FEBS Letters 450 (1999) 306–311 FEBS 21970

Effects of frontotemporal dementia FTDP-17 mutations on heparin-induced assembly of tau filaments

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Received 2 April 1999

Abstract Missense mutations and intronic mutations in the gene for microtubule-associated protein tau cause frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17). Most missense mutations have as likely primary effect a reduced ability of tau to interact with microtubules. We report here an additional effect of several missense mutations, namely the stimulation of heparin-induced filament assembly of recombinant tau, despite the absence of any change in structure indicated by circular dichroism. These findings indicate that missense mutations in tau lead to frontotemporal dementia through potentially multiple mechanisms.

Key words: Tau protein mutation; Tau filament assembly; Frontotemporal dementia

1. Introduction

Abundant filamentous deposits made of hyperphosphorylated microtubule-associated protein tau constitute one of the defining neuropathological characteristics of Alzheimer's disease [1,2]. Similar deposits are found in a number of other neurodegenerative diseases, such as Pick's disease, progressive supranuclear palsy and corticobasal degeneration. An autosomal-dominantly inherited form of frontotemporal dementia and Parkinsonism (known as FTDP-17) is also characterised by an abundant filamentous tau pathology and is linked to chromosome 17q21–22, the same region that contains the tau gene [3,4]. Recent work describing a number of exonic and intronic mutations in the tau gene has shown that the FTDP-17 locus is the tau gene [5–15]. The known mutations are either missense or deletion mutations in the coding region of tau, or intronic mutations located close to the splice-donor site of the intron following exon 10 of the tau gene.

In adult human brain, six tau isoforms are produced from a single gene by alternative mRNA splicing [16–18]. They differ from each other by the presence of 29- or 58-amino acid inserts located in the amino-terminal half and a 31-amino acid repeat located in the carboxy-terminal half. Inclusion of the latter, which is encoded by exon 10 of the tau gene, gives rise to the three tau isoforms with four repeats each; the other three isoforms have three repeats each. The repeats and some adjoining sequences constitute the microtubule-binding domains of tau [19,20].

Most missense mutations lead to a reduced ability of tau to interact with microtubules, thus probably setting in motion the mechanisms that lead to the formation of tau filaments [15,21–23]. By contrast, the primary effect of the intronic mutations and of some missense mutations in exon 10 is at the RNA level [6,24]. Intronic mutations and at least one missense mutation destabilise a stem-loop structure at the boundary between exon 10 and the intron following exon 10, resulting in a net overproduction of four-repeat tau isoforms [7,22]. In addition, some of the mutations that destabilise the stem-loop structure are predicted to increase binding of U1snRNA [7,24].

Besides leading to a reduced ability of tau to interact with microtubules or to increase alternative mRNA splicing of exon 10, missense and deletion mutations in tau may have additional effects. The mutations are located in the microtubule-binding repeat region of tau or close to it. It is well established that this region of the tau molecule forms the core of the paired helical filament of Alzheimer's disease [25–27]. In the present study, we have determined the circular dichroism (CD) spectra of purified recombinant four-repeat wild-type and mutant human tau proteins. We also used a previously described method for assembling synthetic tau filaments [28–30], in order to determine the effects of seven tau mutations on filament assembly. We show that the mutant proteins investigated had CD spectra indistinguishable from the CD spectrum of wild-type tau. By contrast, several mutations produced a marked stimulatory effect on the heparin-induced filament assembly of recombinant tau protein.

