresponding to S1 fraction was used for SDS-PAGE analysis.

Transmission Electron Microscopy

To visualise the presence of tau filaments in the insoluble preparations, the fractions were adhered to carbon-coated 400 mesh copper grids (Christine Gröpl, Austria). Grids were washed with pure water for 2 min and were negatively stained with 2% uranyl acetate for 1 min (Sigma-Aldrich, St. Louis, Missouri, USA). After multiple washings, the stained grids were examined using an FEI Morgagni 268 electron microscope (Prague, Czech Republic).

Guanidine Hydrochloride Disaggregation of Disease Tau Isolated from Human Brains

Guanidine hydrochloride disaggregation of the sarkosyl insoluble fraction (P2) was performed as previously published with minor modifications [22, 23]. Briefly, 500 μg of insoluble fraction (corresponding to concentration of tau) was solubilised and reduced in 6 M guanidine hydrochloride (GdnHCl, Sigma-Aldrich, St. Louis, Missouri, USA) and 5 mM dithiothreitol with gentle homogenisation for 1 h and alkylated with 15 mM indole acetic acid in the dark for 30 min. After brief centrifugation, the samples were subjected to dialysis in 1xPBS, centrifuged at high speed, and concentrated using a microcon centrifugal filter (Millipore, Slovakia).

SDS-PAGE and Western Blotting

Proteins were resolved on 12% SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked with 5% non-fat free milk in 1×TBS-Tween for 1 h. Following blocking, the membranes were incubated with primary pan-tau antibody DC25 (1:1 dilution in blocking buffer) overnight at 4 °C. Following incubation using horseradish peroxidase (HRP)-conjugated secondary antibody (Dako, Glostrup, Denmark), blots were developed using SuperSignal West Pico Chemiluminescent detection kit (Thermo Scientific, USA) on Image Reader LAS-3000 (Fuji Photo Film Co., Ltd., Japan). Immunoblots were quantified using AIDA Biopackage (Advanced Image Data Analyzer software; Raytest, Germany).

Semi-quantitative estimation of sarkosyl-insoluble tau or solubilised tau was performed using immunoblotting as previously published [16, 24]. Briefly, known amounts of recombinant tau 2N4R and tau fractions were subjected to immunoblotting using pan-tau antibody DC25 and were quantified by densitometry using AIDA Biopackage (Advanced Image Data Analyzer software; Raytest, Germany). The concentration of insoluble tau protein was estimated using a standard curve with reference intensities of known concentrations of recombinant tau 2N4R.

Animals

The experiments were performed on transgenic SHR72 rats [19] which express human truncated tau aa151–391. All animals were housed under standard laboratory conditions with free access to water and food and were kept under diurnal lighting conditions. The animals were anesthetised and sacrificed according to ethical guidelines to minimise pain and suffering of experimental animals. Efforts were made to minimise the number of animals utilised. All experiments were approved by the State Veterinary and Food Committee of Slovak Republic, and by the Ethics Committee of the Institute of Neuroimmunology, Slovak Academy of Sciences.

Experimental Groups

Animals (females, $n = 60$) were allocated to experiments as follows:

- Assessment of the capacity of exogenous tau to induce local pathology: three groups of six animals were injected bilaterally either with 600 ng AD brain insoluble tau fraction (AD1), PBS, or control brain extract. Sacrifice 4 months after application.
- Assessment of the capacity of induced tau pathology to spread beyond the injection site: four groups of three animals, injected unilaterally either with 600 ng of one of three AD brain insoluble tau fraction (AD3, AD4, or AD5), or PBS. Sacrifice 2 months after application.
- Assessment of the impact of solubilisation of PHF tau on its seeding potency: three groups of six animals, injected bilaterally either with 600 ng solubilised or AD brain insoluble tau fraction (AD4), or PBS. Sacrifice 4 months after application.
- Assessment of the impact of dose on spreading: two groups of six animals, injected unilaterally either with 400 or 600 ng AD brain insoluble tau fraction (AD1). Sacrifice 4 months after application.

Stereotaxic Administration

Rats were anesthetised with an intraperitoneal administration of Zoletil (30 mg/kg) and Xylariem (10 mg/kg) and fixed to a stereotaxic apparatus (Kopf Instruments, California, USA); 600 or 400 ng (200 ng/μL) of tau proteins dissolved in PBS were injected using an UltraMicroPump III (UMP III) Microsyringe injector and Micro4 Controller (World Precision Instruments, Florida, USA). Stereotaxic coordinates for the anterodorsal hippocampal injection (A/P: −3.6 mm, M/L: ±2.0 mm, D/V: −2.3 mm) were obtained from a stereotaxic atlas [25]. The left hemisphere was used for unilateral injections.

Immunohistochemistry

Rats were anesthetised and perfused transcardially with phosphate-buffered saline (PBS). Brains were fixed in sucrose solutions (15, 25, and 30% for 24 h each) followed by freezing in 2-methyl butane and stored. Frozen brains were serially cut into 40-μm-thick sections using a cryomicrotome (Leica CM1850, Leica Biosystems). We used coronal sections to examine contralateral spread of the pathology and rest of the studies were performed using sagittal sections. The sections were blocked with Aptum section block (Aptum, UK) followed by incubation with primary antibodies (Table 2) overnight at 4 °C. Sections were stained with biotin-conjugated secondary antibodies and developed using Vectastain ABC Kit (Vector Laboratories, CA, USA). After mounting, sections were evaluated using Olympus BX51 microscope equipped with Olympus DP27 digital camera (Olympus microscope solutions).

Tangle Quantification

For the assessment of the hippocampus, sections were collected from the first section where the hippocampal formation becomes apparent, to the last section where it is still present. For assessment of the brainstem, its entire width was sectioned. For quantification, every 8th section was used (resulting distance of sections 320 μm). Quantification was performed manually, with every tangle in the respective region of interest being counted.

Table 2 List of antibodies used for immunohistochemical analysis in the study

Antibody	Clonality	Dilution	Source
Anti-tau DC39C (epitope aa 434–441)	Mouse monoclonal	1:100	Axon Neuroscience (Bratislava, Slovak Republic)
Anti-tau DC39N1 (epitope aa45–68)	Mouse monoclonal	1:100	Axon Neuroscience (Bratislava, Slovak Republic)
Anti-human phospho tau AT8 (pS202/pT205)	Mouse monoclonal	1:1000	Thermo Scientific (IL, USA)
Anti-phospho tau pT205	Rabbit polyclonal	1:1000	Invitrogen (California, USA)
Anti-phospho tau pT212	Rabbit polyclonal	1:1000	Invitrogen (California, USA)
Anti-phospho tau pS214	Rabbit polyclonal	1:1000	Invitrogen (California, USA)
Anti-tau DC25 (epitope aa 347–353)	Mouse monoclonal	1:1*	Axon Neuroscience (Bratislava, Slovak Republic)

*Supernatent from cultured hybridoma cells was used

Statistical Analysis

Experimental data were analysed using Graph Pad Prism version 6 (GraphPad Software, CA, USA). One-way ANOVA with Tukey's post-test was used for multiple comparisons. An unpaired *t* test was used for comparisons between two groups, and a paired *t* test for within-animal comparisons (e.g., brainstem vs. hippocampus, or contralateral vs. ipsilateral side). Data are presented as dot plots, with error bars denoting mean ± SD. Differences were considered to be statistically significant if $p < 0.05$. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ are used to denote statistical significance.

