Dementia Risk in Parkinson Disease

Disentangling the Role of MAPT Haplotypes

Núria Setó-Salvia, BS; Jordi Clarimon, PhD; Javier Pagonabarraga, MD, PhD; Berta Pascual-Sedano, MD, PhD; Antonio Campolongo, BS; Ondre Combarros, MD, PhD; Jose Ignacio Mateo, MD, PhD; Daniel Regaña, BS; Merce Martínez-Corral, MD; Marta Marquié, MD; Daniel Alcolea, MD; Marc Suárez-Calvet, MD; Laura Molina-Porcel, MD; Oriol Dols, BS; Teresa Gómez-Isla, MD, PhD; Rafael Blesa, MD, PhD; Alberto Lleó, MD, PhD; Jaime Kulisevsky, MD, PhD

Background: Dementia in Parkinson disease (PD) causes nursing home placement, caregiver distress, higher health care burden, and increased mortality.

Objectives: To determine whether the microtubule-associated protein tau (MAPT) H1 haplotype and MAPT subhaplotypes play a role in the risk of PD and Parkinson disease–dementia (PDD) complex.

Design: Case-control genetic analysis.

Setting: Movement Disorders and Memory Units, Hospital de Sant Pau, Barcelona, Spain.

Participants: Two hundred two patients with PD (48 of whom developed dementia >2 years after disease onset), 41 patients with Lewy body dementia (LBD, pathologically confirmed in 17), 164 patients with Alzheimer disease (AD), and 374 controls.

Methods: The MAPT haplotype was determined by testing for a 238-base pair deletion between exons 9 and 10, which is characteristic of the H2 haplotype. Haploview was used to visualize linkage disequilibrium relationships between all genetic variants (5 single-nucleotide polymorphisms and the del-In9 variant) within and surrounding the MAPT region.

Results: The H1 haplotype was significantly overrepresented in PD patients compared with controls (P = .001). Stratifying the PD sample by the presence of dementia revealed a stronger association in PDD patients (sex- and age-adjusted odds ratio, 3.73; P = .002) than in PD patients without dementia (sex- and age-adjusted odds ratio, 1.89; P = .04). Examination of specific subhaplotypes showed that a rare version of the H1 haplotype (named H1p) was overrepresented in PDD patients compared with controls (2.3% vs 0.1%; P = .003). No positive signals for any of the MAPT variants or H1 subhaplotypes were found in AD or LBD.

Conclusions: Our data confirm that MAPT H1 is associated with PD and has a strong influence on the risk of dementia in PD patients. Our results also suggest that none of the MAPT subhaplotypes play a significant role in other neurodegenerative diseases, such as LBD or AD.

Arch Neurol. 2011;68(3):359-364

One of the most common nonmotor symptoms in Parkinson disease (PD) is the co-occurrence of dementia, with a mean prevalence of 30%. Dementia in PD is an important cause for nursing home placement, caregiver distress, higher health care burden, and increased mortality. The combination of dementia and parkinsonism is also a key feature that characterizes dementia with Lewy bodies (LBD), the second most common cause of neurodegenerative dementia in the elderly after Alzheimer disease (AD). A recent genome-wide association study has provided unequivocal evidence for a genetic association of the microtubule-associated protein tau (MAPT) (GenBank NG_007398.1 and OMIM 157140) locus with PD in the population with European origin. However, these results were not replicated in an independent genomewide association study performed in an Asian population. With up to 21 studies performed to date, MAPT seems to be undoubtedly associated with PD risk (http://www.pdgene.org). In particular, the MAPT H1 variant, an extended haplotype that results from a common genomic inversion of approximately 800 kb in chromosome 17q21 containing the MAPT gene, has been found to be related to PD risk. Interestingly, the H1 variant has also been associated with cognitive decline in a longitudinal study of PD with a 3½-year follow-up. In a subsequent study by some of the same authors, the MAPT H1 allele was the strongest independent predictor of dementia among PD patients, with an
odds ratio (OR) of 12.1 during 5 years of follow-up. The research addressing the effect of MAPT in other neurodegenerative disorders, such as AD, has yielded inconclusive results. A fine-mapping study of the MAPT H1/H2 clades described the H1c subhaplotype, a version of the H1 haplotype, to be the specific variant associated with AD risk. However, this association has been inconsistently found in subsequent studies. The specific MAPT subhaplotype linked to PD, Parkinson disease–dementia (PDD) complex, or LBD remains unknown.

