

# Neurofibrillary Degeneration in Amyotrophic Lateral Sclerosis/Parkinsonism-Dementia Complex of Guam

## *Immunochemical Characterization of Tau Proteins*

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**Neurofibrillary tangles are observed in several neurodegenerative disorders including Alzheimer's disease, progressive supranuclear palsy, and amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam. The major components of neurofibrillary tangles are hyperphosphorylated tau proteins that can be directly detected in brain homogenates, using immunoblotting with specific immunological probes. To investigate whether tau proteins differ biochemically among various neurodegenerative disorders, we analyzed a series of brain samples from Guamanian patients in comparison with Alzheimer's disease, progressive supranuclear palsy, and normal aging. In Alzheimer's disease, these hyperphosphorylated tau proteins are composed of a triplet referred to as tau 55, 64, and 69, whereas in progressive supranuclear palsy, neurofibrillary degeneration is characterized by a tau doublet (tau 64 and 69). In the present study, characterization of tau proteins was performed by immunoblotting, on different cortical and subcortical regions of postmortem brain specimens from Guamanian natives. In all of the cases, biochemical data were always consistent with neuropathological findings. In contrast to Alzheimer's disease patients where the tau triplet is**

**found mostly in cortical regions, a similar triplet was strongly detected in both cortical and subcortical areas in Guamanian patients. The tau profile differed quantitatively from case to case demonstrating that the Alzheimer's disease-related tau triplet had a heterogeneous regional distribution. These data suggest that the tau triplet found in amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam is similar to that observed in Alzheimer's disease, and the regional distribution of tau proteins differs in these disorders. (Am J Pathol 1995, 68:924-932)**

Amyotrophic lateral sclerosis/parkinsonism-dementia complex (ALS/PDC) is a chronic neurodegenerative disorder highly prevalent in the native Chamorro population of Guam in the Western Pacific ocean.<sup>1-3</sup> The etiopathogenesis of this disorder has not yet been elucidated, although it has been hypothesized that environmental factors such as aluminum or neurotoxins might be involved.<sup>4</sup> Clinically, Guamanian amyotrophic lateral sclerosis (ALS) is undistinguishable from sporadic ALS and presents with fasciculations and lower and upper motor neuron signs. Parkinsonism-dementia (PD) is characterized by an insidious progressive mental decline and extrapyramidal signs.<sup>3,5</sup> Both aspects of the disease are frequently associated, but they are known to occur separately.<sup>6</sup>

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Neuropathologically, Guamanian ALS/PDC is characterized by a severe cortical atrophy and neuronal loss. The neuropathological hallmark of ALS/PDC is the widespread formation of neurofibrillary tangles (NFTs), especially in the neocortex and hippocampal formation.<sup>3,7-9</sup> Although NFTs are present in large numbers in both Alzheimer's disease (AD) and ALS/PDC, these two conditions are distinguished by differential NFT laminar distribution patterns and densities in neocortex. NFTs are preferentially distributed within layers II and III in the neocortical areas of Guamanian ALS/PDC patients and are relatively sparser in layers V and VI, whereas NFTs are denser in layers V and VI than layers II and III in AD cases.<sup>10,11</sup> Immunohistochemical studies have also revealed that several cytoskeletal proteins (neurofilaments, microtubule-associated protein 2, tau, and ubiquitin) are present in NFTs of ALS/PDC patients.<sup>12</sup> Ultrastructurally, NFTs in Guamanian ALS/PDC consist of straight and paired helical filaments (PHF),<sup>13</sup> which have been shown to be essentially similar to those observed in AD.<sup>8</sup>

The basic component of NFT can be directly detected and quantified in total brain homogenates from different areas, using Western blotting and specific immunological probes. Using this approach, several investigators have detected a triplet of hyperphosphorylated tau proteins, referred to as tau 55, 64, and 69, A68 or tau-PHF in the cerebral cortex of AD patients.<sup>14-16</sup> Similarly, the pathological tau triplet has been found within the hippocampal formation of nondemented elderly people,<sup>17</sup> which contains NFTs.<sup>18</sup> It has also been demonstrated that tau proteins are present in NFTs in progressive supranuclear palsy (PSP) and are composed of a tau protein doublet known as tau 64 and 69.<sup>19,20</sup>

In the present study, the biochemical characteristics of tau proteins were analyzed in different cortical and subcortical regions of brains from Guamanian natives, using specific immunological probes against pathological tau proteins, to investigate the possible differences in the profile of tau proteins between ALS/PDC and related dementing disorders.

