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Letter to the editor

# Cardiac $^{123}$I-meta-iodobenzylguanidine scintigraphy in patients with amyotrophic lateral sclerosis and parkinsonism–dementia complex of the Kii peninsula, Japan

*Keywords:*
Amyotrophic lateral sclerosis
Parkinsonism–dementia complex
Muro disease
Meta-iodobenzylguanidine scintigraphy
Kii peninsula

## 1. Introduction

The Kii peninsula of Japan and the island of Guam are the areas with high incidence of amyotrophic lateral sclerosis and parkinsonism–dementia complex (ALS/PDC). ALS/PDC is a distinct endemic disease that is characterized by clinical findings involving parkinsonism, dementia and motor neuron symptoms, and numerous tau protein deposits in the central nervous system (CNS). The high incidence rates of ALS/PDC have been persistent in the Kii peninsula [1].

Meta-iodobenzylguanidine (MIBG) is an analog of norepinephrine. Cardiac $^{123}$I-MIBG scintigraphy measures the function of the cardiac sympathetic nerve terminals. Thus, MIBG can be a biological marker for cardiac sympathetic nerve function [2]. Recently, we reported on a patient with Kii PDC who showed a marked decrease in cardiac MIBG uptake and Lewy bodies in both the CNS and the cardiac sympathetic plexus neuropathologically [3]. In this study, we performed MIBG scintigraphy in six patients with Kii ALS/PDC in order to evaluate their sympathetic nervous system function and to determine whether low cardiac MIBG uptake is a common finding in Kii ALS/PDC patients.

## 2. Methods

### 2.1. Subjects

Six patients with Kii ALS/PDC (one with Kii ALS, one with Kii ALS accompanied by dementia, and four with Kii PDC; two men and four women) were included in this study. All of them were natives of Hohara village, which is an ALS/PDC high prevalence of the Kii peninsula. The mean age at examination was 66.3 years and the mean duration of the illness was approximately 3 years and 9 months. The diagnosis of Kii ALS was made according to Airlie House criteria since the clinical symptoms of Kii ALS are essentially the same as those of classical ALS. The diagnosis of Kii PDC was made by a unique combination of levodopa-unresponsive parkinsonism and dementia, which are frequently accompanied by amyotrophy of the extremities. The summaries of the clinical profiles of the patients are shown in Table 1. The severity of the disease in patients with Kii ALS or Kii ALS with dementia was graded according to the ALS Functional Rating Scale Revised [4]. In the patients with Kii PDC, the severity of the illness was assessed by modified Hoehn–Yahr staging. The patients were not treated with antidepressants or other drugs that might influence the MIBG uptake and none of them had heart disease, arrhythmia, or diabetes mellitus. This study was approved by the Ethics Committee of Mie University Graduate School of Medicine and an informed consent was obtained from the patients or their families. There was no industrial affiliation.

### 2.2. Apparatus and protocol

Cardiac $^{123}$I-MIBG scintigraphy was performed using a single-photon emission computed tomography scanner (Model GCA-901A, Toshiba Corporation, Tokyo, Japan). The early image was taken 15 min and the delayed image was taken 3 h, after administration of 111 MBq $^{123}$I-MIBG injection. Global MIBG uptake was assessed using the ratio of $^{123}$I-MIBG uptake in the heart to the upper mediastinum (H/M) on planar scintigraphic data. The washout rate from the heart was calculated by using early and delayed images without background correction, while all data were subjected to a decay correction.

## 3. Results

Three patients with moderate parkinsonism (patients # 4, 5, and 6 in Table 1) showed a decrease in MIBG uptake (the average H/M ratio of the early image was 1.42 and that of the delayed image was 1.22). While the average H/M ratio was normal in patients # 1, 2, and 3 with no or minimal parkinsonism. MIBG uptake was normal in patient # 1 who showed only ALS features (Fig. 1A), while there was absolutely no MIBG uptake in patients # 5 and 6 (Fig. 1B) with Kii PDC. We defined the normal range of the H/M ratio as those values >2.0 in the early and delayed images and <30% as the normal range for the washout ratio; these values were based on previous reports.

## 4. Discussion

The present study showed that the H/M ratio, which was determined using MIBG scintigraphy, was decreased in the Kii PDC patients with moderate parkinsonism, but not in patients with Kii ALS alone as well as in those with Kii ALS accompanied by dementia, or Kii PDC with mild parkinsonism. The number of patients with Kii ALS, Kii ALS with dementia, and Kii PDC with mild parkinsonism was only one in each; therefore, we could not conclude that cardiac sympathetic nerve function was normal in these phenotypes.

