Human Molecular Genetics, 2012, Vol. 21, No. 15 **3500–3512**
doi:10.1093/hmg/dds161
Advance Access published on May 3, 2012

Evidence for a role of the rare p.A152T variant in *MAPT* in increasing the risk for FTD-spectrum and Alzheimer's diseases

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Received December 24, 2011; Revised April 3, 2012; Accepted April 20, 2012

Rare mutations in the gene encoding for tau (*MAPT*, microtubule-associated protein tau) cause frontotemporal dementia-spectrum (FTD-s) disorders, including FTD, progressive supranuclear palsy (PSP) and corticobasal syndrome, and a common extended haplotype spanning across the *MAPT* locus is associated with increased risk of PSP and Parkinson's disease. We identified a rare tau variant (p.A152T) in a patient with a clinical diagnosis of PSP and assessed its frequency in multiple independent series of patients with neurodegenerative conditions and controls, in a total of 15 369 subjects.

Tau p.A152T significantly increases the risk for both FTD-s (n = 2139, OR = 3.0, CI: 1.6–5.6, P = 0.0005) and Alzheimer's disease (AD) (n = 3345, OR = 2.3, CI: 1.3–4.2, P = 0.004) compared with 9047 controls. Functionally, p.A152T (i) decreases the binding of tau to microtubules and therefore promotes microtubule assembly less efficiently; and (ii) reduces the tendency to form abnormal fibers. However, there is a pronounced increase in the formation of tau oligomers. Importantly, these findings suggest that other regions of the tau protein may be crucial in regulating normal function, as the p.A152 residue is distal to the domains considered responsible for microtubule interactions or aggregation. These data provide both the first genetic evidence and functional studies supporting the role of MAPT p.A152T as a rare risk factor for both FTD-s and AD and the concept that rare variants can increase the risk for relatively common, complex neurodegenerative diseases, but since no clear significance threshold for rare genetic variation has been established, some caution is warranted until the findings are further replicated.

INTRODUCTION

The term frontotemporal lobar degeneration (FTLD) describes a group of dementias distinct from Alzheimer's disease (AD) that are prevalent among presenile cases (1). The clinical syndromes associated with FTLD, collectively named frontotemporal dementias (FTD), comprise 5–10% of neurodegenerative dementias in epidemiologic samples and between 9 and 16% in autopsy series (2). More than a decade of careful clinical and neuropathological characterization has shown that FTLD, corticobasal degeneration (CBD; CBS for corticobasal syndrome), progressive supranuclear palsy (PSP; PSP-S for progressive supranuclear palsy syndrome) and motor-neuron disease share significant clinical and pathological features in many cases and also appear to share many of the same genetic risk factors or causal mutations; hence, they are part of a

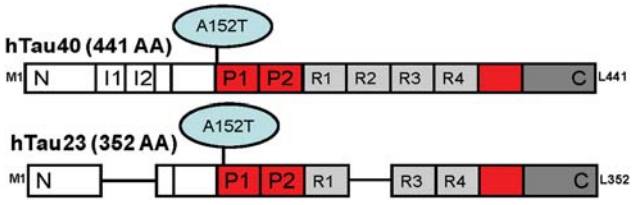

Figure 1. Domain structure of tau. The diagram shows the domain structure of htau40wt and mutation at tau40A152T [largest isoform in the human central nervous system (CNS), 441 residues] and htau23 (smallest isoform in human CNS, 352 residues). Tau domains are broadly divided into the N- terminal 'projection domain' (amino acids M1-Y197) and the C-terminal 'assembly domain' (amino acids Y198-L441). The C-terminal assembly domain includes three or four pseudo-repeats (~31 residues each, R1–R4), which together with their proline-rich flanking regions (P1 and P2) constitute the microtubule-binding region. Repeat R2 and the two near-N-terminal inserts (I1 and I2) may be absent due to alternative splicing. The repeat domain also forms the core of PHFs. The fetal isoform of htau23 has a similar domain structure but lacks the inserts I1, I2 and R2 in the repeat region. The p.A152T substitution is unusual in that it lies far outside the repeat domain, in contrast to most FTDP-17 (frontotemporal dementia and parkinsonism linked to chromosome-17) tau mutations.

spectrum (FTD-spectrum, FTD-s) of related conditions (3). Pathologically, familial and non-familial FTLD are indistinguishable (4), suggesting a final common pathophysiology.

The majority of genetic risks for FTD remain unknown. So far, three common (*MAPT*—microtubule-associated protein tau, *GRN* and *C9ORF72*) and four rare Mendelian (dominant, in the *CHMP2B*, *VCP*, *TARDBP* and *FUS* genes) genetic forms of FTD have been identified (5). Dominantly inherited mutations in the gene encoding microtubule-associated protein tau (*MAPT*) were the first causal mutations identified in familial cases of FTLD and are associated with tau pathology. Most are clustered in the microtubule-binding domain (Fig. 1) and are thought to cause either loss of microtubule stability or enhanced aggregation of tau (6). For this reason, only the last exons of the *MAPT* gene are sometimes sequenced in mutational screens (7). Additionally, a common tau haplotype has been recognized as a major risk factor for PSP, CBD, some variants of FTD and Parkinson's disease (PD), suggesting that common *MAPT* alleles increase the risk for multiple neurodegenerative disorders (reviewed in 8). Together, known mutations account for about half of familial cases and ~10–15% of sporadic cases, leaving the genetic contribution to FTD unknown in most cases. Recently, two common variants increasing risk for FTLD have been identified: (i) the rs5848 polymorphism within the 3′ untranslated region of *GRN*, which has been shown to regulate its expression levels, and possibly the risk for dementia (9); and (ii) variants within the *TMEM106B* gene identified in a recent genome-wide association study (GWAS) as associated with increased risk for FTD (10). In summary, mutations in a few Mendelian genes and three risk factors only explain a fraction of the genetic risk associated with FTD, suggesting that other, undetected risk factors are yet to be identified.

