Neurodegenerative diseases of Guam: Analysis of TAU

Article abstract—Mutations in the tau gene have been described in families affected by frontotemporal dementia with parkinsonism linked to chromosome 17. The authors performed a genetic and biochemical analysis of this gene and its product in the parkinsonism dementia complex of Guam, a disorder characterized by the extensive formation of neurofibrillary tangles. The tau gene is not a primary cause of the parkinsonism dementia complex of Guam.

NEUROLOGY 1999;53:411–413

J. Pérez-Tur, PhD; L. Buée, PhD; H.R. Morris, MRCP; S.C. Waring, DVM, PhD; L. Onstead, BSc; F. Wavrant-De Vriée, BSc; R. Crook, BSc; V. Buée-Scherrer, PhD; P.R. Hof, MD; R.C. Petersen, PhD, MD; P.L. McGeer, MD, PhD; A. Delacourte, PhD; M. Hutton, PhD; T. Siddique, MD; J.E. Ahlskog, PhD, MD; J. Hardy, PhD; and J.C. Steele, MD

ALS and parkinsonism dementia complex of Guam (PDC) are highly prevalent syndromes among the indigenous Chamorro people of the island. A primary genetic basis remains a compelling explanation for Guamanian neurodegenerative disease. Nonetheless, the expression of the disease could be modulated by the presence of other, unknown environmental factors.

Pathologic examination of brains of patients with PDC shows widespread neurofibrillary tangles throughout the brain.1 These structures are mainly composed of filamentous aggregates of the microtubule-associated protein tau in a hyperphosphorylated state, and resemble the equivalent structures found in AD brains, referred to as paired helical filaments, with some differences in their distribution.1 The electrophoretic profile of pathologic tau proteins is similar to that of AD, but different from progressive supranuclear palsy (PSP), with which it shares some clinical features and overlaps in the distribution of the lesions. In PDC and AD, the tangles are composed of a triplet of hyperphosphorylated tau proteins, as seen on Western blots,1 with the same sites being hyperphosphorylated and identical helical structure of the filaments.2 On the contrary, PSP tangles show only two bands on Western blots and the filaments lack the periodicity observed in AD; instead, they are “straight” filaments.1,2

We are searching for genetic variability that could account for the high prevalence of PDC in this ethnic group. During this search, mutations in the tau gene were shown to be associated with the development of frontotemporal dementia with parkinsonism linked to chromosome 17.3-5 The clinical manifestations of this disorder are heterogeneous but usually include extrapyramidal signs similar to those observed in PDC. The fact that the pathologic manifestations of PDC are limited to the presence of tangles makes tau a strong candidate gene for PDC, assuming the disease has a genetic origin.

Methods. For our genome search, 23 unrelated people with PDC (positive family history for PDC or ALS, mean age at onset 65 ± 8 years [range, 48 to 76 years]; 12 women) were identified. In 10 subjects, the diagnosis of PDC was pathologically confirmed in the proband (n = 2) or in a family member (n = 8). PDC was diagnosed on the basis of parkinsonism and dementia. Because the disease might appear with reduced penetrance, the control group consisted of 19 unaffected people with no known family history of PDC or ALS (mean age, 75 ± 6 years [range, 65 to 88 years]; 14 women). The control group was older than the case group to avoid including people in whom the disease could develop. As part of a separate clinical study, a second group of patients with PDC or ALS was available. This group has been previously described.6 All participants in this study are of Chamorro ethnicity. Sequencing of tau was performed on five participants: two with PDC (a 56-year-old woman and a 69-year-old man), one with ALS (a 56-year-old man), and two normal control subjects (a 65-year-old man and an 84-year-old woman) chosen at random among the available subjects.

The association study was performed on the “genome search” group. For immunoblotting experiments, additional study samples were obtained from people with AD and PDC, as described previously.1 DNA was isolated from lymphoblastoid cell lines using standard procedures. Tissue was obtained from primary motor cortex (Brodmann area 4), frontal cortex (Brodmann area 9), and temporal cortex (Brodmann area 20).

Sequencing of the coding exons of the tau gene was performed on both strands from genomic DNA amplified using the primers and conditions described previously. This included only those exons expressed in the brain (1 to

Copyright © 1999 by the American Academy of Neurology
The markers used for the association study and the distances between them are shown in the table. The dinucleotide repeat associated with PSP7 was also included in the study. Statistical analysis of the association data was performed using the program Dislamb.8

Electrophoresis, immunoblotting, and antibody incubation were performed as previously described.9 Briefly, homogenized brain samples (50 mg total protein for AD and 100 mg for PDC) were resolved on a 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, transferred onto nitrocellulose, and incubated with the different antibodies.

Discussion. PDC involves extensive deposition of tau, and therefore we undertook a genetic study of the tau locus in this disorder. No abnormalities in the sequence of the gene were observed. Because direct sequencing of coding exons could miss mutations that alter regulation of protein expression as well as small deletions involving this gene, we performed an association study using markers spanning approximately 30 cM in the chromosomal region where the gene is located (see table). The results of the association study do not point to this locus as the primary cause of PDC. This is consistent with the absence of association observed, by us and others, between PDC and the intronic tau polymorphism associated with PSP.7 This lack of association with tau can be explained in several ways. It is possible that the disease is not genetic but due to an unknown environmental cause. Conversely, the power of the sample may not be great enough to allow the detection of such an association, although this does not seem to be the case (an analysis of the power of this sample can be found in http://www.mayo.edu/papersjax/perez-tur/powerguam/powerguam.htm). It is also possible that tau is not the locus implicated in the etiopathogenesis of PDC, and that another locus is responsible for the disease.