2. Materials and methods

2.1. Expression and purification of wild-type and mutant tau proteins

Site-directed mutagenesis was used to change G272 to valine, to delete K280, to change P301 to either leucine or serine, S305 to asparagine, V337 to methionine or R406 to tryptophan in the four-repeat 412-amino acid isoform of human tau (expressed from cDNA clone htau46) (in the numbering of the 441 amino acid isoform of human tau). The three-repeat 381-amino acid isoform of human tau (expressed from cDNA clone htau37) was used to change G272 to valine, V337 to methionine or R406 to tryptophan (using the numbering of the 441-amino acid isoform of human tau). Wild-type and mutant tau proteins were expressed in *Escherichia coli* BL21(DE3), as described [31]. Bacterial pellets were resuspended in 50 mM Tris-HCl, pH 7.4, 0.5 mM EDTA, 0.1 mM DTT, 0.1 mM PMSF and sonicated. Supernatants were passed onto a DE cellulose column and the flow-through applied to a phosphocellulose column equilibrated in the same buffer. The column was step-washed with NaCl, with tau protein eluting at 0.5 M NaCl. Following precipitation with 50% ammonium sulphate, protein was dissolved in 50 mM MES, pH 6.25, 50 mM NaCl, 0.1 mM DTT, 0.1 mM PMSF and applied to a Sephacryl S-200 column. Tau protein peak fractions were then applied directly to a Mono S column equilibrated in the same buffer and protein was eluted using a NaCl gradient. Peak tau protein fractions were combined and concentrated using an Amicon diaflo ultracentrifugation apparatus (PM30 membrane), followed by dialysis against 40 mM HEPES, pH 7.4, 0.1 mM DTT. All purification steps were monitored by SDS-PAGE. In some

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PII: S0014-5793(99)00508-6

experiments, 10 μg amounts of tau proteins were fractionated on an Aquapore RP-300 7 μm C_8 column using a gradient of 0.1% TFA to 0.04% TFA/90% acetonitrile at a flow rate of 0.5 ml/min. Peak detection was at 225 nm.

2.2. Circular dichroism spectroscopy

Wild-type and mutant four-repeat tau proteins were used at 0.2–0.4 mg/ml in phosphate-buffered saline (PBS). Protein concentrations were determined by quantitative amino acid analysis. Spectra were collected on a Jobin Yvon CD6 spectrophotometer using a 1-mm cell at 25°C. Curve smoothing made use of algorithms provided by the manufacturer.

2.3. Tau filament assembly

Purified wild-type or mutant forms of the 381- and 412-amino acid isoforms of human tau (3 mg/ml) were incubated in the absence or the presence of various concentrations of heparin (100, 200 and 400 μg/ml; British Drug House) in 25 μl of 30 mM MOPS, 1 mM 4-(2-aminoethyl)benzenesulphonylfluoride (AEBSF, Calbiochem), pH 7.4, at 37°C for 48 h, as described [28,30]. Aliquots were placed onto carbon-coated 400-mesh grids and stained with 1% lithium phosphotungstate. Micrographs were recorded at a nominal magnification of ×40 000 on a Philips model EM208S electron microscope. Each filament assembly experiment made use of newly prepared batches of recombinant wild-type and mutant tau proteins that had been purified in parallel. The numbers of separate experiments for each four-repeat mutant were as follows: G272V ($n=3$); ΔK280 ($n=3$); P301L ($n=5$); P301S ($n=4$); S305N ($n=3$); V337M ($n=3$); R406W ($n=4$). They were as follows for the three-repeat tau mutants: G272V ($n=2$); V337M ($n=2$); R406W ($n=2$).

3. Results

The CD spectrum of bacterially expressed recombinant tau protein (412-amino acid isoform of human tau) showed no evidence of significant secondary structure, in confirmation of previous reports using native and recombinant tau proteins [32,33]. The FTDP-17 mutants G272V, ΔK280, P301L, P301S, S305N, V337M and R406W (Fig. 1) had CD spectra that were indistinguishable from those of the wild-type protein, compatible with a 'natively unfolded' structure of the mutant tau proteins and showing no evidence of any α-helical structure (Fig. 2). Similarly, wild-type and mutant tau proteins had identical elution patterns on reverse-phase HPLC, where they all eluted at 33–34% acetonitrile.