Results

Characterisation of the Insoluble Tau Fraction Isolated from Human AD Brain Tissue

Analysis of the sarkosyl insoluble tau fraction by immunoblotting using the pan-tau antibody DC25 (epitope aa347–354; Fig. 1a) revealed tau in all fractions isolated from human AD brain tissues (brains AD1–5), but not in the control human brain (not shown). Recombinant 6 human tau isoforms (6i) and human truncated tau (4R) were used as positive control. The A68 tau triplet, which is characteristic of insoluble tau from human AD, was observed in all insoluble tau fractions isolated from AD brains. Using transmission electron microscopy, we confirmed the presence of paired helical filaments (PHF) in all preparations from AD brains (representative image in Fig. 1b, c).

Insoluble Tau from Alzheimer's Disease Brain Induced Tau Pathology in Rat Hippocampus

When injected bilaterally in the hippocampi of transgenic rats, insoluble tau from Alzheimer's disease brain induced full-blown tau pathology. The hippocampus, in this rodent model, does not develop neurofibrillary pathology despite the expression of human truncated tau. Immunohistochemistry revealed phospho-tau immuno-positive structures (antibody AT8) only in the group injected with insoluble AD tau (Fig. 2a, d), but not in animals injected with non-AD control material (Fig. 2b, e) or PBS (Fig. 2c, f). Furthermore, this effect was dose

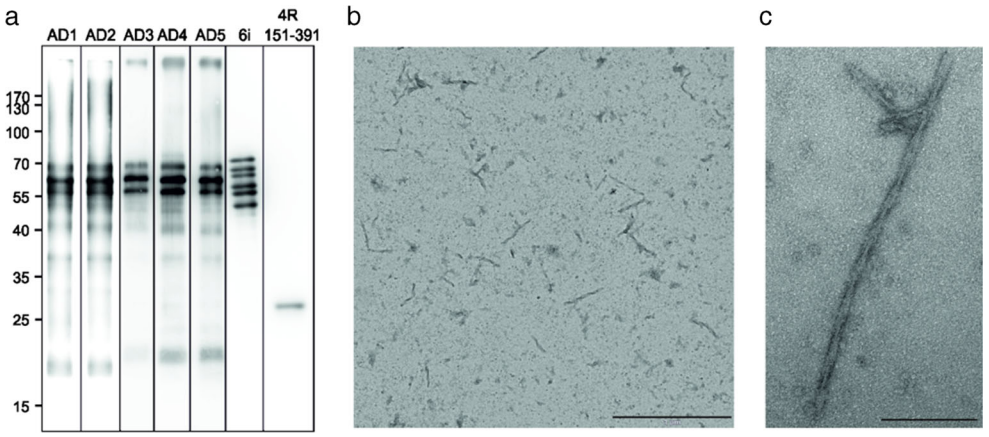

Fig. 1 Characterisation of the sarkosyl-insoluble tau fraction from AD brains. **a** Western blot. Insoluble tau fractions were isolated from AD brain (AD1–5) using sarcosyl 1% *w/v*. Pan-tau antibody DC25 was used for immunostaining. Recombinant human 6 tau isoforms (6i) and truncated tau 4 repeat (4R 151–391) were used as positive controls. **b** Lower magnification electron micrograph show the predominant presence of paired helical filaments (PHF) in insoluble tau preparation. **c** Higher magnification image of paired helical filament. Scale bar: **b** 1 μm and **c** 200 nm

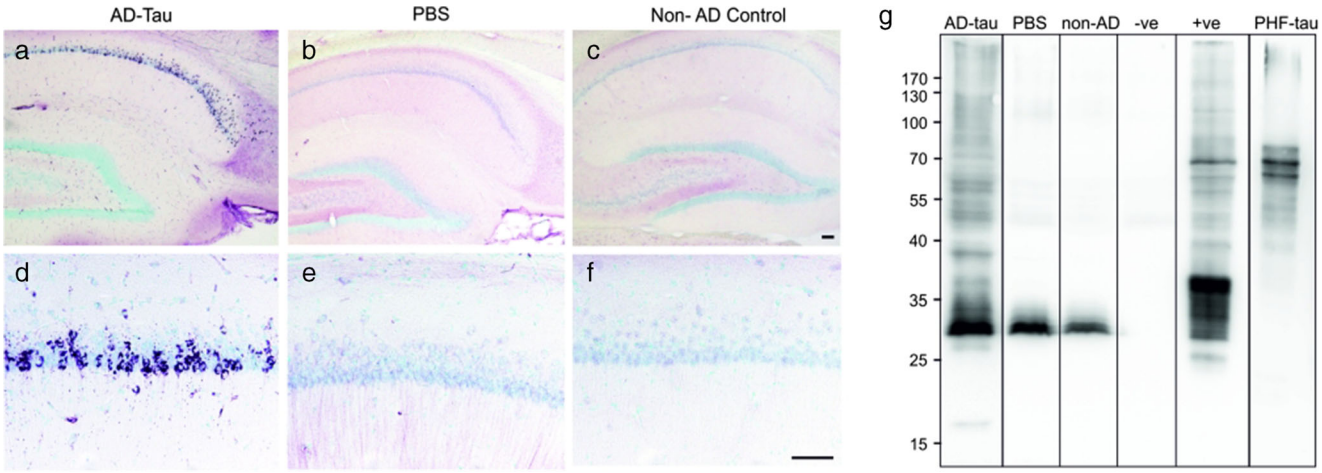

Fig. 2 Analysis of tau pathology in injected groups. Immunohistochemical staining using AT8 antibody reveal the presence of neurofibrillary tangles in groups injected with sarkosyl-insoluble tau from AD brain (**a**, **d**), but not in control PHF (**b**, **e**) or PBS-injected group (**c**, **f**). **g** Western blot analysis of sarkosyl-insoluble tau extracts from hippocampus of injected transgenic rat model. Sarkosyl-insoluble tau was isolated from injected animal groups ($n = 3$). Hippocampus from non-injected transgenic rat model was used as control (PBS). Insoluble tau fraction from brainstem of transgenic rat model (+ve) and PHF-tau were used as positive controls. Insoluble fraction from hippocampus from non-transgenic littermates was used as negative controls (−ve). Scale bar: 100 μm

dependent, we observed numerically higher levels of AT8-positive tangles in animals injected with 600 ng than in those injected with 400 ng (injected side: $p = 0.0018$; uninjected side: $p = 0.93$; Suppl fig. 1). In line with results obtained by immunohistochemistry, robust tau pathology in the hippocampus of animals injected with sarkosyl-insoluble AD tau was observed by immunoblotting using the pan-tau antibody DC25 (Fig. 2g), and no pathology in non-AD control or PBS-injected groups. As positive controls insoluble tau fraction from brainstem from transgenic rat model and AD brains (AD tau) were used. Ponceau staining confirmed that the difference in tau levels measured by immunoblot was not due to differences in loading of proteins (Suppl fig. 2).

