Association Between the Extended tau Haplotype and Frontotemporal Dementia

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Background: Recent studies have shown an association between an extended tau haplotype (H1) that covers the entire human tau gene and progressive supranuclear palsy or, more inconsistently, other neurodegenerative disorders, such as corticobasal degeneration, Parkinson disease, Alzheimer disease, and frontotemporal dementia (FTD). In addition, disease-causing mutations in the tau gene on chromosome 17 have been detected in some families with autosomal dominant FTD and parkinsonism. In FTD, the pathological accumulation of the microtubule-associated protein tau suggests that the tau gene may be a genetic risk factor for this disorder.

Objective: To confirm or refute the association between the H1 haplotype or the H1H1 genotype of the tau gene and FTD.

Methods: Tau genotype (contiguous polymorphisms in exons 1, 7, and 13 and in intron 9 used to reconstruct the extended haplotypes H1 and H2). Clinical examination, psychometric testing, laboratory tests, computed tomography and magnetic resonance imaging, single-photon emission computed tomography, and electroencephalography for patients with FTD.

Results: The H1H1 genotype was significantly overrepresented in patients with FTD compared with controls (62% vs 46%; P = .01, 1-sided; odds ratio adjusted for age and sex, 1.95). After stratification according to apolipoprotein E (APOE) genotype, we found a significant interaction between APOE and tau genotypes (P = .03).

Conclusions: This study of the largest series of patients with FTD confirms the primary role of tau in FTD and establishes that the H1 haplotype of the tau gene and the E2 allele of APOE interact by an unknown mechanism that increases the risk of FTD.

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ONTOTEMPORAL dementia (FTD) is a neurodegenerative disorder characterized by progressive personality change and breakdown in social conduct. Since the Lund and Manchester consensus conference, the clinical criteria for FTD diagnosis have become increasingly more precise, facilitating discrimination between FTD and Alzheimer disease, the most frequent misdiagnosis of FTD. A major neuropathologic characteristic of FTD is filamentous inclusions containing hyperphosphorylated tau protein. Tau is a microtubule-associated protein that binds to microtubules and promotes microtubule assembly. Aggregates of hyperphosphorylated forms of tau protein participate in the formation of neurofibrillary tangles, which characterize a number of tauopathic conditions, such as Alzheimer disease, progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), prion diseases, and amyotrophic lateral sclerosis/parkinsonism-dementia complex. Recently, mutations in the tau gene, localized to 17q21, have been identified in several families with autosomal dominant inheritance of FTD, designated as FTD with parkinsonism linked to chromosome 17. To date, 10 missense mutations, 2 deletions, and 3 transition mutations that do not alter the encoded amino acid sequence have been identified in exons of the tau gene. In addition, 6 intronic mutations have also been found in the 5’ splice donor site of exon 10. This gene accounts for 25% to 40% of families with FTD that have autosomal dominant inheritance.
PARTICIPANTS AND METHODS

PARTICIPANTS

After excluding all patients with autosomal dominant inheritance and mutation of tau (n=6),14 our sample was composed of 100 unrelated patients with FTD (+4% men) admitted consecutively to 12 hospitals in France in a 3-year period. All patients with FTD underwent a thorough clinical examination, including personal and familial medical history, neurologic and psychiatric investigations, psychometric testing (Mini-Mental State Examination,35 Mattis Dementia Rating Scale,36 Verbal Learning Test,37 and Frontal Assessment Battery38), laboratory tests, computed tomography and magnetic resonance imaging, regional cerebral blood flow measurement (single-photon emission computed tomography), and electroencephalography. The diagnosis of FTD was established according to the Lund-Manchester clinical consensus criteria for FTD,4 revised in 1998.39 Age at onset was assessed by interviewing 1 or 2 next of kin and was defined as the age at which relevant symptoms first appeared according to the family (mean ± SD age at onset, 60.6 ± 9.3 years; range, 35-77 years). We identified 40 patients with at least 1 first- or second-degree relative with FTD but without autosomal dominant inheritance and mutation in the tau gene (30% of men; mean ± SD age at onset, 58.7 ± 9.0 years; range, 35-77 years).

Postmortem neuropathologic examination was performed in 3 patients. Blocks of frontal, temporal, parietal, and occipital cortex; amygdala; hippocampus; basol ganglia; thalamus; and cerebellum were stained with usual stains (hematoxylin-eosin and Bodian silver method associated with luxol fast blue). By gross examination, the frontal lobe was moderately to severely atrophic. All 3 patients showed degeneration of the cerebral cortex, which was severe in the frontal cortex, less severe in the temporal and parietal cortices, and generally absent in the occipital cortex. Degeneration was characterized by microvacuolation, considerable neuronal loss, and astrocytic gliosis, especially in layers I and II of the frontotemporal cortices and in the CA1 and subiculum regions. Swollen neurons, Pick bodies, Lewy bodies, neurofibrillary tangles, and senile plaques were absent. Therefore, frontal lobe atrophy lacking distinctive histologic characteristics or features was defined for each patient subjected to autopsy.20

Patients with FTD were compared with 79 age-matched controls (30% men; mean ± SD age at examination, 60.0 ± 8.8 years; range, 38-77 years). Control subjects were the patients’ spouses, healthy blood donors, or individuals living in nursing homes. All participants in this study were white and were living in France. Informed written consent was obtained from all participants, either directly or from the legal tutor.

