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Untangling tau-related dementia

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Abundant cytoplasmic inclusions consisting of aggregated hyperphosphorylated protein tau are a characteristic pathological observation in several neurodegenerative disorders such as Alzheimer's disease, Pick's disease, frontotemporal dementia, cortico-basal degeneration and progressive supranuclear palsy. The recent finding that mutations in the tau gene are responsible for frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) has provided convincing evidence that tau protein plays a key role in neurodegeneration. In the short period since the identification of pathogenic mutations in tau, remarkable progress has been made in understanding some of the mechanisms by which these mutations lead to neurodegeneration. Understanding the disease processes will hopefully provide us with new leads in developing effective therapies for dementia.

INTRODUCTION

In our aging population, dementia is posing a growing social and economic problem. Prevalence values for dementia rise from 5 to more than 30% between the ages of 65 and 85 years, affecting more than 20 million people world-wide, a number expected to double within the next 30 years. Progress in therapeutic approaches has been limited but genetic studies have resulted in the identification of genes involved in the pathogenesis of dementia. Although mutations in these genes explain only a small proportion of cases, the findings have been of great importance in our understanding of the disease process.

Alzheimer's disease (AD) is the most frequent form of dementia. In brains of deceased patients extracellular neuritic plaques and intracellular neurofibrillary tangles (NFTs) can be observed. Abnormal aggregates of amyloid-β peptide form the main component of neuritic plaques, and amyloid metabolism plays an important role in the etiology and pathogenesis of AD. The abundance of amyloid deposits, however, does not correlate well with observed neurodegeneration. In contrast, the abundance of NFTs, consisting of hyperphosphorylated microtubule-associated protein tau, correlates well with observed neurodegeneration.

Tau pathology is also a characteristic observation in several other neurodegenerative disorders, and the recent finding that mutations in the tau gene are responsible for frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) has provided convincing evidence that tau protein plays a key role in neurodegeneration.

Tau promotes tubulin polymerization, reduces microtubule instability and plays a role in maintaining neuronal integrity, axonal transport and axonal polarity. Tau protein is abundant in both the central and peripheral nervous systems. In brain it is predominantly found in neurons concentrated in axons. In adult human brain six tau isoforms are produced from a single gene through alternative mRNA splicing (Fig. 1) (1–3). In the C-terminal part of tau, three or four tandem imperfect repeats are present, containing domains important for binding to microtubules (4). The alternatively spliced exon 10 encodes the additional fourth repeat. Alternative splicing of tau is developmentally regulated in that in immature brain only the transcript encoding the shortest isoform with three repeats is expressed but in adult cerebal cortex all six isoforms are present (2,3,5). Tau is post-translationally modified by phosphorylation in a dynamic process, and it has been suggested that this is an additional mechanism to regulate tau function (6).

TAU MUTATIONS ARE ASSOCIATED WITH FRONTOTEMPORAL DEMENTIA (FTD)

FTD describes a group of presenile dementias, including FTDP-17, characterized by progressive behavioral changes that account for 3–10% of total dementia (7–11). In contrast to AD, there is relative preservation of memory and visual spatial orientation early in the disease. In approximately half of FTD cases, a positive family history has been observed (7–10,12) and many families show filamentous pathology made of hyperphosphorylated tau protein in the absence of amyloid pathology (11,13).

A substantial proportion of FTD is caused by mutations in the tau gene. For most FTDP-17 families a mutation has been described (14–18) (Table 1) and, in a nationwide population study on FTD in The Netherlands, Rizzu *et al.* (19) found pathogenic mutations in ~40% of familial cases. Other studies report frequencies of 13.6 and 29% for familial cases (20,21). Mutations for sporadic FTD, early onset AD and Parkinson's disease have not been detected (19,22,23). Furthermore, in a large community-based series of dementia cases, no mutations were found, showing that tau mutations are not a common cause of general dementia (20).

