Neurogenetics (2005) 6: 91–95
DOI 10.1007/s10048-005-0210-y

LETTER TO THE EDITORS

Manuela Neumann · Silvia Diekmann · Uwe Bertsch · Ben Vanmassenhove · Bernhard Bogerts · Hans A. Kretzschmar

Novel G335V mutation in the *tau* gene associated with early onset familial frontotemporal dementia

Received: 14 October 2004 / Accepted: 27 December 2004 / Published online: 12 March 2005

Abstract Mutations in the *tau* gene cause familial frontotemporal dementia and parkinsonism linked to chromosome 17. Here we describe a novel missense mutation in exon 12 of the *tau* gene, G335V, in a German family with frontotemporal dementia of early age at onset, in the third decade of life. Functional analysis of recombinant tau protein with the G335V mutation showed a dramatically reduced ability to promote microtubule assembly and a more rapid and accelerated tau filament formation, suggesting that the primary effect of the mutation might be the provision of a pool of unbound tau making it available for aberrant tau aggregation.

Keywords FTDP-17 · Tauopathy · Frontotemporal dementia · Tau

Introduction

The identification of mutations in the *tau* gene in familial frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) has established the important role of the tau protein as a cause of neurodegeneration [1–3]. The neuropathological hallmarks of FTDP-17 with *tau* gene mutations are prominent filamentous deposits consisting of the microtubule-associated protein tau in neuronal and glial cells. So far, 34 different pathogenic *tau* gene mutations have been described. These include missense, deletion or silent mutations in the coding region, or intronic mutations located close to the splice-donor site of the intron following exon 10 [4]. Functionally, the mutations fall into two categories. All intronic and some exon 10 mutations influence the alternative splicing of *tau* pre-mRNA, thereby disturbing the normal splicing balance of exon 10 [2, 3, 5]. By contrast, most missense mutations reduce the ability of tau to interact with microtubules (MTs) [6–8], except for the S305N and the recently identified Q336R mutation, which lead to an increased ability of tau to promote MT assembly [9, 10]. Moreover, several missense mutations also stimulate the in vitro assembly of tau into filaments [8, 11–13].

Here we report a novel missense mutation (G335V) in the *tau* gene in a German family with early onset frontotemporal dementia. In contrast to the effect of the adjacent Q336R mutation [10], the G335V mutation results in a dramatically reduced ability of tau to promote MT assembly. In addition, the G335V mutation leads to an increased heparin-induced assembly of recombinant tau into filaments.

Materials and methods

Genetic analysis

Genomic DNA was extracted from whole blood using the Wizard Genomic DNA Purification Kit (Promega, Mannheim, Germany). All exons of the *tau* gene were amplified by polymerase chain reaction (PCR) using primers from the intronic sequences surrounding the exons [1]. The PCR products were checked for size by agarose gel electrophoresis and purified using QIAquick spin columns (Qiagen). DNA sequencing was carried out using the SequiTherm EXCEL long-read kit LC (Epicenture Technologies, Madison, Wis.) and 5′ 41-IRD labelled oligonucleotides. The labelled products were separated by denaturing gel electrophoresis on a 4.3% Long-Ranger gel (AT Biochem, Malvern, Wis.) and monitored using an automated DNA sequencing system (Model 4000L, LI-COR, Lincoln, N.

M. Neumann and S. Diekmann contributed equally to this work.

M. Neumann · U. Bertsch · B. Vanmassenhove · H. A. Kretzschmar (✉)
Center for Neuropathology and Prion Research,
Ludwig Maximilians University,
Munich, Germany
e-mail: Hans.Kretzschmar@med.uni-muenchen.de
Tel.: +49-89-218078000
Fax: +49-89-218078037

S. Diekmann · B. Bogerts
Department of Psychiatry,
University of Magdeburg,
Magdeburg, Germany

E.). The G335V mutation was confirmed by PCR restriction fragment length polymorphism (RFLP) analysis by *Msc*I digestion of the exon 12 PCR products; the mutation eliminates an *Msc*I site.

Informed consent for genetic testing of the *tau* gene was obtained from either the subjects or their next of kin.

Microtubule assembly

Site-directed mutagenesis was used to change glycine 335 to valine (using the numbering of the 441-amino acid isoform of human tau) in the three-repeat 381 amino acid isoform and the four-repeat 412 amino acid isoform of human tau, expressed from cDNA clones *htau37* and *htau46*, respectively. Wild-type and mutant tau proteins were expressed in *Escherichia coli* BL21(DE3) as described previously [14]. Tau proteins were purified and their concentration determined by densitometry and Coomassie staining.

