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Novel mutation in *MAPT* exon 13 (p.N410H) causes corticobasal degeneration

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Abstract

In order to determine the frequency of microtubule associated protein tau gene (*MAPT*) mutations and rare variants in CBD, we performed a systematic sequence analysis of *MAPT* coding and 3' untranslated region (3'UTR) in a large cohort of autopsy-confirmed CBD patients ($N=109$). This identified a novel *MAPT* mutation in exon 13, p.N410H, in a case that is neuropathologically indistinguishable from sporadic CBD. On immunoblot, the p.N410H mutation carrier had the same insoluble tau profile as seen in CBD. Additionally, tau expression analysis in brain tissue found a significant increase in the 4R/3R tau mRNA ratio ($P=0.04$), indicating that p.N410H disrupts tau isoform homeostasis. Biochemically, recombinant tau protein with p.N410H showed a marked increase in tau filament formation compared to wild-type tau ($P<0.001$), had a 19.2% decrease in rate of microtubule assembly ($P<0.05$), and a 10.3% reduction in the extent of total microtubule polymerization ($P<0.01$). Sequence analysis of the complete *MAPT* 3'UTR in autopsy-confirmed CBD cases further identified two rare variants with nominally significant association with CBD. An ATC nucleotide insertion ("MAPTv8") was found in 4.6% of CBD patients compared to 1.2% of controls ($P=0.031$, OR=3.71), and rs186977284 in 4.6% CBD patients, but only 0.9% of controls ($P=0.04$, OR=3.58). Rs186977284 was also present in 2.7% of a large cohort of autopsy-confirmed PSP patients ($N=566$) and only 0.9% of an additional control series ($P=0.034$, OR=3.08), extending the association to PSP. Our findings show that mutations in *MAPT* can cause CBD and *MAPT* non-coding variants may increase the risk of complex 4R tauopathies.

Keywords

Corticobasal degeneration; progressive supranuclear palsy; tauopathy; microtubule associated protein tau; TDP-43; *MAPT* mutations

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INTRODUCTION

Corticobasal degeneration (CBD) is a sporadic neurodegenerative disorder pathologically classified as a primary tauopathy due to neuronal and glial aggregates of hyperphosphorylated microtubule-associated protein tau throughout the brains of these patients [11]. CBD is associated with focal cortical atrophy and because of this, patients can present with a wide range of clinical syndromes depending on the location of the most marked atrophy. Most commonly, CBD patients present with corticobasal syndrome, Richardson syndrome, or frontotemporal dementia [21]. Progressive supranuclear palsy (PSP) is a related tauopathy that has some overlapping clinical and pathologic features with CBD, yet is considered a distinct disease entity [3,10,23].

Microtubule associated protein tau encoded by the *MAPT* gene binds to microtubules and is important for maintaining neuronal morphology and function. Mutations in *MAPT* disrupt tau splicing and/or the binding of tau to microtubules, often increase the aggregation properties of tau, and lead to frontotemporal dementia with parkinsonism (FTDP-17), unequivocally demonstrating that tau dysfunction is sufficient to cause neurodegeneration [18,29,33]. Though there are rare familial cases [13], CBD and PSP are considered sporadic disorders. Yet, despite their sporadic nature, genetic variants at the *MAPT* locus are a strong risk factor for the development of CBD and PSP. Conrad, *et al.* reported an association of risk of PSP with a dinucleotide repeat located in intron 9 of *MAPT* [7]. Subsequently, this association was extended to include CBD and shown to include multiple polymorphisms in complete linkage disequilibrium that are part of the extended *MAPT* H1 haplotype [4,12,17,28,31]. Findings from the recently completed PSP genome-wide association study confirmed that the H1 *MAPT* haplotype confers risk for developing PSP ($P=1.5 \times 10^{-116}$, OR=5.5) [16].

In this study, we performed systematic *MAPT* sequencing analyses in a large cohort of pathologically-confirmed CBD. To understand the role of rare variants, we performed sequence analysis of the *MAPT* coding region in 109 CBD patients as well as the entire ~4kb 3'UTR in 85 CBD patients. Excluding H1/H2-defining polymorphisms, a subset of 3'UTR variants were further tested for association in CBD and PSP case-control series.

MATERIALS AND METHODS

Subjects and samples

Patients with a neuropathologic diagnosis of corticobasal degeneration [11] and frozen tissue were identified from the Mayo Clinic Jacksonville brain bank between 1999 and 2010. Control series were ascertained at the Mayo Clinic Florida (MCF) and Mayo Clinic Arizona (MCA) and were diagnosed by a neurologist to be cognitively normal at the time of blood draw.