The effects of the FTDP-17 mutations G272V, ΔK280, P301L, P301S, S305N, V337M and R406W on filament assembly of four-repeat tau were investigated in the absence and the presence of the sulphated glycosaminoglycan, heparin (Table 1, Fig. 3). In addition, the effects of the G272V, V337M and R406W mutations in the context of a three-repeat tau isoform were also studied. Neither wild-type tau nor any of the seven FTDP-17 tau mutants investigated formed filaments in the absence of heparin over the 48-h incubation period. No clear evidence of filament formation was observed in the absence of heparin when the samples were left for up to 10 days at 37°C, with or without agitation. As observed before [28,30], incubation of the four-repeat-containing tau isoform with heparin gave predominantly straight filaments with a morphology similar to the straight filaments from Alzheimer's disease brain. Incubation of the three-repeat-containing tau isoform with heparin gave twisted filaments with a morphology reminiscent of the paired helical filaments from Alzheimer's disease brain, in confirmation of our previous findings [28,30]. An additional class of filament, appearing as thinner, wavy structures, was also seen, as described [30]. Since the relative numbers of each type of filament (straight versus wavy or twisted versus wavy) are strongly dependent on the tau:heparin ratios [30], we used three different heparin concentrations in each experiment.

Table 1
Relative effects of FTDP-17 mutations on heparin-induced assembly of tau filaments

	Number of filaments
Four-repeat tau isoform (TAU 46)	
Wild-type	+
G272V	++
ΔK280	+
P301L	+++
P301S	+++
S305N	+
V337M	+
R406W	+
Three-repeat tau isoform (TAU 37)	
Wild-type	+
G272V	++
V337M	++
R406W	+

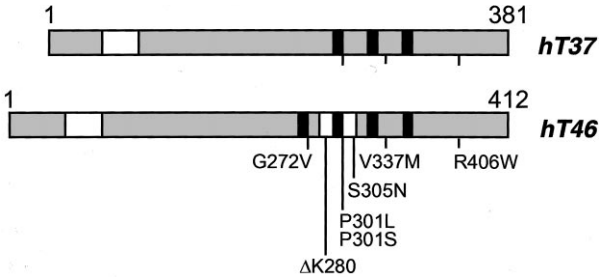

Fig. 1. Schematic diagram of the 381- (three-repeat isoform) and 412-amino acid (four-repeat isoform) human tau proteins. Recombinant proteins were expressed from cDNA clones htau37 and htau46. The positions of the FTDP-17 mutations (G272V, ΔK280, P301L, P301S, S305N, V337M and R406W) are indicated. Alternatively spliced exons 2 and 10 are shown in white and the microtubule-binding repeats are represented as black bars.

The relative effects of the FTDP-17 mutations on heparin-induced tau filament assembly were assessed semi-quantitatively by electron microscopy (Table 1). A large stimulatory effect was observed with the P301L and P301S mutations located in exon 10 (Fig. 3). No stimulatory effect was seen with the ΔK280 and S305N mutations which are also located in exon 10 of the tau gene. This contrasted with the G272V mutation in exon 9 that led to increased tau filament assembly, when compared with either wild-type three-repeat or four-repeat tau protein (Fig. 3). The V337M mutation in exon 12 gave a small increase in filament numbers in the three-repeat, but not the four-repeat tau isoform. The R406W mutation in exon 13 gave similar numbers of filaments to the wild-type protein, whether in the three-repeat or the four-repeat tau isoform (Fig. 3).

4. Discussion

The discovery of tau gene mutations segregating with disease in FTDP-17 has shown that tau protein dysfunction causes neurodegeneration. This is relevant, not only for FTDP-17, but also for other diseases with a filamentous tau pathology, such as Alzheimer's disease, Pick's disease, progressive supranuclear palsy and corticobasal degeneration [2]. Accordingly, there is great interest in the molecular mech-