We report the identification of the rare tau p.A152T substitution, located outside the microtubule-binding domain, as a novel risk for both FTD-s and AD. The significance of this variant was previously unknown, as it had also been found in normal subjects. We assessed the frequency of MAPT p.A152T in multiple large series of patients with neurodegenerative diseases and controls, and performed functional experiments, indicating that p.A152T causes a pronounced decrease in microtubule stability, a moderate decrease in paired helical filaments' (PHFs) stability and an increase in the fraction of tau oligomers. Although the statistical evaluation of risk associated with very rare variants can be challenging, this genetic screen and functional data provide reasonable support for the notion that tau p.A152T is a rare variant associated with increased risk for FTD-s and for AD, possibly representing the first *MAPT* variant associated with AD. This finding has broader implications related to the role of rare variants in altering the risk for neurodegenerative disease.

RESULTS

Variant discovery via MAPT re-sequencing

During routine sequencing of coding exons in *MAPT* in 73 FTD-s cases, we identified the sequence variant p.A152T, within exon 7 of *MAPT,* in a patient with PSP-S. We ascertained that this variant had been detected in previous cases [(11), (12)], but had also been found in controls (M.B. and R.R., unpublished data), and therefore was considered a variant of unknown significance. Occurrence of p.A152T was checked in the Exome Variant Server [NHLBI Exome Sequencing Project (ESP), Seattle, WA, USA, http://evs.gs.washington.edu/EVS/, last accessed 16 March 2012], where it is reported with a minor allele frequency (MAF) of 0.27% in Caucasians ($n = 7020$ alleles) and 0.08% in African Americans ($n = 3738$ alleles), and an overall allelic frequency of 0.20% ($n = 10\,758$ alleles), corresponding to a heterozygote frequency of ~0.41% ($n = 5379$ subjects).

To further assess the potential role in increasing risk for disease, we checked the occurrence of MAPT p.A152T in the entire GIFT cohort and in a large series of 5059 normal controls obtained from NIMH (Table 1; Materials and Methods). In total, we identified 5 carriers in 447 FTD-s cases (1.1%), 0 carriers in 549 AD cases and 15 carriers in 5782 controls (0.26%)—an odds ratio (OR) of 4.3 (CI: 1.2–12.7, Fisher's P-value = 0.012) for FTD-s versus controls.

In the second step, we screened three additional independent series (MAYO, PENN, KCL, see Materials and Methods). Basic demographic information of the cohorts studied is reported in Table 1. Analyses on the individual series indicated an OR for a variety of related neurodegenerative conditions ranging between 1.9 (in the MAYO PD series) and 3.4 (in the MAYO FTD-s series). A combined analysis performed on 15 369 subjects placed the estimated OR at 3.0 (CI: 1.6–5.6, $P = 0.0005$) for FTD-s and 2.3 (CI: 1.3–4.2, $P = 0.004$) for AD versus controls. To ensure that this was not caused by population stratification, we limited the analysis to only individuals of self-reported Caucasian ancestry and found similar results [OR for FTD-s versus controls: 3 (CI: 1.5–6.1, $P = 0.001$), $n = 7779$; AD versus controls: 2.5 (CI: 1.3–4.8, $P = 0.004$), $n = 9008$; Supplementary Material, Table S1]. We also performed principal component and IBD

Table 1. Demographic characteristics and MAPT p.A152T frequencies in four series including patients with FTD-s, AD, PD/LBD and controls (total = 15 369 samples)

	GIFT	MAYO	PENN	KCL	Total
FTD-s	447[a]	1276	416		2139
Carriers (%)	5 (1.12)	11 (0.86)	3 (0.72)		19 (0.89)
OR (CI)	4.3 (1.2–12.7)	3.4 (1–14.9)	1.6 (0.2–8.3)		3.0 (1.6–5.6)
P-value (Fisher's test)[b]	0.012	0.03	0.45		0.0005
FTD (carriers)	426 (2)	552 (6)	0		
PSP (carriers)	4 (1)	594 (4)	340 (3)		
CBD (carriers)	17 (2)	130 (1)	76 (0)		
Age at onset	58 ± 8	65 ± 9	64 ± 10		
%Female	46	49	45		
%Caucasian	87	98	96		
%Neuropath	NA	65	100		
AD	549	889	1367	540	3345
Carriers (%)	0	5 (0.56)	14 (1.02)	4 (0.74)	23 (0.69)
OR (CI)	NA	2.2 (0.5–11)	2.3 (0.8–8.2)	1.4 (0.2–9.5)	2.3 (1.3–4.2)
P-value (Fisher's test)[b]	NA	0.30	0.11	0.72	0.004
Age at onset	66 ± 10	NA	71 ± 8	75 ± 7	
%Female	57	57	62	51	
%Caucasian	32	95	97	99	
%Neuropath	NA	100	54	NA	
PD/LBD		838			838
Carriers (%)		4 (0.48)			4 (0.48)
OR (CI)		1.9 (0.3–10)			1.6 (0.4–4.6)
P-value (Fisher's test)[b]		0.46			0.33
Age at onset		65 ± 12			
%Female		40			
%Caucasian		100			
%Neuropath		21			
Controls	5782[c]	1587	1118[d]	560	9047
Carriers (%)	15 (0.26)	4 (0.25)	5 (0.45)	3 (0.54)	27 (0.30)
Age at examination	50 ± 16	70 ± 12	75 ± 13	75 ± 6	
%Female	48	52	57	55	
%Caucasian	71	100	97	99	
%Neuropath	NA	46	18	NA	

FTD-s, FTD-spectrum, including FTD, PSP-S, CBS; AD, Alzheimer's disease; PD, Parkinson's disease; LBD, Lewy body disease; GIFT, Genetic Investigation in FrontoTemporal dementia study.
Age at onset/draw available in 5046/6778 (GIFT/NIMH series), 3241/4590 (MAYO), 2649/2901 (UPENN), 518/1100 (KCL). Gender available in 6147/6778 (GIFT/NIMH), 4589/4590 (MAYO), 2858/2901 (UPENN), 1086/1100 (KCL). Ethnicity available in 5546/6778 (GIFT/NIMH), 4577/4590 (MAYO), 2640/2901 (UPENN), 1081/1100 (KCL).
[a]Including 206 patients sampled by the French clinical and genetic Network on FTLD/FTLD-ALS.
[b]Compared with the controls available in each series (e.g. GIFT FTD-s versus GIFT controls, MAYO FTD-s versus MAYO controls, etc.).
[c]Including 184 NCRAD and 5059 NIMH controls.
[d]Including 613 LOAD controls and 122 NIMH controls.

sharing analyses in a subset of samples for which SNP data were available and found no particular clustering within groups of samples of Caucasian descent, nor cryptic relatedness among p.A152T carriers (Supplementary Material).