### **Materials and Methods**

The brains from eight demented Guamanian ALS/PDC patients (G1, G2, G6 to G11), six Guamanian ALS/PDC patients with mild dementia (G3, G4, G12 to G15), two patients with Guamanian ALS (G16, G17), and two Chamorro patients with no clinical sign of ALS/PDC (G5, G18) were used in the present study (43 to 101 years of age). In addition, the brains from

four Caucasian patients (one young Caucasian control (C1), one elderly control patient, (C2), one AD patient, and one PSP patient) were included for comparison with the Guamanian patients (Table 1). The clinical histories of the Guamanian cases were obtained from the medical records and neuropathological evaluation (including data from Bielschowsky's staining), of the Marianas Health Study, the Guam Memorial Hospital, and the Division of Neuropathology, Mount Sinai Medical Center, New York. The brains of the Caucasian patients used in this study were obtained from the Department of Neurology, Lille Regional Hospital B, France. In five Guamanian cases (G1 to G5), the basal ganglia, amygdala, hippocampal formation, thalamus; and Brodmann areas 4 (primary motor cortex), 9 and 11 (superior frontal and orbitofrontal cortex), 17/18 (primary and secondary visual cortex), 20 (inferior temporal cortex), and 24 (anterior cingulate cortex) were analyzed. For all of the other cases, only area 9 and basal ganglia (putamen) were available. These regions were sampled from fresh-frozen whole hemispheres that were kept at -80 C. Similar materials were obtained from the Caucasian cases. The other hemisphere was fixed by immersion in cold 4% paraformaldehyde for immunohistochemical studies (cases G1 to G5) or in 10% formalin (cases G6 to G18).

For characterization of tau proteins, a polyclonal antibody raised against PHF isolated from AD brains was used at a dilution of 1:1000. This antibody (anti-PHF) labels normal and pathological tau proteins.<sup>21,22</sup> To label only pathological tau proteins, a specific antibody was prepared by an overnight incubation of the anti-PHF with normal tau proteins. Unbound antibodies to normal tau proteins were shown to label only pathological tau proteins and referred to as abs. anti-PHF.<sup>15</sup> Other immunological probes were also used to ascertain this characterization, including monoclonal antibodies directed against phosphorylation-dependent epitopes, AD2<sup>23</sup>, AT8 (Innogenetics, Gent, Belgium),<sup>24</sup> SMI34 (Sternberger Monoclonals, Baltimore, MD)<sup>25,26</sup> and Tau-1 (Boehringer, Mannheim, Mannheim, Germany),<sup>27</sup> and polyclonal antibodies (M19G, S28T) directed against the amino- and carboxy-terminal regions respectively of tau proteins.<sup>28,29</sup> A tau-PHF preparation<sup>30</sup> was used as the immunogen for the production of monoclonal antibodies including AD2. AD2 is a phosphatase-sensitive monoclonal antibody, directed against a phosphorylated epitope located in the carboxy-terminal domain, and that specifically recognizes NFTs, PHF, and the abnormal tau triplet in total AD brain homogenates<sup>23</sup> (V. Buée-Scherrer, submitted).

Table 1.

Case	Age (years old)	PMI (hours)	Clinical status	Neocortical NFT	Subcortical NFT	SP
G1	75	4	ALS/PDC*	+	+	+
G2	76	3	ALS/PDC*	+	+	-
G3	76	4	ALS/PDC†	+	+	-
G4	78	5	ALS/PDC†	+	+	-
G5	101	5	Control‡	-	-	+
G6	72	7	ALS/PDC*	+	+	-
G7	66	10	ALS/PDC*	+	+	-
G8	60	45	ALS/PDC*	+	+	-
G9	72	3	ALS/PDC*	+	+	+
G10	68	19	ALS/PDC*	+	+	-
G11	53	5	ALS/PDC*	+	+	-
G12	52	4	ALS/PDC†	+	+	+
G13	70	18	ALS/PDC†	+	+	-
G14	66	?	ALS/PDC†	+	+	-
G15	68	?	ALS/PDC†	+	+	-
G16	52	3	ALS§	+	+	-
G17	?	?	ALS§	+	+	-
G18	43	16	Control‡	-	-	-
PSP	71	10	PSP	+	+	-
AD	70	6	AD	+	-	+
C1	34	16	Control	-	-	-
C2	83	4	Control	-	-	-

\* Severely demented Chamorro patients presenting with ALS/PDC.