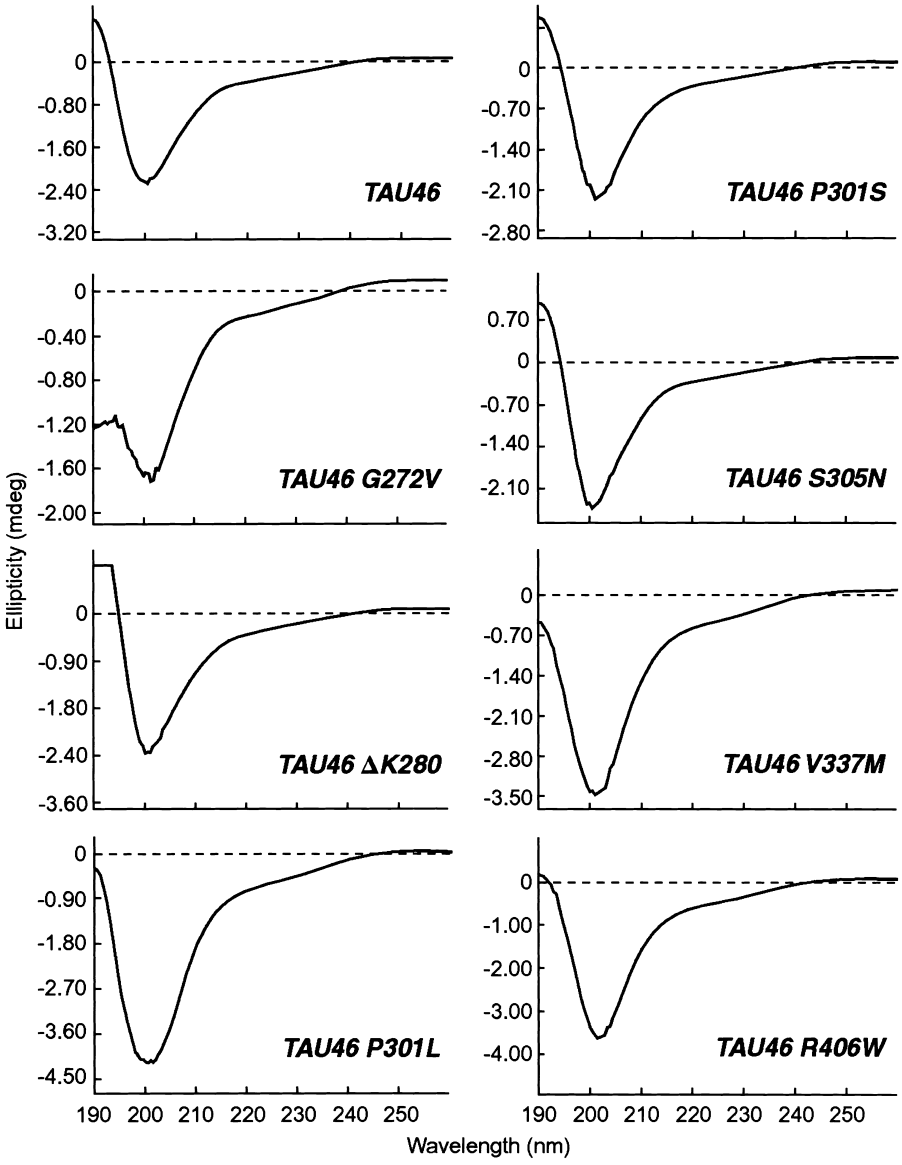

Fig. 2. Circular dichroism spectra of wild-type and mutant four-repeat tau proteins (TAU 46). Wild-type tau and the tau mutants were mainly random coil in phosphate-buffered saline. The seven mutant proteins (G272V, ΔK280, P301L, P301S, S305N, V337M and R406W) were indistinguishable from wild-type tau under these conditions.

anisms by which tau mutations lead to frontotemporal dementia.

Most missense mutations lead to a reduced ability of tau to interact with microtubules, suggesting that this may be their primary effect [15,21–23]. Alternatively, intronic mutations and some missense mutations lead to increased splicing of exon 10, resulting in the overproduction of four-repeat-containing tau isoforms [6,24]. This may result in an excess of four-repeat tau over available binding sites on microtubules and result in a reduced ability of excess four-repeat tau to interact with microtubules. To date, all the mutations examined belong to one of these two groups. A reduced ability to interact with microtubules has thus been proposed as a necessary step for the hyperphosphorylation of tau and assembly of tau into filaments [7,34].