The additional 53 p.A152T carriers identified in the confirmation series had diagnoses of FTD-s ($n = 14$), AD/MCI ($n = 23$), PD ($n = 4$), or were asymptomatic normal controls ($n = 12$, Supplementary Material, Table S1). Overall, p.A152T carriers did not have a significantly different age at onset, compared with non-carriers. Of note, when we considered the FTD series where neuropathological data were available (MAYO), the variant was not enriched in 162 FTD-s cases with TDP-43 pathology, which had a carrier frequency of 0.62% (1/162, versus 0.25% in 1587 controls, $P = 0.38$). This is a potentially important finding, as TDP-43 cases do not have tau pathology, further indicating that the increased risk may be specific for tau-related pathology; however, due to the small sample size, this finding requires follow-up in future studies.

Assessment of the functional consequences of the p.A152T substitution

One difficulty with rare variants of an intermediate effect size such as p.A152T identified here is that it can be difficult to assess functional effects. However, demonstration of potentially pathogenic alterations in tau protein function would provide another line of evidence beyond association with disease, supporting its pathogenic role.

The tau p.A152T variant was introduced by site-directed mutagenesis into the normal coding region of human tau cDNA (htau40 isoform, '2N4R', containing 441 residues, Fig. 1). Wild-type and mutant tau were subjected to two *in vitro* assays diagnostic of the cellular functions of tau: (i) formation

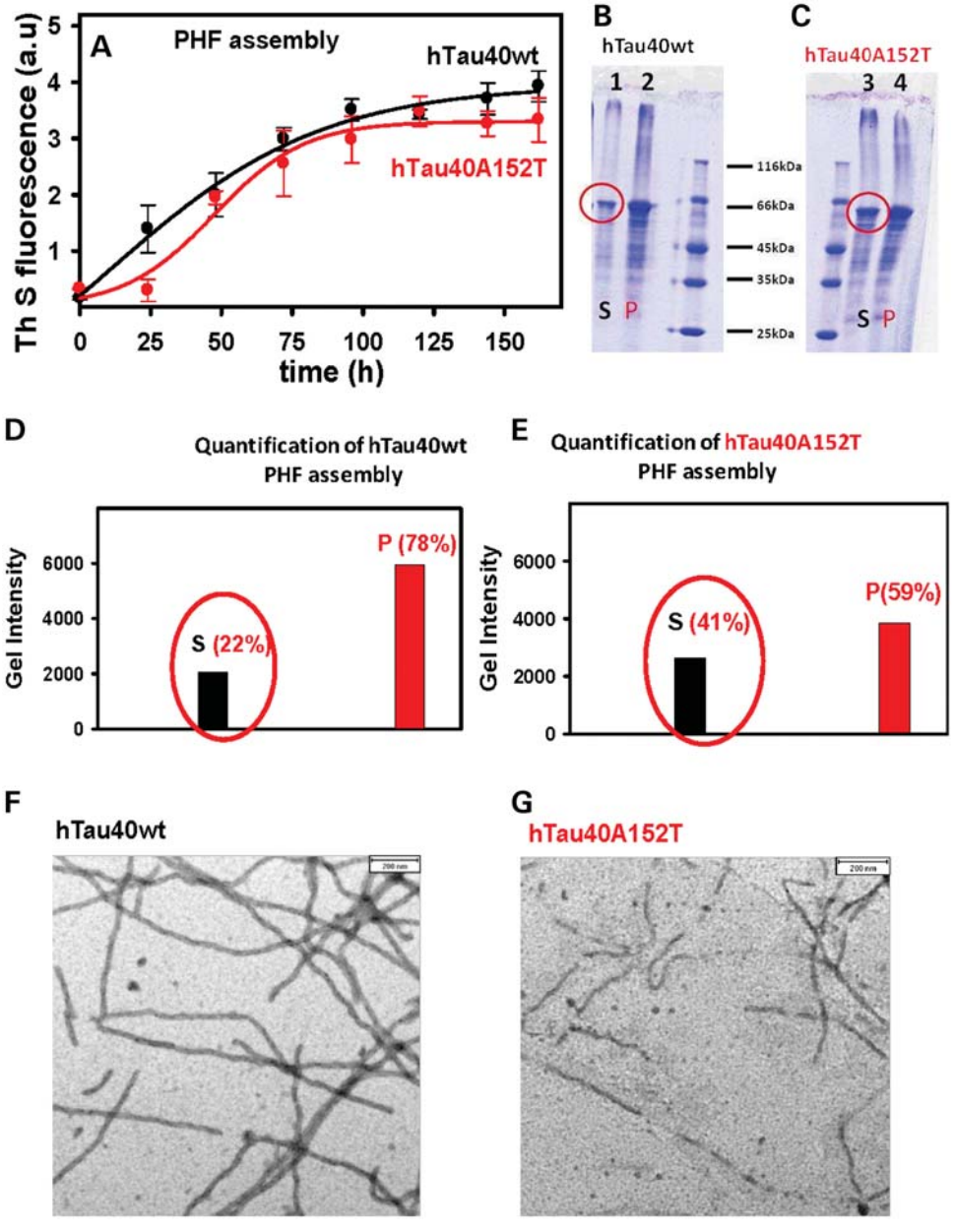

Figure 2. Aggregation of tau and the p.A152T mutant. (**A**) Aggregation of htau40wt and p.A152T mutant monitored by the ThS fluorescence assay in the presence of the cofactor heparin. The aggregation of tauA152T is somewhat slower and reaches a somewhat lower final level of aggregation, but overall the assembly characteristics are comparable. (**B** and **C**) SDS gels showing soluble and aggregated tau (S, supernatant; P, pellet), along with molecular weight markers. Both tau and tauA152T show a major band around 60 kDa and some fragments at lower molecular weights. (**D** and **E**) Quantification of the proteins of (B) and (C). Note that mutant tau (E) aggregates less extensively than the wild-type protein (D) by this assay. (**F** and **G**) Electron micrographs of filaments formed from htau40wt and the mutant p.A152T. Note that the filament preparations from mutant tau are more fragile and contain more oligomers.

