Guadeloupean parkinsonism: a cluster of progressive supranuclear palsy-like tauopathy

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Summary

An unusually high frequency of atypical Parkinson syndrome has been delineated over the last 5 years in the French West Indies. Postural instability with early falls, prominent frontal lobe dysfunction and pseudo-bulbar palsy were common and three-quarters of the patients were L-dopa unresponsive. One-third of all patients seen had probable progressive supranuclear palsy (PSP). This new focus of atypical parkinsonism is reminiscent of the one described in Guam and may be linked to exposure to tropical plants containing mitochondrial complex I inhibitors (quinolines, acetogenins, rotenoids). Two hundred and twenty consecutive patients with Parkinson's syndrome seen by the neurology service at Pointe à Pitre, Guadeloupe University Hospital were studied. Currently accepted operational clinical criteria for Parkinson's syndromes were applied. The pathological findings of three patients who came to autopsy are reported. Fifty-eight patients had probable PSP, 96 had undetermined parkinsonism and 50 had Parkinson's disease, 15 had amyotrophic lateral sclerosis with parkinsonism and one had probable multiple system atrophy. All three PSP patients in whom post-mortem study was performed had early postural instability, gaze palsy and parkinsonian symptoms, followed by a frontolimbic dementia and corticobulbar signs. Neuropathological examination showed an accumulation of tau proteins, predominating in the midbrain. There was an exceptionally large accumulation of neuropil threads in Case 1. Biochemical studies detected a major doublet of pathological tau at 64 and 69 kDa in brain tissue homogenates. All cases were homozygous for the H1 tau haplotype, but no mutation of the *tau* gene was observed. Clinical, neuropathological and biochemical features were compatible with the diagnosis of PSP, although some unusual pathological features were noted in Case 1. A cluster of cases presenting with atypical parkinsonism is reported. Guadeloupean parkinsonism may prove to be a tauopathy identical or closely related to PSP.

Keywords: atypical parkinsonism; Guadeloupe; PSP; tauopathy

Abbreviations: ALS = amyotrophic lateral sclerosis; CBD = corticobasal degeneration; PCR = polymerase chain reaction; PDC = parkinsonism dementia complex; PSP = progressive supranuclear palsy; UP = undetermined parkinsonism; UPDRS = Unified Parkinson's Disease Rating Scale

Introduction

An unexpectedly high frequency of atypical parkinsonism has been reported on the island of Guadeloupe in the French West Indies, with only 20% of cases being diagnosed as classical Parkinson's disease (Caparros-Lefebvre *et al.*, 1999). Such a distribution contrasts with the one encountered in Europe and North America, where classical Parkinson's disease is by far the commonest diagnosis among patients with Parkinson's

disease (de Rijk *et al.*, 1997; Golbe *et al.*, 1988; Tanner and Goldman, 1996). The characteristic spectrum of Guadeloupean atypical parkinsonism includes symmetrical bradykinesia, predominantly axial rigidity, early postural instability, cognitive decline with prominent features of frontal lobe dysfunction, and with a negligible or at best transient and poor response to L-dopa. Several causes for this

atypical parkinsonism may be suggested. Guadeloupe has 422 000 inhabitants, ~80% of which have African or admixed origins, 15% have an Indian background and 5% are Caucasian—raising the possibility that atypical parkinsonism may be commonest in African-Caribbeans. However, atypical parkinsonism was found in people of diverse ethnic origin, making this possibility unlikely. One environmental factor may be of special interest: herbal medicine intake has a deep-rooted cultural tradition in the West Indies and most patients reported regular use of herbal tea and/or fruits of tropical plants, which are known to contain alkaloid toxins. These include benzyltetrahydroisoquinolines, tetrahydroprotober-berines (Leboeuf *et al.*, 1982) and acetogenins (Alali *et al.*, 1999). They are potent mitochondrial complex I inhibitors and some have an affinity for dopaminergic receptors and inhibit dopamine reuptake (Nagatsu, 1997).

We report here an extension of our original observations on the clinical characteristics of Guadeloupean parkinsonism, confirming the high prevalence of atypical presentations. We also report the results of pathological and biochemical studies performed on the brains of three patients, included in our initial report and diagnosed clinically as progressive supranuclear palsy (PSP) (Caparros-Lefebvre *et al.*, 1999). Our findings indicate that Guadeloupean parkinsonism is a tauopathy with some unusual pathological features, possibly different from the currently known tau disorders.

Methods
Examination and inclusion of patients
Two hundred and twenty consecutive patients with Parkinson's syndrome referred to the Department of Neurology of the French West Indies University Hospital (Pointe à Pitre, Guadeloupe) between September 1996 and May 2001 have been studied. Three-quarters of them were referred by primary care physicians, and the rest by neurologists. Patients with known previous stroke, treatment with neuroleptics during the 2 years prior to evaluation, a history of severe head trauma or encephalitis were not included in the study. Attempts were made to classify the patient's neurological disorder as Parkinson's disease, PSP, corticobasal degeneration (CBD), multiple system atrophy (MSA), dementia with Lewy bodies (LBD) or frontotemporal lobar degeneration (FTD) (Steele *et al.*, 1964; Hughes *et al.*, 1992; Litvan *et al.*, 1996a, 1998; McKeith *et al.*, 1996; Gilman *et al.*, 1998; Neary *et al.*, 1998). Patients who did not fulfil the operational criteria for these established disorders were labelled as undetermined parkinsonism (UP). Patients were examined by at least two of us (D.C.-L. and A.L.) and 145 of them have been either examined or studied on videotape records by movement disorder specialists in the CAPAS group (see Appendix I) (Caparros-Lefebvre *et al.*, 1999).

Evaluation of acute and chronic response to L-dopa, electromyography when motor neurone disease was clinically suspected, brain computed tomography (CT) scan or MRI, and formal cognitive evaluation were carried out.