Purified wild-type and mutant htau37 (0.3 mg/ml) and htau46 (0.2 mg/ml) were incubated with bovine tubulin (1 mg/ml; Cytoskeleton, Denver, Colo.) in assembly buffer (80 mM PIPES, 0.2 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 1 mM GTP) at 37°C as described [7]. Assembly of tubulin into microtubules was monitored over time by the change in turbidity at 350 nm using a Specord S100 photometer (Analytic Jena, Jena, Germany). Estimates of the initial rate of MT assembly and of the extent of assembly for each tau protein sample were based on results from four separate assembly reactions.

Heparin-induced tau assembly

Purified wild-type and mutant htau37 and htau46 (0.2 mg/ml) were incubated in 30 mM MOPS buffer at pH 7.4 in the presence of 0.02 mg/ml heparin (Sigma, St. Louis, Mo.) at 37°C as described previously [15]. Assembly was monitored quantitatively at intervals from 0–48 h by thioflavin S fluorescence as previously described [16] using the LS55 Luminescence Spectrometer (Perkin-Elmer Instruments, Shelton, Conn.) with excitation at 440 nm and emission at 490 nm. Measurements were carried out at room temperature in the presence of 10 μM thioflavin S (Sigma). Values were analyzed for significance by a two-tailed Student's *t* test.

Results

Clinical description and family history

The pedigree of the kindred is shown in Fig. 1. To maintain patient confidentiality, their sexes are not shown and birth orders have been modified. Both sexes were affected with female to male and male to female transmission of the disorder.

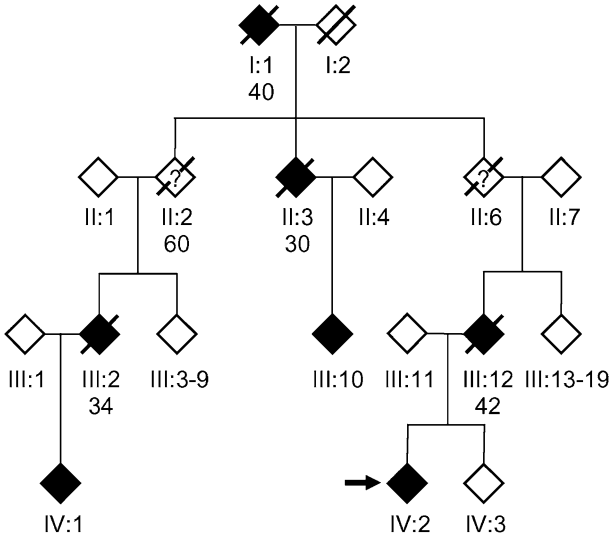

Fig. 1 Pedigree of the family. *Blackened symbols* denote affected individuals. *Slashes* indicate deceased individuals. Age at death is indicated *below symbols*. The index patient is marked by an *arrow*. The *question mark* indicates uncertainty as to whether the individual was affected or not

In the index patient (IV:2), family members noticed early personality changes including lack of interest, unsocial behaviour and personal neglect at age 25 years. About 1 year later the patient was suspected of being schizophrenic and was admitted to a psychiatric hospital. The patient had no past medical or psychiatric history and no history of illegal drug abuse; developmental milestones had been normal. The patient had worked successfully until the onset of the disease. Neuropsychological examination revealed obvious deficits in attention and in executive functions, such as abstraction, planning and mental set-shifting. Orientation and memory functions were relatively preserved. Reduced speech output was observed with a tendency to answer "don't know". No neurologic abnormalities, especially no signs of parkinsonism have been noted to date. A wide range of serum and cerebrospinal fluid investigations including metabolic studies as well as an EEG were normal. MRI and CCT revealed marked bilateral fronto-temporal atrophy.

There was a history of a similar dementing disorder in several other family members IV:1 (onset at 26 years), III:10 (onset not known), III:2 (onset at 26 years), III:12 (onset at 22 years), II:3 (onset at 28 years); I:1 (onset not known) with a mean age at onset of 25.4±2.1 years.

No autopsies had been performed on the deceased family members.

