FTDP-17 mutations N279K and S305N in tau produce increased splicing of exon 10

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Abstract Missense mutations and intronic mutations in the tau gene cause frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17). Known missense mutations reduce the ability of tau to promote microtubule assembly. Intronic mutations lead to increased mRNA splicing of the alternatively spliced exon 10, resulting in an overproduction of tau isoforms with four microtubule-binding repeats. We show here that the recently identified FTDP-17 missense mutations N279K and S305N do not reduce the ability of tau to promote microtubule assembly. Instead they lead to increased splicing of exon 10, like the intronic mutations. The N279K and S305N mutations define a class of missense mutations in tau whose primary effects are at the RNA level.

Key words: Tau protein mutation; Microtubule assembly; RNA splicing; Frontotemporal dementia

1. Introduction

Mutations in the gene for microtubule-associated protein tau cause familial frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17) [1–4]. Four missense mutations and four intronic mutations have been identified in approximately 20 FTDP-17 families. Neuropathologically, all the FTDP-17 cases examined to date are characterised by an abundant filamentous tau pathology [5,6].

In adult human brain, six tau isoforms are produced from a single gene by alternative mRNA splicing [7]. They differ by the presence or absence of inserts of 29 and 58 amino acids 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 [7–9], gives rise to tau isoforms with four microtubule-binding repeats.

Intronic mutations are located close to the splice-donor site of the intron following exon 10, where they destabilise a predicted stem-loop structure [2,3]. They are found at positions +3, +13, +14 and +16, with the first nucleotide of the splice-donor site taken as +1. Intronic mutations have been shown to lead to increased production of exon 10-containing transcripts [2] and to increased levels of the three tau isoforms with four microtubule-binding repeats [3]. Overproduction of four-repeat tau isoforms may lead to an excess of tau over available binding sites on microtubules [3], resulting in the assembly of four-repeat tau isoforms into wide twisted ribbons [10]. The known exonic mutations in tau are located in the microtubule-binding repeat region or close to it [1,2,4]. They are G272V in exon 9, P301L in exon 10, V337M in exon 12 and R406W in exon 13 (using the numbering of the longest human brain tau isoform). Mutations in exon 10 only affect four-repeat tau isoforms, whereas missense mutations located outside exon 10 affect all six tau isoforms [1,2,4].

We have previously shown that the four exonic mutations all lead to a reduced ability of tau to promote microtubule assembly, which was more marked for three-repeat than for four-repeat isoforms [11]. The P301L mutation produced the largest effect, the R406W mutation the smallest effect and the G272V and V337M mutations intermediate reductions. A reduced ability of tau to interact with microtubules may be the primary effect of these missense mutations and may be necessary for setting in motion the mechanisms that lead to the assembly of tau into narrow twisted ribbons, as is the case of mutations in exon 10 [12], or into paired helical and straight filaments, as is the case of missense mutations located outside exon 10 [13]. A subsequent study using only four-repeat tau isoforms has also reported a reduced ability of mutant recombinant tau to promote microtubule assembly [14]. However, the relative effects of the various mutations were very different from those obtained in our study [11]. The reasons for these significant discrepancies remain to be established.

Recently, two additional missense mutations in exon 10 have been identified in two FTDP-17 families [15,16]. The N279K mutation (AAT to AAG) of pallido-ponto-nigral degeneration (PPND) [15] creates a purine-rich stretch (AAGAAGAAG), which resembles an exon splice-enhancer consensus sequence [17,18], suggesting that it may affect splicing in of exon 10 [15]. The S305N mutation (AGT to AAT) is located in the last amino acid of exon 10 [16]. It forms part of the predicted stem-loop structure at the exon 10-5′ intron boundary, where the G to A transition is found at position −1. As a result of this mutation, the predicted stem-loop is destabilised, suggesting that it may also lead to increased splicing of exon 10.