In order to characterise the neurofibrillary inclusions in the AD tau injected groups, we performed histological staining using antibodies against tau phospho-epitopes pT212 (Fig. 3c, d), and pS214 (Fig. 3e, f); robust staining was observed with both phospho-tau antibodies. In addition, staining using the DC39C antibody specific for the C-terminus of tau (Fig. 3g, h) which does not recognise transgenic human truncated tau (aa151–391) showed that either endogenous or seeded human misfolded tau or both may be incorporated in the neurofibrillary inclusions in these animals. Gallyas silver staining, a hallmark of tauopathies, confirmed the presence of mature argyrophilic tau lesions in the hippocampus in AD tau injected animals (Fig. 3i, j).

Exogenous Misfolded Tau Protein Is a Component of Neurofibrillary Pathology in Experimental Model

To investigate whether misfolded human tau protein from AD brains was incorporated in the neurofibrillary inclusions in rats, we performed immunostaining of brain sections using the human-specific N-terminal tau antibody DC39N1 [26]; widespread DC39N1 staining of neurofibrillary tangles generated in the hippocampus shows this to be the case (Fig. 4b, d). The DC39N1 staining was less pronounced than AT8 staining (Fig. 4a, c). Interestingly, AT8 revealed degenerating neurons with early and late neurofibrillary tangles, as well as neuropil threads. On the contrary, DC39N1 antibody recognised solely mature tangles; the staining was mainly restricted to the soma (Fig. 4e, f). However, the number of AT8-positive hippocampal tangles was significantly higher than the number of DC39N1-positive tangles ($p = 0.021$; Fig. 4i).

In order to confirm that the specificity of the antibody to human tau N-terminus in transgenic rat brain, we immunostained hippocampus, that contains injected human AD tau and brainstem, where we did not inject AD tau. The transgenic line innately develops AT8-positive inclusions in the brainstem early on, and they are DC39N1 negative. As suspected, we did not observe any DC39N1-positive tau inclusions (Fig. 4h) in the brainstem, while numerous AT8-positive neurofibrillary tangles were present (Fig. 4g). Mapping the topographical distribution of neurofibrillary pathology (Fig. 4j) revealed propagation of pathology from the site of injection (CA1) to adjacent regions both in the rostral (i.e., towards CA2) and caudal direction (towards the subiculum). Interestingly, the distribution of human tau-specific DC39N1-positive inclusions closed mimicked the distribution of AT8-positive tangles suggesting the dissemination of exogenous human misfolded tau from the site of injection and its inclusion in neurofibrillary lesions at distant sites.

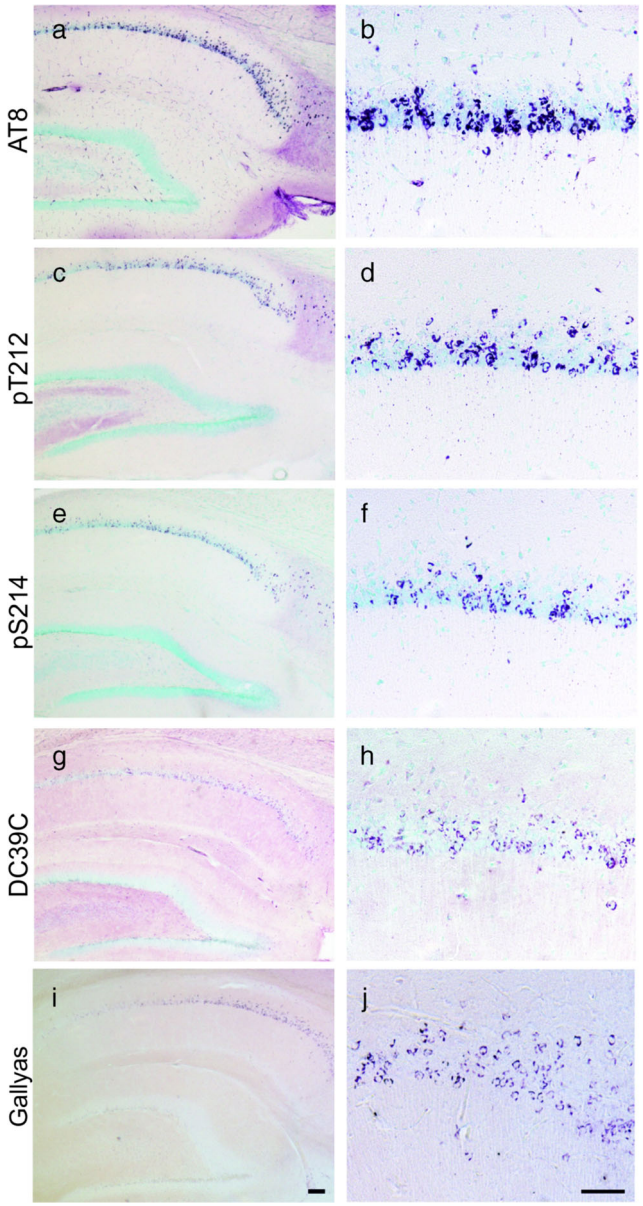

Fig. 3 Neurofibrillary pathology in AD tau injected group is mature and is phosphorylated in AD-specific tau phospho-sites. Immunohistographs showing neurofibrillary tangles immunoreactive with AT8 (**a**, **b**), pT212 (**c**, **d**), pS214 (**e**, **f**), and antibody DC39C (**g**, **h**). **i**, **j**. Mature neurofibrillary tangles in the form of Gallyas-positive argyrophilia were observed in these animals. Scale bar: 100 μm