Previous reports have shown that MIBG cardiac uptake is decreased in Parkinson's disease (PD), Parkinson's disease with dementia (PDD), diffuse Lewy body disease (DLBD), and pure autonomic failure compared to the normal uptake seen in corticobasal degeneration, progressive supranuclear palsy, and Alzheimer's disease. An autopsy-proven PD patient with a marked decrease in cardiac MIBG uptake showed not only Lewy body

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**Table 1**
Clinical features, heart to upper mediastinum (*H/M*) ratio, and washout ratios of cardiac Meta-iodobenzylguanidine (MIBG) scintigraphy in patients with amyotrophic lateral sclerosis/parkinsonism–dementia complex (ALS/PDC) of Kii.

| Kii ALS-PDC | Age | Sex | Clinical diagnosis | Duration of the illness | Motor neuron signs | Dementia | Parkinsonism | ALSFRS-R*/ Modified H–Y** | Autonomic dysfunction | *H/M* ratio (early/delay) | Wash out (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| #1 | 63 y | Female | ALS | 5 y | BP, PTS, MA | None | None | 23* | None | 2.68/2.68 | n.e. |
| #2 | 64 y | Male | ALS with dementia | 1 y | BP, PTS, MA | MD | None | 37* | None | 2.18/2.35 | 25.0 |
| #3 | 75 y | Female | PDC | 1 y 6 m | PTS | MD | Rgt | 1** | constipation | 2.36/2.01 | n.e. |
| #4 | 65 y | Female | PDC | 3 y | PTS, MA | MD, DO | Rgt, BK, GD | 3** | constipation | 1.47/1.0 | 33.0 |
| #5 | 65 y | Female | PDC | 5 y | PTS, MA | MD | Rgt, BK, GD | 3.5** | constipation | 1.51/1.39 | 34.8 |
| #6 | 66 y | Male | PDC | 7 y | PTS, MA (small hand muscles) | MD | Rgt, BK, GD | 3.5** | OHT | 1.29/1.27 | 37.0 |

H/M ratios were generally correlated with the modified Hoehn–Yahr staging of each patient. Parkinsonism of the patient #5 that showed severe ALS symptoms at the terminal stage was not evaluated.
BP; bulbar palsy, PTS; pyramidal tract signs, MA; muscle atrophy, MD; memory disturbance, DO; disorientation; Hypb, hypobulia; Rgt, rigidity; BK, bradykinesia; GD, gait disturbance; OHT, orthostatic hypotention.
y, year(s); m, months; ALSFRS-R, ALS Functional Rating Scale Revised; H–Y, Hoehn & Yahr; n.e, not examined.

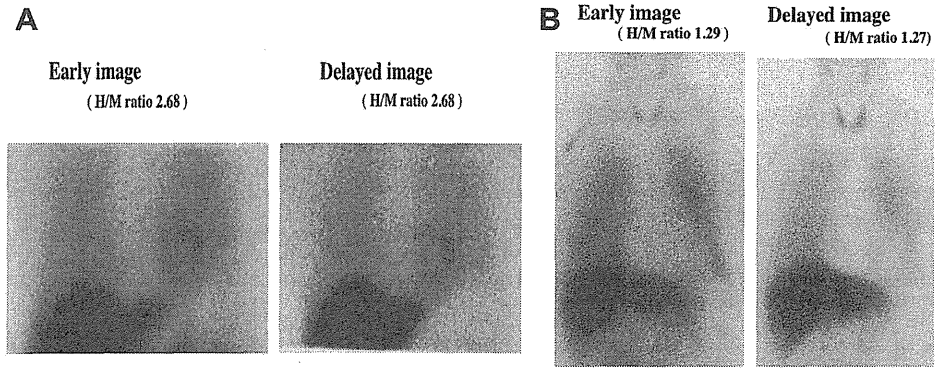


**Fig. 1.** Cardiac Meta-iodobenzylguanidine (MIBG) scintigram of Kii patients with amyotrophic lateral sclerosis/parkinsonism–dementia complex (ALS/PDC). (1A) Patient # 1 showed ALS symptoms without parkinsonism or dementia; the uptake of $^{123}$I-MIBG was normal. (1B) In patient # 6 who showed moderate parkinsonism, dementia, and ALS symptoms; there was almost no uptake of $^{123}$I-MIBG.

pathology in the CNS, but also Lewy axons in the epicardial fatty tissue. MIBG cardiac uptake was normal in patients with the parkin mutation. Lewy bodies do not appear in the CNS of patients with the parkin mutation in general; therefore, the decreased MIBG cardiac uptake is thought to be related to Lewy body pathology. Furthermore, one-third of the patients with multiple system atrophy showed low cardiac MIBG uptake. The presence of Lewy bodies may be related with low cardiac MIBG uptake [5]. Taken together, Lewy body pathology may affect cardiac sympathetic nerves and result in a reduction in the MIBG cardiac uptake.