Patients gave informed consent to participate in the study, which was approved by the Comite consultatif de protection des personnes pour la recherche biomédicale (CCPPRB).

Neuropathological study
Autopsy was performed in all three cases <12 h after death. The brains were divided into two parts: the left hemisphere was fixed in formaldehyde; the right one was frozen for biochemical study. Microscopical examination was performed on paraffin-embedded material after haematoxylin and eosin staining, Gallyas silver impregnation for neurofibrillary tangles and immunohistochemistry with the following antibodies: tau (polyclonal antibody; Dako®, Trappes, France, raised against the C-terminal part of the recombinant human protein, including the four repeats), ubiquitin (polyclonal; Chemicon®, Temecula, USA), α-synuclein, Dako®, Trappes, France, glial fibrillary acidic protein (GFAP) (clone 6F2; Dako®, Trappes, France), MAP2 (microtubule associated protein) (B9 polyclonal antibody; a gift from J. P. Brion, Brussels, Belgium).

Biochemical study
Antibody used for Western blot
AD2, Bio-Rad, Hercules, USA, a monoclonal antibody directed against the dually phosphorylated site 396 and 404 of tau protein (numbering according to the longest tau isoform), was used. It does not cross-react with unphosphorylated tau protein.

Brain tissue samples
The frozen brain tissue samples were dissected and homogenized in lysis buffer, provided by U.K. Laemmli, University of Genova, Switzerland at the ratio 1 : 10 (w/v), boiled for 10 min and kept at –80°C until used.

SDS–PAGE and Western blot
The protocol described by Delacourte *et al.* (1999) was used. Briefly, an equal volume (30 µl) of brain tissue samples was loaded onto 10–20% gradient SDS–PAGE (sodium docecyl sulphate polyacrylamide gel electrophoresis). After migration, the proteins were transferred on a nitrocellulose membrane with the semi-dry Novablot blotting system (Amersham Pharmacia, Piscataway, NJ, USA). The blot was blocked for 30 min in TNT (Tris–HCl 50 mM pH 8.0, 150 mM NaCl, 0.5% Tween-20) supplemented with 5% skimmed milk. AD2 was incubated for 2 h at ambient temperature at a final dilution of 1 in 20 000 in TNT. The

Table 1 *Primers and restriction enzymes used to genotype* tau *polymorphisms in exons 1, 7 and 13*

Exon	Primer sequence	Polymorphism	PCR product (bp)	Restriction enzyme	Digestion product in bp (allele)
1					
F	CCCAACACTCCTCAGAACTT	−13A/G	231	*Alu*I	231 (A), 185/46 (G)
R	CAGTGATCTGGGCCTGCTGT				
7					
F	GGTGGCAGTAACTTTTCCCA	528G/A	182	*Fok*I	182 (G), 155/27 (A)
R	AGCTGGGTGGTGTCTTTGGAGCGGA				
13					
F	CTTTCTCTGGCACTTCATCT	+34T/C	315	*Tsp*509I	287/30 (T), 315 (C)
R	GTCCCAGGTCTGCAAAGTGG				

F = forward; R = reverse

membrane was rinsed three times with TNT and incubated with an anti-mouse horseradish peroxidase-conjugated antibody. The immune complexes were visualized with an ECL™ Western blot kit (Amersham Pharmacia, Piscataway, NJ, USA) using Hyperfilms (Amersham Pharmacia). The films were digitized and the signal was quantified using ImageMaster 1-D software (Amersham Pharmacia, Piscataway, NJ, USA).

Genetic study

Exons 1–5, 7 and 9–14 of the microtubule-associated protein *tau* gene and the corresponding flanking intronic sequences were polymerase chain reaction (PCR) amplified using the primer pairs described by Dumanchin *et al*. (1998). PCR reactions were performed in a final volume of 50 µl containing 0.5 µM of each primer and 1 U of *Taq* DNA polymerase. The PCR consisted of 35 cycles of 30 s at 96°C, 30 s at 50–62°C and 45 s at 72°C, preceded by a 10 min denaturation at 96°C and followed by a final extension of 5 min at 72°C. PCR products were purified by centrifugation using a 96-well filter plate (Dutscher®, Brumath, France) and sequenced directly on both strands using the Big Dye Terminator Cycle Sequencing Ready Reaction DNA sequencing kit (Perkin Elmer®, Wellesley, MA, USA) and an ABI377 automated sequencer (ABI, Foster City, CA, USA). Sequences were analysed with Sequence analysis 3.0 (ABI Prism, Foster City, CA, USA) software.

Polymorphisms −13 A/G, 528 G/A and +34 T/C [previously described by Dumanchin *et al*. (1998) and Poorkaj *et al*. (1998)], located in exons 1, 7 and 13 of the *tau* gene, respectively, were analysed by PCR amplification using the primer pairs used for sequencing the corresponding exons and followed by digestion of the product (Table 1). For the 528 G/A polymorphism, a mismatch primer was used for genotyping. PCR conditions were as described for sequencing of the *tau* gene except that the final volume was 25 µl and that exon 7 was amplified in 10% dimethyl sulphoxide (DMSO). Two units of the restriction enzymes and 1 in 10 volume of the appropriate ×10 buffer were directly added to the PCR

product, except for exon 13, for which ethanol precipitation was performed before digestion in the appropriate buffer. The dinucleotide repeat polymorphism located on intron 8 was analysed by PCR amplification, as described previously by Conrad *et al*. (1997). One of each primer pair was labelled and was analysed on the ABI377 sequencer using the Genotyper software (Perkin Elmer®, Wellesley, MA, USA). H1 and H2 haplotypes of the *tau* gene were assigned as described by Baker *et al*. (1999).