DNA sequencing

DNA from 109 CBD cases was screened for mutations in *MAPT* for all CNS-expressed coding exons (exons 1-7 and 9-13). To determine the genetic variability in the *MAPT* 3'UTR, 69 CBD cases homozygous for the *MAPT* H1 haplotype were further sequenced over the region encompassing ~4 kb of the *MAPT* 3'UTR (UCSC genome browser, chr17:44,101,538- 44,105,704, Feb. 2009 assembly) plus 200bp flanking the 3'UTR. Sixteen H1/H2 heterozygote CBD patients were also sequenced, but the presence of numerous insertion and deletion polymorphisms prohibited Sanger sequencing of the entire 3'UTR for these patients. Two individuals homozygous for *MAPT* H2 haplotype were included as a reference for haplotype-defining variants. PCR reactions of approximately 500bp fragments

were performed in 15µl reactions in 384-well plates. PCR products were purified using AMPure (Agencourt Biosciences), then sequenced in both directions using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, USA). Sequencing reactions were purified using CleanSEQ (Agencourt Biosciences) and analyzed on an ABI 3730 Genetic Analyzer (Applied Biosystems). Base calling, sequence alignments, and heterozygote detection were performed using Sequencher (Gene Codes).

Genotyping analysis

Genetic variants identified by sequence analysis were genotyped using MassArray iPLEX technology (Sequenom) and genotype calls were made using Typer 4.0 software following manufacturer's instructions. One H1/H2 haplotype-defining SNP, rs1052553, was included in the iPLEX SNP panel in order to use *MAPT* haplotype as a covariate in association analyses. The variants that did not multiplex in Sequenom assay design (rs11331969, rs186977284, and rs75182761) were genotyped using custom Taqman SNP genotyping assays (Applied Biosystems), read on 7900HT Fast Real Time PCR system, and genotype calls were made using SDS v2.2 software.

Tissue sampling and neuropathologic assessment

Primary and association cortices, basal ganglia, diencephalon, brainstem and cerebellum were evaluated with tau immunohistochemistry as previously described [21]. Thioflavin S fluorescent microscopy was used to assess Alzheimer-type pathology, and haematoxylin and eosin (H&E) stained sections were used to evaluate neuronal loss and gliosis. For tau and TDP-43 immunohistochemistry, sections were processed using a DAKO Autostainer (Universal Staining System, Carpinteria, CA) using 3,3'-diaminobenzidine (DAB) as the chromogen and a phospho-tau antibody (CP13, mouse IgG1, 1:1000, kind gift of Peter Davies, Albert Einstein College of Medicine, Bronx, NY, USA), 3R tau antibody (RD3, Millipore, Temecula, CA), 4R tau antibody (RD4, Millipore, Temecula, CA), or a monoclonal phospho-TDP-43 antibody (ps409/410, 1:5000, Cosmobio Co., Tokyo, Japan). After immunostaining, the sections were counterstained with haematoxylin.

Recombinant tau purification

Recombinant tau was expressed and purified as previously described [1]. Wild type and the p.N410H mutant 4R0N tau cDNA were cloned into pET30a and expressed in competent BL21 (DE3) cells. Briefly, overnight cultures were used to inoculate bulk media at 1/100, and these were grown to an OD (600) of 0.5 and induced by adding 0.5 mM IPTG for 2.5 hours. Cell pellets were collected, washed in 1× PBS and stored at −80°C. The cells were lysed with three freeze and thaw cycles, and the tau proteins were then purified by heating the lysates for 10 minutes at 80°C and isolating the tau proteins from clarified supernatants using ion exchange chromatography. The fractions containing tau proteins were then dialyzed overnight in 80 mM PIPES, 2mM MgCl₂ and 0.5mM EGTA at pH 6.8 with two buffer changes. These samples were further purified by HPLC C8 reverse phase chromatography if degraded fragments were detected following purification [9]. The purity of the tau preparations was analyzed by SDS-polyacrylamide gel electrophoresis and Coomassie blue staining, and protein concentrations were determined using the BCA protein assay kit with bovine serum albumin as a standard (Pierce, Rockford, IL).

Microtubule Assembly

Microtubule assembly with recombinant tau proteins was performed in a 96 well plate in a final volume of 100 µl. Ice-cold tubulin at 1.5 mg/ml (30 µM) (Cytoskeleton Inc.) was added to 0.12 mg/ml (3 µM) of recombinant tau protein in assembly buffer (80 mM PIPES, 2mM MgCl₂, 0.5mM EGTA 1 mM GTP) also on ice and immediately transferred to a 96

well plate equilibrated to 37°C. The extent of microtubule assembly was monitored by turbidity assay per the manufacturer's recommendation, and the absorbance (optical density) was measured at 340 nm on a SpectraMax M5 Multi-Mode Microplate Readers (Molecular Devices, California). Reactions were run in quadruplicate, and this allowed both the rate and extent of microtubule polymerization to be assessed.