We have examined the effects of the N279K and S305N mutations on the splicing of exon 10 and the ability of tau to promote microtubule assembly. We show here that, unlike other missense mutations, the N279K and S305N mutations produce no reductions in the ability of tau to promote microtubule assembly. However, like the intronic mutations, they lead to increased splicing of exon 10, demonstrating that their primary effects are at the RNA level.

2. Materials and methods

2.1. Production of tau proteins and microtubule assembly

Site-directed mutagenesis was used to change N279 to lysine and

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S305 to asparagine (using the numbering of the 441 amino acid isoform of human brain tau) in the four-repeat 412 amino acid isoform (expressed from cDNA clone htau46). All constructs were verified by DNA sequencing. The P301L htau46 construct has been described [11]. Wild-type and mutated tau proteins were expressed in *Escherichia coli* BL21(DE3), as described [19]. Recombinant tau proteins were purified as described [11]. Tau protein concentrations were determined using densitometry (Molecular Dynamics) and calibration against tau protein of known concentration. Bovine serum albumin was used as the standard for densitometry. In each experiment, wild-type and mutant tau proteins were expressed and purified in parallel. Purified recombinant wild-type and mutant htau46 proteins (0.1 mg/ml, 2.3 μM) were incubated with bovine brain tubulin (1 mg/ml, 20 μM, cytoskeleton) in assembly buffer at 37°C, as described [11]. The assembly of tubulin was monitored over time by a change in turbidity at 350 nm.

2.2. Exon trapping

The wild-type construct and constructs with the N279K mutation, the P301L mutation, the S305N mutation and the +16 intronic mutation were used. For making the wild-type construct, PCR products were obtained from genomic DNA of a control individual. They included the 93 nucleotides of exon 10, as well as 34 nucleotides at the 3′ intron-exon 10 boundary and 85 nucleotides at the exon 10-5′ intron boundary, with *Sac*I sites at each end. Site-directed mutagenesis was used to introduce the N279K, P301L and S305N missense mutations in exon 10, as well as the +16 intronic mutation in the intron following exon 10. All the constructs were verified by DNA sequencing. PCR products were digested with *Sac*I and subcloned into the *Sst*I site of the splicing vector pSPL3 (Life Technologies). For exon trapping, COS7 cells were transiently transfected with 5 μg/ml plasmid DNA using DEAE-dextran/chloroquine. Cells were harvested 24 h later and RNA extracted using the Trizol reagent (Life Technologies). First-strand cDNA synthesis and PCR were done using a commercially available system (Life Technologies), following the manufacturer's instructions. The nested PCR reactions used 30 cycles (denaturation 95°C, 1.5 min; annealing 60°C, 1.5 min; extension 72°C, 2.5 min). The proportion of exon 10-containing transcripts was determined by image analysis of photographs of ethidium bromide-stained agarose gels of the PCR products. Various amounts of the primary PCR reactions and different numbers of amplification cycles were used in control experiments, to ensure that the PCR reactions were quantitative. In each experiment, all five constructs were run in parallel. The identities of the PCR products were verified by DNA sequencing.

3. Results

Recombinant htau46 proteins with the N279K or S305N mutation in exon 10 (Fig. 1) were expressed and their ability to promote microtubule assembly examined. When compared with wild-type htau46, N279K htau46 had a similar ability to promote microtubule assembly (Fig. 2). S305N htau46 showed a slightly increased ability to promote microtubule assembly, when compared with wild-type protein; the increase was approximately 15%, when expressed as the optical density at 2 min (Fig. 2). These findings contrast with those obtained with P301L htau46, which showed a greatly reduced ability to promote microtubule assembly, in confirmation of our previous findings (Fig. 2) [11].

Exon trapping was used to investigate the effects of the N279K and S305N mutations on the splicing of exon 10-containing transcripts, with the P301L mutation and the +16 intronic mutation being used as controls. The results were expressed as the ratios of exon 10-containing over exon 10-lacking transcripts (Fig. 3). The P301L mutation produced no change, whereas the +16 intronic mutation produced an approximately 11-fold increase in the ratio of exon 10-containing over exon 10-lacking transcripts, in confirmation of a previous study (Fig. 3) [2]. Both the N279K and S305N mutations produced large increases in the splicing in of exon 10 (Fig. 3).