Studies have shown that the neurofibrillary tangles in several neurodegenerative diseases have different biochemical signatures, and that these qualitative differences can be observed using specific antibodies.1,2,9,10 Differences in electrophoretic mobility are due to the aggregation of particular tau isoforms into filaments, modified according to the isoform concerned and the degree of protein phosphorylation.9 Brain tau constitutes a family of six isoforms resulting from alternative splicing. It is possible that diseases with different origins show common or overlapping clinical features because of the set of neurons affected by the disease process, and that the molecular pathologic processes reflect the different pathways that come into play during the course of the disease.

We have characterized the biochemical profile of the tau isoforms involved in tangle formation. Previous reports showed that AD tangles and PDC tangles shared the same profile when probed with antibodies recognizing hyperphosphorylated tau. In addition, the antibodies are described in Mailliot et al.9

<table>
<thead>
<tr>
<th>Marker</th>
<th>Distance, cM</th>
<th>LRT*</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D17S1294</td>
<td>0.000</td>
<td>11.27</td>
<td>0.500</td>
</tr>
<tr>
<td>D17S1299</td>
<td>0.000</td>
<td>1.15</td>
<td>0.139</td>
</tr>
<tr>
<td>D17S579</td>
<td>0.000</td>
<td>0.46</td>
<td>0.500</td>
</tr>
<tr>
<td>D17S1804†</td>
<td>0.000</td>
<td>0.54</td>
<td>0.500</td>
</tr>
<tr>
<td>D17S791</td>
<td>0.000</td>
<td>2.69</td>
<td>0.500</td>
</tr>
<tr>
<td>D17S2180</td>
<td>0.000</td>
<td>8.14</td>
<td>0.500</td>
</tr>
<tr>
<td>D17S787</td>
<td>0.000</td>
<td>7.01</td>
<td>0.500</td>
</tr>
<tr>
<td>D17S1290</td>
<td>0.000</td>
<td>0.000</td>
<td>0.500</td>
</tr>
</tbody>
</table>

The distance between markers was obtained from the Marshfield Center for Medical Genetics (http://www.marshmed.org/genetics/).

* Likelihood ratio test, a measure of the degree of association.
† Closest marker to tau.
electron microscopy studies showed that the ultrastructure of the tangle filaments in PDC was identical to that of AD. More recent reports, however, suggest the existence of some “disease-specific” sets of tau isoforms that become hyperphosphorylated and are deposited into tangles in different neurodegenerative diseases. We wished to identify which isoforms are involved in tangle formation in PDC, to accomplish this, we compared the electrophoretic pattern observed when probing brain extract from cases of PDC and AD using newly developed antibodies directed against exons that are subjected to alternative splicing. The figure shows the results of such an analysis. All six tau isoforms are involved in tangle formation in PDC, in a pattern identical to that in AD.

Our data do not support the involvement of tau as a primary cause for the disease, although they do not rule out the possibility of tau being important downstream in the process. We remain focused on genetic factors as playing a major etiologic role in Guamanian neurodegenerative disease.

Acknowledgment

The authors thank the patients and their families for their support, and Dr. D. Perl for neuropathologic evaluation of the cases. AD2 was developed through a collaboration between UMR 9921 CNRS, Sandofi/Diagnostic Pasteur and INSERM.

Regional cerebral blood flow difference between dementia with Lewy bodies and AD

Dementia with Lewy bodies (DLB) is an increasingly recognized form of dementia in elderly people. In 1996, the Consortium on DLB International Workshop (CDLBIW) proposed criteria for clinical and pathologic diagnosis. Studies have delineated neuroimaging features that distinguish DLB from AD. Features of DLB that contrast with those of AD include relatively well preserved medial temporal glucose metabolism and profound occipital hypometabolism on $[^{18}F]$-2-fluoro-deoxy-D-glucose (FDG) PET. In a preliminary study by Donnemiller et al., occipital hypoperfusion resembling a horseshoe defect was noted in patients with DLB on visual inspection of regional cerebral blood flow (CBF) images by SPECT and different radionucloids. However, most SPECT studies have not demonstrated features of regional CBF pattern that distinguish DLB from AD.

The aim of this study was to delineate the features of regional cerebral blood flow of DLB with N-isopropyl-$[^{123}I]$iodoamphetamine (IMP) and SPECT.

References


K. Ishii, MD; S. Yamaji, MD; H. Kitagaki, MD; T. Imamura, MD; N. Hirono, MD; and E. Mori, MD

Article abstract—The authors studied 14 patients with dementia with Lewy bodies (DBL), 14 patients with AD, and 14 healthy control subjects with N-isopropyl-$[^{123}I]$iodoamphetamine SPECT. Comparison with the statistical parametric mappings revealed that relative cerebral blood flow was lower in the occipital lobes and higher in the right medial temporal lobe in the DLB group than in the AD group. Decreased occipital perfusion and relatively well preserved medial temporal perfusion are features that distinguish DLB from AD.