Tau filament formation

Polyglycosaminoglycan-induced tau aggregation reactions were performed as previously described [1]. Briefly, 8 μ M of tau and 0.04 mg/ml of low molecular weight heparin or dextran sulfate were set up in 10mM HEPES at pH 7.4, 100mM NaCl. Samples were incubated at 37°C and analyzed at 30 and 90 minute time points. Tau filament polymerization was measured directly by adsorption of 10 μ l of reaction mixture onto a carbon/Formvar grid (EM Sciences Inc.) for 60 seconds and staining with 2% uranyl acetate for 60 seconds. Electron micrographs were captured using a Phillips EM208S electron microscope and camera. Tau filament length measurements and quantification were performed blinded to genotype by one analyzer using ImageScope software (version 11.2; Aperio Technologies). Each genotype had nine electron micrograph images captured from a set of predetermined grid regions per time point. This allowed for the number of tau filaments to be counted for each field, and their individual length and the total polymer mass could then be determined. Tau aggregation properties were confirmed independently using the diagnostic thioflavin-S fluorescence which provides a measure of the cross -sheet secondary structure that is formed as tau proteins polymerize into filaments.

Western blot

Sarkosyl insoluble protein fractions were extracted from frontal cortex of sporadic CBD cases and the p.N410H mutation carrier, samples were prepared and separated on 10% Tris-glycine gels (Invitrogen Life Technologies, USA), transferred to PVDF membrane, and immunoblotted as previously described [21]. The primary antibody used against phosphorylated tau was PHF-1 (1:1000, from Peter Davies, Albert Einstein College of Medicine, NY), which recognizes the C-terminal region of phosphorylated tau (p-Ser396/p-Ser404) and anti-mouse IgG secondary antibody (1:5000).

Tau Quantitative Real-Time PCR

Total RNA was extracted from gray matter of the frontal cortex with the RNeasy Plus Mini Kit (Qiagen, USA) and RNA integrity was checked on an Agilent 2100 Bioanalyzer (Agilent Technologies, USA). The Power SYBR Green RNA-to-CT 1-Step RT-PCR kit (Invitrogen Life Technologies, USA) was used according to manufacturer protocol. Isoform-specific primers were used for 3R tau (F: AGGCGGGAAGGTGCAAATAG; R: TCCTGGTTTATGATGGATGTT) and 4R tau (F: GAAGCTGGATCTTAGCAACG; R: GACGTGTTTGATATTATCCT) [19]. Total RNA (30 ng) was used in 10 μ l reactions and qRT-PCR was performed on a 7900HT Fast Real-Time PCR System (Applied Biosystems) and data were imported and analyzed in SDS v2.2. All samples were run in triplicate and tau mRNA 4R:3R tau ratios in the p.N410H carrier were compared to a pathologically normal individual. Relative expression was determined using the $\Delta\Delta$ Ct method after normalization to the geometric mean of GAPDH and RPLPO expression.

Statistical analysis

All genetic association analyses were performed with PLINK software available at <http://pngu.mgh.harvard.edu/purcell/plink/> [30]. Association of *MAPT* variants with CBD or PSP was tested under an additive model adjusting for age at death, sex, and for the number of

MAPT H1 haplotypes (0, 1, or 2). Statistical analyses of all functional studies were performed using Student's t-test.

RESULTS

Sequencing and association studies of *MAPT* in CBD

Sequence analysis of the entire coding region of the *MAPT* gene in 109 pathology-confirmed CBD cases identified one novel coding *MAPT* mutation in exon 13, c.1228A>C (relative to *MAPT* isoform 2 NM_005910) predicted to result in p.N410H substitution (Fig. 1). This mutation has not been previously reported in dbSNP or the 1000 Genomes databases and genotyping using a custom-designed ABI Taqman assay excluded this mutation from 1224 healthy controls and 566 PSP cases. Sequence analysis of the *MAPT* coding region additionally identified the rare p.A152T polymorphism in *MAPT* exon 7 in one pathologically-confirmed CBD case, which has been previously reported [8].