A 18-fold increase in the ratio of exon 10-containing over exon 10-lacking transcripts was observed for the N279K mutation and a 26-fold increase for the S305N mutation (Fig. 3).

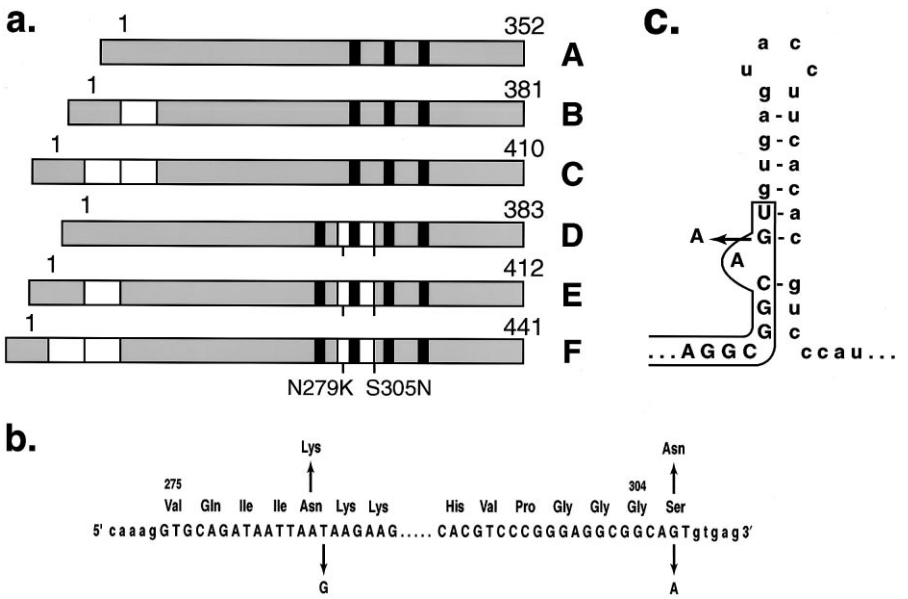

Fig. 1. The N279K and S305N mutations of the tau gene in FTDP-17. a: Schematic diagram of the six tau isoforms (A–F) that are expressed in adult human brain. Alternatively spliced exons 2, 3 and 10 are shown in white. The N279K and S305N mutations in the alternatively spliced exon 10 are indicated. They only affect tau isoforms with four microtubule-binding repeats (D–F) (the repeats are shown as black bars). b: Nucleotide sequence of the exon 10-intron junctions. c: Structure of the predicted stem-loop in the pre-mRNA of tau. Exon sequences are shown in capital letters and intron sequences in small letters. The N279K and S305N mutations are indicated in panel b and the G to A transition of the S305N mutation in panel c.