Genetic analysis

Sequence analysis of genomic DNA from the index patient (IV:2) and the affected second cousin (IV:1) identified a G→T transition at the second base position in codon 335 in exon 12 of the *tau* gene (Fig. 2), resulting in a glycine to valine substitution in the repeat-binding domain of tau. The

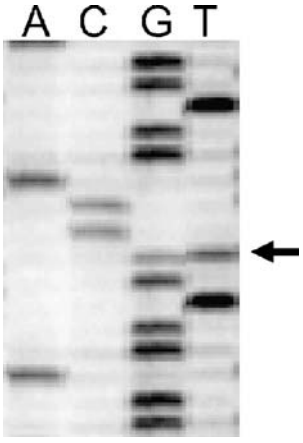

Fig. 2 Sequence analysis of exon 12 of the *tau* gene in the index patient. The position of the heterozygous G→T substitution leading to an amino acid change from glycine to valine at codon 335 is marked by an *arrow*

presence of the mutation was confirmed by PCR-RFLP. The mutation was absent in the tested healthy family members (III:3, III:13) and in 100 controls, checked by PCR-RFLP.

Microtubule assembly

Recombinant three-repeat and four-repeat human tau isoforms with the G335V mutation showed a markedly reduced ability to promote MT assembly when compared with the corresponding wild-type proteins (Fig. 3a, b). The rate of MT assembly was reduced to ~5% for the three-repeat isoform and ~30% for the four-repeat isoform when expressed as the optical density at 2 min (Fig. 3c).

Heparin-induced tau assembly

The effect of the G335V mutation on the heparin-induced assembly of recombinant three-repeat and four-repeat tau isoforms was quantified with the thioflavin-S assay (Fig. 4). Both G335V mutants showed an increased extent of aggregation when compared with the corresponding wild-type proteins, leading to an ~1.5-fold higher thioflavin-S fluorescence after a 48 h incubation time (htau37-G335V