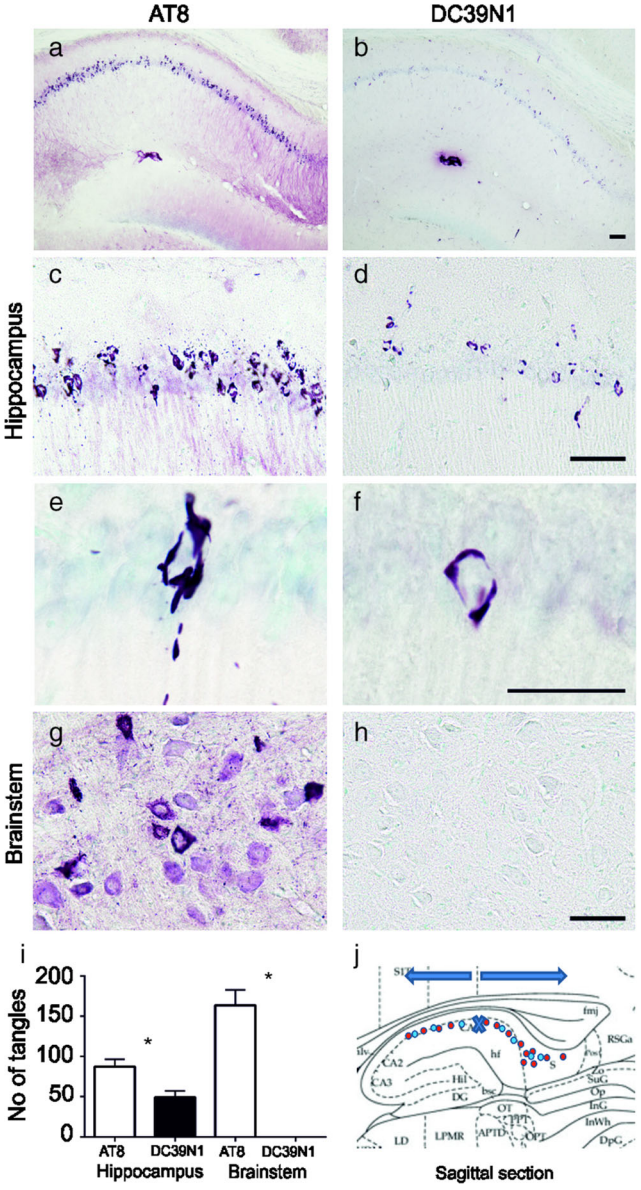

Fig. 4 Human tau is a component of hippocampal neurofibrillary inclusions in the AD tau injected animals. Lower and higher magnification immunohistographs showing staining using AT8 antibody (**a**, **c**, and **e)** or human tau specific antibody DC39N1 (**b**, **d**, and **f**) in the hippocampus (**a**–**f**) and brainstem (**g**, **h**). Histographs reveal the presence of human tau in the NFTs in hippocampus (injected region); intrinsic tangles in the brainstem of AD tau-injected animals shown for comparison (**g**, **h**). **i** Graphs showing the number of AT8 or DC39N1-positive tangles in the hippocampus and brainstem of injected animals. Higher numbers of AT8-positive tangles are evident in both regions; tangles in the brainstem do not contain DC39N1-positive injected AD tau at all. **j** Depiction of the distribution of AT8 (red) and DC39N1 (green)-positive tangles in the hippocampus and adjacent regions in the groups injected with insoluble tau

Misfolded Tau from Different AD Patients Displayed Different Seeding and Spreading Properties

We were intrigued to find out whether tau protein from different AD brains differs in its seeding potency. We isolated sarkosyl-insoluble tau from three different AD brains (AD3, 4, 5; Fig. 5a) with similar post-mortem delay (Table 1); 600 ng of insoluble tau was injected (unilaterally) into hippocampus (CA1) of SHR72 transgenic rats and spreading assessed 2 months later. We observed AT8-positive structures in all groups inoculated with AD tau (Fig. 5b–g), in and around the site of injection, albeit to a varied degree (Fig. 5h). Insoluble tau from AD4 showed higher numbers of tangles around the site of injection (AD3 vs AD4: $p < 0.05$). The

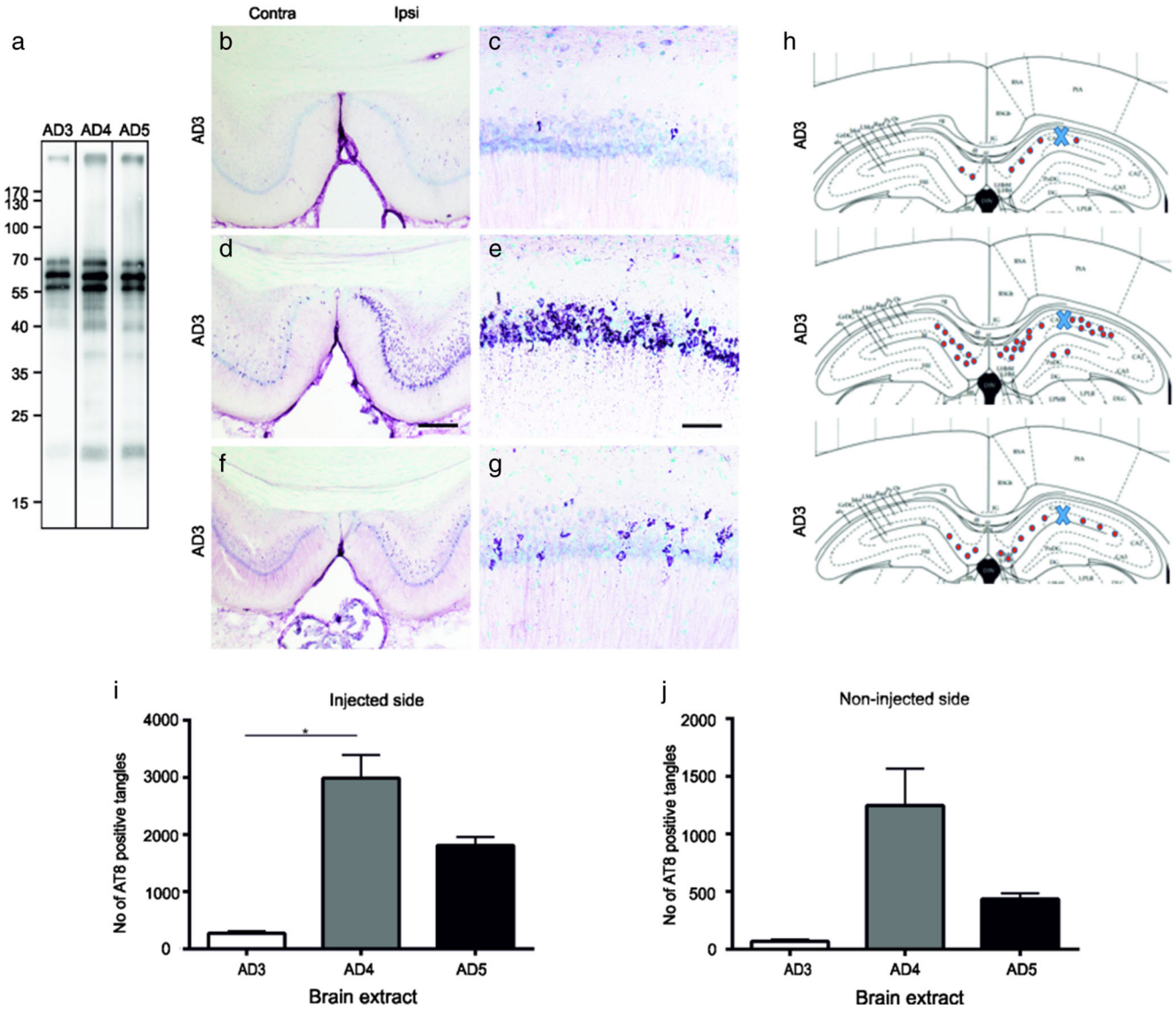

Fig. 5 Comparative analysis of pathology in animals injected with insoluble tau isolates from three different AD brains. **a** Extract of blot from Fig. 1b showing insoluble tau fraction used for comparative analysis. Coronal sections showing AT8-positive structures were observed in injected as well as non-injected sites (marked by *) of hippocampus with AD tau tau extracts from **b** AD3, **d** AD4, and **f** AD5, scale bar: 200 μm. **c**, **e**, **g** Higher magnification images are shown in B, D, and F, respectively, scale bar: 100 μm. **h** Scheme showing the distribution of tau pathology in injected and non-injected regions after injection of AD tau extract from three different brains. **i**, **j** Graph showing differences between the numbers of AT8-positive tangles in groups injected with different brain extracts. *p* values: $*p < 0.05$

capacity of the AD brain extract to induce tau spreading was confirmed by the fact that we also observed AT8-positive structures in the non-injected (contralateral) hippocampal regions in these animals (asterisk), yet, as expected, at a significantly lower level than on the injected side (paired *t* test; $p = 0.0047$).