Here we describe an additional effect of some of the missense mutations in tau, namely a stimulatory effect on heparin-induced filament assembly of recombinant tau. Of the seven mutations tested, by far the strongest effects were seen with the P301L and P301S mutations. Recombinant four-repeat tau proteins with these mutations gave rise to a large increase in the number of filaments when compared with the wild-type protein. Proline residue 301 is located in exon 10 of the tau gene and these mutations therefore only affect four-repeat tau isoforms. Two other mutations in exon 10, namely ΔK280 and S305N, failed to increase heparin-induced tau filament formation. The G272V mutation in exon 9 produced an increase in tau filaments in both the three-repeat and four-repeat isoforms. A lesser effect was seen for the V337M mutation in exon 12 which had a small stimulatory effect in the three-repeat tau isoform. No stimulatory effects were observed for the R406W mutation in exon 13. A recent study using synthetic peptides derived from each of the four microtubule-binding domains of tau has shown increased heparin-induced filament formation for the P301L mutation in repeat 2, but not for the G272V mutation in repeat 1 [35].

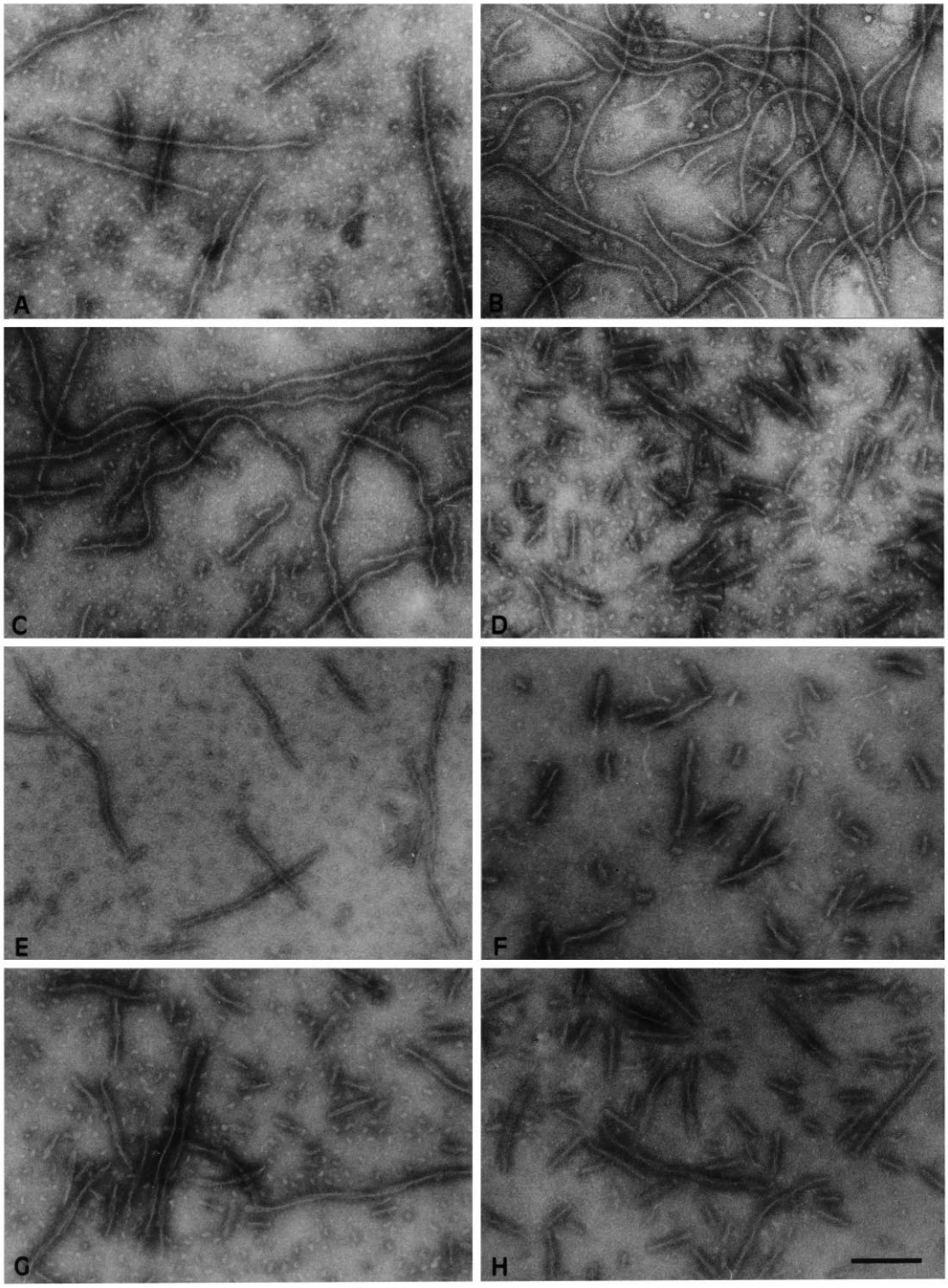

Fig. 3. Representative fields of filaments assembled from various wild-type and mutant tau proteins. A–E: Four-repeat tau (TAU 46). F–H: Three-repeat tau (TAU 37). A: Wild-type; B: P301S; C: P301L; D: G272V; E: R406W; F: wild-type; G: G272V; H: V337M. Scale bar, 200 nm.

The effects of tau mutations on filament assembly were not paralleled by changes in their CD spectra, where both wild-type and mutant tau proteins showed a 'natively unfolded' structure. These findings are at variance with a recent report describing an increase in α-helical content of recombinant tau proteins with four of the missense mutations investigated here [36]. The reasons for this discrepancy are unknown. However, the fact that an overproduction of normal four-repeat tau resulting from some mutations can lead to FTDP-17 indicates that a significant change in the structure of tau is not necessary for the development of pathology. Our CD spectra indicate that the same is true of the missense and deletion mutations of tau studied here.

The magnitude of the stimulatory effects of tau mutations on heparin-induced filament assembly is comparable to their relative inhibitory effects on tau-promoted microtubule assembly [15,21]. Thus, the P301L and P301S mutations produce a strong inhibitory effect on microtubule assembly, with inter-