PDC is fundamentally a tauopathy, but we found α-synuclein pathology in the CNS in over 80% and Lewy body pathology in approximately 56% of the patients with Kii ALS/PDC (unpublished data). Recently, we reported on a patient with Kii PDC who showed a marked decrease of cardiac MIBG uptake and Lewy bodies in both the CNS and the cardiac sympathetic plexus [3]. Taken together, Lewy body pathology is correlated with dysfunction of the postganglionic sympathetic nerves in the heart of Kii ALS/PDC patients. Thus, MIBG measurements might be a useful adjunctive tool for diagnosing not only DLBD and PD, but also very rare cases of PDC.

## Conflicts of interest

None declared.

## Disclosure

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Yasumasa Kokubo*
*Department of Neurology,*
*Mie University Graduate School of Medicine, 2-174, Edobashi,*
*Tsu City, Mie prefecture 514-8507, Japan*

Yoshiyuki Nomura
*Department of Radiology,*
*Mie Prefectural Shima Hospital, Shima city,*
*Mie prefecture, Japan*

Satoru Morimoto
*Department of Neuropathology,*
*Metropolitan Gerontology Hospital,*
*Tokyo, Japan*

Shigeki Kuzuhara
*Department of Medical Welfare,*
*Suzuka University of Medical Science, Suzuka city,*
*Mie prefecture, Japan*

* Corresponding author.
Tel.: +81 59 231 5107;
fax: + 81 59 231 5082.
*E-mail address:* kokubo-y@clin.medic.mie-u.ac.jp (Y. Kokubo)

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Chapter 3

# Amyotrophic lateral sclerosis–parkinsonism–dementia complex in the Kii Peninsula of Japan (Muro disease): a review on recent research and new concept

Shigeki Kuzuhara and Yasumasa Kokubo

More than a century ago, Kin-no-suke Miura, Professor of Internal Medicine of the University of Tokyo discovered a high prevalence of amyotrophic lateral sclerosis (ALS) in the Muro district, the mountainous areas of the southern Kii Peninsula (including the southern parts of the Mie and Wakayama prefectures) (Figure 3.1) (1). In the early 1960s, Kimura, Yase and their colleagues performed a door-to-door survey on patients with ALS in the whole of the Muro district, and discovered two foci of extremely high ALS incidence (i.e. Hohara in the Mie prefecture and Kozagawa in the Wakayama prefecture; see Figure 3.1) (2–4). ALS in these foci showed clinical signs and symptoms produced by selective anatomical involvement of the upper and lower motor neurones in the same manner as Charcot had described in sporadic ALS (5), unique neuropathological features were characterized by the presence of many neurofibrillary tangles (NFTs) throughout the central nervous system (4,6) as in ALS of the Chamorro people in Guam, another high-incidence ALS focus (7,8). In the 1960s, Gajdusek discovered a new ALS focus among the Auyu and Jakai people of southeastern West New Guinea (9). Foci of high-incidence ALS in the Kii Peninsula was thus segregated from classical ALS and classified as 'Kii ALS' or 'Muro disease', a member of the hyperendemic western Pacific ALS family (10).

In Guam, another peculiar neurodegenerative disease or 'parkinsonism–dementia complex' (PDC) was discovered (11,12). PDC was characterized clinically by a combination of progressive dementia and atypical progressive parkinsonism poorly responsive to drugs, and neuropathologically by frontotemporal brain atrophy with presence of many NFTs in the whole central nervous system. Mixed ALS and PDC in which both features were clinically and neuropathologically overlapped were also identified, and ALS and PDC in these high-incidence foci were thus regarded as a spectrum of one disease entity or ALS/PDC (13). Extensive studies failed to solve the cause and pathogenesis of