Sequence analysis of *MAPT* 3'UTR identified 31 genetic variants of which 27 were known SNPs and 21/27 variants were *MAPT* H1/H2 haplotype-defining SNPs. Of the remaining 6 novel SNPs, three were common and occurred only on the H1 haplotype, and three were rare variants (MAF<5%). The 4 novel variants were rare and identified in 5 CBD patients or less. To determine whether any of the none H1/H2 defining SNPs in the *MAPT* 3'UTR were implicated in CBD risk, we genotyped these 10 variants in 643 controls and a total of 108 autopsy-confirmed CBD patients, including the 85 patients we used for sequencing (Table 1). The variants are listed in Table 2 and genotype counts and frequencies are provided in Online Resource Table 1. When controlling for *MAPT* haplotype (using rs1052553), the common variants that occur on the H1 haplotype did not show association with CBD. In contrast, two of the rare variants, an ATC insertion ("MAPTv8"; chr17:44104155:44104157; UCSC Genome Browser February 2009 (GRCh37/hg19) assembly) and rs186977284 showed nominally significant association with CBD as assessed by logistic regression analysis using an additive model with age, sex, and number of H1 alleles as covariates (Table 2). The effects observed for the two variants were similar, with overlapping odds ratio (OR) and 95% confidence intervals (95% CI), indicating an increased risk of CBD in the presence of their minor allele. Although the MAPTv8 and rs186977284 association with CBD does not surpass the significance threshold for multiple testing correction, MAPTv8 was present in 4.6% of CBD compared to 1.2% frequency in Control Series 1 ($P=0.031$, $OR=3.71$) and rs186977284 was present in 4.6% CBD patients compared to 0.9% frequency in Control Series 2 ($P=0.045$, $OR=3.58$). To provide additional evidence for the involvement of these two non-coding variants in tauopathies, we next tested for association in 566 autopsy-confirmed PSP patients and Control Series 2 consisting of 659 individuals. Logistic regression analysis using an additive model with age, sex, and number of H1 alleles as covariates found rs186977284 to also associate with PSP ($OR=3.07$, 95% $CI=1.08-8.71$, $P=0.035$), but MAPTv8 did not show evidence of an association with PSP (Table 2).

p.N410H *MAPT* mutation in pathologically-confirmed CBD

Clinical history—The female patient carrying this mutation had an age at onset of 63 years, with the chief complaint being forgetfulness and problems with mood and memory. There was a positive family history for dementia in an aunt. On initial neurological examination one year after onset, she had mild cognitive impairment and parkinsonism. She later developed a flat affect. Her movements became slowed; she lost facial expression; and her voice softened. Three years after disease onset, memory impairment became more severe, and she began falling. The following year, she had gait freezing, and she became aggressive, with increasingly poor insight, concentration, and comprehension. On

examination, she was abulic; her gait was unsteady; and her gait was stooped with small steps. Ocular exam was noted for abnormal saccades and smooth pursuit. Four years into the disease, she was practically aphonic with impaired eye movement, and some postural hand tremor. She developed obsessive use of hands either picking her nose or scratching her hair. A year later, she became mute and did not answer questions or follow commands. She had hypokinesia and progressive rigidity on the left more than right side. Behavioral dyscontrol progressed and she became incontinent of urine. Her antemortem differential diagnosis was progressive supranuclear palsy syndrome versus corticobasal syndrome. She died at age 67 after a five year disease course.

Neuropathology—The fixed brain weighed 1000 grams. Macroscopic examination revealed mild atrophy of the frontal convexity (Fig. 2a) and the midbrain showed marked loss of pigment in the substantia nigra (Fig. 2b). On coronal sections there was mild atrophy of the superior frontal cortex (Fig. 2c) and mild enlargement of frontal and temporal horns of the lateral ventricle. On microscopic examination, tau immunohistochemistry revealed fine, granular inclusions, or tau pretangles, most notably in small neurons of layer II. There was a high density of 4R tau-immunoreactive and argyrophilic astrocytic plaques throughout affected cortical regions (Fig. 3a, b) and accompanied by thread-like tau pathology in both gray and white matter (Fig. 3d, e). Superior frontal gyrus had spongiosis, gliosis, and ballooned neurons (Fig. 3c), most numerous in superior frontal and cingulate gyri. The frontal white matter had myelin staining pallor and patchy gliosis. Putamen and globus pallidus had numerous 4R tau-immunoreactive pretangles, astrocytic plaques, and thread-like processes. White matter structures, including anterior commissure, internal capsule, and pencil fibers in the putamen had tau positive thread-like processes and oligodendrocyte coiled bodies. Globus pallidus had neuronal loss and gliosis with hemosiderin-like pigment and axonal spheroids. Thalamus was histologically unremarkable on H&E, but had extensive tau-immunoreactive lesions in the anterior nuclei, medial and dorsal nuclei, as well as ventral lateral nuclei. Subthalamic nucleus had moderate neuronal loss and gliosis in the medial portion of the nucleus and to a lesser extent in the lateral portion of the nucleus. There were numerous tau neuronal pretangles in the amygdala, basal nucleus of Meynert, and hypothalamus. Amygdala also had many tau-immunoreactive threads, grains, astrocytes, and ballooned neurons. The substantia nigra had marked pigment loss due to dopaminergic nerve cell degeneration. There were significant tau-immunoreactive lesions in the pons and cerebellum, especially a high density of oligodendroglial coiled bodies and tau pretangles in the cerebellar dentate.

Immunohistochemistry for 3R tau was negative in superior frontal, motor, cingulate, temporal, and somatosensory cortices, posterior hippocampus, putamen, globus pallidus, amygdala, nucleus basalis, and hypothalamus. Neocortical and hippocampus sections were negative for senile plaques and neurofibrillary tangles by thioflavin-S fluorescent microscopy, but there were numerous threads and grains in the CA1 and subiculum.