mediate effects of the G272V and V337M mutations and a smaller effect of the R406W mutation. Although we failed to detect a stimulatory effect of the R406W mutation on tau filament assembly, we cannot exclude a small effect that may have gone undetected. The S305N mutation produces no reduction in the ability of tau to promote microtubule assembly and its primary effect is at the RNA level [24]. It had no detectable effect on tau filament assembly. The ΔK280 mutation appears to be an exception, since it has a strong inhibitory effect in microtubule assembly experiments [10], without an obvious effect on tau filament assembly.

In view of the strong inhibitory effects of the P301L and P301S mutations on tau-promoted microtubule assembly [15,21] and their stimulatory effects on heparin-induced tau filament assembly, one might expect these mutations to lead to a more severe clinical phenotype than mutations with lesser effects, such as R406W. This is borne out to some extent, with the P301S mutation leading to an early age of onset of disease and a relatively short disease duration, with an extremely severe tau pathology [15]. It contrasts with the R406W mutation which produces a later age of onset and gives rise to a more protracted disease course, with a less severe tau pathology [37]. However, the P301L mutation leads to disease only in mid-life, although the progression of disease can be rapid and the tau pathology is severe [9,14,38,39].

Additional effects, for example on the phosphorylation state of tau, of the missense and deletion mutations in tau possibly remain to be discovered. Where studied, pathological tau from FTDP-17 brain is hyperphosphorylated, in a similar manner to the pathological tau from Alzheimer's disease brain [4,34]. With the possible exception of the P301S mutation, the known mutations in tau do not create additional phosphorylation sites. Therefore, in most cases, hyperphosphorylation of tau must be an event downstream of the primary effects of the tau mutations and may be a consequence of the partial loss of function. It probably enhances the effects of the missense and deletion mutations, since it is well established that hyperphosphorylated tau is unable to bind to microtubules [40,41]. Thus, an indirect effect of the mutations on the phosphorylation of tau could contribute to disease. A number of interactions between tau and several non-microtubule proteins have been described [42–45]. It is possible that tau mutations may interfere with some of these interactions.

In conclusion, the present study shows that some missense mutations in tau lead to a marked stimulation of heparin-induced formation of tau filaments. Together with the previously described effects of these mutations on microtubule assembly, it suggests that potentially multiple mechanisms underlie the pathogenic effects of the missense mutations in tau.

Acknowledgement: We thank Claudio Villa for help with photography.

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