of aggregates (one of the pathological effects of tau), (ii) promotion of microtubule assembly (the physiological role of tau in neurons) and the corresponding microtubule affinity. Figure 2A illustrates the aggregation assay based on the fluorescence of the dye thioflavin S (ThS), which increases when amyloid-like structures assemble by interaction of β-sheets. In this assay, the p.A152T mutant appears to aggregate with somewhat lower efficiency than the wild-type protein. A clearer picture emerges when large aggregates and soluble species are separated by centrifugation and quantified by SDS–PAGE (Fig. 2B–E). In these examples, 78% of wild-type tau is aggregated, and only 22% remains soluble. In contrast, 41% of mutant tau remains soluble. This fraction contains not only monomeric tau, but also oligomers (up to roughly 70 monomers in these experimental conditions) which are not pelleted, but also contribute to the ThS signal (13). Electron microscopy (Fig. 2F and G) reveals extended filaments for aggregated wild-type tau, whereas the filaments of mutant tau show frequent breaks and a background of smaller oligomers. Since tau oligomers are considered to be more toxic than filaments (14), this finding points

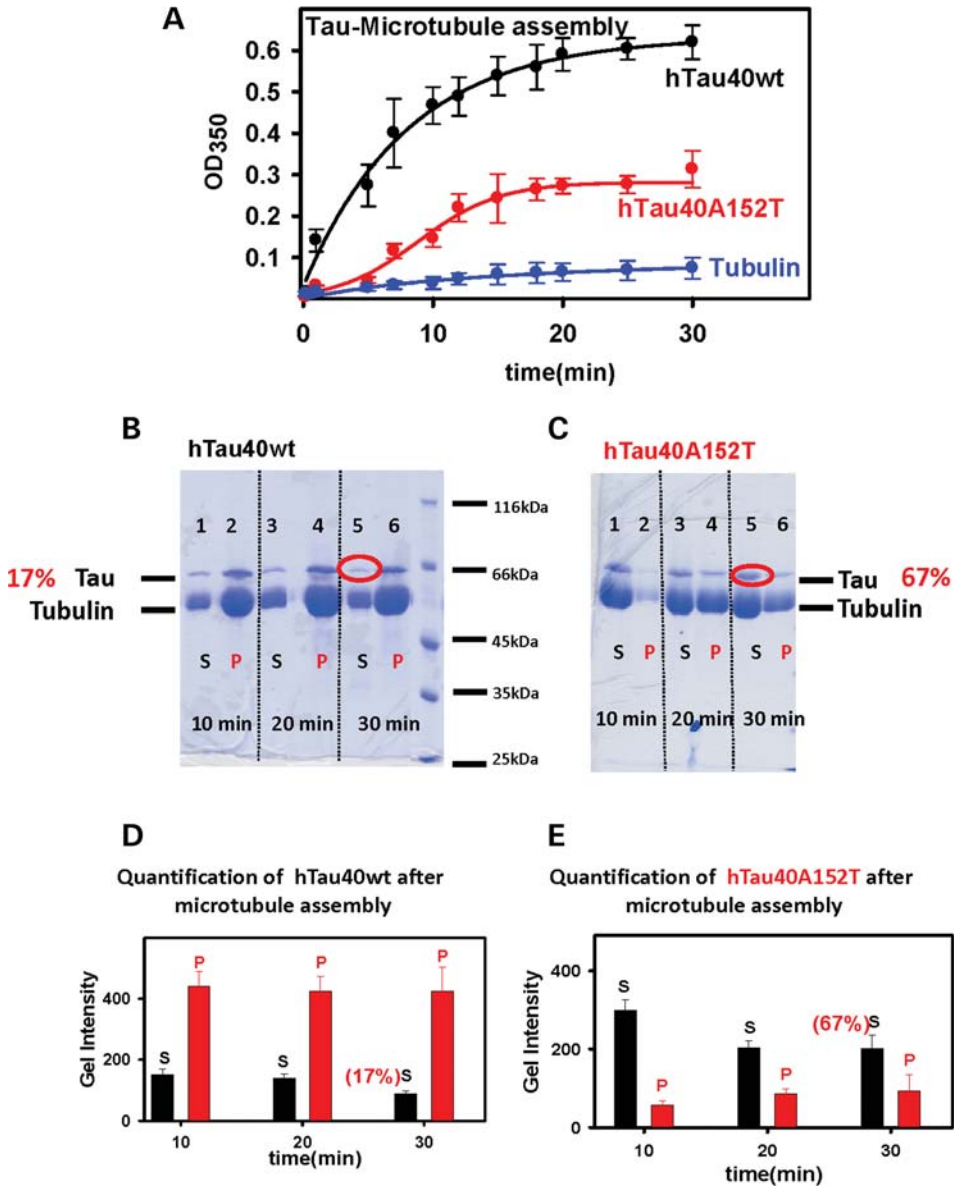

Figure 3. Microtubule assembly induced by htau40wt and the p.A152T mutant. (**A**) Microtubule assembly induced by wild-type tau (black curve, top) and mutant tau (red curve, middle) monitored by light scattering at 350 nm. Note that mutant tau is much less efficient in stabilizing microtubules than wild-type tau. As a control, tubulin alone without tau does not assemble in these conditions (blue curve, bottom). (**B** and **C**) Binding of tau to polymerized microtubules in (A). The proteins were incubated for several time periods (10–30 min), separated by pelleting and analyzed by SDS–PAGE. S, supernatant; P, pellet. Tau is visible as a sharp band above the broad band of tubulin. (**D** and **E**) Quantification of (B) and (C). Note that wild-type tau is mostly bound to microtubules (~75%) and therefore appears in the pellet (red bars in D). In contrast, mutant tau binds much more weakly (only ~20–30%) and remains mostly in the supernatant (black bars in E).

to a possible gain of toxic function of the mutant, even though the overall tendency for aggregation appears somewhat lower. We also tested the three-repeat isoform, htau23wt (smallest isoform, 352 residues), and its mutant, htau23A152T. As in the case of htau40, the rate of PHF aggregation is similar (somewhat slower for the mutant, Fig. 5A).