Therefore, in this study, we sought to determine whether MAPT H1/H2 is associated with PD and whether it influences the occurrence of dementia during the PD clinical course. We also checked whether variability in the H1 background, ie, specific variants of the H1 clade, could lead to PD and PDD. To assess the role of the MAPT gene in other neurodegenerative diseases, we explored MAPT genetic variability in LBD and AD patients from a well-defined clinical and pathologic series from the north of Spain.

METHODS

SUBJECTS

A total of 390 unrelated patients were prospectively recruited from the outpatient Movement Disorders and Memory Units at the Hospital de Sant Pau, Barcelona. All patients were examined by neurologists with expertise in neurodegenerative diseases. A total of 202 patients fulfilled the diagnostic criteria described by Hughes et al for idiopathic PD and were prospectively recruited from among outpatients regularly attending the Movement Disorders Unit. Motor symptoms and disease severity were assessed in accordance with the Unified Parkinson’s Disease Rating Scale (UPDRS) and the Hoehn and Yahr scale. Of the total group of PD patients, 48 fulfilled current diagnostic criteria for probable PDD. Diagnosis of dementia was based on a score of 1 or more on the Clinical Dementia Rating scale and fulfillment of the 294.1 criteria for PDD in the Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition, Text Revision). Patients with PD without any cognitive decline were classified as the PdnD group (n = 154). Twenty-four patients with current consensus diagnostic criteria for LBD were included. In accordance with published research criteria, we used the 1-year rule to distinguish between PDD and LBD. We also included 17 patients with neuropathologically confirmed LBD. Brain tissue was provided by the Neurological Tissue Bank, Hospital Clinic de Barcelona at the University of Barcelona. Neuropathologic staging of Lewy body–related pathology was performed in accordance with proposed diagnostic criteria for LBD and PD-related pathology. We assessed AD-related pathology according to Alzheimer’s Disease Neuroimaging Initiative criteria and populationspecific database recommendations for the postmortem diagnosis of AD. We used consensus recommendations to estimate the likelihood that AD lesions underlie dementia. In order to include only those patients whose death was most likely due to LBD, we assessed Lewy bodies and AD pathology in accordance with McKeith et al’s recommendations.

A total of 164 patients fulfilled clinical diagnostic criteria for possible or probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association criteria for AD.

We recruited 374 unrelated controls from the Department of Neurology at Hospital Universitari Marqués de Valdecilla (Santander, Spain). All had complete neurologic and medical examination findings that showed they were free of significant illness. They also had Mini-Mental State Examination scores of 28 or higher (corrected for age), verified by at least 1 annual follow-up assessment. Although we did not perform a formal screening process regarding patients’ descent from the data available concerning their origin (name and place of birth), it was deduced that all were of European ancestry.

All participants or their families provided written informed consent, and the study was approved by the respective ethics committees.

GENOTYPING

The MAPT haplotype was determined by testing for the presence of a 238-base pair deletion between exons 9 and 10, which is characteristic of the H2 haplotype. Genotyping of the surrounding tagging single-nucleotide polymorphisms (SNPs) rs1467967, rs242557, rs3785883, rs2471738, and rs7521, within the 17q21 region, was conducted by Taqman Assays-on-Demand on the ABI PRISMS 7900HT Sequence Detection System (Applied Biosystems, Foster City, California), followed by analysis with SDS 7500 software, version 2.0.1 (Applied Biosystems, Carlsbad, California).

STATISTICAL ANALYSIS

Hardy-Weinberg equilibrium was assessed by the χ² test. Haplotype was used to visualize linkage disequilibrium relationships between all genetic variants (5 SNPs and the del-ln9 variant) within and surrounding the MAPT region. Linkage disequilibrium blocks were constructed following the D’ method by Gabriel et al, also implemented in Haploview. Genotype and allele frequencies were estimated by direct counting and were compared between patients and controls by means of χ² analysis with 1 degree of freedom. Multiple logistic regression models were used to adjust for covariants, such as age and sex. Data were analyzed using the Statistical Package for the Social Sciences, version 17.0.0 (SPSS Inc, Chicago, Illinois). Haplotypes were reconstructed and population frequencies were estimated using the program PHASE, version 2.1 (Matthew Stephens Lab, Chicago, Illinois). Global differences in haplotype frequencies between controls and cases were assessed using 1000 permutations for each comparison. Individual haplotype frequency differences were assessed by haplotype trend regression analysis, with 10 000 permutations. Haplotype-specific score statistics adjusted for age and sex were assessed with the R package Haplo.score, with 10 000 simulations for each empirical P value. The t test was performed to compare means between groups.