GENOTYPING AND HAPLOTYPE RECONSTRUCTION

Polymorphisms of exons 1 (+5 A/G), 7 (528 G/A), and 13 (+34 T/C) were analyzed by polymerase chain reaction amplification, followed by digestion of the product with the diagnostic restriction enzyme. For polymorphism of exon

### Table 1. Haplotype and Genotype Frequencies in 100 Patients With FTD and 79 Controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients With FTD, No. (%)</th>
<th>Controls, No. (%)</th>
<th>1-Sided P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1H1</td>
<td>(n = 100)</td>
<td>(n = 79)</td>
<td></td>
</tr>
<tr>
<td>H1H2 + H2H2</td>
<td>38 (38)</td>
<td>36 (46)</td>
<td>0.01</td>
</tr>
<tr>
<td>Alleles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H1</td>
<td>(n = 200)</td>
<td>(n = 158)</td>
<td>0.57</td>
</tr>
<tr>
<td>H2</td>
<td>154 (77)</td>
<td>110 (70)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>46 (23)</td>
<td>48 (30)</td>
<td></td>
</tr>
</tbody>
</table>

*FTD indicates frontotemporal dementia.

The 6 major isoforms of tau found in the normal adult brain are generated by alternative splicing of exons 2, 3, and 10.14 These isoforms differ by the presence of 3 or 4 tandem repeats of 31 to 32 amino acids in the carboxy-terminal region, in conjunction with 0, 1, or 2 inserts of 29 amino acids in the amino-terminal region. Sequencing of the tau gene revealed the existence of at least 16 polymorphisms in different exons and introns.15 All of these polymorphisms are in complete linkage disequilibrium with each other.14 As a result, there are only 2 common extended haplotypes, H1 and H2, that cover the entire tau gene (approximately 100 kilobases). Analysis of these 2 haplotypes revealed no recombinant event in more than 500 chromosomes.16-18 Association studies have been performed on polymorphisms of the tau gene and in disorders in which the tau protein is variably involved in the physiopathology. The A0 allele of a dinucleotide polymorphism in intron 9 of the tau gene (included in the H1 haplotype) was first found to be associated with PSP.19 This association was replicated in different populations20,23-24 and extended to the entire H1 haplotype.16,20,27 The haplotype has also been found to be associated with CBD,17,24,28 Parkinson disease,23,24,29 and Alzheimer disease,30,31 but with inconsistent results.23,24,29,32,33

Morris et al24 found no significant difference in the frequency of the A0 allele and the A0A0 genotype in 32 patients with FTD and in 75 control subjects. However, in 36 clinically ascertained patients with FTD, Ingelson et al13 recently found that the H1 haplotype, in combination with the apolipoprotein E (APOE) ε4 allele, was a genetic risk factor for FTD.

The present study was initiated to determine whether an association between the H1 haplotype and FTD could be detected in a larger independent population of clinically ascertained patients with FTD and a different genetic background (French population) than in previous studies.

RESULTS

Assuming a 1-sided significance level of α = .05 and a power (1 - β) of 80%, the size of our sample (100 patients with FTD and 79 controls) was sufficient to detect an OR of at least 2.10 for carriers of the HIHI1 genotype and at least 1.85 for carriers of the H1 haplotype.
Haplotypes were reconstructed as previously described.16

TABLE 1. Combined Analysis of tau Genotypes and APOE2 Alleles in 100 Patients With FTD and 79 Controls*

Table 1: Combined Analysis of tau Genotypes and APOE2 Alleles in 100 Patients With FTD and 79 Controls

<table>
<thead>
<tr>
<th>APOE Genotypes</th>
<th>Patients With FTD</th>
<th>Controls</th>
<th>Patients With FTD</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2-</td>
<td>7 (7)</td>
<td>7 (9)</td>
<td>10 (10)</td>
<td>4 (5)</td>
</tr>
<tr>
<td>E2+</td>
<td>55 (55)</td>
<td>29 (37)</td>
<td>28 (28)</td>
<td>39 (49)</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>36</td>
<td>38</td>
<td>43</td>
</tr>
</tbody>
</table>

*Data are given as number (percentage). APOE indicates apolipoprotein E; FTD, frontotemporal dementia; E2+, the presence of at least 1 E2 allele; and E2-, the absence of E2 allele.

in Table 3. The results remained significant, or borderline significant, even after Bonferroni correction for multiple tests.