An analogous, but more pronounced difference emerges from the microtubule interaction studies. Wild-type tau induces the efficient assembly of microtubules in the light scattering assay, whereas mutant tau reaches only much lower levels of assembly (~30%) and shows a longer lag time (Fig. 3A). In co-sedimentation assays, 83% of wild-type tau is attached to microtubules, compared with only 33% for mutant tau (Fig. 3B–E). Furthermore, if microtubules are stabilized by taxol independently of tau and then probed for tau binding, 74% of wild-type tau is bound to microtubules and 26% remains detached, compared with 43% of mutant tau (Fig. 4A–D). Thus, there is a close correspondence between the ability of the two tau species to bind to microtubules and to promote their assembly. The effect of htau23wt and htau23A152T on microtubule assembly was also tested. As in the case of htau40, the efficiency of microtubule assembly

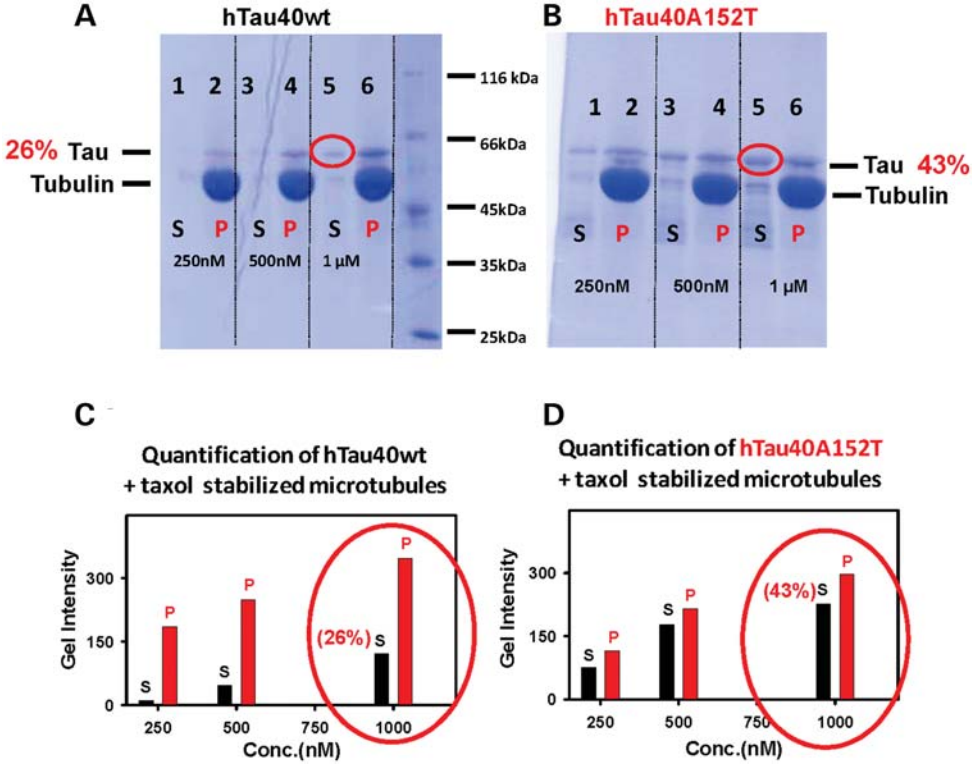

Figure 4. Binding of tau to preformed taxol-stabilized microtubules. Stable microtubules were first assembled in the presence of 30 μM taxol and then incubated with wild-type or mutant tau at different concentrations of tau (250 nM to 1 μM) (with tubulin fixed at 30 μM). The microtubules with bound tau were separated from soluble tau by pelleting and analyzed by SDS–PAGE. (**A** and **B**) SDS gels showing soluble and assembled fractions of tau and tubulin. (**C** and **D**) Quantitation of (A) and (B). Note that wild-type tau is mostly bound to microtubules (red bars), whereas a large fraction of mutant tau does not bind and remains soluble.

is clearly lower for the mutant (Fig. 5B). The data illustrate that mutant tau is strongly impaired in its physiological function of stabilizing microtubules, a mechanism that has also been implicated in dominant forms of tauopathy (15).

DISCUSSION

Whether the genetic contribution to common complex diseases comes from common or rare variants (or a combination of both) is a major issue in complex disease genetics. Identification of genetic variants predisposing to common disease has focused on the identification of rare, highly-penetrant Mendelian genetic variants in small numbers of families, or common variants in large populations. These approaches have been successful, identifying many risk variants, but also revealing a large territory of missing heritability (16). A recent provocative report suggested that at least some of the signals detected in large GWAS could be due to rare variants (17), but the relative weight of this phenomenon is still unclear (18), and large-scale resequencing studies are expected to clarify this issue (19). Re-sequencing at the gene level provides an efficient method for identifying rare variants in disease, many of which may be of an intermediate effect size, rather than causal Mendelian loci, as has been the typical assumption for rare variants (20).