For some of the described FTDP-17 families the genetic defect is still unknown. Three families (HFTDIII, Karolinska

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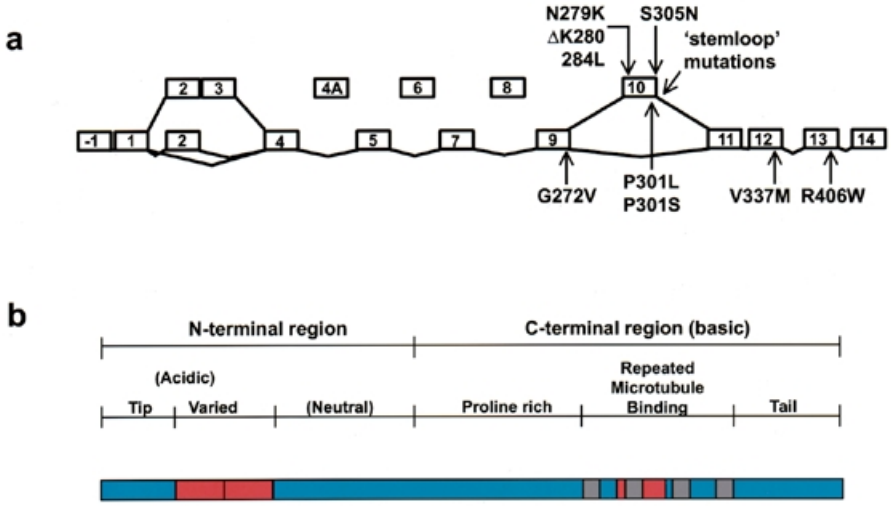

Figure 1. (**a**) Schematic diagram of the genomic organization of the tau gene. Exons are indicated by boxes. Six distinct mRNA transcripts are generated in brain by alternative splicing of exons 2, 3 and 10. Exons 4a, 6 and 8 are not present in mRNA from brain. Arrows indicate the position of known pathogenic mutations. (**b**) Representation of the longest isoform of tau protein (441 amino acids). Alternatively spliced exons are indicated in red. The six distinct tau isoforms vary in length from 352 to 441 amino acids. Grey boxes indicate microtubule-binding repeats.

and Seattle B) do not show obvious tau pathology and this distinguishes them from the other FTDP-17 families (11; unpublished data; S. Froehlich, personal communication). The HDDD2 family, however, has tau pathology, and although memory loss is a prominent feature in this family there are also behavioral symptoms and therefore a clear distinction from other FTDP-17 families cannot be made (24). For these four families either mutations in the tau gene have gone undetected, or the disorder is caused by a defect in another gene on chromosome 17. A second locus for FTD has been identified on chromosome 3 in a single family (25) but for the majority of familial FTD cases (between 60 and 75%) the genetic defect remains unknown.

TAU-RELATED DEMENTIA

The finding of tau mutations in FTD generated renewed interest in the role of tau in other forms of neurodegeneration showing extensive tau pathology such as Pick's disease (PiD), cortico-basal degeneration (CBD), progressive supranuclear palsy (PSP) and the amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam. Genetic association studies for several of these disorders found no evidence for the involvement of tau, with the clear exception of PSP. PSP is a form of parkinsonism characterized by akinetic-rigid syndrome, supranuclear gaze palsy, pyrimidal tract dysfunction, pseudobulbar signs and cognitive decline of the frontal lobe type (26). It usually occurs as a sporadic disorder but familial forms have also been described. Neuropathologically, PSP is characterized by NFTs and neuropil treats consisting of hyperphosphorylated tau protein in neurons and glia of subcortical and cortical structures.

Linkage to chromosome 17 for familial forms of PSP was excluded (27) but several studies have reported an allelic association between an intronic polymorphism after exon 9 of the tau gene and sporadic PSP cases (28–34). More detailed studies using multiple tau polymorphisms (32,34) showed association with an extended haplotype (H1/H1) spanning the whole tau gene including the promoter region (35), making it difficult to pinpoint the exact location of the biologically relevant defect.

One explanation for these findings could be that the H1/H1 haplotype harbors a mild risk factor since it is also present in >50% of the healthy control population. Alternatively, the result could be explained by the possibility that a subtype of the H2 allele, which is virtually absent in PSP cases, harbors a protective effect. The search for the biologically relevant defect has so far been unsuccessful. Mutation analyses have not revealed pathogenic mutations (32,34,36). However, the finding that NFTs found in PSP are almost exclusively composed of four-repeat tau suggests a disturbance of the normal splicing ratio of exon 10 (E10) (37). This is in agreement with the disturbed ratio of $E10^{+}/E10^{-}$ mRNA transcripts in PSP brains (38). The relevant defect could be localized in unknown elements that regulate splicing and a similar mechanism might be important in other disorders where tau pathology consists of mainly three- (PiD) or four-repeat (CBD) isoforms (39–41).