Guanidine Hydrochloride Disaggregation Mitigates Seeding Properties of Tau

One of the aims of the study was to investigate the property of misfolded tau protein which contributes to the spreading of pathology in the affected brain. We speculated that insolubility of the misfolded tau protein could be one of the key properties that confer its seeding potency. To test this hypothesis, we treated the insoluble tau preparation using guanidium hydrochloride (GdnHCl) and injected the resulting material in the hippocampus of transgenic rats. Post-disaggregation, we confirmed the presence of tau protein in the fraction using pan-tau antibody DC25 (Fig. 6a). The pattern of treated insoluble tau protein exhibited smear of high and low molecular tau species as previously described, with a doublet at 64 kDa and reduced intensities at 60 and 68 kDa [27, 28]. However, we observed that in comparison to the insoluble tau fraction, the disaggregated tau fraction was devoid of high molecular tau aggregates (asterisk).

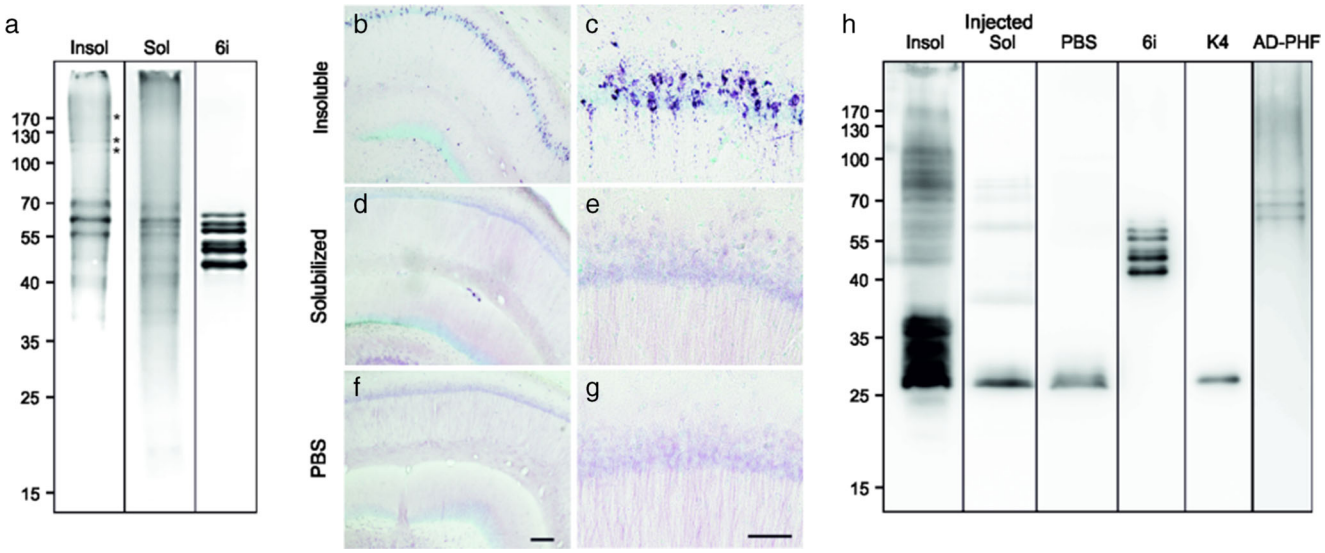

Fig. 6 Comparison of tau pathology after injection of insoluble and disaggregated tau. **a** Western blot analysis of insoluble and solubilised tau fractions using pan-tau antibody DC25. Note the absence of high molecular weight tau species (*) in the solubilised fraction and presence of low molecular weight bands in the solubilised groups. Six recombinant human tau isoforms (6i) were used as control. Immunohistographs using AT8 antibody reveal the presence of neurofibrillary tangles only in groups injected with insoluble tau (**b**, **c**) but not in those injected with solubilised tau (**d**, **e**) or PBS (**f**, **g**). **h** *Hippocampus* extracts from injected animals. Injection of insoluble tau induced tau pathology results in aggregation of tau protein in the hippocampus; such pathology was absent in the groups injected with PBS or solubilised tau. Recombinant 6 human tau isoforms, truncated 4R tau 151–391, and PHF-tau were used as positive control. Scale bar: 100 μm

We injected 600 ng (200 ng/μL) of insoluble or disaggregated tau fraction (unilaterally) in the hippocampus of transgenic line expressing human truncated tau at 2 months of age and assessed spreading 4 months post-injection. Interestingly, using immunohistochemistry we did not observe AT8-positive neurofibrillary structures in hippocampus (in CA1) of transgenic rats injected with treated tau (Fig. 6d, e) or PBS injected groups (Fig. 6f, g) when compared to the group injected with insoluble tau (Fig. 6b, c). Using the pan-tau antibody DC25 in immunoblotting, we observed high molecular tau species in the hippocampus of transgenic rats only in the group injected with insoluble tau, but not in in animals who received disaggregated tau or PBS (Fig. 6h).

Discussion

Progressive accumulation of tau protein and transmission of tau pathology across anatomically connected regions forms correlates well with cognitive decline in Alzheimer's disease [3, 29, 30]. The process of tau transmission is mainly regulated by several mechanisms such as exocytosis [31], synaptic discharge [32, 33], or other means of release into the extracellular milieu and subsequent uptake by neighbouring neurons [34]. Several independent studies demonstrated that diseased forms of tau isolated from human AD brains can induce and drive neurofibrillary degeneration either in transgenic mouse models expressing mutant tau or in non-transgenic mice. Various forms of tau protein, like fibrils [11, 14, 15], filament forms [7, 8], oligomers [13] either insoluble [7, 11, 14–16] or soluble [6, 13], differ in their seeding and transmission properties. Moreover, intracerebral injection of misfolded tau protein from different tauopathies led to the formation of fibrillary lesions characteristic for individual tauopathies [7, 8].