Our data suggest that rare variants can increase the risk for complex diseases with heterogeneous phenotypes, likely in synergy with other (common or rare) polymorphisms. The variant reported here occurs in a gene (*MAPT*) where rare Mendelian, disease-causing mutation can also occur, suggesting that both Mendelian pathogenic and susceptibility variants can occur in the same gene (21,22). In the future, it will be challenging to prioritize rare variants occurring in genes that have not been yet linked to neurodegeneration, in order to perform large screens and demonstrate that they increase the risk for disease. Large-scale resequencing projects will facilitate this by providing frequencies of rare variants that can be used for *in silico* screens. It should be noted that the statistical evaluation of the role of very rare sequence variants poses a challenge (23), as no thresholds for rare variant significance have been established (24); several studies of rare variant detection have provided either no statistical support for individual gene variants (25) or a threshold of $P < 0.05$, which has been used for aggregate rare variant signals (26). We expect that novel statistical methods will be developed, possibly more powerful than the traditional methods applied here, and a more solid rubric for rare variant significance will be established. Our large cohort provides the first evidence for a specific rare tau variant, increasing risk for AD, and FTD-s disorders including PSP. As sample sizes grow, it will be important to continue to re-evaluate its effect size and contribution to disease. In addition, population stratification is a potential

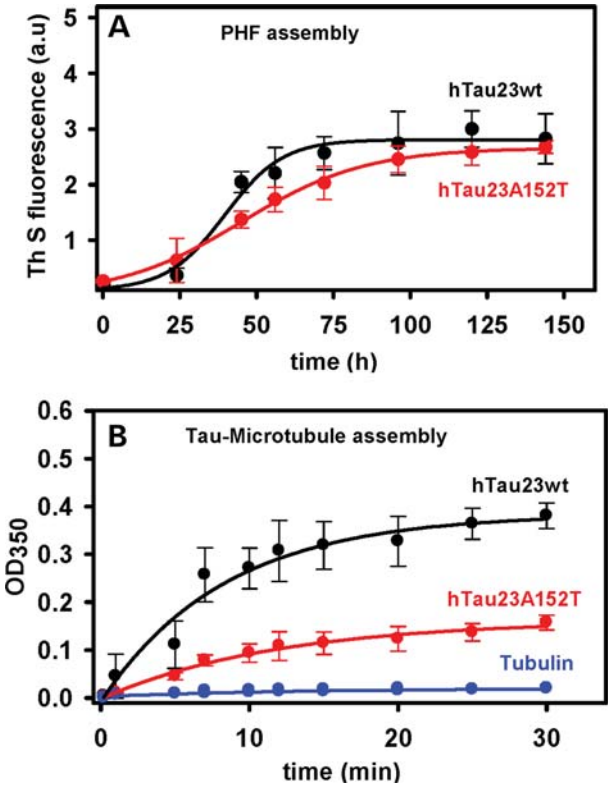

Figure 5. Aggregation propensity and microtubule assembly of htau23wt and htau23A152T. (**A**) Aggregation of htau23wt and p.A152T mutant monitored by the ThS fluorescence assay in the presence of the cofactor heparin. The aggregation of tau23A152T is somewhat slower and reaches a somewhat lower final level of aggregation, but overall the assembly characteristics are comparable. (**B**) Microtubule assembly induced by wild-type tau (black curve, top) and mutant tau (red curve, middle) monitored by light scattering at 350 nm. Note that mutant tau is much less efficient in stabilizing microtubules than wild-type tau. As a control, tubulin alone without tau does not assemble in these conditions (blue curve, bottom).

confounder in association studies, and, although we did not find evidence for population bias in a subset of our samples, the very small numbers of p.A152T carriers do not allow us to exclude this possibility conclusively.

Tau is a key component of AD pathology, and tau levels, isoform ratios or function may influence AD risk (27). A genetic contribution from sub-haplotypes at the 17q21.31 *MAPT* locus has been reported (28), although this association has not been consistently observed (29,30). Our combined analysis indicates that p.A152T is the first genetic risk factor for AD reported in *MAPT*. The estimated OR for this variant in AD is less than *APOE*, but greater than other common variants, such as in *SORL1* (31) and *CLU* (32). The same is true for FTD and PSP; the OR for FTD is nearly as large as that for the ApoE4 allele in AD, so this is the first susceptibility factor with a moderate effect size in an FTD-s condition. It should also be noted that a significant number of controls in this study (i.e. the NIMH samples within the GIFT series) were overall younger, possibly leading to an underestimation of the risk effect, since younger p.A152T carriers might still develop disease.

The identification of this *MAPT* rare variant, among the first with an intermediate effect size, suggests that a broad sequencing approach targeted at such forms of rare genetic variation in neurodegenerative dementia may be useful, as very rare variants are not likely to generate a GWAS association signal. The frequency of the tau p.A152T variant was also higher in PD patients than in controls, but this association did not reach statistical significance in our mega-analysis. PD has no tau inclusions, but tau-positive FTD is associated with parkinsonism in a significant number of patients with FTLD (33), and PD-related genes, such as DJ-1, colocalize with tau inclusions (34). Finally, recent, large GWAS in PD (e.g. 20) detected an association signal over the *MAPT* region, suggesting that *MAPT* and genes involved in tau metabolism and function may be worthwhile candidates for study in PD. Thus, larger PD association studies with rare *MAPT* variants may be worthwhile.

The functional data on tau–microtubule and tau–tau binding reveal that p.A152T tau has decreased potential for normal functional interactions. In the case of the physiological interaction with microtubules, this amounts to an impaired stability of microtubules, equivalent to a loss of function of mutant tau. In the case of tau aggregation, the seemingly similar level reached by the two species in the ThS fluorescence assay would suggest a somewhat lower tendency of aggregation for mutant tau. However, this view must be weighed against the fact that mutant tau is more prone to form oligomers, which are thought to be more toxic than either filaments or monomers (14). This is equivalent to a toxic gain of function. The parallel changes in the two assays, microtubule assembly versus PHF assembly, are reminiscent of changes observed with several other dominantly acting tau mutants, where an impaired microtubule binding is accompanied by a higher tendency to form pathological aggregates (15,35), suggesting that the change in protein conformation caused by the p.A152T substitution decreases the interaction with microtubules and at the same time exposes the domains of tau that are prone to aggregate. The isoform htau40wt (441 residues, with 4 repeats) can bind strongly to microtubules, whereas isoform htau23wt (352 residues, with 3 repeats) has only three repeats and binds less strongly, which results in a lower stabilization of microtubules (36).