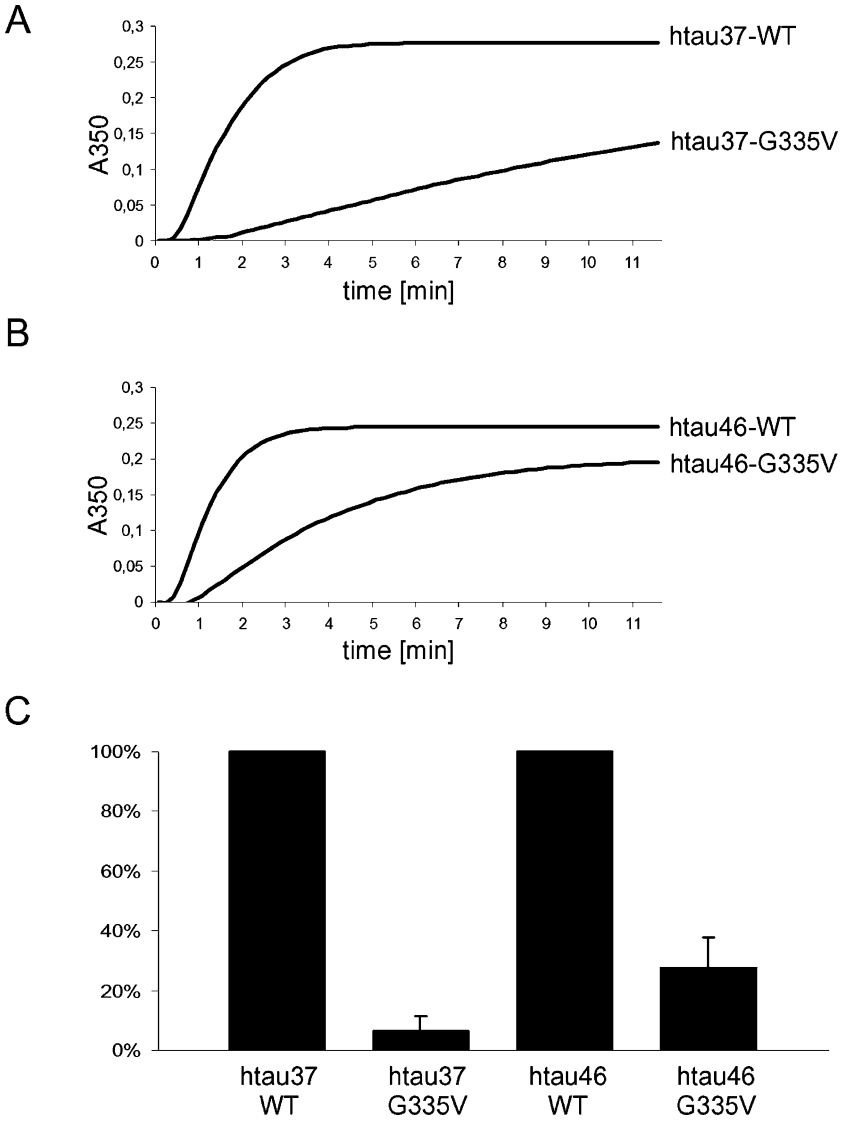

Fig. 3 Effects of the G335V mutation on the ability of three-repeat tau (htau37) and four-repeat tau (htau46) to promote microtubule assembly. Polymerization of tubulin induced by wild-type htau37 and htau37-G335V (**a**) and wild-type htau46 and htau46-G335V (**b**) as monitored over time by turbidity at 350 nm. **c** Optical densities for wild-type and mutant three-repeat and four-repeat tau proteins at 2 min (expressed as percentage of wild-type three-repeat and four-repeat tau, taken as 100%). Each result is expressed as the mean±SEM (*n*=4)

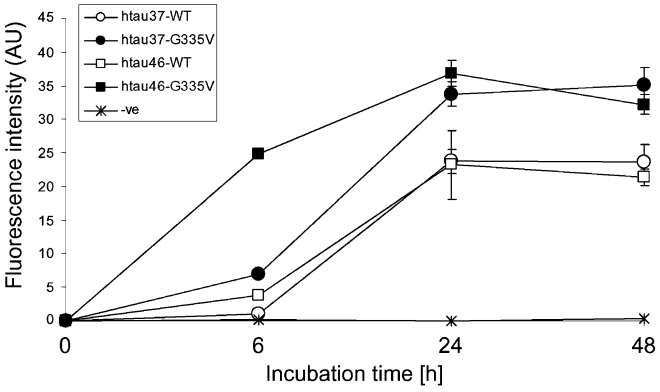

Fig. 4 Heparin-induced assembly of wild-type and G335V recombinant three-repeat tau (htau37) and four-repeat tau (htau46) measured by thioflavin-S fluorescence at indicated time intervals. *-ve* no heparin control for htau-G335V recombinant protein. The fluorescence is given in arbitrary units (AU). Each result is expressed as the mean±SEM (n=4)

1.48-fold, P=0.0019; htau46-G335V 1.51-fold, P=0.00002). Particularly remarkable was the more rapid and accelerated aggregation rate of the G335V mutants, as demonstrated by a ~6.5-fold higher thioflavin-S fluorescence after a 6-h incubation period (htau37-G335V 6.49-fold, P=0.0002; htau46-G335V 6.41-fold, P=0.00002).

Discussion

This study describes a novel mutation, G335V, in exon 12 of the *tau* gene in a family with frontotemporal dementia. It is highly likely that this mutation is pathogenic since it segregates with the clinical phenotype in the family and this genetic variation has not been found in 100 unaffected individuals.

The clinical features in the G335V family with early signs of personality changes and frontotemporal dementia are consistent with other clinical phenotypes described in FTDP-17 families. Interestingly, the initial diagnosis in the proband with the G335V mutation that we studied was schizophrenia, which has also been described in patients with the V337M mutation [17]. The age at onset in this family, with a mean of 25.4 years, differs from the later onset in most other FTDP-17 families, with an average age at onset of 49 years [18, 19]. So far, a similar early onset of disease has only been reported in families with the P301S mutation [20, 21]. Because no autopsies have been performed on deceased family members, no neuropathological data are available for the G335V mutation.