The p.N410H carrier had significant TDP-43 pathology (Fig. 3f). The severity and neuroanatomical distribution of tau and TDP-43 immunoreactivity are summarized in Table 3. There were scarce TDP-43 immunoreactive lesions in neocortical regions in contrast to sections from the basal ganglia, which had numerous TDP-43 neuronal cytoplasmic inclusions, especially in striatum, globus pallidus, and subthalamic nucleus. Additionally, TDP-43-immunoreactive oligodendroglial inclusions and thread-like processes were found in several brain regions, including striatum, diencephalon, midbrain tectum, and pontine tegmentum (Fig. 3f). There were numerous tau-positive neurons in the granule cells of the dentate fascia and pyramidal layer, but no TDP-43 positive inclusions were observed. In a larger CBD cohort we screened a section of basal forebrain with TDP-43 immunohistochemistry and found TDP-43 pathology 34/117 (29%) cases. Semiquantitative

lesion scores in four sporadic CBD cases revealed that TDP-43 inclusion density was greatest in caudate/putamen, globus pallidus, hypothalamus, subthalamic nucleus, substantia nigra, and locus ceruleus. There were no TDP-43 inclusions in the dentate gyrus of the hippocampus or subiculum (Table S2. Cases were previously described in [22]). When compared to the p.N410H case, TDP-43 pathology in sporadic CBD did not significantly differ in severity or distribution.

In vivo characterization of p.N410H mutant tau in patient brain samples—

Immunoblot analysis was performed on sarkosyl-insoluble fractions prepared from superior frontal cortex in three sporadic CBD cases and the p.N410H mutation carrier (protein extracted from three different frontal cortex sections). Using the phosphorylation-dependent PHF-1 antibody, the typical doublet at 64 and 68 kDa composed of 4R tau was observed in all cases (Fig. 4a). Most importantly, the p.N410H case showed the same lower molecular weight tau species, two bands at ~37 kDa, as those found in sporadic CBD that characterize the disease biochemically with regard to aggregated lower molecular weight tau species (Fig. 4a, asterisks). To determine the effect of p.N410H on tau expression, quantitative real-time PCR for 4R tau and 3R tau was performed on total RNA isolated from the frontal cortices of a neuropathologically normal individual and the p.N410H carrier. A p.N279K mutation carrier was included as an enhancer of exon 10 splicing positive control. Expression analysis showed that the p.N410H mutation carrier had a significant increase in the 4R:3R tau mRNA ratio compared to the normal case ($P=0.04$) (Fig. 4b).

p.N410H mutant tau has impaired ability to promote microtubule assembly in vitro—

In order to assess the functional consequences of this increased 4R tau with the p.N410H mutation, the effects on tubulin polymerization and tau aggregation properties of mutant tau were compared to wild-type (WT) tau using *in vitro* assays. Microtubule assembly and tau filament formation assays were performed comparing WT and p.N410H tau in the 4R0N isoform which aggregates preferentially in CBD. Tau protein with the p.N410H mutation showed a decrease in its ability to promote microtubule assembly when compared to WT tau as both the rate and extent of tubulin polymerization were shown to be modestly, but significantly reduced (Fig. 5a). The rate of tubulin polymerization decreased from 0.00032 ± 0.000030 OD340/min in WT tau to 0.000260 ± 0.000043 OD340/min ($P<0.05$) when the p.N410H mutation was introduced, and the steady state levels of polymerized microtubules decreased from 0.0722 ± 0.0028 OD340 to 0.0648 ± 0.0031 OD340 ($P<0.01$) with the p.N410H mutation. These represent a significant loss of normal tau function, as the rate of microtubule assembly decreased by 19.2% and the extent of total microtubule polymerization decreased 10.3% with mutated p.N410H tau.

p.N410H induces tau filament formation—The effects of the p.N410H mutation in the 4R0N isoform on tau filament assembly were also examined as this toxic gain of function is commonly observed in tauopathies, and it appears the tau aggregates themselves or their precursors are specifically toxic [32]. Upon inducing filament formation *in vitro*, the extent of p.N410H tau filament assembly after 30 minutes incubation was assessed directly using electron microscopy and observed to be statistically unchanged compared to WT tau with no effects on tau filament nucleation or total polymer mass per field observed (data not shown). However as shown in Fig. 5b and 5c, by 90 minutes both the rate of nucleation and the extent of aggregation were markedly increased as the number of tau filaments observed per field increased from 41 ± 14 to 179 ± 21 ($P<0.001$) when the p.N410H mutation was introduced and the total tau filament length per field increased from 5200 ± 1540 nm/field to 22110 ± 2060 nm/field ($P<0.001$). Representative electron micrographs of WT tau (Fig. 5d) and p.N410H tau (Fig. 5e) at 90 minutes illustrate the extent of tau filament formation. These p.N410H filament assembly effects appeared to be largely nucleation driven as the

average filament length was unchanged between WT (126 ± 36 nm) and p.N410H tau (123 ± 50 nm) at the 90 minute time point. These large increases in tau aggregation with the p.N410H mutation were supported by data collected using thioflavin S fluorescence as a measure of tau folding into fibrillogenic forms (Online Resource Fig. 1).