† Chamorro patients presenting with ALS/PDC and mild dementia.

‡ Chamorro control cases.

§ Chamorro patients presenting with predominant ALS symptomatology.

|| Caucasian cases. NFT and senile plaques (SP) after neuropathological examination were indicated by either presence (+) or absence (-). G1 to G18 are Chamorro cases. AD is a typical AD patient. PSP is a typical PSP patient. C1 and C2 are young and elderly Caucasian control cases respectively. Postmortem intervals (PMI) are indicated in hours. Some data about age and/or PMI were not always available and referred to as (?).

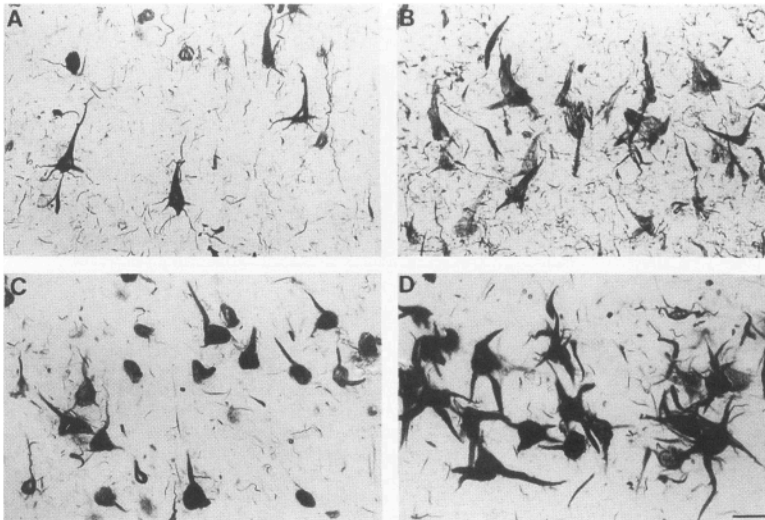
Brain tissues were homogenized in Laemmli sample buffer 1:10 (w/v) containing 5% sodium dodecyl sulfate (SDS) and 0.25% dithiothreitol, and heat treated.<sup>31</sup> For immunoblot, total brain homogenates were loaded and 150 µg total proteins were resolved on 10 to 20% polyacrylamide gel gradients in presence of SDS and transferred onto nitrocellulose sheets. After transfer, membranes were saturated with 5% skim milk. Primary antibody was then added. After a 2-hour incubation, the primary antibody was detected with a peroxidase conjugated antibody (Diagnostics Pasteur, Marnes la Coquette, France). Finally, peroxidase activity was visualized on Hyperfilm using the Enhanced Chemiluminescence Kit (Amersham France, Les Ulis, France).

For immunohistochemical purposes, blocks from the hemisphere fixed in cold 4% paraformaldehyde, corresponding topographically to the frozen samples from the five Guamanian brains (G1 to G5), were post-fixed as required.<sup>32,33</sup> The specimens were then washed in a series of graded sucrose solutions (12%, 16%, and 18%), frozen and sectioned at 40 µ on a cryostat. Sections were incubated at 4 C with either the same polyclonal antibody against PHF (dilution 1:800) described above or the purified monoclonal antibody AD2 (dilution: 0.6 µg/ml). After incubation, the sections were processed with an avidin-biotin kit (Vector Laboratories, Burlingame, CA), incubated in

diaminobenzidine, and intensified in 0.005% osmium tetroxide.<sup>32</sup> Similar protocol and use of Aβ polyclonal antibody to perform Aβ immunohistochemistry were previously described.<sup>33,34</sup> Neuropathological data on the remaining Guamanian cases were obtained from their medical records.