THREE VERSUS FOUR REPEATS: A DELICATE BALANCE

Mutations in the tau gene lie within the C-terminal region of the protein where the microtubule-binding domains are located. Mutations in the intron following exon 10 influence its alternative splicing, leading to a change in the ratio of protein isoforms with three or four microtubule-binding repeats (15–17). The mechanism by which most of these mutations act is probably by destabilizing a short stem–loop structure that spans the splice site (Fig. 2). The stem–loop is proposed to compete with the U1 snRNP for binding to the 5′ splice site of exon 10 (15). Exon trap assays with constructs containing tau mutations, gel migration assays and RNase mapping are all in agreement with this model (15,19,42,43). The exact structure and length of the stem–loop, however, has been difficult to determine and different RNA conformations and stem lengths

Table 1. Tau mutations

Mutation	Family no.	Age at onset (years)	Duration (years)	Characteristic symptoms	Tau pathology	Western blot	Filaments	References
Clinical and pathological characteristics of different tau mutations								
G272V (exon 9)	1	47.6 ± 3.8	8.3 ± 2.3	Disinhibition, initiative loss, cognitive decline, mutism	Pretangles in neurons and occasional glial cells	NA	NA	60,64,68
P301L (exon 10)	3	51.4 ± 5.7	8.6 ± 1.8	Disinhibition, initiative loss, cognitive decline, mutism	Pretangles in neurons and glial cells	4R	STFs/straight	60,64,68
	1	57 ± 2.0	5–10	Behavioral changes	Neurons and glial cells	4R	NA	62
	1	49	5.1	Behavioral changes, disinhibition	NFTs, neuron and glial cells	NA	STFs/straight	61
	2	64	7.3–8.0	Language deficits, mutism	Neurons and glial cells	NA	NA	61
	6	41–62	NA	Disinhibition, initiative loss, cognitive decline	NA	NA	NA	21
P301S (exon 10)	1	27/29	7	Depression, parkinsonism, memory loss	High in neurons and glial cells	NA	Straight	65
	1	25–40	±6	Behavioral changes, parkinsonism, epileptic seizures	NA	NA	NA	66
V337M (exon 12)	1	54.3 ± 7.9	12.8 ± 5.7	Anti-social, psychotic behavior	NFTs, no glial deposits	3R/4R	PHFs/straight	69
R406W (exon 13)	1	55 (45–75)	>20	Memory loss, personality change, parkinsonism	NFTs, no glial deposits	3R/4R	PHFs/straight	67
	1	59.5 ± 5.5	12.7 ± 1.7	Memory loss, personality change, parkinsonism	NFTs, no glial deposits	3R/4R	PHFs/straight	64
Mutations affecting alternative splicing exon 10								
Shift towards three repeat								
ΔK280	1	53	NA	Initiative loss, disinhibition	NA	NA	NA	64
Shift towards four repeat								
284L	1	51.8 ± 4.8	9–10	Word finding, visual spatial abilities, behavioral changes, Aβ pathology, executive function	NFTs, glial deposits	NA	NA	45
S305N	1	NA	NA	Personality change, cognitive function, memory loss	NFTs, neurons and glial cells	NA	Straight	70
N279K	2	32–58	8.4 ± 3.3	Parkinsonism, personality change, memory loss	Inclusions in neurons and glial cells	4R	STF	71,72
	1	40–45	7					76
+3	1	49 ± 10	11	Memory, bradykinesia, rigidity	Neurons and glial cells	4R	STF	73
+13	1	NA	NA	NA	NA	NA	NA	15
+14	1	45	13	Disinhibition, dementia, parkinsonism	Neurons and glial cells	4R	STF	74
+16	1	53 ± 8.9	4–15	Personality changes	NA	NA	NA	75
	1	49 ± 17	12 ± 10	Behavioral changes, cognitive impairment	Neurons and glial cells	4R	STF	18

have been proposed. An elegant study using UV melting assays and NMR spectroscopy of oligonucleotides confirmed that a stem–loop is indeed formed with a stem of six base pairs separated by an unpaired residue, possibly followed by another paired residue. Longer stem structures, however, could not be excluded since the length of the oligonucleotides used in these experiments was limiting (44).