DISCUSSION

In a cohort of autopsy-confirmed CBD patients, we report the identification of a patient with a novel *MAPT* mutation in exon 13, p.N410H, located at a highly conserved amino acid residue. Although we were unable to test other family members for segregation, 1224 normal controls and 566 PSP cases, and genome databases were all negative for p.N410H. We showed that sporadic CBD and p.N410H have the same neuropathologic features and aggregated tau protein species on immunoblot analysis, further illustrating the similarities between p.N410H and sporadic CBD. To the best of our knowledge, this is the first case meeting neuropathologic diagnostic criteria for CBD harboring a *MAPT* mutation.

Our *in vivo* and *in vitro* studies of p.N410H strongly support the pathogenic nature of this novel mutation. We showed that the p.N410H *MAPT* mutation in exon 13 disrupts tau alternative splicing, as evidenced by a more than two-fold increase in the 4R/3R tau mRNA ratio compared to a normal individual. Comparably, the p.E342V exon 12 tau mutation has also been shown to increase 4R tau mRNA [25], demonstrating that it is possible to alter 3R and 4R tau mRNA levels through a mutation located outside of exon 10. This suggests that even though the p.N410H mutation is located distal to exon 10, it may contribute to disease through a tau alternative splicing mechanism that disturbs the normal tau 3R and 4R ratios. Alternatively, differential pathogenic effects of p.N410H on 3R tau isoforms versus 4R tau isoforms may be contributing to the altered 4R/3R tau ratio in the frontal cortex of the p.N410H carrier. In addition to altered tau mRNA expression levels in brain tissue, our *in vitro* studies showed that p.N410H has concomitant microtubule assembly impairment and increased tau aggregation properties. Tau protein with the p.N410H mutation showed a significant loss of normal tau function, as the rate of microtubule assembly decreased by 19.2% and the extent of total microtubule polymerization decreased 10.3% with mutated p.N410H tau compared to WT tau. Additionally, p.N410H caused a marked increase in tau filament formation, demonstrating that this mutation also acts through a toxic gain of function mechanism. Filament assembly effects appeared to be largely nucleation driven since the average filament lengths were unchanged between WT and p.N410H tau. There were significant increases in thioflavin S fluorescence at 30 minutes when changes in filament number and mass were not detectable by electron microscopy which further supports the hypothesis that p.N410H accelerates the folding of tau proteins into assembly-competent subunits. The thioflavin S data also show that tau polymerization with the p.N410H mutation persist, as they are still observed with steady state readings at 960 minutes. In conclusion, the p.N410H mutation seems to affect tau on multiple levels, leading to an increased 4R/3R tau mRNA ratio, a reduced ability to promote microtubule assembly, and an increased propensity to aggregate into filaments compared to WT tau.

The p.N410H mutation carrier and sporadic CBD were indistinguishable on neuropathologic examination using tau and TDP-43 immunohistochemistry. TDP-43 pathology has been described in CBD pathology and other tauopathies, but the significance of co-occurring tau and TDP-43 pathology is not clear [14,26,35]. Of the 117 pathologically-confirmed CBD cases we screened, 29% had TDP-43 pathology. Upon mapping the distribution of TDP-43 pathology in four sporadic CBD cases, we observed a similar distribution as in the p.N410H mutation carrier. The neuroanatomical distribution of TDP-43 pathology observed in CBD was greatest in basal ganglia, thalamus, midbrain, and pons. The distribution of TDP-43 pathology in CBD has not been previously described, but one study found TDP-43

immunoreactivity in the dentate fascia as in FTLT-TDP as well as TDP-43 colocalization with tau pathology in astrocytic plaques and oligodendroglial white matter pathology [35]. In the present study, we did observe oligodendroglial TDP-43 immunoreactive inclusions, yet we did not find TDP-43 pathology in granule cells of the dentate fascia or astrocytic inclusions in any of the 117 CBD cases.

A review of the literature for FTDP-17 cases with CBD resulted in a limited number of reported cases, none of which met neuropathologic criteria for CBD. The most convincing *MAPT* mutation carrier with CBD pathology is a patient with an exon 9 mutation, p.I260V, who presented clinically with FTD and pathologically had a 4R tauopathy [15]. This p.I260V carrier had tau-immunoreactive astrocytic processes and argyrophilic thread-like lesions in white matter, yet swollen achromatic or ballooned neurons were absent. Other *MAPT* mutations have been described to have features of CBD pathology, but because of additional pathologic features, these cases do not meet research diagnostic criteria for CBD [27,34,38]. Some members of the pallidopontonigral degeneration family harboring the p.N279K mutation present with corticobasal syndrome, but they have neuropathologic features not fitting with CBD [37]. A p.P301S carrier presented with corticobasal syndrome, but a pathologic assessment was not performed [6]. Due to the heterogeneity of pathology underlying corticobasal syndrome, this raises the question if this case was in fact CBD [5]. Taken together, we believe that the p.N410H is the first *MAPT* mutation carrier that meets neuropathologic diagnostic criteria for CBD.