## Results

The distribution of pathological changes within cortical and subcortical regions in the Chamorro cases reported in the present study was compatible with previous analyses of Guamanian ALS/PDC.<sup>8,10,11,34,35</sup> As indicated in the neuropathological examination (Table 1), all of these patients were devoid of senile plaques, except three PD cases (G1, G9, G12) including two severely demented patients, and the oldest Chamorro patient (G5, 101-year-old). Cases G1 to G5 were extensively studied for Aβ immunohistochemistry, because they were aged. G1 and G5 displayed diffuse deposits in most of the cortical areas (data not shown). Cases G2 to G4 were devoid of amyloid deposition including preamyloid deposits. NFTs were observed in most of the cortical regions analyzed (Figure 1, A and B). In the neocortex, the temporal regions were generally more severely affected (Figure 1A) than were the frontal, parietal, and occipital regions, and NFTs displayed the



**Figure 1.** Comparison of NFTs between case G1 (A, B) and a typical AD patient (C, D) in layer V of area 20 (A, C), and entorhinal cortex (B, D). Note the intense staining of pyramidal neurons in both areas. NFTs in both ALS/PDC and AD cases were stained with the monoclonal antibody AD2. Scale bar = 25  $\mu$ .

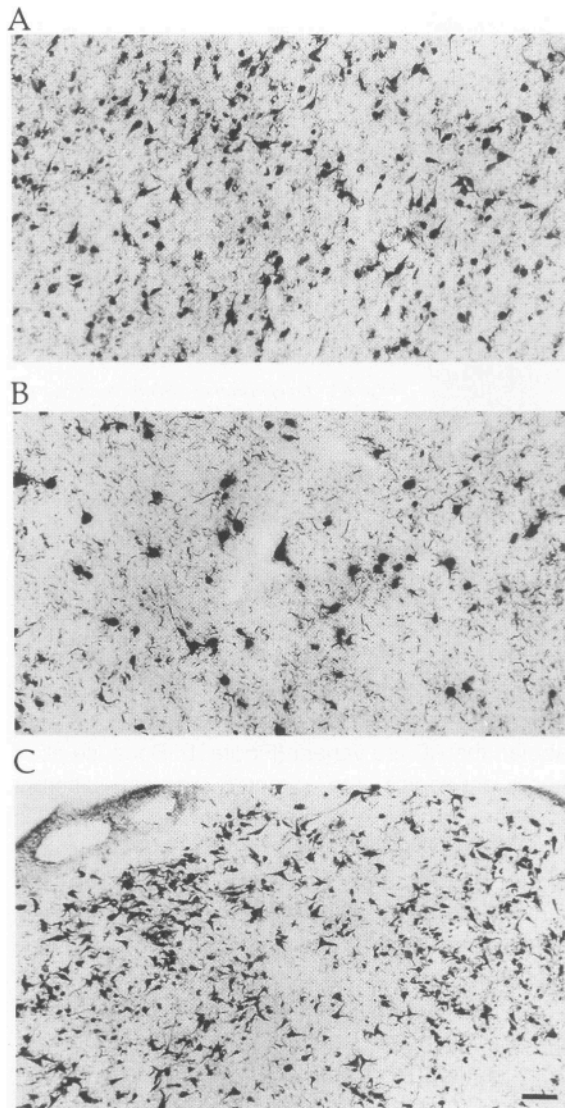
laminar distribution typical of Guamanian ALS/PDC with higher densities in supragranular layers than in infragranular layers.<sup>10,11,34</sup> It should be noted that the AD2 monoclonal antibody exhibited similar NFT-immunoreactivity in both ALS/PDC and AD (Figure 1, A to D). Subcortical structures such as amygdala, thalamus, basal ganglia, and substantia nigra were also consistently affected in all of the Guamanian ALS/PDC cases. In particular, the basolateral nucleus of the amygdala, the nucleus reuniens of the thalamus, and the caudate nucleus showed in certain cases very high NFT densities (Figure 2, A to C). It was noted that similar structures were labeled by both antibodies (AD2 and anti-PHF). To investigate further the similarities between NFTs in AD and ALS/PDC, we analyzed by immunoblotting tau proteins and some of their state of phosphorylation in both disorders using several immunological probes.