In the adult human brain, six tau isoforms are generated by alternative mRNA splicing, which differ by having either no, one or two 29-amino acid inserts in the amino-terminal half and either three or four carboxy-terminal repeat motifs in the MT-binding domain [22]. These motifs are composed of highly conserved 18-amino acid long binding elements with an invariant PGGG motif separated by flexible, but less conserved, 13–14 amino acids inter-repeat sequences [23, 24]. The G335V mutation is the fifth identified mutation in exon 12 (Q336R, V337M, E342V, K369I) [1, 10, 25, 26]. It affects a highly conserved residue within the repeat sequence by changing the invariant PGGG motif in the third MT-binding repeat (numbering according to the longest tau isoform) to PGGV. A glycine is found in all known tau species [27, 28]. So far, three other mutations (P301L, P301S and G272V) located in the PGGG motif have been described, affecting adjacent positions. Thus, for G272V the PGGG motif in the first repeat becomes PGVG and for P301L and P301S in the second repeat it becomes LGGG and SGGG, respectively [2, 21]. Functionally, the G335V mutation leads to a dramatic decrease in the ability of recombinant tau to promote MT assembly, consistent with results obtained with other mutations in the PGGG motif and most other missense mutations in the MT-binding domains [2, 7, 21]. Remarkably, this is in contrast to the inverse effect of the Q336R mutation affecting the adjacent amino acid residue, where an increased ability to interact with MT has been reported [10]. In addition, the G335V mutation leads to a highly significant increase in the heparin-induced tau filament formation for both the three-repeat and the four-repeat tau isoforms, as described for several other missense tau mutations including the Q336R mutation [8, 10–13].

In summary, the description of the novel G335V mutation in a family with early onset of FTDP-17 extends the spectrum of *tau* gene mutations whose primary pathological effect might be the reduced MT binding affinity. Consequently, this might increase the cytosolic concentration of unbound mutant tau making it available for aberrant tau aggregation and inclusion body formation.

Acknowledgements We thank M. Goedert for the *tau* cDNAs. This work was supported by the Deutsche Forschungsgemeinschaft grant KFO 113/1 to H.A.K. and M.N.

References

1. Poorkaj P, Bird TD, Wijsman E, Nemens E, Garruto RM, Anderson L, Andreadis A, Wiederholt WC, Raskind M, Schellenberg GD (1998) *Tau* is a candidate gene for chromosome 17 frontotemporal dementia. Ann Neurol 43:815–825
2. Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, Pickering-Brown S, Chakraverty S, Isaacs A, Grover A, Hackett J, Adamson J, Lincoln S, Dickson D, Davies P, Petersen RC, Stevens M, de Graaff E, Wauters E, van Baren J, Hillebrand M, Joosse M, Kwon JM, Nowotny P, Heutink P (1998) Association of missense and 5′ splice site mutations in *tau* with the inherited dementia FTDP-17. Nature 393:702–705
3. Spillantini MG, Murrel JR, Goedert M, Farlow MR, Klug A, Ghetti B (1998) Mutation in the *tau* gene in familial multiple system tauopathy with presenile dementia. Proc Natl Acad Sci USA 95:7737–7741
4. Rademakers R, Cruts M, Van Broeckhoven C (2004) The role of tau (MAPT) in frontotemporal dementia and related tauopathies. Human Mutat 24:277–295
5. Grover A, Houlden H, Baker M, Adamson J, Lewis J, Prihar G, Pickering-Brown S, Duff K, Hutton M (1999) 5′ Splice site mutations in *tau* associated with the inherited dementia FTDP-17 affect a stem-loop structure that regulates alternative splicing of exon 10. J Biol Chem 274:15134–15143