In addition to the *MAPT* coding regions, our study also systematically assessed the *MAPT* 3'UTR in pathologically-confirmed CBD patients. We identified two rare genetic variants (MAPTv8 and rs186977284) that nominally associate with risk of developing CBD. Furthermore, rs186977284 was found to be associated with an increased risk of developing PSP. Although this association of *MAPT* 3'UTR variants with CBD and PSP does not hold up upon correction for multiple testing, confirmatory studies in other CBD and PSP cohorts are warranted. We did note an unusually high number of neuropathologically atypical CBD and PSP cases harboring the MAPTv8 and rs186977284 risk alleles. One case had CBD with olivopontocerebellar atrophy (CBD-OPCA) [22] and three cases had PSP with pallido-nigro-luysial degeneration which is an atypical pathologic variant of PSP [2,36]. The contribution of rare *MAPT* variants to the development of neurodegenerative disease is currently not clear, but there is evidence that the p.A152T variant causes tauopathy, including CBD [8,20,24]. CBD and PSP are considered to lie on a 4R tauopathy spectrum, and the fact that we find association of rare variants in the *MAPT* 3'UTR with risk of CBD and PSP further supports this notion. On the other hand, the MAPTv8 association was unique to CBD, providing additional evidence of how CBD and PSP are distinct clinicopathologic disorders.

In conclusion, performing an in-depth sequence analysis of *MAPT* in a large autopsy-confirmed CBD cohort identified the novel p.N410H tau mutation, indicating that *MAPT* sequencing should be considered in CBD patients. Functional characterization of this mutation demonstrated that p.N410H disrupts tau isoform homeostasis and has a reduced ability to promote microtubule assembly, with a potentially toxic gain of function demonstrated by a four-fold increase in its ability to polymerize into tau filaments. Additional functional characterization of this mutation may lead to a better molecular understanding of CBD pathogenesis and models for studying CBD-specific propagation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Tau mutation p.Asn410His (N410H)

Fig. 1. Novel MAPT p.N410H mutation in exon 13
Chromatogram of mutant and wild-type sequence of the MAPT exon 13 p.N410H mutation (top). Evolutionary conservation of MAPT is shown across species (bottom)

Fig. 2. p.N410H macroscopic features

On macroscopic examination of the left hemibrain there was mild atrophy of the frontal convexity, in a parasagittal distribution extending into the parietal cortex (**a**). The midbrain had marked loss of neuromelanin pigment in substantia nigra (**b**). Coronal sections of p.N410H revealed mild atrophy of superior frontal cortex and enlargement of lateral ventricles (**c**)

Fig. 3. Neuropathology of a CBD case with p.N410H *MAPT* mutation

Sections from motor cortex of p.N410H mutation carrier showed 4R tau-immunoreactive astrocytic plaques (a) and numerous threads in gray and white matter (d), both inclusion types were argyrophilic on Gallyas silver stain (b, e). Ballooned neurons were abundant in superior frontal cortex (c). Sections from the caudate nucleus of the p.N410H case had TDP-43 immunoreactive neuronal cytoplasmic and oligodendroglial cytoplasmic inclusions with thread-like processes (f). Bar=50 μ m

Fig. 4. Tau biochemical analysis in sporadic CBD and p.N410H mutation carrier

(a) Sarkosyl-insoluble tau fractions were prepared from superior frontal cortex in three sporadic CBD cases and three different samplings of p.N410H and immunoblotted with anti-PHF-1 to detect phosphorylated tau. Tau lower molecular weight doublet at ~37 kDa (asterisks) is a characteristic finding in CBD. (b) Tau 3R and 4R mRNA expression was measured from total RNA extracted from frontal cortex gray matter in a neuropathologically normal individual, p.N410H mutation carrier, and p.N279K mutation carrier as a positive control for increased 4R:3R tau ratio. Data represent the average of triplicate measurements and error bars are the SEM. * $P < 0.05$

Fig. 5. Microtubule assembly and tau filament formation

(a) Mutant p.N410H tau had a reduced ability to promote microtubule assembly compared to WT tau *in vitro*. The rate of tubulin polymerization decreased by 19.2% and the extent of total microtubule polymerization decreased 10.3% with mutated p.N410H tau. Ninety minutes after stimulating aggregation, tau with p.N410H mutation caused a marked increase in total number of tau filaments formed compared to WT tau (b) and total filament length per field representing the total polymer mass (c). Representative electron micrographs of WT tau (d) and p.N410H tau (e) at 90 minutes show the extent of filament formation. Bar graphs represent the average of nine EM images per genotype per time point and error bars are the SEM. * $P < 0.001$