For biochemical analyses, areas 9 and/or 20 from a Caucasian AD patient were used as an internal standard to visualize the pathological tau triplet on each blot. With different monoclonal antibodies directed against phosphatase-sensitive epitopes, all of the affected regions from Guamanian ALS/PDC showed the hyperphosphorylated AD tau triplet at the same molecular weight as in the AD case in both cortical and subcortical structures, area 9 and basal ganglia respectively, whereas the PSP case showed the typical tau doublet (Figure 3). It is interesting to note that the tau triplet was always present in patients exhibiting NFTs, even if postmortem interval (PMI) was very long. For instance, the tau triplet was found in brain homogenates from cases G13 (PMI = 18 hours) (Figure 3), and G8 (PMI = 45 hours, data not shown). Similar data were obtained with the abs. anti-PHF. The tau triplet-immunoreactivity was intense in AD and

Guamanian ALS/PDC brain homogenates using AD2 monoclonal antibody (Figure 4). Other phosphorylation-dependent monoclonal antibodies, including AT8 and SMI34, also labeled the tau triplet (Figure 4). In contrast to these antibodies, Tau-1 never labeled the AD tau variant (Figure 4). Because all of these antibodies are directed against different epitopes, hyperphosphorylation of tau proteins is likely to be similar in both disorders. Polyclonal antibodies M19G and S28T directed against amino- and carboxy-terminal regions of tau proteins respectively labeled both normal and pathological tau proteins (Figure 4).

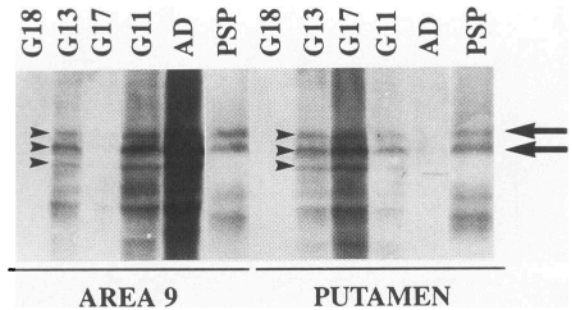
Extensive regional brain sampling was available in only five Guamanian cases (G1 to G5). All of the regions studied biochemically from two severely demented Guamanian patients (G1, G2) exhibited the tau triplet at the same molecular weight as in the Caucasian AD case, except in areas 17/18 (Figure 5A). The amygdala and hippocampal formation demonstrated the strongest labeling, followed by area 20. Areas 9 and 11, as well as area 4, were less affected than area 20 as reflected by the weaker pathological tau triplet staining. The tau triplet was also present in subcortical areas such as basal ganglia and thalamus. The presence of tau-PHF in nearly all of the cerebral regions indicates that the brains of these patients were severely affected by the degenerating process. These biochemical data were correlated with the neuropathological findings, since the two cases displayed high densities of NFT in the hippocampal formation, and areas 9, 11, and 20 as well as in subcortical structures (Figures 1 and 2).

There was a strong immunolabeling of tau 55, 64, and 69 in the hippocampal formation, amygdala, and area 20 of the Guamanian case (G3). The pathologi-



**Figure 2.** Presence of NFTs in amygdala (A) nucleus reuniens of the thalamus (B) and caudate nucleus (C) in case G1. Note that these subcortical structures are also severely affected by the degenerating process. Materials were stained with the anti-PHF antibody. Scale bar = 100  $\mu$ .

cal tau triplet was present, but with a much lower concentration in the primary motor area (area 4), area 9, and basal ganglia (Figure 5B). In contrast to cases G1 and G2, the tau triplet was not observed in area 11, and in the thalamus. These results indicated that in this case, the cerebral cortex was differently affected by the neurodegenerative process. These biochemical data were well correlated with neuropathological findings that showed that this patient was much less severely affected than patients in cases G1 and G2. In case G3, NFTs were mainly observed in the hippocampal formation and temporal areas, and to a lesser degree in orbitofrontal cortex and basal gan-