All of abovementioned studies were performed exclusively on mouse models suggesting that this specific feature of diseased tau can be influenced by mouse brain microenvironment. In this study, we utilised a rat model for human tauopathy that expresses human 4-repeat truncated tau protein [19]. In general, rats are more resistant to amyloid or tau-induced neurodegeneration in comparison to mice. While mice expressing either mutant forms of APP, PS1, PS2 or tau usually developed a large number of plaques or tangles, rats needs higher expression in order to develop pathological lesions. Several transgenic rat strains that express either human wild type [35] or mutant APP [36–41] have been generated. Strikingly, the majority of these models failed to develop β-amyloid plaques. The only transgenic rat model showing amyloid pathology is based on the expression of human amyloid precursor protein (APP) with the familial AD (FAD) mutations K670N/M671L and K670N/M671L/V717I and human presenilin-1 (PS-1) transgene with the FAD M146V mutation [38]. The similar situation is also in the case of mutated tau where no single rat model expressing mutant tau developed tau pathology [42]. This means that replication of mouse phenotype in rats is not as straightforward as one might expect.

The model used in the study is characterised by the presence of tau neurofibrillary lesions in the brainstem, but not in the hippocampus despite expression of human truncated tau. Through intracerebral administration of human AD tau, we induced full-blown tau pathology in the hippocampus. The neurofibrillary inclusions contained human transgenic tau and rat endogenous tau; the majority of tau is hyperphosphorylated at multiple phospho-sites pS202-pT205 (AT8), pT212, and pS214. Importantly, at least a portion of NFTs was argyrophilic, i.e. at the late stage of tangle development, which suggests that the full tau neurodegenerative cascade is replicated in the model.

The rat model showed several interesting and potentially very valuable specifics in the present study. For example, about 50% of induced hippocampal neurofibrillary tangles contain besides transgenic and endogenous rat tau also human tau originated from AD brain. The presence of exogenous human tau was identified with an antibody recognising a human-specific portion of the N-terminus, which is absent on the transgene. The antibody recognised hippocampal NFTs following injection of exogenous AD tau. This is in sharp contrast to earlier reports, which showed the absence of injected tau in the neurofibrillary inclusions [6, 8]. This could be explained by the fact that the rat tau proteome consists of six isoforms, identically to humans, and has high sequence homology with human tau [43], thus allowing stable interactions between human and rat tau proteins. Our results showed that the misfolded human tau protein can act as a driver in the initial stage of neurodegeneration; later on, it becomes a stable component of the diseased tau proteome.

We tested three different AD brain isolates for their ability to induce and spread tau pathology. In terms of the ability to seed and propagate tau pathology, the insoluble tau from different AD brains displayed quantitative differences. We found that tau extracted from the brain AD4 induced the highest number of NFTs at the injection site when compared to two other insoluble tau extracts (AD2 and AD4) despite of the same amount of diseased tau injected into the hippocampus. Our results suggest the existence of multiple tau species or strains–tauons–in various AD brains. It is known that the rate of progression of AD between patients can vary pronouncedly [44, 45]. We speculate that besides several other factors (such as genetic pre-dispositions, the pattern and rate of brain atrophy, and vascular changes), the difference in the seeding and propagation potency of misfolded tau strains could be one of the reasons for this heterogeneity in the patients. Investigating the spreading properties of various tau strains and understanding the underpinning mechanisms that allow some strains to spread more efficiently is a promising approach towards the development of therapeutics that seek to arrest the progression of the disease process in tauopathies.

This study and many others [7, 11, 15, 16] demonstrated that sonicated insoluble tau protein contains a group of tau species displaying seeding properties. The stability of the sarkosyl-insoluble tau is dependent on the salt bridges reinforced by hydrophobic interactions [46]. We therefore treated insoluble tau by using GdnHCl, a weak interacting molecule widely used as chemical denaturant to study the native folding and refolding state of proteins [47–49]. Disaggregation of insoluble tau reduced the number of high molecular weight tau aggregates, and concomitantly diminished the seeding and transmissivity potency of tau protein in the brain. The treatment of insoluble tau by GdnHCl induces a dynamic state of tau protein, which consists of a structurally diverse ensemble of unfolded conformations [50]. It does so by interacting with the hydrophobic regions of the protein, thereby exposing it to the solvent. Therefore, direct electrostatic interaction—but not covalent modification—is responsible for the structural transition of proteins [51]. Furthermore, the phosphorylation sites on the tau protein remain intact after GdnHCl treatment [52]. This indicates that the conformation (rather than phosphorylation) of insoluble tau protein determines the seeding potency. Our results are consistent with an earlier study reporting that high molecular weight sarkosyl-insoluble tau proteins are key mediators of tau pathology [50]. Recent research also shows that high molecular weight tau aggregates are key mediators in seeding and propagation of tau pathology [53]. We observed an absence of high molecular weight tau after disaggregation of insoluble tau, indicating a lack of seed competent tau species in our preparation. Our results suggest that the insolubility and stability of tau aggregates is a key pathological property of diseased tau that is essential for seeding and propagation of misfolded tau protein in human neurodegenerative maladies.

The insoluble tau preparation from AD brain contains both filaments and oligomers. It has been previously shown that paired helical filaments or oligomers contribute to seeding competency in rodents [8, 10, 13, 53, 54]. Moreover, it is also argued that tau oligomers from AD are more efficient that filaments in seeding phenomenon [13] and vice versa [53]. We speculate that oligomers, which arise during brief sonication employed in the preparation of insoluble tau fraction, may be partly responsible for the seeding potency in the animal model. However, based on our results, it is difficult to conclude the species of tau or 'tauons' are specifically responsible for the seeding and spreading potency in this study.

To summarise, we have successfully performed in vivo tau spreading experiments in a rat model via hippocampal injection of tau protein isolated from human AD brains. The exogenous human AD tau protein not only initiates and propagates tau pathology but also forms an inherent component of neurofibrillary inclusions in this transgenic rat model of tauopathy. We have demonstrated that the pathological tau proteomes from different AD brains vary in their seeding and propagation properties. In addition, we show that insolubility of misfolded tau protein is a key predisposition for

seeding and propagation properties. Taken together, these data highlight the rat model as a useful tool for further identification of mechanisms of tau transmission, whether by using imaging technologies or proteomic approaches.

Acknowledgements The authors wish to thank to the brain banks from Newcastle, London and Kuopio for providing the brain tissues.

Funding The work is supported by research grants JPND ReFRAME, EU structural fund 26240220046, APVV-14-0872, VEGA 2/0164/16 and 2/0181/17, and Axon Neuroscience R&D Services SE.