Table 1
Cohort description

Cohort	<i>N</i>	Age ^a (years)	Females (%)	Sample Source
CBD cases	109	69.7 ± 8.7	47.7	Mayo Clinic Florida
Control series 1	643	73.7 ± 11.8	52.3	Mayo Clinic Florida
PSP cases	566	75.1 ± 7.9	48.2	Mayo Clinic Florida
Control series 2	660	70.4 ± 12.0	58.3	Mayo Clinic Arizona

^a Age is shown as the mean ± standard deviation representing the age at death of cases in the autopsy-confirmed CBD and PSP cohorts and the age at blood draw of subjects in the control series. Subjects included in the control series were healthy non-Hispanic white individuals in whom clinical history of neurological disease and cognitive impairment were excluded. Subjects from all cohorts agreed to be in the study and biological samples were obtained after informed consent with ethical committee approval from the respective institution.

Table 2
Description of variants in *MAPT* and logistic regression results for association test with CBD and PSP

Variant name	Chr	Position	Location	Major allele	Minor allele	dbSNP MAF	CBD vs Control Series 1		
							OR (95% CI)	P-value	
MAPTv4	17	44101605:44101607	3'UTR	AAT	-	NA	2.52 (0.22-28.28)	0.454	
rs5820605	17	44102682:44102683	3'UTR	-	T	NA	0.88 (0.60-1.29)	0.499	
rs16940802	17	44103704	3'UTR	G	A	0.050	0.58 (0.20-1.69)	0.318	
MAPTv7	17	44103803:44103804	3'UTR	CT	-	NA	NA	NA	
MAPTv8	17	44104155:44104157	3'UTR	-	ATC	NA	3.71 (1.12-12.25)	0.031	
rs11331969	17	44104616:44104617	3'UTR	-	G	NA	0.94 (0.67-1.33)	0.734	
rs186977284	17	44104972	3'UTR	T	C	NA	3.58 (1.03-12.43)	0.045	
MAPTv11	17	44105107	3'UTR	T	C	NA	NA	NA	
rs114213384	17	44105635	3'UTR	A	G	0.025	1.43 (0.27-7.49)	0.680	
rs7521	17	44105395	3'UTR	G	A	0.429	1.24 (0.90-1.70)	0.193	
PSP vs Control Series 2									
MAPTv8	17	44104155:44104157	3'UTR	-	ATC	NA	2.39 (0.38-15.17)	0.356	
rs186977284	17	44104972	3'UTR	T	C	NA	3.07 (1.08-8.71)	0.035	

Chromosome position is relative to the UCSC Genome Browser February 2009 (GRCh37/hg19) assembly. The dbSNP minor allele frequency (MAF) based on the 1000 genomes project (NA, not available). Genotype counts and frequency in our study population are shown in Online Resource Table 1. Logistic regression analyses were performed using an additive model with age, sex, and number of H1 haplotype alleles as covariates. *MAPTv7* and *MAPTv11* were not identified in controls precluding statistical analyses on these variants.

Table 3
Semiquantitative assessment of tau and TDP-43 pathology in p.N410H case

Region	Tau immunohistochemistry				TDP-43 Immunohistochemistry		
	NFT & pre-NFT	Coiled bodies	Astrocytic plaques	Tau+ threads	NCI	GCI	TDP+ threads
Temporal cortex	+++	++	++	+++	0 – +	–	–
Superior frontal cortex	+++	++	+++	+++	+	+	–
Motor cortex	++	+	++	++	–	–	–
Caudate/putamen	+++	++	++	+++	+	+	+
Globus pallidus	++	+++	–	+++	+	++	+
Basal nucleus	++	+	–	+++	+	–	–
Hypothalamus	+++	+	–	+++	+	+	–
Ventral thalamus	+++	+	+	+++	+	++	+
Subthalamic nucleus	++	+	–	+++	++	++	+
Thalamic fasciculus	–	+	–	+++	–	–	–
Red nucleus	+	+	–	+	–	–	–
Substantia nigra	+	+	–	+++	+	+	+
Oculomotor complex	++	+	–	+++	–	+	–
Midbrain tectum	+++	+	+	+++	+	+	+
Locus ceruleus	+++	–	–	+++	+	–	+
Pontine tegmentum	+++	+	–	+++	+	++	+
Pontine base	++	++	–	+++	0 – +	–	–
Medullary tegmentum	NA	NA	NA	NA	NA	NA	NA
Inferior olive	NA	NA	NA	NA	NA	NA	NA
Dentate nucleus	++	–	–	+	–	–	–
Cerebellar white matter	–	++	–	+++	–	0 – +	–

NFT neurofibrillary tangles, *NCI* neuronal cytoplasmic inclusions, *GCI* glial cytoplasmic inclusions, *NA* not available