Results

Using established clinical diagnostic criteria, patients were classified into four main groups: Parkinson's disease, PSP, parkinsonism-amyotrophic lateral sclerosis (ALS) syndrome and UP. The latter group of patients did not fulfil operational criteria for the clinical diagnosis of any of the well-defined Parkinson's syndromes. Fifty-eight patients (26.5%) had dopa-resistant parkinsonism, associated with postural instability and early falls, ophthalmoplegia, pseudo-bulbar palsy and frontal lobe signs compatible with probable PSP. The UP group comprised 96 patients (43.8%), who presented with a relatively symmetrical akinetic-rigid syndrome predominating in axial muscles, unresponsiveness to L-dopa in the long-term, frequent early cognitive impairment, frontal lobe signs and pseudo-bulbar palsy. None of the UP patients had peak-dose L-dopa related dyskinesias. Four had dystonia, which occurred before L-dopa treatment in two. The main differences with classical PSP were the relative mildness of postural instability and late occurrence of falls after >2 years of duration. Gaze palsy was not present at disease onset but sometimes occurred in the very late stages, leading to a redesignation as PSP in four patients who were examined in the last 4 months prior to their death. In the UP group, no case fulfilled the clinical criteria of multiple system atrophy, dementia with Lewy bodies, or corticobasal degeneration. Thirty-five per cent of UP patients had mild to moderate treated and stabilized hypertension. Seven per cent of UP cases had diabetes mellitus. None fulfilled criteria for vascular parkinsonism (Winikates and Jankovic, 1999). The
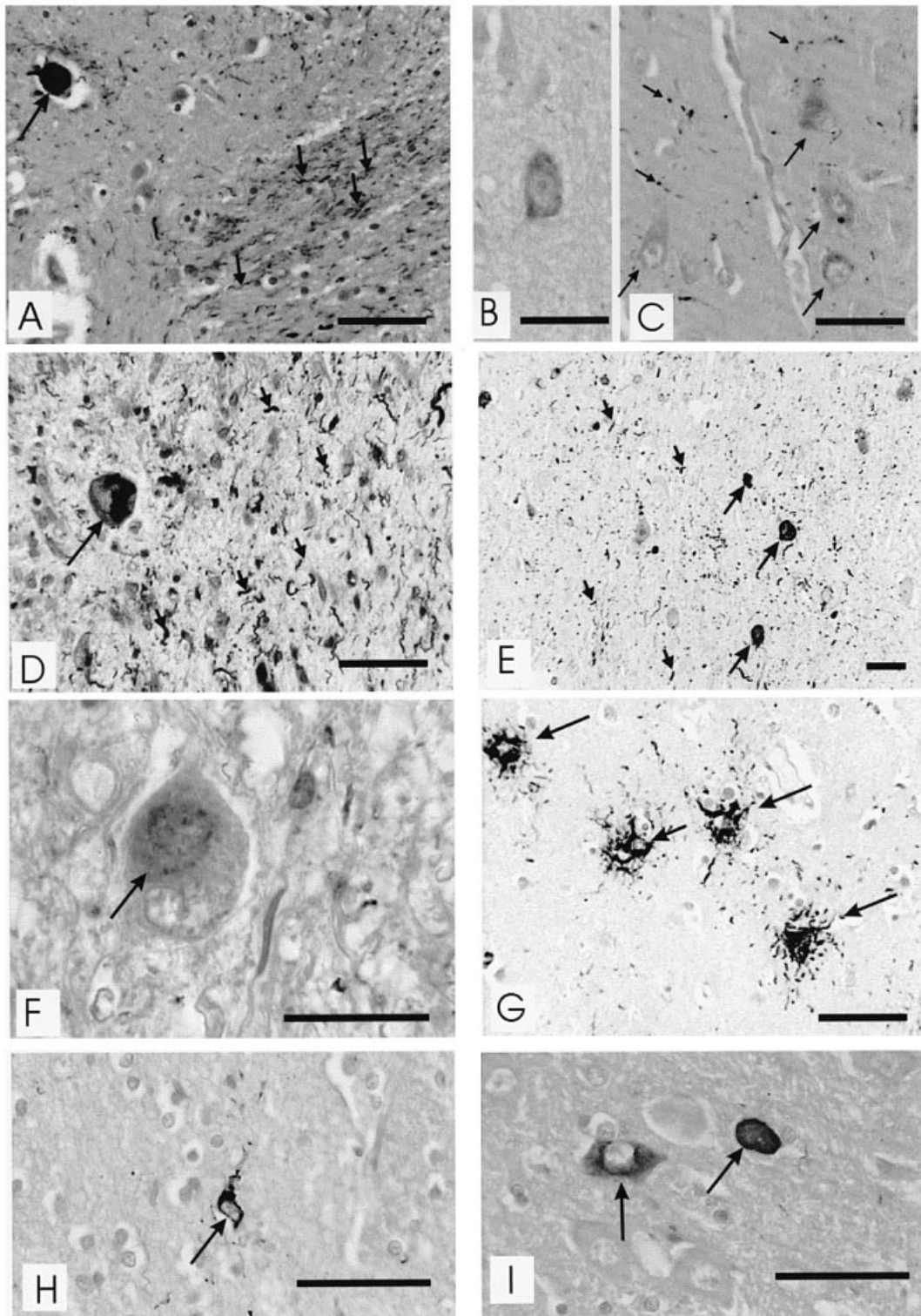
Fig. 1 Main histopathological aspects of fibrillary pathology in Case 1 (**A–F**) and Case 3 (**G–I**). Case 1 (**A**) Tau immunohistochemistry: striatum. One neurofibrillary tangle is present in the upper left part of the picture (large arrow). The small arrows indicate bundles of tau-positive fibres in the fascicles that normally cross the striatum. (**B**) Tau-positive neurone (pretangle) in the parietal isocortex. (**C**) Tau-positive neurones (big arrows) and threads or grains (small arrows) in the subiculum. (**D**) Gallyas staining of the substantia nigra. The neuronal loss was severe. The big arrow points to a neurofibrillary tangle, the small arrows indicate threads which were particularly abundant. (**E**) Midbrain tegmentum: numerous tangles (big arrows) and threads (small arrows). (**F**) A tangle in a neurone of the subthalamic nucleus. Case 3 (all the pictures after tau immunohistochemistry) (**G**) Four astrocytic tufts (arrows) in the striatum. (**H**) A coiled body (arrow) in a fascicle of the striatum. (**I**) Two tangles (arrows) in the nuclei pontis. All bars = 50 μ, except (**F**) = 20 μ.