Compliance with Ethical Standards

Conflicts of Interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Williams DR (2006) Tauopathies: classification and clinical update on neurodegenerative diseases associated with microtubule-associated protein tau. Intern Med J 36(10):652–660. https://doi.org/10.1111/j.1445-5994.2006.01153.x
2. Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 82(4):239–259
3. Nelson PT, Alafuzoff I, Bigio EH, Bouras C, Braak H, Cairns NJ, Castellani RJ, Crain BJ et al (2012) Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature. J Neuropathol Exp Neurol 71(5):362–381. https://doi.org/10.1097/NEN.0b013e31825018f7
4. de Calignon A, Polydoro M, Suárez-Calvet M, William C, Adamowicz DH, Kopeikina KJ, Pitstick R, Sahara N et al (2012) Propagation of tau pathology in a model of early Alzheimer's disease. Neuron 73:685–697. https://doi.org/10.1016/j.neuron.2011.11.033
5. Liu L, Drouet V, Wu JW, Witter MP, Small SA, Clelland C, Duff K (2012) Trans-synaptic spread of tau pathology in vivo. PLoS One 7:e31302. https://doi.org/10.1371/journal.pone.0031302
6. Clavaguera F, Bolmont T, Crowther RA, Abramowski D, Frank S, Probst A, Fraser G, Stalder AK et al (2009) Transmission and spreading of tauopathy in transgenic mouse brain. Nat Cell Biol 11:909–913. https://doi.org/10.1038/ncb1901
7. Boluda S, Iba M, Zhang B, Raible KM, Lee VM, Trojanowski JQ (2015) Differential induction and spread of tau pathology in young PS19 tau transgenic mice following intracerebral injections of pathological tau from Alzheimer's disease or corticobasal degeneration brains. Acta Neuropathol 129:221–237. https://doi.org/10.1007/s00401-014-1373-0
8. Clavaguera F, Akatsu H, Fraser G, Crowther RA, Frank S, Hench J, Probst A, Winkler DT et al (2013) Brain homogenates from human tauopathies induce tau inclusions in mouse brain. Proc Natl Acad Sci U S A 110:9535–9540. https://doi.org/10.1073/pnas.1301175110
9. Novak M (1994) Truncated tau protein as a new marker for Alzheimer's disease. Acta Virol 38:173–189
10. Hu W, Zhang X, Tung YC, Xie S, Liu F, Iqbal K (2016) Hyperphosphorylation determines both the spread and the morphology of tau pathology. Alzheimers Dement 12(10):1066–1077. https://doi.org/10.1016/j.jalz.2016.01.014
11. Iba M, Guo JL, McBride JD, Zhang B, Trojanowski JQ, Lee VM (2013) Synthetic tau fibrils mediate transmission of neurofibrillary tangles in a transgenic mouse model of Alzheimer's-like tauopathy. J Neurosci 33:1024–1037. https://doi.org/10.1523/JNEUROSCI.2642-12.2013
12. Kfoury N, Holmes BB, Jiang H, Holtzman DM, Diamond MI (2012) Trans-cellular propagation of tau aggregation by fibrillar species. J Biol Chem 287:19440–19451. https://doi.org/10.1074/jbc.M112.346072
13. Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Guerrero-Munoz MJ, Kiritoshi T, Neugebauer V, Jackson GR, Kayed R (2012) Alzheimer brain-derived tau oligomers propagate pathology from endogenous tau. Sci Rep 2:700. https://doi.org/10.1038/srep00700
14. Peeraer E, Bottelbergs A, Van Kolen K, Stancu IC, Vasconcelos B, Mahieu M et al (2015) Intracerebral injection of preformed synthetic tau fibrils initiates widespread tauopathy and neuronal loss in the brains of tau transgenic mice. Neurobiol Dis 73:83–95. https://doi.org/10.1016/j.nbd.2014.08.032
15. Wu JW, Herman M, Liu L, Simoes S, Acker CM, Figueroa H, Steinberg JI, Margittai M et al (2013) Small misfolded tau species are internalized via bulk endocytosis and anterogradely and retrogradely transported in neurons. J Biol Chem 288:1856–1870. https://doi.org/10.1074/jbc.M112.394528
16. Levarska L, Zilka N, Jadhav S, Neradil P, Novak M (2013) Of rodents and men: the mysterious interneuronal pilgrimage of misfolded protein tau in Alzheimer's disease. J Alzheimers Dis 37:569–577. https://doi.org/10.3233/JAD-131106
17. Ahmed Z, Cooper J, Murray TK, Garn K, McNaughton E, Clarke H, Parhizkar S, Ward MA et al (2014) A novel in vivo model of tau propagation with rapid and progressive neurofibrillary tangle pathology: the pattern of spread is determined by connectivity, not proximity. Acta Neuropathol 127:667–683. https://doi.org/10.1007/s00401-014-1254-6
18. Guo JL, Narasimhan S, Changolkar L, He Z, Stieber A, Zhang B, Gathagan RJ, Iba M et al (2016) Unique pathological tau conformers from Alzheimer's brains transmit tau pathology in nontransgenic mice. J Exp Med 213(12):2635–2654
19. Koson P, Zilka N, Kovac A, Kovacech B, Korenova M, Filipcik P, Novak M (2008) Truncated tau expression levels determine life span of a rat model of tauopathy without causing neuronal loss or correlating with terminal neurofibrillary tangle load. Eur J Neurosci 28:239–246. https://doi.org/10.1111/j.1460-9568.2008.06329.x
20. Greenberg SG, Davies PA (1990) A preparation of Alzheimer paired helical filaments that displays distinct tau proteins by polyacrylamide gel electrophoresis. Proc Natl Acad Sci U S A 87:5827–5831
21. Jadhav S, Katina S, Kovac A, Kazmerova Z, Novak M, Zilka N (2015) Truncated tau deregulates synaptic markers in rat model for human tauopathy. Front Cell Neurosci 9:24. https://doi.org/10.3389/fncel.2015.00024
22. Roher AE, Palmer KC, Chau V, Ball MJ (1998) Isolation and chemical characterization of Alzheimer's disease paired helical filament cytoskeletons: differentiation from amyloid plaque core protein. Js Cell Biol 107:2703–2716
23. Zilka N, Kovacech B, Barath P, Kontsekova E, Novak M (2012) The self-perpetuating tau truncation circle. Biochem Soc Trans 40:681–686. https://doi.org/10.1042/BST20120015
24. Forest SK, Acker CM, d'Abramo C, Davies P (2013) Methods for measuring tau pathology in transgenic mouse models. J Alzheimers Dis 33:463–471. https://doi.org/10.3233/JAD-2012-121354
25. Paxinos G, Watson C (1996) The rat brain in stereotaxic coordinates. Academic Press, Chicago
26. Soltys K, Rolkova G, Vechterova L, Filipcik P, Zilka N, Kontsekova E, Novak M (2005) First insert of tau protein is present