The results of this case-control study, to our knowledge the largest performed to date in FTD, suggest that patients who are H1H1 homozygotes for the tau gene are at increased risk for developing FTD. The different results observed between patients with FH+ and those with FH− are probably owing to the difference in the sample size (40 patients with FH+ and 60 with FH−), and thus in the study power.
Table 3. Odds Ratios (ORs), 95% Confidence Intervals (CIs), and P Values When Combining APOE and tau Genotypes*

<table>
<thead>
<tr>
<th>APOE Genotype</th>
<th>tau Genotype</th>
<th>OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2−</td>
<td>H1H2 + H2H2</td>
<td>1.00 (Referent)</td>
<td>. . .</td>
</tr>
<tr>
<td>E2−</td>
<td>H1H1</td>
<td>2.64 (1.52-4.60)</td>
<td>.002</td>
</tr>
<tr>
<td>E2+</td>
<td>H1H2 + H2H2</td>
<td>3.48 (1.21-10.00)</td>
<td>.02</td>
</tr>
<tr>
<td>E2+</td>
<td>H1H1</td>
<td>1.39 (0.53-3.67)</td>
<td>.28</td>
</tr>
</tbody>
</table>

*APOE indicates apolipoprotein E; E2−, the absence of E2 allele; and E2+, the presence of at least 1 E2 allele.

Previous studies have reported an association between this haplotype in the tau gene and PSP,16,21 and CBD,17,28 which are also characterized by neuronal accumulation of tau protein. It must be added that earlier studies10-24 of an association with the most common allele of the dinucleotide polymorphism (A0) in intron 9 of the tau gene reflect in fact the association with the broader haplotype. The A0 allele for this polymorphism is indeed included in the H1 haplotype.36 Two previous studies15,24 have analyzed this haplotype in patients with FTD, but the results were not significant. Because of the small size of the samples (36 and 32 patients with FTD, respectively), these studies probably did not have sufficient a priori statistical power to demonstrate a nonmajor effect of the H1 haplotype. Our study of 100 patients with FTD was sufficiently powerful to detect an effect (OR) of at least 2.10 for the H1H1 genotype and 1.85 for the H1 haplotype.

A possible confounding factor in our study could be “contamination” by patients with PSP or CBD. Therefore, we decided to use strict criteria for the diagnosis of FTD: (1) clinical diagnosis according to the Lund-Manchester clinical consensus criteria for FTD,2,39 (2) neuropsychologic confirmation of frontal lobe dysfunction, (3) frontal or frontotemporal atrophy on computed tomographic or magnetic resonance images, and (4) frontotemporal hypoperfusion on single-photon emission computed tomographic images. Furthermore, we used age-matched controls, and ORs were adjusted for age and sex to eliminate these possible confounding factors. So, although only a small proportion of the patients were examined neuropathologically (3%), which allowed confirmation of the diagnosis of FTD, misdiagnosis as PSP or CBD is unlikely given the criteria used to assess patients.

The recent findings of pathogenic mutations in the tau gene in 25% to 40% of patients with FTD and autosomal dominant inheritance2,10,13 and the association of an extended haplotype that covers the entire tau gene indicate that tau plays a primary role in FTD and also in other neurodegenerative disorders with altered tau profiles, such as PSP, CBD, and Parkinson disease. Although our findings clearly suggest that the tau gene is a genetic risk factor for FTD, the molecular mechanisms underlying this effect are not known yet. It is possible to speculate, however, that the H1 haplotype is associated with a different level of tau gene expression than the H2 haplotype. However, the biologically relevant polymorphisms that are responsible for the risk of the disease remain to be determined.

The interaction between the tau and APOE genes was significant. The logistic regression coefficient for the interaction term was negative, which explains why the estimated OR for carriers of both at-risk genotypes (carriers of the H1H1 tau genotype and of APOE2 alleles) was lower than the OR estimated for only one of the at-risk genotypes. Therefore, the effects of these 2 risk factors do not seem to be synergistic but rather alternative: each at-risk genotype has an effect when individuals are carriers of one of them, but this effect disappears when individuals are carriers of both. The interaction found in our study (with APOE2) is different from that found by Ingelson et al15 (with APOE4). Divergent results have also been observed among studies of APOE in FTD. Indeed, several studies have suggested that APOE might be a risk factor in FTD, but APOE2 was associated with FTD in 2 studies16,21 and APOE4 in 2 other studies.42,44 The size of the case-control sample reported in the article by Ingelson et al15 (n = 36) could be another possible point of difference to explain why their results are different from ours. However, the molecular mechanisms for the interaction between tau and APOE in the pathway to tau physiopathology seen in FTD are still unknown and should be specified by molecular studies.

The fact that the H1 haplotype in the tau gene seems to be a risk factor for several neurodegenerative disorders with different clinical presentations, including FTD, suggests that these disorders share a common pathogenic mechanism that involves tau dysfunction. Further studies are needed to determine how the H1 haplotype in the tau gene affects the neurodegenerative processes to better understand the pathogenesis of such disorders.

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REFERENCES