pathology of one of these UP cases is currently being studied, and preliminary results suggest an association of an unusual tauopathy with the presence of diffuse Lewy bodies. Fifteen patients (6.8%) had predominant parkinsonism-ALS. In one of them, the neuropathological examination revealed the features of classical ALS and additional severe neuronal loss in the substantia nigra (Caparros-Lefebvre *et al.*, 1999). Overall, 77.1% of the patients had dopa-resistant parkinsonism as defined by a failure to respond to a minimum daily dose of 600 mg of L-dopa with decarboxylase inhibitor for >6 months. Compliance was monitored through the family, and L-dopa response was assessed 1 month after the introduction of a dose of 600 mg/day. If patients did not improve, the dose was increased incrementally up to 2 g/day for at least 3 months, unless precluded by adverse events which occurred in 20%. Only 50 (22.9%) had L-dopa responsive Parkinson's disease and one patient had probable multiple system atrophy. Twenty patients died during the follow-up in the PSP group, and nine in the UP group. Three patients fulfilling the diagnostic criteria of PSP came to autopsy.

Case 1 (Patient 18-99)

This African-Caribbean man, born and living on Marie-Galante (a small island included in the archipelago of Guadeloupe), developed mental and physical slowing in 1995 when he was 53 years old. He had no risk factors for stroke, nor a family history of parkinsonism, tremor or dementia. He was described as apathetic and had been unable to behave appropriately when a fire broke out in his house. His gait and movements were slow, but no falls were reported during the first year. Before disease onset, he drank herbal tea and herbal liquors containing Annonaceae leaves for aphrodisiac effects. He moved to his sister's house on Guadeloupe and later to France where he continued to drink herbal liquors. A frontal lobe syndrome became increasingly intrusive with urinary incontinence, sexual disinhibition, visual hallucinations and delusions. He was referred to a neurological unit in France in May 1998. At this time, he had obvious frontal dementia with euphoria, echolalia, impaired verbal fluency, disorientation, memory loss and frontal lobe type bladder incontinence. He had postural instability with occasional falls, parkinsonism with prominent nuchal rigidity, and vertical supranuclear palsy involving upgaze and downgaze.

In December 1998, he returned to Guadeloupe and was referred to our department. There was severe impairment of postural reflexes, with occasional falls. He had a complete vertical gaze palsy with preserved oculocephalic reflexes. Horizontal gaze movements were full, but very slow. He had developed a pseudo-bulbar palsy, with severe dysarthria and moderate dysphagia. His blink reflex did not extinguish (glabellar tap), while spontaneous blinking was extremely rare (<1 per min). A palmo-mental reflex was present bilaterally and there was a brisk jaw jerk with absent palatal response. He was still able to walk unsteadily but with ignition failure and marked axial rigidity and bradykinesia. None of the parkinsonian signs responded to L-dopa (1000 mg daily). His Unified Parkinson's Disease Rating Scale (UPDRS) score was 62. Bradykinesia and rigidity in the limbs was symmetrical and there was no tremor. He had no orthostatic hypotension. A brain CT scan was normal. By February 1999, the gaze palsy was complete in both vertical and horizontal planes, with preserved oculocephalic reflexes. He had developed blepharospasm and his stretch reflexes were very brisk. In July 1999, he became mute but he was still able to perform simple movements to command. Death occurred in August 1999, from aspiration pneumonia after 4 years of disease.

Pathology

The pathology carried out for Case 1 is shown in Fig. 1A–F. The right hemisphere (weight 660 g) was formalin-fixed for 2.5 months. The external surface of the brain was normal. Ventricles were not enlarged. The putamen, pallidum, thalamus and hippocampus were macroscopically normal. The substantia nigra was pale while the locus coeruleus was still pigmented. The pons, medulla oblongata and cerebellum were unremarkable.

On microscopical examination, a severe loss of neurones with intense astrocytosis was observed in the substantia nigra and, to a lesser degree, in the midbrain tegmentum. Moderate neuronal loss was also noticed in the septal nuclei and in the thalamus (centrum medianum and nucleus parafascicularis). Astrocytosis was marked in the pallidum. Spongiosis of layer II and gliosis were seen in the cerebral cortex, particularly Brodmann area 9 (prefrontal cortex). Immunohistochemistry revealed a diffuse and severe accumulation of tau protein in processes (threads) and in cellular bodies (pretangle, i.e. diffuse immunostaining of the cell body) or true fibrillary tangles. Threads were the most abundant lesion and were seen in all the examined samples—in the deep layers of the isocortex, in the hippocampus and in the parahippocampal gyrus. They were also abundant in the striatum where they were found not only in the grey matter but also in the fascicles that cross the nucleus. They were present in large numbers in the thalamus, mesencephalic tegmentum, locus coeruleus, transverse fibres of the pons, white matter of the cerebellum and dentate nucleus. Some threads were also seen in the white matter of the cerebral hemisphere and in the pes pedunculi. Double immunofluorescence (examined by confocal microscopy) revealed that they were not co-localized with GFAP (glial fibrillary acidic protein), phosphorylated neurofilaments (present in axons) or MAP2 (present in dendrites). Pretangles in cells with neuronal morphology were observed in the cerebral cortex (prefrontal areas), dentate gyrus, pyramidal neurones of the hippocampus and parahippocampal gyrus, thalamus, striatum and nucleus basalis of Meynert. They were particularly abundant in the mesencephalic tegmentum, where they involved the nucleus raphe dorsalis and the griseum centrale mesencephali. They were also

present in the nuclei pontis. They were absent from the dentate nucleus, although threads were abundant. Compared with the abundance of pretangles, true neurofibrillary tangles were rare. They involved the areas with pretangles and were most abundant in the mesencephalic tegmentum, substantia nigra and lenticular nucleus. Labelling of the cellular bodies of astrocytes was also seen as well as fibrillary tangles. However, no astrocytic tufts were observed. Only one lesion had the appearance of an astrocytic plaque. Aβ peptide and anti-alphasynuclein immunohistochemistry were negative. Ubiquitin antibodies labelled only a small fraction of tau accumulation (in the fibres and in the cell bodies).