In the case of most *MAPT* mutations, the majority of sites lie in or near the repeat domain; since this domain determines both microtubule binding and PHF assembly, the dual consequences of a given mutation are plausible. In contrast, the enigma of the p.A152T mutation lies in the fact that it is far away from regions implicated in tau's established cellular functions. Three ideas come to mind regarding possible functions. (i) Residue p.152 is just upstream of the motif Thr-Pro (residues p.153–154), one of the numerous SP or TP motifs in tau that are targets of proline-directed kinases and whose elevated phosphorylation is a diagnostic marker of AD and other tauopathies. Indeed, p.T153 is phosphorylated during the cell cycle in neuronal cell lines (37), in parallel to other SP/TP motifs, but functional consequences are not known. It is possible that the mutation p.A152T interferes with phosphorylation in a cellular context. (ii) Even though the N-terminal half of tau is traditionally considered a 'projection domain' which does not bind to microtubules, this picture is oversimplified since a number of residues reveal microtubule interactions by nuclear magnetic resonance

(NMR) spectroscopy analysis (38). This includes p.I151, just upstream of the mutation site, which might therefore explain the weakening of the microtubule interaction. (iii) The region around residues p.160–180 shows an extended character in tau [beta strand, followed by poly-Pro helix (38)], whose direction and, thus, conformation might be altered by the mutation at residue p.152 or phosphorylation at p.T153. These ideas are currently under experimental investigation.

In conclusion, genetic evidence from multiple large series and functional studies indicate the tau p.A152T as a risk factor for FTD-s and possibly AD. The effect size for FTD is remarkable relative to known common variants, and similar to that for ApoE4 heterozygotes in AD, although the effect of the A152T for AD appears about half. The functional studies show that the p.A152T tau (i) binds less tightly to microtubules, and (ii) forms aggregates which are less stable, but favor fragments of filaments and smaller units. Both properties would enhance the level of tau oligomers, for which accumulating experimental evidence supports as a more toxic species (39,40). Additional genetic (including co-segregation studies within large families) and functional studies will be needed to clarify the role of this variant in the pathogenesis of neurodegenerative disease. As is becoming clear in other complex diseases, additional genetic variants are likely be at play in promoting neurodegeneration in tau p.A152T carriers. Exome sequencing in mutation carriers compared with controls may yield a source of potential interacting loci that can be followed up by studies in cellular and animal models.

MATERIALS AND METHODS

Ethics statement

All subjects and/or their proxies signed informed consents for genetic studies.

Subjects

We screened four series for a total of 15 369 subjects (Table 1). In the first screen, patients were enrolled as part of a large genetic study in neurodegenerative dementia [Genetic Investigation in Frontotemporal Dementia, GIFT (41)] at the Alzheimer's Disease Research Centers (ADRCs) of UC San Francisco, Davis, Irvine, Los Angeles, University of South California, Emory University, and including 206 samples collected by a French research network on FTLD/FTLD-ALS. A control set of 5059 normal subjects was obtained from NIMH. Additional confirmation series included samples with FTD-s, AD, PD and controls recruited at (i) University of Pennsylvania (PENN series), (ii) Mayo Clinic Jacksonville and Mayo Clinic Rochester (MAYO series) and (iii) King's College London (KCL series). Part of the PENN and MAYO series have been included in previous reports (42,43).

Genetic studies

Genotyping of the sequence variant in *MAPT* exon 7 NM_005910.5:c.454G>A (p.A152T), and *APOE* (rs429358 and rs7412) and *MAPT* H1/H2 (rs1560310) defining variants, was conducted using a TaqMan Allelic Discrimination Assay on an ABI 7900HT Fast Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Sanger Sequencing was used to confirm identified variant carriers. All primer and probe sequences are available on request. Frequencies were compared using a two-sided Fisher's exact test, as implemented in the fisher.test function in the statistical environment R (www.r-project.org) with default parameters.

Tau-dependent microtubule assembly and aggregation

Thioflavine S and heparin were obtained from Sigma (Steinheim, Germany). The human full-length tau isoform htau40wt (441 residues) and mutant htau40A152T, and the shortest full-length isoform of three-repeat tau (htau23wt, 352 residues) and its mutant htau23A152T were expressed in BL21 (DE3) *Escherichia coli* as described (44,45). For terminology of Tau isoforms, see Goedert *et al*. (46). Tau mutations were created by site-directed mutagenesis using the Quik-change site-directed mutagenesis kit (Stratagene, Amsterdam, the Netherlands) and the plasmid pNG2. The assembly was induced by incubating soluble tau in the range of 50 μM in volumes of 20 μl at 37°C in 20 mM BES (*N*,*N*,-bis [2-hydroxyethyl]-2-aminoethanosulfonic acid), pH 7.4, plus 25 mM NaCl buffer with the anionic cofactor heparin 5000 (molar ratio of tau to heparin = 4:1). The formation of aggregates was monitored by ThS fluorescence and confirmed by electron microscopy. After the ThS aggregation assay, solutions of aggregated tau were centrifuged at 100 000*g* for 30 min to separate aggregated tau pellet and non-aggregated tau supernatant [in these conditions, oligomers up to ~70 tau monomers would remain in solution, estimated from the equation $S = K/T$, where $K \approx 70$ is the clearing factor of the centrifuge rotor, and $T = 0.5$ is the centrifugation time; this yields a cutoff of $S \approx 140$, equivalent to 70 times the Svedberg value of hTau40 monomers of ~2 (47)], and analyzed by SDS–PAGE (10% polyacrylamide gels). Gels were stained with Coomassie Blue R-250 and quantified (AIDA IMAGE software). All experiments were performed at least five times, and four batches of proteins were purified.

Tau-induced microtubule polymerization and binding

Microtubule assembly was monitored by UV light scattering in the presence and absence of tau. For the binding of tau to preassembled microtubules, tubulin assembly was performed in microtubule assembly buffer. Tubulin (30 μM) was incubated with 30 μM taxol at 37°C for 20–30 min to induce microtubule formation. The suspension of the samples was fractionated by ultracentrifugation at 28 000*g* for 20 min. The stabilized microtubule solutions were then diluted to the desired concentration and titrated with different concentrations of tau to measure the interaction by co-sedimentation assay. The samples were fractionated by ultracentrifugation, analyzed by SDS–PAGE and the percentages of tau protein in supernatants and pellets were quantified by densitometry of the Coomassie Blue R-250-stained gels.