RESULTS

A total of 390 patients (comprising PD, AD, and LBD patients) and 374 controls were recruited (Table 1 for sample details). Of the 202 PD patients, 48 developed dementia (PDD subgroup) and 154 remained free of dementia (PdnD subgroup). The mean (SD) interval from PD onset to dementia was 11.3(7.1) years, ranging from 2 to 30 years. Therefore, none of the PDD patients included in our series developed dementia within 2 years of PD onset. The mean (SD) age of dementia onset was 74.3(7.0) years. Comparison of clinical characteristics

©2011 American Medical Association. All rights reserved.
between PDnD and PDD patients revealed that disease duration was longer in PDD patients (13.5 years in PDD patients vs 11.2 years in PDnD patients; \( P = .03 \)). Also, age of onset was later in PDD patients compared with PDnD patients (63.0 vs 56.6 years; \( P < .001 \)). Finally, total UPDRS motor score and Hoehn and Yahr scale scores were higher in PDD patients compared with scores in PDnD patients (37.2 vs 27.1 for the UPDRS; \( P < .001 \); and 3.4 vs 2.4 for the Hoehn and Yahr scale; \( P < .001 \)). No differences in sex were found between PD patients with and without dementia.

Genotype frequencies did not differ significantly from the predictions of the Hardy-Weinberg equilibrium in controls. Single-locus analysis of the genetic variants analyzed within the MAPT region disclosed a highly significant overrepresentation of the H1 allele in the entire PD group (comprising both PDD and PDnD) compared with controls (\( P = .002 \) (Table 2). Interestingly, although this association remained significant in both subgroups of PD patients, the effect was greater in PDD patients than in PDnD patients (OR, 2.69; 95% confidence interval [CI], 1.47-4.95; \( P = .001 \) in the PDD subgroup; and OR, 1.38; 95% CI, 1.02-1.86; \( P = .04 \) in the PDnD subgroup). Because PD patients were not age- and sex-matched with controls, we performed a logistic regression analysis adjusting the H1 risk for these 2 variables. Again, although the H1-adjusted effect persisted in the overall PD group (OR, 2.47; 95% CI, 1.44-4.22; \( P = .001 \)), the risk-adjusted H1 for the PDD subgroup was twice that for the PDnD subgroup (OR, 4.95; 95% CI, 2.69-8.46; \( P = .001 \) in the PDD subgroup; and OR, 1.48; 95% CI, 1.04-2.12; \( P = .04 \) in the PDnD subgroup).

When we focused on other SNPs surrounding the H1/H2 allele, a protective effect was detected for the rs1467967-A allele on both the PD risk (\( P = .007 \)) (Table 2). This polymorphism did not have any effect on the PDnD group. Only 1 SNP (rs3785883) was marginally significant in the PDnD group (OR, 1.23; 95% CI, 0.75-2.00; \( P = .12 \)). None of the 6 variants analyzed within the MAPT region had any role in either LBD or AD risk.
relative positions of the tagging single-nucleotide polymorphisms (SNPs) are shown below, with the genomic length between flanking SNPs in base pairs (bp) indicated. Numbers within the diamonds are D' values for the respective SNP pairs.

**Figure.** Linkage disequilibrium plot across the microtubule-associated protein tau (MAPT) gene in control individuals from Spain. The gene structure of MAPT is shown (top), with vertical bars representing exons. Relative positions of the tagging single-nucleotide polymorphisms (SNPs) are shown below, with the genomic length between flanking SNPs in base pairs (bp) indicated. Numbers within the diamonds are D' values for the respective SNP pairs.

### Table 3. Association of MAPT Haplotypes

<table>
<thead>
<tr>
<th>Haplotype ID</th>
<th>Haplotype Variants</th>
<th>Haplotype Frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2a</td>
<td>AGG/AGC/AGC</td>
<td>CO: 27.2, PD: 20.5, PDD: 22.8, LBD: 12.5, AD: 27.4</td>
</tr>
<tr>
<td>H1b</td>
<td>GSG/AGC/AGC</td>
<td>CO: 11.9, PD: 15.4, PDD: 11.3, LBD: 6.3, AD: 11.2</td>
</tr>
<tr>
<td>H1d</td>
<td>AAGC/AGC/AGC</td>
<td>CO: 7.8, PD: 8.4, PDD: 8.3, LBD: 7.3, AD: 7.1</td>
</tr>
<tr>
<td>H1e</td>
<td>AGGC/AGC/AGC</td>
<td>CO: 7.9, PD: 8.4, PDD: 8.3, LBD: 7.3, AD: 7.1</td>
</tr>
<tr>
<td>H1p</td>
<td>GGGT/AGG/AGG</td>
<td>CO: 0.1, PD: 0.9, PDD: 0.2, LBD: 2.3, AD: 0.2</td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer disease; CO, controls; LBD, Lewy body dementia; MAPT, microtubule-associated protein tau; PD, Parkinson disease; PDD, Parkinson disease–dementia; PDnD, Parkinson disease without any cognitive decline.