Case 2 (Patient 498-99)

This African-Caribbean man was referred because of walking difficulties when he was 66 years old. He had no family history of neurodegenerative disease and no risk factor for stroke. He was accustomed to drinking herbal tea containing various medicinal plants, including Annonaceae leaves, and he ate Annonaceae fruits weekly. When first seen, he had no evidence of parkinsonism, or gaze abnormality but presented with mild cognitive blunting, apathy and poor balance. Six months later, he had developed vertical gaze slowing and a frontal lobe syndrome, while imbalance was increasingly disabling. A diagnosis of PSP was made. His frontal dementia worsened with severe apathy, impaired verbal fluency, disorientation, memory loss, urinary incontinence and occasional hallucinations. He had reduced speech output and developed a symmetrical bradykinesia and limb rigidity, without tremor. Axial rigidity was pronounced and he had bilateral Babinski signs. His UPDRS score was 53 1 year after disease onset, and was unchanged after 6 months of L-dopa therapy (1000 mg daily). Progressive gait impairment, daily falls and subcortical dementia precluded an independent existence. Eighteen months after disease onset, he had a complete vertical gaze palsy with preserved doll's eye movements. He then developed a severe pseudo-bulbar palsy, with dysarthria, dysphagia, brisk palmo-mental reflex, jaw jerks and an absent gag reflex. Blink reflex did not extinguish and spontaneous blinking frequency was <1/min. He became chairbound. A brain CT scan showed mild cerebellar atrophy. In July 1999, he was confined to bed after he fractured his right leg. At this time, he was still able to perform simple movements on request. Death occurred in October 1999 due to septicaemia 2 years after disease onset.

Pathology

The right hemisphere (weight 642 g) was formalin-fixed for 5 months. The external surface of the brain was normal and the ventricles were not enlarged. The pallidum and sub-thalamus appeared atrophic. The whole section area of the substantia nigra was devoid of pigment while the locus coeruleus remained identifiable. The pons, medulla oblongata and cerebellum were unremarkable.

At microscopic examination, a severe loss of neurones was observed in the substantia nigra, pallidum and subthalamic nucleus. There was pronounced gliosis in the periaqueductal grey and the pallidum. There were only minor changes in the cerebral neocortex. In the cerebellum, only occasional Purkinje cell axon torpedoes were seen in the granule cell layer. Immunohistochemistry revealed an accumulation of tau protein, with neuropil threads, pretangles and fibrillary tangles. Occasional tangles and pretangles were seen in the deep layers of the isocortex, the limbic areas of the anterior cingulate gyrus, the hippocampus and the parahippocampus. They were also present in the caudate and the putamen, where they were associated with glia immunoreactivity for tau and neuropil threads. Tangles were more abundant in the nucleus basalis of Meynert, tegmental nuclei, nuclei pontis and dentate nucleus, where grumose degeneration was seen. In the medulla, tangles were numerous in the inferior olive, in addition to the lateral, medial and dorsal nuclei. Anti-alphasynuclein immunohistochemistry was negative. The accumulation, type and topography of tau was suggestive of PSP.

Case 3 (Patient 45-00)

This 68-year-old Indian woman was referred in May 1997 because of difficulty in walking. In January 1996, she had been admitted for weakness of the right arm lasting a few hours. Her brain CT scan was normal and a transient cerebral ischaemic attack was diagnosed. She had no parkinsonian features nor gait impairment at this point. Before disease onset, she used to drink herbal tea containing various medicinal plants, including Annonaceae leaves, and ate Annonaceae fruits weekly. By 1997, she had developed postural instability with falls, urinary incontinence and mild cognitive impairment interfering with some daily activities such as cooking. On examination, she had vertical upgaze palsy and downgaze slowing, intermittent myoclonic jerks of the hand and mild symmetrical bradykinesia with axial rigidity. Neuropsychological testing revealed impairment of reasoning, calculation and executive functions. She was unable to follow the rules of the GO–NO test as well as the anti-saccades test. By January 1998, she had developed a dystonic posture of the left foot and by August of the same year, blepharospasm, a complete vertical supranuclear gaze palsy and Babinski signs. She experienced auditory and visual hallucinations. Echolalia was present. Diagnosis of frontal type dementia was made. A pseudo-bulbar palsy developed with progressive dysarthria and dysphagia. During 1999, the pseudo-bulbar syndrome worsened. She had severe and distressing swallowing difficulties. She was able to stand unsupported, but was unable to walk. She developed severe nuchal dystonia with retrocollis, requiring injections of botulinus toxin in the neck extensor muscles. Her general

status declined with weight loss. A few days before death, she was still able to perform simple movements on command. Death occurred after 3.5 years of disease and was due to aspiration pneumonia.

Pathology

The pathology carried out for Case 3 is shown in Fig. 1G–I.

Gross examination of the brain: only the left hemisphere (weight 454 g) was examined. There was moderate atrophy of the cerebral cortex, mainly in the anterior part of the temporal lobe. The pallidum and the subthalamic nucleus were atrophic and perivascular cavities were noticed in the external pallidum. The substantia nigra was pale. The locus coeruleus was pigmented. There was no atrophy of the pons. The medulla and cerebellum appeared macroscopically normal.