6. Hong M, Zhukareva V, Vogelsberg-Ragaglia V, Wszolek ZK, Reed L, Miller BI, Geschwind DH, Bird TD, McKeel D, Goate A, Morris JC, Wilhelmsen KC, Schellenberg GD, Trojanowski JQ, Lee VMY (1998) Mutation-specific functional impairments in distinct tau isoforms of hereditary FTDP-17. Science 282:1914–1917
7. Hasegawa M, Smith MJ, Goedert M (1998) Tau proteins with FTDP-17 mutations have a reduced ability to promote microtubule assembly. FEBS Lett 437:207–210
8. Barghorn S, Zheng-Fischhofer Q, Ackmann M, Biernat J, von Bergen M, Mandelkow EM, Mandelkow E (2000) Structure, microtubule interactions, and paired helical filament aggregation by tau mutants of frontotemporal dementias. Biochemistry 39:11714–11721
9. Hasegawa M, Smith MJ, Iijima M, Tabira T, Goedert M (1999) FTDP-17 mutations N279K and S305N in tau produce increased splicing of exon 10. FEBS Lett 443:93–96
10. Pickering-Brown SM, Baker M, Nonaka T, Ikeda K, Sharma S, Mackenzie J, Simpson SA, Moore JW, Snowden JS, Silva RR, Revesz T, Hasegawa M, Hutton M, Mann DM (2004) Frontotemporal dementia with Pick-type histology associated with Q336R mutation in the *tau* gene. Brain 127:1415–1426
11. Nacharaju P, Lewis J, Easson C, Yen S, Hackett J, Hutton M, Yen SH (1999) Accelerated filament formation from tau protein with specific FTDP-17 missense mutations. FEBS Lett 447: 195–199
12. Goedert M, Jakes R, Crowther RA (1999) Effects of frontotemporal dementia FTDP-17 mutations on heparin-induced assembly of tau filaments. FEBS Lett 450:306–311
13. Grover A, DeTure M, Yen SH, Hutton M (2002) Effects on splicing and protein function of three mutations in codon N296 of tau in vitro. Neurosci Lett 323:33–36
14. Goedert M, Jakes R (1990) Expression of separate isoforms of human tau protein: correlation with the tau pattern in brain and effects on tubulin polymerization. EMBO J 9:4225–4230
15. Grover A, England E, Baker M, Sahara N, Adamson J, Granger B, Houlden H, Passant U, Yen SH, DeTure M, Hutton M (2003) A novel *tau* mutation in exon 9 (1260V) causes a four-repeat tauopathy. Exp Neurol 184:131–140
16. Friedhoff P, Schneider A, Mandelkow EM, Mandelkow E (1998) Rapid assembly of Alzheimer-like paired helical filaments from microtubule-associated protein tau monitored by fluorescence in solution. Biochemistry 37:10223–10230
17. Sumi SM, Bird TD, Nochlin D, Raskind MA (1992) Familial presenile dementia with psychosis associated with cortical neurofibrillary tangles and degeneration of the amygdala. Neurology 42:120–127
18. Ghetti B, Hutton ML, Wszolek ZK (2003) Frontotemporal dementia and parkinsonism linked to chromosome 17 associated with *tau* gene mutations (FTDP-17T). In: Dickson D (ed) Neurodegeneration: the molecular pathology of dementia and movement disorder. ISN Neuropath, Basel, pp 86–102
19. Reed LA, Wszolek ZK, Hutton M (2001) Phenotypic correlations in FTDP-17. Neurobiol Aging 22:89–107
20. Sperfeld AD, Collatz MB, Baier H, Palmbach M, Storch A, Schwarz J, Tatsch K, Reske S, Joosse M, Heutink P, Ludolph AC (1999) FTDP-17: an early-onset phenotype with parkinsonism and epileptic seizures caused by a novel mutation. Ann Neurol 46:708–715
21. Bugiani O, Murrell JR, Giaccone G, Hasegawa M, Ghigo G, Tabaton M, Morbin M, Primavera A, Carella F, Solaro D, Grisoli M, Savoiardo M, Spillantini MG, Tagliavini F, Goedert M, Ghetti B (1999) Frontotemporal dementia and corticobasal degeneration in a family with a P301S mutation in *tau*. J Neuropathol Exp Neurol 58:667–677
22. Goedert M, Spillantini MG, Potier MC, Ulrich J, Crowther RA (1989) Cloning and sequencing of the cDNA encoding an isoform of microtubule-associated protein tau containing four tandem repeats: differential expression of tau protein mRNAs in human brain. EMBO J 8:393–399
23. Lee G, Neve RL, Kosik KS (1989) The microtubule binding domain of tau protein. Neuron 2:1615–1624
24. Butner KA, Kirschner MW (1991) Tau protein binds to microtubules through a flexible array of distributed weak sites. J Cell Biol 115:717–730
25. Lippa CF, Zhukareva V, Kawarai T, Uryu K, Shafiq M, Nee LE, Grafman J, Liang Y, George-Hyslop PH, Trojanowski JQ, Lee VM (2000) Frontotemporal dementia with novel tau pathology and a Glu342Val *tau* mutation. Ann Neurol 48:850–858
26. Neumann M, Schulz-Schaeffer W, Crowther RA, Smith MJ, Spillantini MG, Goedert M, Kretzschmar HA (2001) Pick's disease associated with the novel *Tau* gene mutation K369I. Ann Neurol 50:503–513
27. Goedert M, Baur CP, Ahringer J, Jakes R, Hasegawa M, Spillantini MG, Smith MJ, Hill F (1996) PTL-1, a microtubule-associated protein with tau-like repeats from the nematode *Caenorhabditis elegans*. J Cell Sci 109:2661–2672
28. Heidary G, Fortini ME (2001) Identification and characterization of the Drosophila tau homolog. Mech Dev 108:171–178