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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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 htau40 and mutation at tau40A152T (largest isofrom in the human central nervous system (CNS), 441 residues) and htau23 (smallest isofrom 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). Domiinantly 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 FTLD (10). In summary, mutations in a few Mendelian genes possibly the risk for dementia (9); and (ii) variants within which has been shown to regulate its expression levels, and common variants of FTD and Parkinson’s disease (PD), suggesting that has been recognized as a major risk factor for PSP, CBD, some mutations accounts 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 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 challeng- ing, 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 allele 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
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

### 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)

<table>
<thead>
<tr>
<th></th>
<th>GIFT</th>
<th>MAYO</th>
<th>PENN</th>
<th>KCL</th>
<th>Total</th>
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<tr>
<td><strong>FTD-s</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Carriers (%)</td>
<td>4.47 ± 0.12</td>
<td>11 ± 0.86</td>
<td>3 ± 0.72</td>
<td>19 ± 0.89</td>
<td>2139</td>
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<tr>
<td>OR (CI)</td>
<td>4.3 (1.2–12.7)</td>
<td>3.4 (1–14.9)</td>
<td>1.6 (0.2–8.3)</td>
<td>3.0 (1.6–5.6)</td>
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<tr>
<td>P-value (Fisher’s test)</td>
<td>0.012</td>
<td>0.03</td>
<td>0.45</td>
<td>0.0005</td>
<td></td>
</tr>
<tr>
<td><strong>FTD (carriers)</strong></td>
<td>46 ± 2</td>
<td>130 ± 1</td>
<td>76 ± 0</td>
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<tr>
<td>Age at onset</td>
<td>65 ± 9</td>
<td>64 ± 10</td>
<td>64 ± 10</td>
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<td></td>
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<tr>
<td>%Female</td>
<td>46</td>
<td>49</td>
<td>45</td>
<td></td>
<td></td>
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<tr>
<td>%Caucasian</td>
<td>87</td>
<td>98</td>
<td>96</td>
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<td></td>
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<tr>
<td>%Neuropath</td>
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<td>65</td>
<td>100</td>
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<tr>
<td><strong>AD</strong></td>
<td>549 ± 0.56</td>
<td>1367 ± 0.74</td>
<td>540 ± 0.74</td>
<td>3345 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Carriers (%)</td>
<td>0 ± 0.3</td>
<td>5 ± 0.56</td>
<td>14 ± 0.12</td>
<td>23 ± 0.69</td>
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</tr>
<tr>
<td>OR (CI)</td>
<td>NA</td>
<td>2.2 (0.5–11)</td>
<td>2.3 (0.8–8.2)</td>
<td>2.3 (1.3–4.2)</td>
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<tr>
<td>P-value (Fisher’s test)</td>
<td>NA</td>
<td>0.30</td>
<td>0.72</td>
<td>0.004</td>
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<tr>
<td>Age at onset</td>
<td>66 ± 10</td>
<td>71 ± 8</td>
<td>75 ± 7</td>
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<td></td>
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<tr>
<td>%Female</td>
<td>57</td>
<td>57</td>
<td>51</td>
<td></td>
<td></td>
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<tr>
<td>%Caucasian</td>
<td>32</td>
<td>95</td>
<td>99</td>
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<td></td>
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<tr>
<td>%Neuropath</td>
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<td>100</td>
<td>54</td>
<td>NA</td>
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<tr>
<td><strong>PD/LBD</strong></td>
<td>838 ± 0.48</td>
<td>838 ± 0.48</td>
<td>1.6 (0.4–4.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carriers (%)</td>
<td>4 ± 0.46</td>
<td>4 ± 0.48</td>
<td>0.33</td>
<td></td>
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</tr>
<tr>
<td>OR (CI)</td>
<td>1.9 (0.3–10)</td>
<td>1.6 (0.4–4.6)</td>
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<tr>
<td>P-value (Fisher’s test)</td>
<td>0.46</td>
<td>NA</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Age at onset</td>
<td>65 ± 12</td>
<td>75 ± 13</td>
<td>75 ± 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%Female</td>
<td>40</td>
<td>57</td>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%Caucasian</td>
<td>100</td>
<td>97</td>
<td>99</td>
<td></td>
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<tr>
<td>%Neuropath</td>
<td>21</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td><strong>Controls</strong></td>
<td>5782 ± 0.26</td>
<td>1118 ± 0.45</td>
<td>560 ± 0.54</td>
<td>27 ± 0.30</td>
<td>9047</td>
</tr>
<tr>
<td>Carriers (%)</td>
<td>15 ± 0.26</td>
<td>4 ± 0.25</td>
<td>3 ± 0.54</td>
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<td></td>
</tr>
<tr>
<td>Age at examination</td>
<td>50 ± 16</td>
<td>70 ± 12</td>
<td>75 ± 13</td>
<td>75 ± 6</td>
<td></td>
</tr>
<tr>
<td>%Female</td>
<td>48 ± 0.23</td>
<td>52</td>
<td>57</td>
<td>55</td>
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<tr>
<td>%Caucasian</td>
<td>71 ± 0.28</td>
<td>97</td>
<td>99</td>
<td></td>
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<tr>
<td>%Neuropath</td>
<td>NA</td>
<td>46</td>
<td>18</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

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).

aIncluding 206 patients sampled by the French clinical and genetic Network on FTLD/FTLD-ALS.

bCompared with the controls available in each series (e.g. GIFT FTD-s versus GIFT controls, MAYO FTD-s versus MAYO controls, etc.).

cIncluding 184 NCWAD and 5059 NIMH controls.

dIncluding 613 LOAD controls and 122 NIMH controls.
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
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...
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...
isoform ratios or function may influence AD risk (27). A
this possibility conclusively.
small numbers of p.A152T carriers do not allow us to exclude
evidence for population bias in a subset of our samples, the very
these conditions (blue curve, bottom).
Aggregation propensity and microtubule assembly of htau23wt and
and mutant tau (red curve, middle) monitored by light scattering at 350 nm.
B (A) Aggregation of htau23wt and p.A152T mutant monitored
by the ThS fluorescence assay in the presence of the cofactor heparin. The ag-
gration of tau23A152T is somewhat slower and reaches a somewhat lower
final level of aggregation, but overall the assembly characteristics are compar-
are. (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 vari-
ants, 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 associ-
ation 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 me-
tabolism 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 physiologi-
cal interaction with microtubules, this amounts to an impaired sta-
bility 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 fluores-
cence assay would suggest a somewhat lower tendency of ag-
grgregation for mutant tau. However, this view must be weighed
against the fact that mutant tau is more prone to form oligo-
mers, which are thought to be more toxic than either filament
so or monomers (14). This is equivalent to a toxic gain of func-
tion. The parallel changes in the two assays, microtubule as-
sembly 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), sugges-
ting that the change in protein conformation caused by
the p.A152T substitution decreases the interaction with microtu-
bules 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 conse-
quences 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 func-
tions. (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
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

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 ag-
gration of tau23A152T is somewhat slower and reaches a somewhat lower
final level of aggregation, but overall the assembly characteristics are compar-
able. (B) Microtubule assembly induced by wild-type tau (black curve, top)
and mutant tau (red curve, middle) monitored by light scattering at 350 nm.
(NMR) spectroscopy analysis (38). This includes p.1151, 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-aminoethanesulfonic 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 000g 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 000g 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 H2O, 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.

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