*The allele for each particular single-nucleotide polymorphism is shown in the following order: rs1467967, rs242557, rs3785883, rs2471738, del-in9 and rs7521. Numbers on the last line indicate P values that result from haplotype frequency comparisons between each group of patients and controls. PHASE, version 2.1 (Matthew Stephens Lab, Chicago, Illinois) was used to reconstruct haplotypes and compare haplotype frequencies. Logistic haplotype trend regression was performed for each particular haplotype frequency comparison, and only those that differed significantly from controls are indicated as P < .05 (a) and P < .01 (b). Haplotype IDs were assigned using nomenclature previously proposed by Myers et al.18 Of all uncommon H1 subhaplotypes (frequencies < 5%), only the H1p variant is presented.

This study supports previous evidence that the MAPT H1 haplotype is associated with PD and furthers our understanding of its role in PDD. Our data augment the findings of recent studies indicating that the MAPT genotype has a clear effect on the development of dementia among PD patients.15 The mechanism linking MAPT variants and dementia in PD remains unknown. However, several lines of evidence support the biologic basis of this association. First, mutations in MAPT lead to frontotemporal dementia with parkinsonism linked to chromosome 17q21.31; second, atypical parkinsonian syndromes, such as progressive supranuclear palsy and corticobasal degeneration, both accompanied with a progressive cognitive decline leading to dementia, have been consistently associated with the MAPT H1 haplotype; third, tau and α-synuclein, the pathologic hallmark of PD, can interact and fibrillize synergistically in vitro; and fourth, tau and α-synuclein are known to colocalize in brains with Lewy body pathology.
To our knowledge, our study represents the first comprehensive analysis of MAPT subhaplotypes and their role in PD, PDD, and LBD. We found a striking protective effect between the H2a subhaplotype and the risk of PDD. Interestingly, when we investigated uncommon subhaplotypes, we found that up to 2% of PDD chromosomes had the H1p haplotype, whereas only 0.1% of control chromosomes carried this variant. This is pivotal because it refines the MAPT association and delineates the specific variant that could be related to the disease. There is a clinical and pathologic overlap between PDD and LBD, and current criteria use the timing of the onset of cognitive symptoms in relation to motor symptoms to differentiate the 2 entities: 1 year or less defines LBD and more than 1 year defines PDD.25,46 Therefore, the question arises as to whether PD, LBD, and PDD are different stages along the continuum of a single disorder with different genetic backgrounds. Our data suggest that LBD and PDD could be separate clinical entities with different genetic backgrounds. Our data suggest that LBD and PDD could be related to distinct genetic factors because we found no hints of association between MAPT and LBD. Although to our knowledge this is the first study to assess the role of MAPT in LBD risk, our negative results should be interpreted with caution in view of the low sample size.

We were unable to replicate previous associations between the H1c subhaplotype and the risk of AD.18 One explanation for these conflicting data may be related to differences in participants’ origin (the cohort in the study by Myers et al18 were from the United Kingdom and the United States). However, samples in both studies share European ancestry and haplotype frequencies (9% for the H1c haplotype in the Myers et al cohort and 7.4% in our cohort). It is therefore unlikely that the discrepancies between the 2 studies can be attributed to geographic factors.

One limitation of our study is that controls were not age- and sex-matched with our PD and LBD cohorts. However, in view of their age and good cognitive status, we presume our control cohort has genetic protective factors that prevented the development of neurodegenerative disorders. Another limitation is lack of definite neuropathologic diagnoses in most of our samples. However, because all patients were evaluated by neurologists highly specialized in movement disorders or dementia, a low rate of misdiagnosis could be expected.