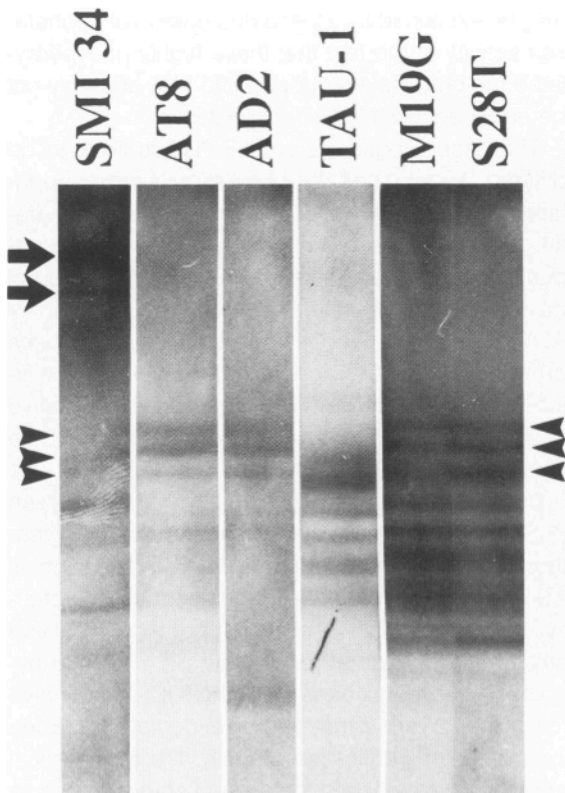
**Figure 3.** Immunoblotting of brain homogenates (frontal cortex (area 9) and basal ganglia (putamen)) from four Chamorro cases (G18, G13, G17, G11) and one typical AD and one PSP case. Note the presence of the abnormal tau triplet in all ALS/PDC cases, whereas no staining is observed in the Chamorro control case G18. Differences in the intensity of the tau triplet immunoreactivity are related to the severity of the degenerative process. For instance typical Guamanian ALS/PDC (G13) exhibits the tau triplet with the same immunoreactivity in both cortical and subcortical structures. Case G17 with a predominant ALS symptomatology shows a more intense tau triplet in putamen than in area 9. Conversely, in a Guamanian ALS/PDC with severe dementia (G11) the labeling of the tau triplet is denser in cortical area (area 9) than in subcortical region (putamen). Blots were stained with the monoclonal antibody AD2.

glia. No or very few NFTs were seen in frontal and motor areas, and thalamus.

Two cases (G4 and G5) had the abnormal tau triplet only in the amygdala and hippocampal formation (Figure 5C). The tau triplet immunoreactivity was more intense in case G4 than G5, and the labeling was denser in the hippocampal formation than in the amygdala. Tau-PHF were not detected in the other regions investigated in these cases. Interestingly, case G4 was clinically considered to be an early PD case, and the biochemical and neuropathological data showed that NFTs were restricted to the hippocampal formation and amygdala. In addition, the distribution of the abnormal tau triplet observed in case G5 was comparable to that of elderly Caucasian control patients.<sup>17</sup> These biochemical results were in good agreement with the neuropathological data, which showed a small number of NFTs restricted to the hippocampal formation in this case.