Several mutations also affect the binding of the U1 snRNP complex to the splice site. The 5′ terminus of the U1 snRNA of this complex has a sequence that base pairs with the splice donor consensus sequence: AGgu(a/g)agu. The N305S mutation increases the base pairing of U1 snRNA since GUgugagua changes to AUgugagua, which is a stronger splice site (43,45). In a similar way, the E10+3 mutation increases U1 snRNP

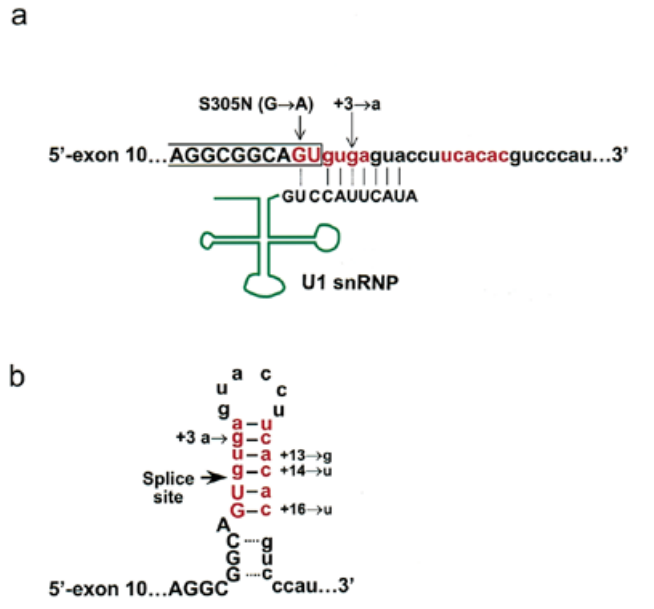

Figure 2. Sequence around the exon 10 3′ splice site. Exonic RNA sequence is indicated with capital letters, intronic RNA sequence with lowercase letters. The U1 snRNP complex binds to splice site sequences by base pairing as indicated by solid lines (**a**). Dashed lines show additional base pairing introduced by mutations (indicated by arrows). In the presence of a stem–loop structure (**b**), binding of the U1 snRNP complex is inhibited. Red indicates bases involved in base pairing of the predicted stem–loop structure.

binding by a better matching sequence: GUguaagua (42,43,45).

Other mutations within the coding region of exon 10 provide evidence for additional exon 10 splicing regulating elements. The N279K and 284L mutations upstream of the stem–loop also increase E10$^+$ transcripts (43,45): the N279K mutation by creating a GAR repeat (AAGAAGAAG), a sequence known to enhance splicing, and 284L by disrupting a UUAG sequence that might act as a splicing silencer (45). The ΔK280 mutation seems to have the opposite effect to that of the N279K mutation by removing an AAG triplet resulting in a reduction of E10$^+$ transcripts (45; unpublished data). ΔK280 is intriguing in that the protein with this mutation has a strong effect on microtubule interactions (see below) but this might be compensated by the possibility that the mutation also reduces the splicing of E10$^+$ transcripts. Thus, on the RNA level the mutation prevents the production of its own mutant protein. It could be that *in vivo* small amounts of four-repeat mutant protein are produced and the net effect might level out, resulting in an average phenotype severity. It has not yet been possible to verify the effects of the mutation in brain material from patients.

The stem–loop sequence is conserved in primates and bovines but not in rodents, and only four-repeat tau isoforms are present in adult rodent brain. During development, however, rodents express three-repeat tau, and therefore the developmental switch from three- to four-repeat tau must be controlled by other regulatory sequences such as, for example, the splicing silencer disrupted by the 284L mutation.

All mutations that affect the alternative splicing of exon 10 have in common the fact that they affect the ratio between three- and four-repeat tau protein isoforms. The proteins themselves are fully normal and functional, showing that a simple disturbance of this balance can result in neurodegeneration.