Of the 6 variants analyzed in the present study, rs1467967 was the second most important genetic variant associated with PDD after the H1 polymorphism. Interestingly, it is located at the most 5’ region of the gene, relatively close to the transcription regulation region. Although it is unlikely that this association is due to a direct functional consequence of this SNP, the most plausible explanation would be that this polymorphism may be in linkage disequilibrium with another functional variant within the 5’ genomic region of the MAPT gene. Therefore, regardless of the exact mechanism underlying the association between this variant and PDD, genetic variability in the expression of tau protein likely increases the risk of developing dementia in PD. Determining precisely which element of the MAPT locus is responsible for the association will be difficult because of the strong and extensive linkage disequilibrium across this genomic region.

The present analyses also replicate previous clinical data that reported higher age and more severe symptoms to be major risk factors for the development of dementia related to PD.47

In summary, our data confirm that the MAPT H1 haplotype plays a significant role in PD patients’ predisposition to developing dementia and strongly suggest that the rs1467967 variant and the H1p subhaplotype could contribute to PDD. This genetic finding, combined with appropriate clinical assessments such as specific neuropsychologic tests,48 may have implications in the early identification of subgroups of PD patients with a high risk of dementia. This knowledge would therefore be an invaluable help in the selection of patients who could benefit from future therapeutic trials aimed at improving or slowing the progression of cognitive impairment in PD.

Accepted for Publication: July 1, 2010.

Author Affiliations: Memory Unit (Ms Setó-Salvia, Drs Clarimón, Marquié, Alcolea, Suárez-Calvet, Molina-Porcel, Gómez-Isla, Blesa, Lleó, and Kulisevsky, and Mr Dols) and Movement Disorders Unit (Drs Pagonabarraga, Pascual-Sedano, and Martínez-Corrál, Ms Campolongo, and Mr Regaña), Neurology Department, Hospital de Sant Pau, Universitat Autònoma de Barcelona, Barcelona; Grupo de Autoecología Humana del Cuaternario, Universitat Rovira i Virgili-IPHES, Tarragona (Ms Setó-Salvia); Center for Networker Biomedical Research in Neurodegenerative Diseases (Drs Clarimón, Pagonabarraga, Pascual-Sedano, Combarros, Mateo, Martínez-Corrál, Gómez-Isla, Blesa, Lleó, and Kulisevsky, Ms Campolongo, and Mr Regaña) and Neurology Department, Hospital Universitario Marqués de Valdecillas, Santander (Drs Combarros and Mateo), Spain.

Correspondence: Jaime Kulisevsky, MD, PhD, and Jordi Clarimón, PhD, Neurology Department, Hospital de Sant Pau, Sant Antoni M. Claret 167, 08025 Barcelona, Spain (jkulisevsky@ santpau.cat and jclarimon@santpau.cat).

Author Contributions: Drs Clarimón and Kulisevsky had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Clarimón, Molina-Porcel, and Kulisevsky. Acquisition of data: Setó-Salvia, Pascual-Sedano, Campolongo, Combarros, Mateo, Regaña, Martínez-Corrál, Marquié, Alcolea, Suárez-Calvet, Dols, Gómez-Isla, Blesa, and Kulisevsky. Analysis and interpretation of data: Pagonabarraga, Combarros, Blesa, Lleó, and Kulisevsky. Drafting of the manuscript: Setó-Salvia, Clarimón, Pagonabarraga, and Lleó. Critical revision of the manuscript for important intellectual content: Pascual-Sedano, Campolongo, Combarros, Mateo, Regaña, Martínez-Corrál, Marquié, Alcolea, Suárez-Calvet, Molina-Porcel, Dols, Gómez-Isla, Blesa, and Kulisevsky. Statistical analysis: Clarimón. Obtained funding: Clarimón and Kulisevsky. Administrative, technical, and material support: Setó-Salvia, Pascual-Sedano, Campolongo, Mateo, Alcolea, Suárez-Calvet, Molina-Porcel, and Gómez-Isla. Study supervision: Clarimón, Pagonabarraga, Combarros, Martínez-Corrál, Blesa, and Kulisevsky.

Financial Disclosure: None reported.
Disclaimer: Ms Seto-Salvia and Dr Clarimón contributed equally to this work.

Additional Contributions: We are indebted to all patients for their participation. This work was supported by the Center for Networker Biomedical Research in Neurodegenerative Diseases and by grant P100/00098 from Fondo de Investigaciones Sanitarias. We thank Ellen Gelpi, MD, PhD, from the Neurological Tissue Bank, Hospital Clinic de Barcelona at the University of Barcelona, for helpful suggestions, and Kenneth Kidd, PhD, and Peter Donnelly, PhD, for their help with data interpretation.

REFERENCES