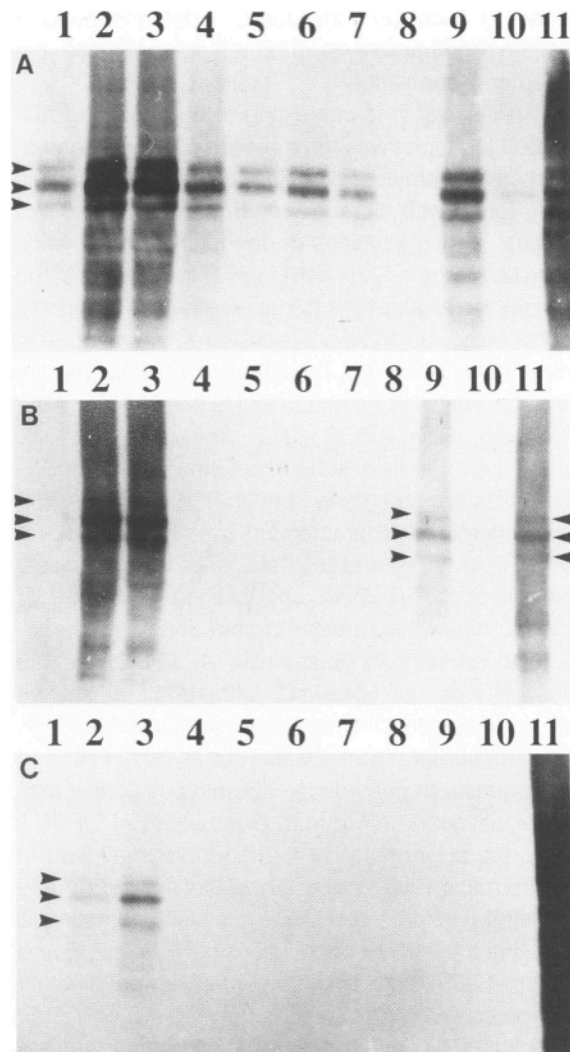
## Discussion

The results of this study reveal that pathological tau proteins are consistently found in Guamanian ALS/PDC. Because the same amount of total brain homogenates was used for each sample, it is possible to compare the tau-PHF immunoreactivity in all of the cases. Moreover, comparable PMIs were found for individuals from each patient's group. Thus, similarities and differences between tau-PHF obtained from patients with ALS/PDC, AD, and PSP do not reflect artifacts due to the PMI in the different groups of patients. The tau profile differs quantitatively among



**Figure 4.** Immunoblotting of amygdala from case G2 using different antibodies. The triplet (arrowheads) is detected with all phosphatase-sensitive antibodies (AD2, AT8 and SMI34). SMI 34 also labels high and medium molecular weight neurofilament proteins (arrows). Using polyclonal antibodies against amino-terminal (M19G) and carboxy-terminal (S28T) part of tau proteins both normal and pathological tau proteins were revealed. Using Tau-1 antibody the tau triplet was not immunodetected while normal tau proteins were found.

Guamanian cases showing that this disorder is likely to be biochemically more heterogeneous than AD. For instance, the five cases extensively analyzed in the present study exhibit three distinct tau protein profiles depending on their regional distribution. First, the abnormal tau triplet was found in all of the brain regions (in both cortical and subcortical regions) in contrast to AD patients where the triplet is mainly located in the hippocampal formation and neocortical association areas.<sup>36-38</sup> Second, in a patient with rapidly advancing ALS/PDC for 1 year before death, degenerative changes occurred first in limbic and inferior temporal cortex, as observed in AD.<sup>38</sup> In addition, subcortical nuclei were also differently affected in this case, with a relative sparing of the thalamic nuclei, whereas basal ganglia were already severely affected by the neurodegenerative process. Third, one nonaffected elderly Guamanian patient and one patient with early signs of PD displayed the same tau-PHF distribution restricted to the hippocampal formation and amygdala as observed in nondemented



**Figure 5.** (A) Immunoblotting of brain homogenates from case G1 (Guamanian PD case with no sign of ALS) with the abs. anti-PHF antibody: area 20 from the Caucasian AD case was loaded on the same gel and was used as an internal standard to visualize the abnormal tau triplet (lane 11). All of the regions biochemically studied from case G1 contain the abnormal tau triplet (arrowheads) except area 17/18 (lane 8). Case G2 yielded similar results. Amygdala (lane 2) and hippocampal formation (lane 3) demonstrate the strongest labeling followed by area 20 (lane 9), indicating that this association area is affected later than the hippocampus and amygdala. Note that areas 4, 9, and 11 (lanes 5, 6, and 7 respectively) are less affected than area 20, which showed a weaker tau triplet staining. The abnormal tau triplet is also present in the subcortical areas such as basal ganglia (lane 1) and thalamus (lane 4). (B) Immunoblotting of brain homogenates from case G3 (ALS/PDC patient of Guam) with the abs. anti-PHF antibody. Tau 55, 64, and 69 are strongly immunoreactive in the hippocampal formation (lane 3), amygdala (lane 2), and area 20 (lane 9). Note the presence of the abnormal tau triplet with a much lower immunoreactivity than in cases G1 and G2 in areas 4 and 9 and also in basal ganglia (lanes 5, 6, and 1 respectively). The abnormal tau triplet is not found in areas 17/18 (lane 8) and in other remaining association areas such as area 11 (lane 7) and in thalamus (lane 4). (C) Immunoblotting of brain homogenates from cases G4 and G5 with the abs. anti-PHF antibody. These two patients display a distribution of the abnormal tau triplet (arrowheads) restricted to the amygdala (lane 2) and hippocampal formation (lane 3). The intensity of the labeling is stronger in the hippocampal formation than in the amygdala suggesting that the neurofibrillary degeneration affects the hippocampal formation earlier than the amygdala. Similar results were obtained with the monoclonal antibody AD2. Lane 10: area 24.

elderly Caucasian individuals,<sup>17</sup> demonstrating a pattern comparable to "normal aging" within the Chamorro population.