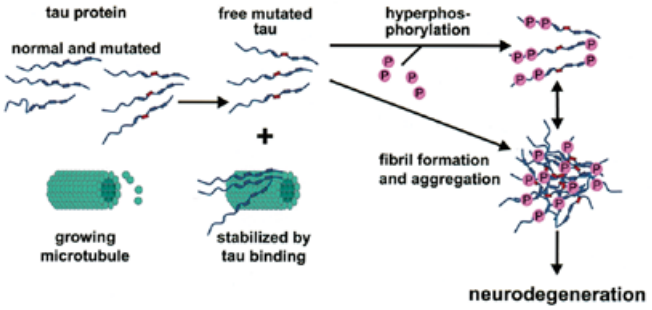

Figure 3. Effect of pathogenic FTD mutations on the tau protein.

IS MICROTUBULE BINDING ESSENTIAL?

Based on their location it can be predicted that most coding mutations might have an effect on the microtubule-binding properties of tau. Several studies have confirmed this using *in vitro* microtubule assembly and/or binding assays (19,46,47). Although there are discrepancies between studies on the relative effect of each mutation, additional arguments that confirm this partial loss of function come from transfection studies with tau constructs carrying mutations in CHO cells (48). After treatment with cytochalasin B, an actin-disrupting agent, or colcemide, a microtubule-depolymerizing drug, mutant tau shows a reduced ability to form extensions and disturbs the microtubule network of cells.

Mutations in exons 9, 12 and 13 affect all six tau isoforms produced from one of the two gene copies present, affecting 50% of the available protein. Mutations in exon 10 affect only the four-repeat isoforms from a single gene copy, and thus the majority of tau protein is normal. The question therefore arises whether the relatively mild loss of function observed for most mutations is the most likely explanation for the disease. FTD is a late age of onset disease and one could propose that the partial loss of function has a limited effect that will prove fatal only over time, but how then do we explain the fact that most splice mutations that increase the amount of four-repeat isoforms lead to a very similar clinical phenotype? Four-repeat tau binds more strongly to microtubules than does three-repeat tau, and therefore the effect of the splicing mutations seems to be the opposite to the coding mutations. Furthermore, mice with a targeted disruption of the tau gene are fully viable and fertile, demonstrating that the microtubule-binding properties of tau are not an essential function (49).

Since the pathological hallmark of FTDP-17 is the filamentous deposits of hyperphosphorylated tau protein, a more probable explanation for the effect of the pathogenic mutations would be that they induce a ‘gain of toxic function’ (Fig. 3). Three-repeat tau binds microtubules at sites different to those bound by four-repeat tau (50). The splicing mutations cause an excess of specific isoforms of the protein and the result could be a shortage of available binding sites for the overexpressed tau isoform leading to an excess of free tau.

The partial loss of function observed for the coding mutations could also result in an excess of free (mutated) tau. This free tau would then be available for hyperphosphorylation and/or assembly into filaments and/or aggregates (Fig. 3).

Recent findings indeed point towards this explanation: tau filaments can be generated *in vitro* and four-repeat tau aggregates more readily than does three-repeat tau. Compared with this wild-type protein, tau with missense mutations aggregates even faster (51–53). Not all studies on mutated tau are in complete agreement; one study tested both three- and four-repeat protein isoforms for 48 h and found a strong effect for the P301L and P301S mutations and a smaller effect for other mutations such as R406W (53). The ΔK280 mutation did not affect fibril formation. A second study with a longer experimental duration found similar results for the P301L mutation but showed that although the R406W mutation initially has a small effect, it had a fast polymerization rate later in the process (51).

It will be important to see whether these effects can be reproduced in cultured cells or animal models. Changes in the phosphorylation of tau have been reported in transfection studies (48) but until now tau filament formation in transfected cells has not been reported.

Several studies have reported transgenic mice expressing specific isoforms of human tau under the control of heterologous promoters (54–57). Mice overexpressing four-repeat tau on pathology showed axonopathy and hyperphosphorylated tau protein but no NFTs (56). More recently, however, a three-repeat tau mouse with a high transgene expression showed axonal degeneration and abundant argyrophilic tau-immunoreactive inclusions in neurons (57).

If the formation of filaments that aggregate into insoluble inclusions is the toxic gain of function caused by the pathogenic mutations, why would this then lead to neurodegeneration? The answer might come from findings in other neurodegenerative disorders where aggregates of abnormal protein are found (reviewed in ref. 58).