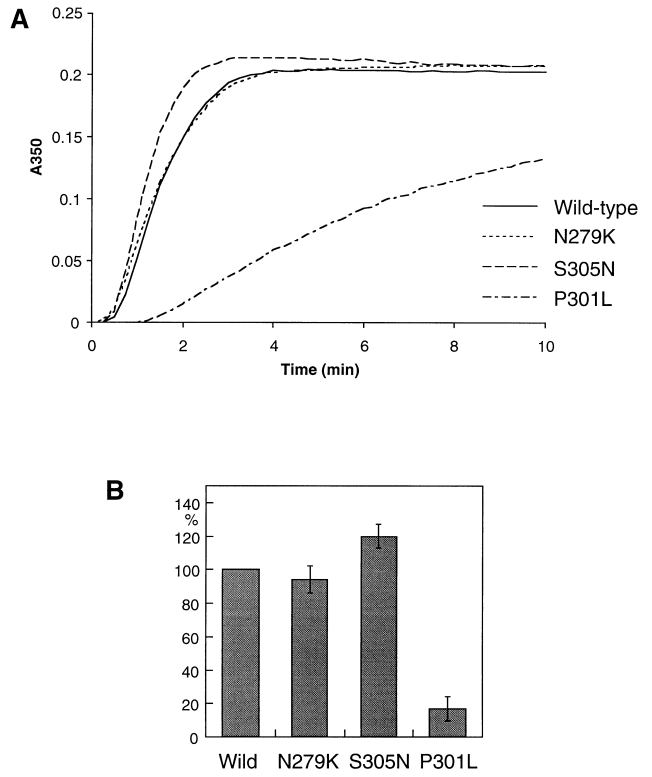

Fig. 2. Effects of the N279K, P301L and S305N mutations on the ability of four-repeat htau46 (412 amino acid isoform of human tau) to promote microtubule assembly. A: Polymerisation of tubulin induced by wild-type htau46, htau46 N279K, htau46 P301L and htau46 S305N, as monitored over time by turbidimetry. A typical experiment is shown; similar results were obtained in three separate experiments. B: Optical densities for wild-type htau46 and the three tau mutants at 2 min (expressed as % of wild-type htau46, taken as 100%). The results are expressed as means ± S.D. ($n = 3$). Amino acid numbering is according to the 441 amino acid isoform of human tau.

4. Discussion

Missense mutations and intronic mutations in the tau gene have recently been identified as the genetic lesions responsible for familial FTDP-17 [1–4]. All the FTDP-17 cases examined to date share an abundant filamentous tau pathology, with characteristic tau isoform patterns and filament morphologies [5,6,20]. Functionally, mutations studied so far fall into two distinct groups. Missense mutations have been shown to produce a reduced ability of tau to interact with microtubules [11]. This contrasts with the intronic mutations, which lead to increased splicing in of exon 10 [2], resulting in a net overproduction of soluble tau isoforms with four microtubule-binding repeats [3].

Here we describe the functional effects of the N279K and S305N mutations in exon 10. The N279K mutation had no significant effect on the ability of four-repeat tau to promote microtubule assembly, in agreement with a recent report [14]. This contrasts with four-repeat tau with the S305N mutation which had a slightly increased ability to promote microtubule assembly. It represents the first example of a FTDP-17 mutation that produces an increased ability of tau to interact with microtubules. It remains to be seen whether this functional effect contributes to the pathogenesis of FTDP-17 in this family. These findings indicate that the primary effects of the N279K and S305N mutations differ from those of the G272V, P301L, V337M and R406W missense mutations, which produce a reduced ability of tau to promote microtubule assembly [11].

It has been suggested that the N279K mutation may lead to increased splicing of exon 10, as it produces a purine-rich stretch [15], which resembles a splice-enhancer consensus sequence [17,18]. We have therefore used exon trapping to investigate the effects of the N279K mutation on the splicing of exon 10. A large increase in exon 10-containing transcripts was observed, demonstrating that the primary effect of this missense mutation is at the RNA level. The S305N mutation changes the last amino acid of exon 10 and destabilises the predicted stem-loop at the exon 10-5′ intron boundary [16]. By exon trapping, it was found to produce a large increase in the splicing in of exon 10, demonstrating that its primary effect is also at the RNA level.

The locations of tau mutations and their primary effects appear to determine the filamentous and cellular pathologies