in all stages of tau pathology in Alzheimer's disease. Neuroreport 16:1677–1681
27. Smith MA, Siedlak SL, Richey PL, Nagaraj RH, Elhammer A, Perry G (1996) Quantitative solubilization and analysis of insoluble paired helical filaments from Alzheimer disease. Brain Res 717:99–108
28. Yang LS, Gordon-Krajcer W, Ksiezak-Reding H (1997) Tau released from paired helical filaments with formic acid or guanidine is susceptible to calpain-mediated proteolysis. J Neurochem 69:1548–1558
29. Brier MR, Gordon B, Friedrichsen K, McCarthy J, Stern A, Christensen J, Owen C, Aldea P et al (2016) Tau and Aβ imaging, CSF measures, and cognition in Alzheimer's disease. Sci Transl Med 8(338):338ra66. https://doi.org/10.1126/scitranslmed.aaf2362
30. Murray ME, Lowe VJ, Graff-Radford NR, Liesinger AM, Cannon A, Przybelski SA, Rawal B, Parisi JE et al (2015) Clinicopathologic and 11C-Pittsburgh compound B implications of Thal amyloid phase across the Alzheimer's disease spectrum. Brain 138(Pt 5):1370–1381. https://doi.org/10.1093/brain/awv050
31. Saman S, Kim W, Raya M, Visnick Y, Miro S, Saman S, Jackson B, McKee AC et al (2012) Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid in early Alzheimer disease. J Biol Chem 287:3842–3849. https://doi.org/10.1074/jbc.M111.277061
32. Calafate S, Buist A, Miskiewicz K, Vijayan V, Daneels G, de Strooper B, de Wit J, Verstreken P et al (2015) Synaptic contacts enhance cell-to-cell tau pathology propagation. Cell Rep 11:1176–1183. https://doi.org/10.1016/j.celrep.2015.04.043
33. Sokolow S, Henkins KM, Bilousova T, Gonzalez B, Vinters HV, Miller CA, Cornwell L, Poon WW et al (2015) Pre-synaptic C-terminal truncated tau is released from cortical synapses in Alzheimer's disease. J Neurochem 133:368–379. https://doi.org/10.1111/jnc.12991
34. Chai X, Dage JL, Citron M (2012) Constitutive secretion of tau protein by an unconventional mechanism. Neurobiol Dis 48:356–366. https://doi.org/10.1016/j.nbd.2012.05.021
35. Clarke J, Thornell A, Corbett D, Soininen H, Hiltunen M, Jolkkonen J (2007) Overexpression of APP provides neuroprotection in the absence of functional benefit following middle cerebral artery occlusion in rats. Eur J Neurosci 26(7):1845–1852
36. Agca C, Fritz JJ, Walker LC, Levey AI, Chan AW, Lah JJ, Agca Y (2008) Development of transgenic rats producing human beta-amyloid precursor protein as a model for Alzheimer's disease: transgene and endogenous APP genes are regulated tissue-specifically. BMC Neurosci 9:28
37. Echeverria V, Ducatenzeiler A, Alhonen L, Janne J, Grant SM, Wandosell F, Muro A, Baralle F et al (2004) Rat transgenic models with a phenotype of intracellular Abeta accumulation in hippocampus and cortex. J Alzheimers Dis 6(3):209–219
38. Flood DG, Lin YG, Lang DM, Trusko SP, Hirsch JD, Savage MJ, Scott RW, Howland DS (2009) A transgenic rat model of Alzheimer's disease with extracellular Abeta deposition. Neurobiol Aging 30(7):1078–1090
39. Folkesson R, Malkiewicz K, Kloskowska E, Nilsson T, Popova E, Bogdanovic N, Ganten U, Ganten D et al (2007) A transgenic rat expressing human APP with the Swedish Alzheimer's disease mutation. Biochem Biophys Res Commun 358(3):777–782
40. Liu L, Orozco IJ, Planel E, Wen Y, Bretteville A, Krishnamurthy P, Wang L, Herman M et al (2008) A transgenic rat that develops Alzheimer's disease-like amyloid pathology, deficits in synaptic plasticity and cognitive impairment. Neurobiol Dis 31(1):46–57
41. Ruiz-Opazo N, Kosik KS, Lopez LV, Bagamasbad P, Ponce LR, Herrera VL (2004) Attenuated hippocampus-dependent learning and memory decline in transgenic TgAPPswe Fischer-344 rats. Mol Med 10(1–6):36–44
42. Korhonen P, van Groen T, Thornell A, Kyrylenko S, Soininen ML, Ojala J, Peltomaa E, Tanila H et al (2011) Characterization of a novel transgenic rat carrying human tau with mutation P301L. Neurobiol Aging 32(12):2314–2315
43. Hanes J, Zilka N, Bartkova M, Caletkova M, Dobrota D, Novak M (2009) Rat tau proteome consists of six tau isoforms: implication for animal models of human tauopathies. J Neurochem 108:1167–1176. https://doi.org/10.1111/j.1471-4159.2009.05869.x
44. Chui HC (1987) The significance of clinically defined subgroups of Alzheimer's disease. J Neural Transm Suppl 24:57–68
45. Thalhauser CJ, Komarova NL (2012) Alzheimer's disease: rapid and slow progression. J R Soc Interface 9(66):119–126. https://doi.org/10.1098/rsif.2011.0134
46. Andronesi OC, von Bergen M, Biernat J, Seidel K, Griesinger C, Mandelkow E, Baldus M (2008) Characterization of Alzheimer's-like paired helical filaments from the core domain of tau protein using solid-state NMR spectroscopy. J Am Chem Soc 130:5922–5928. https://doi.org/10.1021/ja7100517
47. Ahmad F, Yadav D, Taneja S (1992) Determining stability of proteins from guanidinium chloride transition curves. Biochem J 287:481–485
48. Camilloni C, Rocco AG, Eberini I, Gianazza E, Broglia RA, Tiana G (2008) Urea and guanidinium chloride denature protein L in different ways in molecular dynamics simulations. Biophys J 94:4654–4661. https://doi.org/10.1529/biophysj.107.125799
49. England JL, Haran G (2011) Role of solvation effects in protein denaturation: from thermodynamics to single molecules and back. Annu Rev Phys Chem 62:257–277. https://doi.org/10.1146/annurev-physchem-032210-103531
50. Falcon B, Cavallini A, Angers R, Glover S, Murray TK, Barnham L, Jackson S, O'Neill MJ et al (2015) Conformation determines the seeding potencies of native and recombinant tau aggregates. J Biol Chem 290(2):1049–1065. https://doi.org/10.1074/jbc.M114.589309
51. O'Brien E, Dima R, Brooks B, Thirumalai D (2007) Interactions between hydrophobic and ionic solutes in aqueous guanidinium chloride and urea solutions: lessons for protein denaturation mechanism. J Am Chem Soc 129:7346–7353. https://doi.org/10.1021/ja069232+
52. Hanger DP, Gibb GM, de Silva R, Boutajangout A, Brion JP, Revesz T, Lees AJ, Anderton BH (2002) The complex relationship between soluble and insoluble tau in tauopathies revealed by efficient dephosphorylation and specific antibodies. FEBS Lett 531:538–542
53. Jackson SJ, Kerridge C, Cooper J, Cavallini A, Falcon B, Cella CV, Landi A, Szekeres PG et al (2016) Short fibrils constitute the major species of seed-competent tau in the brains of mice transgenic for human P301S tau. J Neurosci 36(3):762–772. https://doi.org/10.1523/JNEUROSCI.3542-15.2016
54. Smolek T, Jadhav S, Valachova B, Vogels T, Legath J, Novak P, Zilka N (2017) Transmission of tau pathology from human to rodent brain: How to humanise animal models for Alzheimer's disease research. J Alzheimers Dis Parkinsonism 7:400. https://doi.org/10.4172/2161-0460.1000400