Microscopical examination: the lesions consisted of various types of tau accumulation within neurones and glia, which could be visualized either by tau immunohistochemistry or by Gallyas staining. In the cerebral cortex, astrocytic tufts were numerous, especially in the prefontal cortex and in the supramarginal gyrus, while tau-positive neurones and threads were sparse. The first temporal gyrus and the motor cortex were less severely affected. The primary visual cortex (area 17) was almost entirely spared.

The most severely affected subcortical nuclei contained numerous neurofibrillary tangles. Glial inclusions were less abundant than in the cortex. Neuronal loss and gliosis were moderate to severe. This pattern of involvement was observed in the pallidum, septal nuclei, nucleus basalis of Meynert and subthalamic nucleus. The involvement of the lateral nuclei of the thalamus was also marked, but involved the glia to a greater extent than the neurones. Neuronal loss was mild. In the caudate and putamen, the astrocytic tufts were abundant, while neuronal involvement was mild to moderate. In the brainstem, the substantia nigra was severely involved. Neuronal loss was marked. Some cavitation of the neuropil was seen in the most affected regions of the nucleus. The tegmentum of the midbrain, pons and medulla contained numerous threads and tangles. The inferior olive and the dentate nucleus contained neurofibrillary tangles and threads. Double immunostaining did not show co-localization of tau accumulation and MAP2. Anti-alphasynuclein immunohistochemistry was negative. The accumulation of tau, its type and topography were compatible with the diagnosis of PSP.

Biochemical studies

Immunopathological study

As described previously by Delacourte *et al.* (1999), AD2 is a useful immunological tool with which to visualize pathological tau in post-mortem brain tissue homogenates because it does not react with dephosphorylated tau proteins of control brain tissue samples. Western blotting was used to determine the distribution of pathological tau in all three cases. As a positive control, an Alzheimer's disease (AD) brain tissue homogenate was loaded on each SDS–PAGE, both to visualize the pathological tau components and for quantification. In this AD sample, AD2 antibody detected the typical pathological tau triplet at 60, 64 and 69 kDa in AD (Fig. 2, lane AD). A major doublet of pathological tau at 64 and 69 kDa was detected in the brain tissue homogenates of the three Guadeloupean cases.

In Case 2, pathological tau was not detected in isocortical brain regions, with the exception of the primary motor cortex where traces of the pathological tau doublet were identified (Fig. 2, BA4bis). In contrast, the hippocampus, entorhinal cortex and subcortical nuclei (including the caudate nucleus, thalamus, pallidum, putamen, amygdala and subthalamus nucleus) showed large amounts of the pathological tau doublet. Traces of pathological tau were detected in the cerebellum and in the centrum ovale. Pathological tau was not detected in the insula and pons.

Conversely, Case 1 showed the pathological tau doublet in all the brain structures analysed. The pathological tau doublet was detected in lower amounts in the cerebellum, medulla oblongata, insula and pons (Fig. 2, Case 1). In contrast, the Western blot was intensively stained in the subcortical nuclei as well as in the motor cortex and the hippocampus, in which the signal intensities were similar to the ones measured in the AD brain tissue samples.

Case 3 showed a similar pathological tau pattern and tau pathology distribution (Fig. 2, Case 3), but the pathological tau components at 64 and 69 kDa were less intensively stained in the isocortical brain areas compared with Case 1. Overall, tau pathology appeared particularly prominent in the subcortical structures in the three Guadeloupean cases studied.

Additional brain structures were investigated, including the spinal cord and the cortical white matter. In both structures, the pathological tau doublet was detected with AD2 antibody. These results suggest that neurofibrillary degeneration extended into the anterior horn cells. The expression of normal tau proteins was investigated in the brain areas unaffected by tau pathology, or in the less affected brain areas where tau pathology was widespread. Tau holoproteins were immunodetected with both anti-amino and carboxy terminal phospho-independent tau antibodies. No overexpression of a specific tau isoform was observed compared with the pattern of controls analysed on the same blots (not shown).