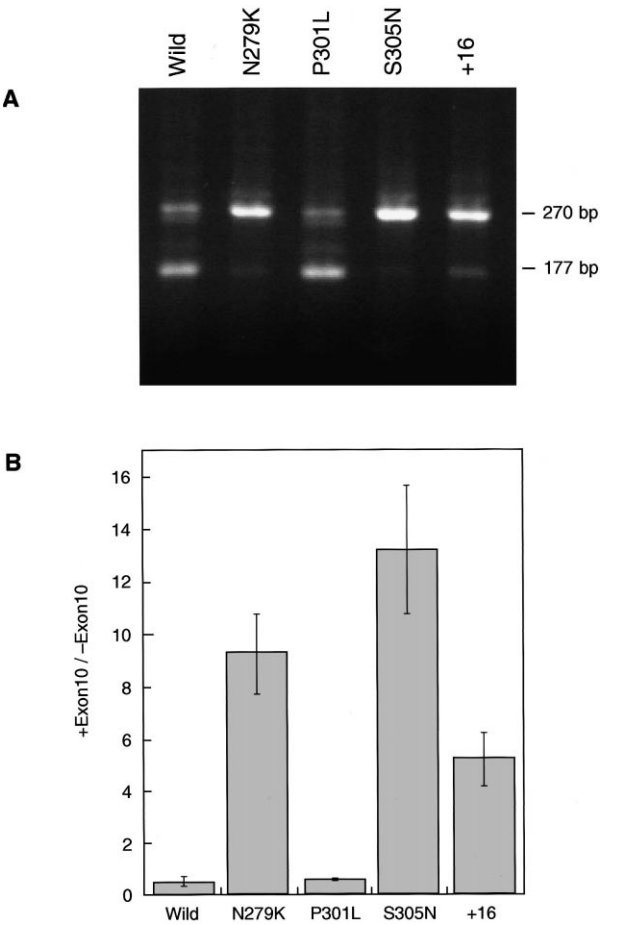

Fig. 3. Exon trapping analysis of the effects of the N279K, P301L and S305N missense mutations and of the +16 intronic mutation on the splicing of exon 10 of the tau gene. A: Ethidium bromide-stained agarose gel of nested PCR products. The 270 bp band contains exon 10, whereas the 177 bp band lacks exon 10. A single experiment is shown. Similar results were obtained in three separate transfection experiments. B: Quantitative analysis of exon trapping of wild-type construct and constructs with the N279K, P301L, S305N missense mutations and the +16 intronic mutation. The results are expressed as means ± S.D. ($n = 3$) of the ratios of exon 10-containing (+Exon 10) over exon 10-lacking (−Exon 10) transcripts.

of FTDP-17 [6,21]. Thus, missense mutations in exon 10, which produce a reduced ability of tau to promote microtubule assembly [1,2,4,11,14], lead to the formation of narrow, twisted ribbon-like filaments that consist predominantly of four-repeat tau isoforms [12]. The tau pathology is neuronal and glial. Intronic mutations lead to an overproduction of soluble four-repeat tau isoforms [2,3] and the assembly of four-repeat tau into wide twisted ribbons [10]. The tau pathology is neuronal and glial, with a more extensive glial pathology than in cases with an exon 10 missense mutation that results in a reduced ability of tau to interact with microtubules [6,11,12]. Missense mutations located outside exon 10 lead to the formation of paired helical and straight filaments which are made of all six tau isoforms [1,13], like those from Alzheimer's disease brain [22].

The N279K mutation is a missense mutation in exon 10 which leads to increased splicing in of exon 10. It is therefore expected to lead to an overproduction of soluble four-repeat tau isoforms and the formation of wide twisted ribbons made of four-repeat tau, as in the FTDP-17 cases with intronic mutations [3,10]. It is also expected to lead to a neuronal and glial tau pathology, with an extensive glial component. The known characteristics of the tau pathology of PPND, a FTDP-17 with the N279K mutation, are entirely consistent with these expectations [14,15,23]. The S305N mutation in tau is predicted to lead to an overproduction of four-repeat tau isoforms, followed by their assembly into wide twisted ribbon-like filaments, as in the FTDP-17 cases with the N279K mutation and the intronic mutations [3,10,14,23]. These studies must await the availability of suitable brain tissue. Like the N279K mutation, the S305N mutation has been shown to lead to a neuronal and glial tau pathology, with an abundant glial component [16].

In conclusion, we have shown that the primary effects of two missense mutations located in exon 10 of the tau gene are at the RNA level. Functionally, the N279K and S305N mutations behave like the known intronic mutations, which also produce large increases in exon 10-containing transcripts.

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