Tau aggregates in neurons stain with antibodies against ubiquitin, suggesting that the deposits are tagged for degradation by the ubiquitin degradation pathway by proteasomes. It has been suggested for other neurodegenerative disorders that the proteolytic pathway in these cells might be altered because the nuclear aggregates may resist degradation and subsequently prevent ubiquitin recycling and/or disrupt the proteasome (59). As a result the ubiquitin-degradation pathway may become disturbed in such a way that its normal function cannot be performed, making the neuron vulnerable for other stress factors.

Now that it has become clear that tau-immunoreactive filaments can be generated in model systems it is important to find out whether this aggregation process is similar to the formation of NFTs and to use these models to test these hypotheses for tau-related dementias. This year we can expect the publication of several studies using transgenic mice overexpressing pathogenic tau mutations that, given the results mentioned above, are likely to show tau-containing inclusions.

GENOTYPE–PHENOTYPE CORRELATION?

Given the large variation of phenotypes caused by tau mutations, a clear correlation between genotype and phenotype is not immediately obvious. A first prerequisite for such a correlation would be that the variation within families or between families with the same mutation is smaller than between families with different mutations.

The P301L mutation is described in most detail (15,17,21,60–64). Most patients show an age at onset of ~50 years but within families there is a relatively large variation. The duration of the disease is ~8 years. Presenting symptoms usually are behavioral changes such as disinhibition, language deficits and mutism. There is relative preservation of visual spatial orientation early in disease. Autopsy reveals neuronal loss and gliosis. Usually no prominent parkinsonian symptoms are present. Tau pathology consists of intracytoplasmic tau deposits in neurons, glial cells and neurites in hippocampus, neocortex and substantia nigra, mainly consisting of slender twisted filaments (STFs) of four-repeat tau isoforms.

In one small family, memory loss seemed to be more prominent than behavioral disturbances (17), and some families had a higher age at onset or a shorter disease duration (61), but in most cases clinical and pathological characteristics were quite similar.

Finding order between different mutations is a more daunting task and for many families clinical and pathological data are scarce. In Table 1 the main characteristics of families with a tau mutation are listed. The observed pathology is consistent with the position of mutations in that mutations affecting all six isoforms show AD-like paired helical filaments (PHFs) consisting of all six tau isoforms in neurons, and mutations affecting only four-repeat tau mostly show mainly STFs consisting of four-repeat tau isoforms in both neurons and glial cells.

Clinically the P301S mutations seem to be on one side of the spectrum; two families have been described with a very early onset (65,66), behavioral changes, memory loss and parkinsonism. One family showed euphoric moods and epileptic seizures. In the other family hallucinations and delusion were reported. Information on the pathology is limited to one family, where neuronal loss, gliosis, spongiosis and very extensive tau pathology in neurons and glial cells was observed. The severe phenotype of this mutation might be explained by the possibility that the mutation might introduce an additional phosphorylation site within the microtubule-binding domains of tau making it more prone to the formation of insoluble filaments.

The other side of the clinical spectrum is shown by families with mutations in the more C-terminal part of the protein. Two R406W families have been described in detail (64,67). The difference with the P301L families is the more prominent memory loss, very late mutism, later age at onset and longer duration of disease. Tau pathology consists of PHFs.

In conclusion, coding mutations within the first two microtubule-binding domains show an earlier onset and shorter disease duration than more C-terminal mutations, which show a more prominent memory loss (Table 1). All splicing mutations that result in the overexpression of four-repeat tau also show similarities in the observed pathology and phenotype. Tau pathology consists mostly of STFs of four-repeat tau, and parkinsonism is a frequent finding (Table 1).

SUMMARY AND PERSPECTIVE

It has become clear that the tau gene is a major locus of autosomal dominant dementia and this has important consequences for understanding the pathogenesis of FTD, but also shows that

tau must play a key role in many other neurodegenerative disorders such as AD, PiD, PSP and CBD.

The mechanism through which tau mutations lead to neurodegeneration remain largely unclear, although there are indications that mutations could result in increased filament formation. The gradual build-up of aggregated tau filaments could be a toxic gain of function, impairing the normal defense mechanisms of the cells, resulting in premature neuronal loss. Now that animal models with tau pathology are becoming available, these hypotheses can be tested and challenged, hopefully leading to the development of novel and effective treatments of dementing illnesses.

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