Qualitatively, it is interesting to note that the AD-related tau triplet was the main feature in all Guamanian cases studied. For instance, similar tau profiles were found with all monoclonal antibodies directed against phosphorylation-dependent epitopes such as AD2, AT8, and SMI34. In Guamanian ALS/PDC, the tau triplet was also strongly labeled by AD2 antibody. Conversely, Tau-1 did not recognize the tau triplet in either AD or ALS/PDC. Thus, the tau triplet seemed to have the same immunochemical features in both ALS/PDC and AD. ALS/PDC is also characterized by the presence of NFTs in subcortical structures. Subcortical NFTs are commonly seen in PSP. However, PSP is biochemically characterized by a tau doublet referred to as tau 64 and 69,<sup>19</sup> also seen in corticobasal degeneration<sup>39</sup> (L. Buée unpublished data). In PSP, this tau doublet is present in both cortical and subcortical areas.<sup>20</sup> In Guamanian ALS/PDC, the tau doublet was not observed. Ultrastructural studies also indicated that filaments within NFT in AD are PHF, whereas straight filaments are observed in PSP and corticobasal degeneration.<sup>13</sup> Both types of filaments are visualized in NFTs from Guamanian ALS/PDC.<sup>8</sup> Thus, the aggregation of the tau triplet may lead to PHF formation, whereas a tau doublet may aggregate into straight filaments. Finally, it is also interesting to note that the laminar distributions of NFTs in PSP and Guam ALS/PDC are similar, whereas they are different compared with AD cases.<sup>10,11,34,35,40</sup> AD and ALS/PDC share biochemical and ultrastructural features whereas PSP and ALS/PDC share laminar and regional NFT distribution. These data suggested that either the neuronal populations involved in these two disorders or the mechanisms involved in NFT formation are distinct.

Highly phosphorylated tau proteins similar to tau-PHF were recently found in rapidly processed human brain biopsies. They were shown to be AT8-immunoreactive.<sup>41</sup> These highly phosphorylated tau variants are not found in classical autopsy-derived materials, because a rapid dephosphorylation post-mortem (within two hours) occurs under the action of phosphatases 2A and 2B, leading to the formation of hypophosphorylated tau proteins, which are Tau-1-immunoreactive.<sup>41,42</sup> In the present study, we could not find these highly phosphorylated tau proteins, because they were likely to be dephosphorylated in control brain autopsic samples with PMI of >4 hours. However, it is interesting to note that tau-PHF are not fully dephosphorylated in autopsy-derived brain samples from ALS/PDC, AD, and PSP cases. This

may be explained by either a decrease in phosphatases activity or the fact that these highly phosphorylated tau proteins are trapped into PHF and may not be easily accessible to phosphatases.

The etiopathogenesis of ALS/PDC is likely to be different from that of AD, given that neuronal populations are differently affected by the neurodegenerative process in these two disorders and amyloid deposits are not usually observed in ALS/PDC. Although pathogenic mechanisms involved in Guamanian ALS/PDC have not yet been determined, it has been proposed that both high levels of aluminum in the island soil and water and the ingestion of a putative toxin from the plant *Cycas circinalis* might contribute to NFT formation.<sup>2,4,43-48</sup>

In conclusion, this study indicates that Guamanian ALS/PDC is a heterogeneous disease sharing features with other neurodegenerative disorders such as AD and PSP. Recent data suggested a neuroprotective role of the variant E2 of apolipoprotein E in AD and other neurodegenerative disorders.<sup>49-51</sup> If this hypothesis can be extended to ALS/PDC, a protective role of the E2 variant may be also expected<sup>51</sup> (L. Buée submitted for publication). Finally, the biochemical approach developed in the present study represents a reliable tool to analyze the molecular components of the neurofibrillary degeneration among different dementing disorders, and provides a valuable means to classify dementing conditions that share certain biochemical and histochemical features.

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