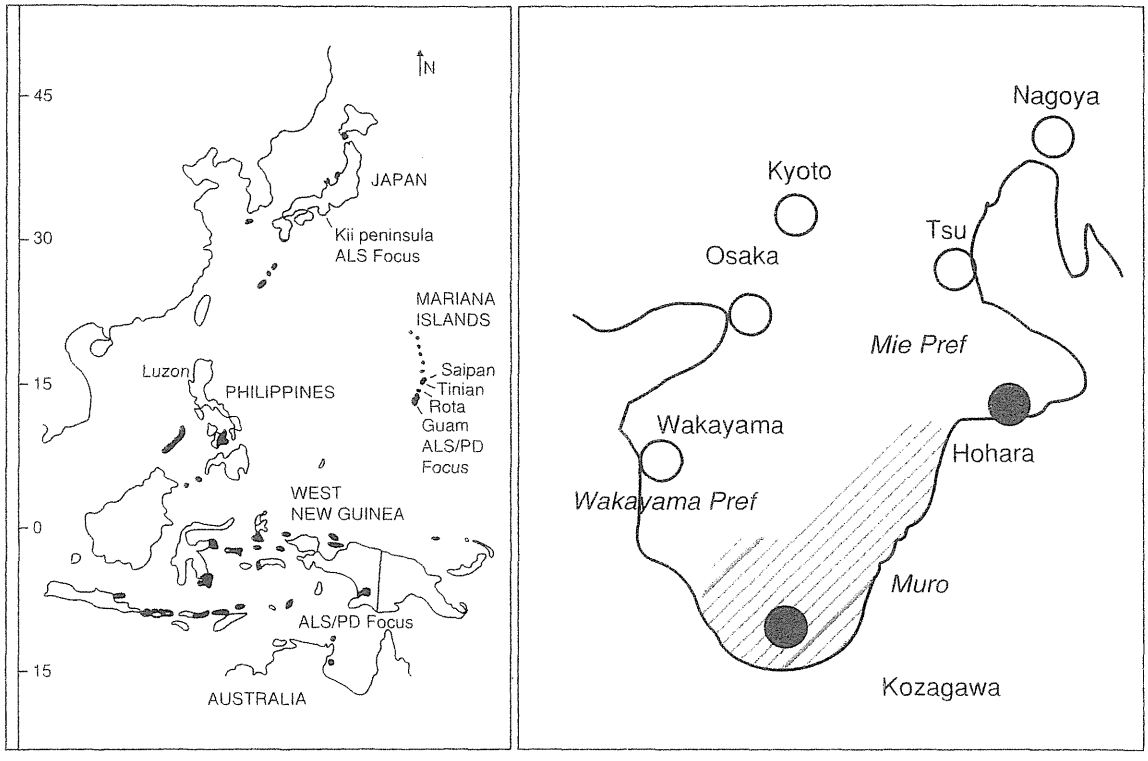


**Fig. 3.1** Western Pacific ALS/PDC foci (left) and enlargement of the Kii Peninsula. The shaded areas are the Muro district. Kozagawa and Hohara were the high-incidence ALS foci during 1950–90. (The left map is a modification of the original map in figure 1 of Garruto *et al.* (14).)

high-incidence ALS/PDC. Meanwhile, high-incidence rates of ALS markedly declined in the 1960s and 1970s, and spontaneously disappeared by the early 1980s in Guam (14). A similar decline of high-incidence rates of ALS during the 1980s was also reported in foci of the Kii Peninsula (15) and West New Guinea (16).

In early 1990s, Kuzuhara paid attention to the reoccurrence of ALS among residents in the Hohara focus. After several years' investigations, we confirmed not only the high-incidence rates of ALS but also the coexistence of a high incidence of progressive parkinsonism and dementia associated with many NFTs in the central nervous system neuropathologically, reminiscent of PDC of Chamorro in Guam (11,12,17,18). The focus of this chapter is to review findings of recent research with respect to the Kii ALS/PDC from the Hohara focus, particularly regarding clinical features, neuroimaging studies, immunohistochemical findings of the brain and spinal cord, genetic studies, and recent epidemiological trends. We will also discuss new disease concepts derived from these findings.