Genetic analysis

No causal mutation was detected in the sequenced exons and exon–intron boundaries in all three cases. All three patients were homozygous for the H1 haplotype and carried the A0 repeat in the dinucleotide polymorphism of intron 8 (Baker *et al.*, 1999).
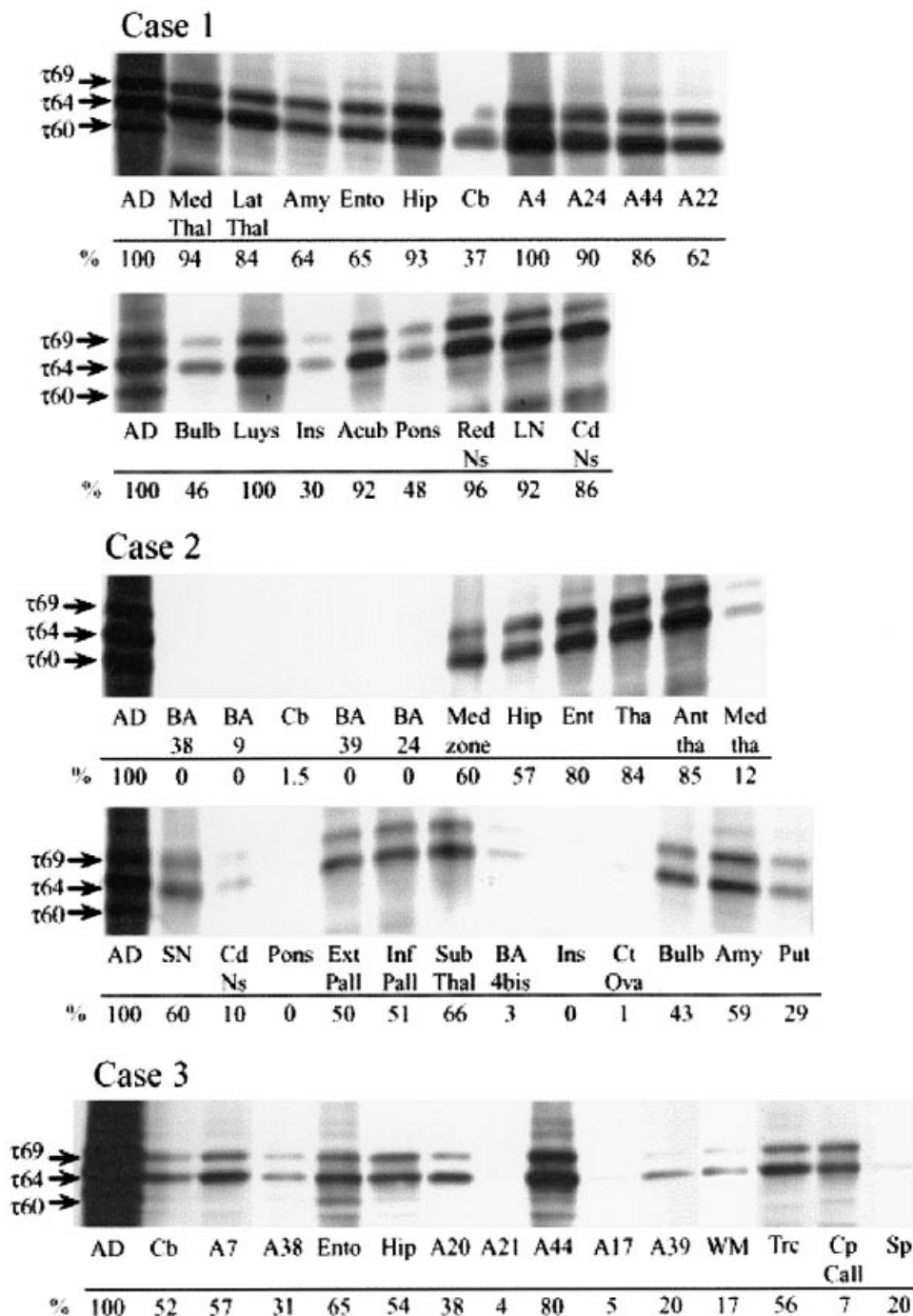
Fig. 2 Cerebral biochemical mapping of pathological tau of three Guadeloupean cases. Twenty-four, 18 and 15 brain regions of Case 1, Case 2 and Case 3 were analysed for their pathological tau content by Western blotting. Antibody AD2 detected a doublet of pathological tau at 64 and 69 kDa in the three cases, whereas the typical triplet of pathological tau is observed in the Alzheimer brain tissue samples (AD lanes). Arrowheads indicate the pathological tau components. The pathological tau immunoreactivity was quantified using ImageMaster 1D software and expressed as the percentage of the signal obtained in AD brain tissue samples. AD = Alzheimer's disease; Accu = nucleus accumbens septi; Amy = amygdala; Ant tha = anterior thalamus; BA = Brodmann area; Bulb = bulbe; Cb = cerebellum; Cd Ns = caudate nucleus; Cp Call = corpus callosum; Ct Ova = centre ovale; Ento = entorhinal cortex; Ext Pall = exterior pallidum; Hip = hippocampus; Inf Pall = inferior pallidum; Ins = insula; LN = locus niger; Lat Thal = ventral lateral nucleus; Med zone = medial zone; Med tha = median thalamus; Put = putamen; Red Ns = red nucleus; SN = substantia nigra; Sub Thal = subthalamic nucleus (nucleus of Luys); Tha = thalamus; Trc = brainstem; WM = white matter.

Discussion

The clinical features of atypical Parkinson syndrome on Guadeloupe include symmetry of bradykinesia and rigidity, with L-dopa unresponsiveness, postural instability, dementia and, after 2–5 years, a pseudobulbar palsy. This picture is reminiscent of the clinical features of bodig (Chamona Indian name for PDC) delineated on Guam over the last 40 years (Elizan *et al.*, 1966).

The early occurrence of postural instability followed by vertical supranuclear gaze palsy allowed the diagnosis of PSP in 58 cases. Interestingly, in the seminal description of PSP (Steele *et al.*, 1964), one of the seven patients was African-Caribbean.

PSP could not be clinically diagnosed in the patients with UP, although we believe a large number of these cases may prove to have the same histopathology as those diagnosed as PSP. Daniel *et al.* (1995) and Dubas *et al.* (1983) demonstrated the lack of supranuclear gaze palsy in histopathologically proven cases of PSP. Clinical heterogeneity, including the presence of postural or rest tremor, is recognized in 12–16% of PSP cases (Masucci and Kurtzke, 1989).

Two of us (A.L., D.C.-L.) have had the opportunity of examining cases with PDC on Guam. PDC has striking clinical similarities with the Guadeloupean UP, including L-dopa unresponsiveness, akinetic rigid syndrome with axial predominance, postural instability and frontal type dementia. The cognitive failure differs obviously from cortical dementia of the Alzheimer type, since Guamanian and Guadeloupean patients have preserved verbal comprehension up to a few days before death. Tremor seems more common in the geographic isolate series, in comparison with PSP, while vertical supranuclear gaze palsy (required for the diagnosis of PSP) is observed in one-third of patients with both atypical dopa-resistant parkinsonism on Guadeloupe and PDC of Guam. However, supranuclear palsy may also develop in UP in the very late stage of the disease (Lepore *et al.*, 1988; Oyanagi *et al.*, 2000). The duration of the disease seems more variable in bodig and Guadeloupean tauopathy than in PSP.