Electron microscopy

Protein solutions (tau filaments or microtubules) were diluted to 1–10 μm, placed on 600-mesh carbon-coated copper grids for 1 min, washed with two drops of H_2O, negatively stained with 2% uranyl acetate for 45 s and examined in a Philips CM12 electron microscope at 100 kV.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

ACKNOWLEDGEMENTS

The authors would like to thank all patients and research subjects for their support of our research. We also thank many study coordinators and auxiliary personnel involved in many aspects of this research.

NCRAD series. We thank contributors, including the Alzheimer's Disease Centers, who collected samples used in this study, as well as patients and their families, whose help and participation made this work possible.

GIFT series. The samples from the French clinical and genetic Network on FTLD/FTLD-ALS were collected by A. Brice (Paris), I. Le Ber (Paris), B. Dubois (Paris), L. Lacomblez (Paris), R. Levy (Paris), C. Duyckaerts (Paris), M.O. Habert (Paris), V. Meininger (Paris), F. Salachas (Paris), D. Hannequin (Rouen), F. Pasquier (Lille), M. Didic (Marseille), B.F. Michel (Marseille), E. Guedj (Marseille), M. Vercelletto (Nantes), V. Golfier (Rennes), Ph. Corcia (Tours), C. Thomas-Antérion (Saint-Etienne), M. Puel (Toulouse), P. Couratier (Limoges), P. Corcia (Tours), F. Sellal (Colmar), F. Blanc (Strasbourg).

NIMH series. Control subjects from the National Institute of Mental Health Schizophrenia Genetics Initiative (NIMH-GI), data and biomaterials are being collected by the 'Molecular Genetics of Schizophrenia II' (MGS-2) collaboration. The investigators and co-investigators are: ENH/Northwestern University, Evanston, IL, MH059571, Pablo V. Gejman, MD (Collaboration Coordinator; PI), Alan R. Sanders, MD; Emory University School of Medicine, Atlanta, GA, MH59587, Farooq Amin, MD (PI); Louisiana State University Health Sciences Center, New Orleans, LA, MH067257, Nancy Buccola APRN, BC, MSN (PI); University of California-Irvine, Irvine, CA, MH60870, William Byerley, MD (PI); Washington University, St Louis, MO, U01, MH060879, C. Robert Cloninger, MD (PI); University of Iowa, Iowa, IA, MH59566, Raymond Crowe, MD (PI), Donald Black, MD; University of Colorado, Denver, CO, MH059565, Robert Freedman, MD (PI); University of Pennsylvania, Philadelphia, PA, MH061675, Douglas Levinson, MD (PI); University of Queensland, Queensland, Australia, MH059588, Bryan Mowry, MD (PI); Mt Sinai School of Medicine, New York, NY, MH59586, Jeremy Silverman, PhD (PI).

We thank the Harvard Brain Tissue Center (contact person: Francine Benes, btrc@mclean.harvard.edu). We would like to thank Ilka Lindner (Hamburg) for expert technical assistance in protein preparation. We also wish to thank the DNA and Cell Bank of the CRicm U975 (Paris) for expert technical assistance in DNA preparation.

Conflict of Interest statement. The authors have no conflicts of interest to declare that might raise the question of bias in the work reported or the conclusions, implications or opinions stated—including pertinent commercial or other sources of funding for the individual author(s) or for the associated department(s) or organization(s), personal relationships or direct academic competition.

FUNDING

This work was supported by grants from the National Institutes of Health (grant numbers R01 AG026938 to D.H.G., RC1AG035610 to G.C., P50 AG-16570 to J.R., AG13854 to E.B., R37 AG11762 to G.D.S., P50-AG05142 to H.C.C., P50 AG025688 to A.I.L., R01 AG038791 to A.L.B., R01 NS078086 to O.A.R., R01 NS065782 to R.R., P50 AG023501 to B.L.M., Mayo Udall Center P50NS072187 to O.A.R., Z.K.W., R.J.U., D.W.D.); the Tau Consortium (G.C., R.O.K., S.E.L., E-M.M., B.L.M., E.M., D.H.G.); a gift from Carl Edward Bolch, Jr, and Susan Bass Bolch (O.A.R., Z.K.W., R.J.U., D.W.D.); the Irene and Abe Pollin Fund for CBD Research (A.L.B. and R.R.); the CurePSP Foundation (G.D.S.); the Peebler PSP Research Foundation (G.D.S.); the NIHR Biomedical Research Centre for Mental Health at SLAM and KCL (http://www.slam.nhs.uk/about-us/biomedical-research-centre/brc-home.aspx); the Medical Research Council (http://www.mrc.ac.uk/index.htm) through PhD studentship funding; the Alzheimer's Research UK (http://www.alzheimersresearchuk.org), the 7th framework program of the European Union (ADAMS project, http://www.adams-consortium.eu/, HEALTH-F4-2009-242257, to S.L. and J.P]; and the Agence Nationale de la Recherche (FTDGenes, R R08104DS, to I.L.B. and A.B.). In the NCRAD series, samples from the National Cell Repository for Alzheimer's Disease (NCRAD), which receives government support under a cooperative agreement grant (U24 AG21886) awarded by the National Institute on Aging (NIA), were used in this study. The National Cell Repository for Alzheimer's Disease (NCRAD) receives government support under a cooperative agreement grant (U24 AG21886) awarded by the National Institute on Aging (NIA). Costs for tissue samples submitted by T.G.B. were supported by the National Institute on Aging (P30 AG19610 Arizona Alzheimer's Disease Core Center), the Arizona Department of Health Services (contract 211002, Arizona Alzheimer's Research Center), the Arizona Biomedical Research Commission (contracts 4001, 0011, 05-901 and 1001 to the Arizona Parkinson's Disease Consortium) and the Michael J. Fox Foundation for Parkinson's Research. The funders had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

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APPENDIX

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