On Guam, the simultaneous high frequency of PDC and ALS could explain the occasional association of both diseases. However, distal muscle atrophy is commonly observed with PDC and Guadeloupean UP. On Guadeloupe, some parkinsonism-ALS patients have an unusual tremor and frontal type dementia. We previously studied the neuropathological features of one case of Guadeloupean ALS (Caparros-Lefebvre *et al.*, 1999), where we reported an association of typical ALS lesions and a severe neuronal loss in the substantia nigra. Tau depositions in the anterior horn of the spinal cord were observed in the cases of PSP coming to autopsy in this series. These observations suggest a link between ALS and parkinsonism. The association of ALS and PDC has also been demonstrated in the Kii peninsula focus (Konagaya, 1999). However, the Western blot of tau protein (exhibiting two bands) in our cases was different from the results obtained with PDC.

We believe this series of patients is representative of Guadeloupean parkinsonism, since most patients were admitted after referral from family doctors who referred all cases of parkinsonism on an equal basis. As on Guam, a high frequency of atypical parkinsonism could be associated with a low frequency of Parkinson's disease. If the susceptibility to parkinsonism was identical in both groups, an environmental cause could influence the phenotype of the disease. The proportion of parkinsonian cases that do not seek advice from medical doctors is difficult to assess. However, it is highly improbable that it is different for atypical parkinsonism and Parkinson's disease. There are 3.4 times more PSP and atypical cases than Parkinson's disease cases in Guadeloupe while, in the UK, the percentage of L-dopa unresponsive parkinsonism is below 10% of all Parkinson syndromes. It should also be stressed that the prevalence of PSP is higher in Guadeloupe; even if we consider only the patients we have examined (we had 58 PSP cases on our files out of a population of 422 000 inhabitants (i.e. 14 out of 100 000), while the known prevalence of PSP in the UK is five out of 100 000) (Schrag *et al.*, 1999; Nath *et al.*, 2001). The percentage of Caucasians with Parkinson's disease was higher (10%) than among other groups (0.6% = one patient with ALS and tremor).

All three PSP cases coming to autopsy had similar clinical features, but the disease duration was different. The pathological data were compatible with PSP (Litvan *et al.*, 1996*b*). In Case 1, however, there were some unusual features. Tau-positive threads were by far the most abundant lesion and tau-positive neurones were much more numerous than true neurofibrillary tangles. The nature of the threads (neuronal or glial, astrocytic or oligodendroglial) remains to be determined. The absence of co-localization of the tau immunoreactivity observed in the threads with neurofilament, MAP2 or GFAP immunoreactivity is compatible with an oligodendroglial topography of the accumulation. Finally, astrocytic tufts, considered to be highly suggestive of PSP (Hauw *et al.*, 1990; Komori *et al.*, 1998) were virtually absent. Alternative diagnoses were considered but excluded: the lack of chromatolytic neurones and astrocytic plaques made the diagnosis of corticobasal degeneration unlikely. The abundance of threads in the white matter and the scarcity of neurofibrillary tangles in the hippocampus distinguished it from Guam ALS–dementia complex. In Case 1, the accumulation of tau took place in the cell body of the neurones but was particularly abundant in threads. The widespread distribution of the accumulation and its unusual characteristics are suggestive of some 'tauopathies' that have been described in FTDP-17, due to mutation of the *tau* gene. However, such a mutation was not found here.

The electrophoretic pattern of tau proteins provides a biochemical signature of diseases with tauopathy. Four classes of tau aggregation are presently defined: (i) Alzheimer's disease and bodig (all six tau isoforms); (ii) PSP and CBD (the three isoforms with exon 10 corresponding sequence); (iii) Pick's disease (the three tau isoforms without

exon 10); and (iv) myotonic dystrophy (the shortest tau isoform) (Delacourte and Buee, 2000). Together, biochemical data suggest that the Guadeloupean diseases presented here are linked to a dysfunction of tau isoforms with exon 10. In the three cases that could be analysed at the biochemical level, the brain mapping of tau pathology matched the topography of the lesions observed at neuropathology. A major doublet at 64 and 69 kDa and a minor 74 kDa pathological tau component characterized the electrophoretic pattern of Guadeloupean parkinsonism in the most severely affected brain regions. This pattern is observed in PSP and CBD. Recent data have shown that this pattern is related to the accumulation and hyperphosphorylation of tau protein isoforms containing the exon 10 sequence (Mailliot *et al.*, 1998; Sergeant *et al.*, 1999). This was confirmed by 2D gel electrophoresis and using exon 10-specific tau antiserum that specifically detected the pathological tau components in the three Guadeloupean cases (data not shown). The presence of the 64–69 pathological tau doublet profile in Guadeloupean parkinsonism is not compatible with the Guam syndrome, in which the tau profile has been shown to be a triplet of pathological tau components at 60, 64 and 69 kDa (Buee-Scherrer *et al.*, 1995). This finding, however, needs to be confirmed in more patients.

None of the patients coming to autopsy had a family history of parkinsonism or dementia, so it is not too surprising that no mutations of the *tau* gene were found—even if a tau mutation is sometimes observed in sporadic FTDP-17. Furthermore, the normal expression of other tau isoforms observed in the three cases also favours the absence of a mutation that would cause abnormal splicing and an increase of 4R isoforms. To firmly exclude this hypothesis, all coding exons expressed in the nervous system and all their exon–intron boundaries were analysed by direct sequencing. Interestingly, all patients were homozygous for the H1 haplotype, which has been described as a risk factor for PSP (Morris *et al.*, 1999; Delacourte and Buee, 2000) and more recently for CBD (Houlden *et al.*, 2001). However, this haplotype is also the most frequent in all tested populations so far, although the frequency of the H1 haplotype has not been assessed in African-Caribbeans. The number of cases is, however, too small to determine whether this haplotype is also a risk factor for Guadeloupean parkinsonism with tauopathy.

We have examined 10 patients with parkinsonism in Martinique, the other main island of the French West Indies, where it seems that at least 50% of patients have atypical parkinsonism. This raises the interesting possibility that atypical parkinsonism may be more frequent in African-Caribbean and Indian populations than in Caucasians (Chaudhuri *et al.*, 2000) and should lead to a search for specific genetic and environmental factors.

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Appendix I

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