## Clinical features of Kii amyotrophic lateral sclerosis/parkinsonism–dementia complex

At onset, patients developed signs and symptoms of motor neurone disease (MND), parkinsonism, or dementia. Of the 26 new cases of ALS/PDC neurologically confirmed during 1996 and 1999 in Hohara, four patients developed MND signs consistent with ALS, while 22 patients developed parkinsonism and dementia almost simultaneously (Figure 3.2) (19). Patients with MND who did not demonstrate features of parkinsonism

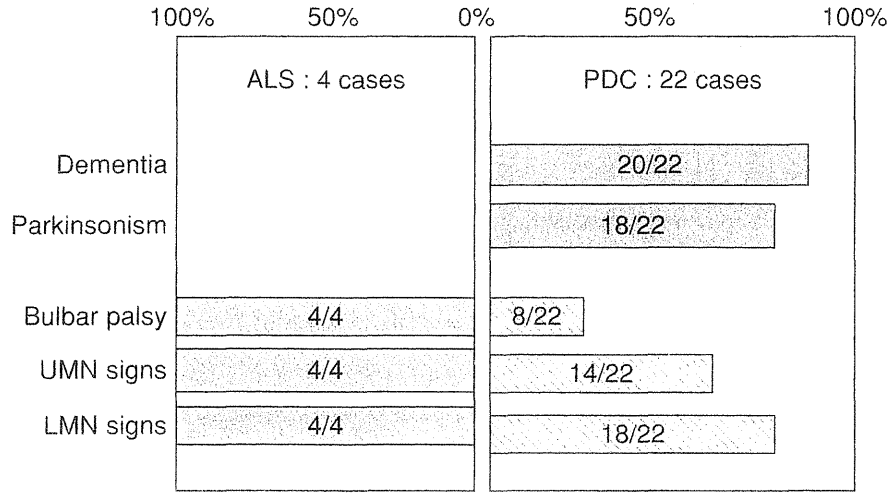


Fig. 3.2 Clinical manifestations of 26 cases of ALS and PDC examined during 1996 and 1999. All four patients with ALS manifested the signs and symptoms of selective motor neurone involvement, while patients with PDC presented with motor neurone disease symptoms several years after the establishment of parkinsonism and dementia (19).

or dementia during their disease progression were classified into 'pure ALS'. Patients with PDC at onset who developed signs of MND during their disease course were classified into 'PDC/ALS'. Rarely, patients who simultaneously developed signs of MND and dementia at onset were classified into the 'ALS–dementia complex (ALSDC)'. Similar clinical overlap of ALS and PDC was described in patients with Guam ALS/PDC in the 1980s (20).

Patients with pure ALS presented with the same clinical signs and symptoms caused by the selective involvement of the upper and lower motor neurones as described by Charcot in sporadic ALS (5). Three main clinical subtypes of ALS were observed: classic ALS, progressive bulbar palsy, and progressive muscular atrophy. Patients with ALS died due to either respiratory paralysis or complications of bulbar palsy. Parkinsonism presented with bradykinesia, rigidity, and postural instability in all cases, and tremor of resting or postural type in some. Parkinsonian motor symptoms responded poorly to levodopa and other antiparkinsonian drugs. Dementia was characterized by deficits of such fundamental frontal functions as basic attention, motivation/initiation, and information processing speed, which were consistent with those of frontal–subcortical dementia (21). Forgetfulness, difficulty in memory retrieval, and slowing in cognition, mental processing, and speech were present, although mild. Patients with PDC fell into akinetic mutism at the late stage. Any patients with PDC and other dementia–ALS/parkinsonism complex (PDC/ALS and ALSDC) did not manifest severe amnesia, aphasia, apraxia, agnosia, disorientation in time and place, and behavioural psychological syndromes frequently seen in Alzheimer's disease, severe personality changes, abnormal behaviours and speech disturbances suggestive of frontotemporal dementia, or severe psychosis and hallucination implying dementia with Lewy bodies.

In summary, Kii ALS/PDC presented clinically with three core features: ALS/MND, frontal–subcortical dementia, and progressive atypical parkinsonism unresponsive to levodopa and other antiparkinsonian drugs. These features occurred individually or in

combination, and pure ALS, PDC, and other dementia–ALS/parkinsonism complex were differentiated on the basis of clinical features at the onset and subsequent overlapping symptoms.

## Neuroimaging

Either computed tomography or magnetic resonance imaging were applied for anatomical studies and single photon emission computed tomography (SPECT) with a tracer technetium ($^{99m}$Tc) ethylcysteinate dimer for cerebral blood flow (22). Patients with pure Kii ALS did not show obvious brain atrophy on computed tomography and magnetic resonance imaging (Figure 3.3a), but demonstrated reductions in cerebral blood flow on SPECT (Figure 3.3b). In contrast, patients with PDC and PDC/ALS developed rapidly progressing symmetrical brain atrophy on computed tomography and magnetic resonance imaging, most prominently at the pole of the frontal and temporal lobes with marked dilatation of the lateral ventricles and inferior horns (Figure 3.3c). This was associated with a marked

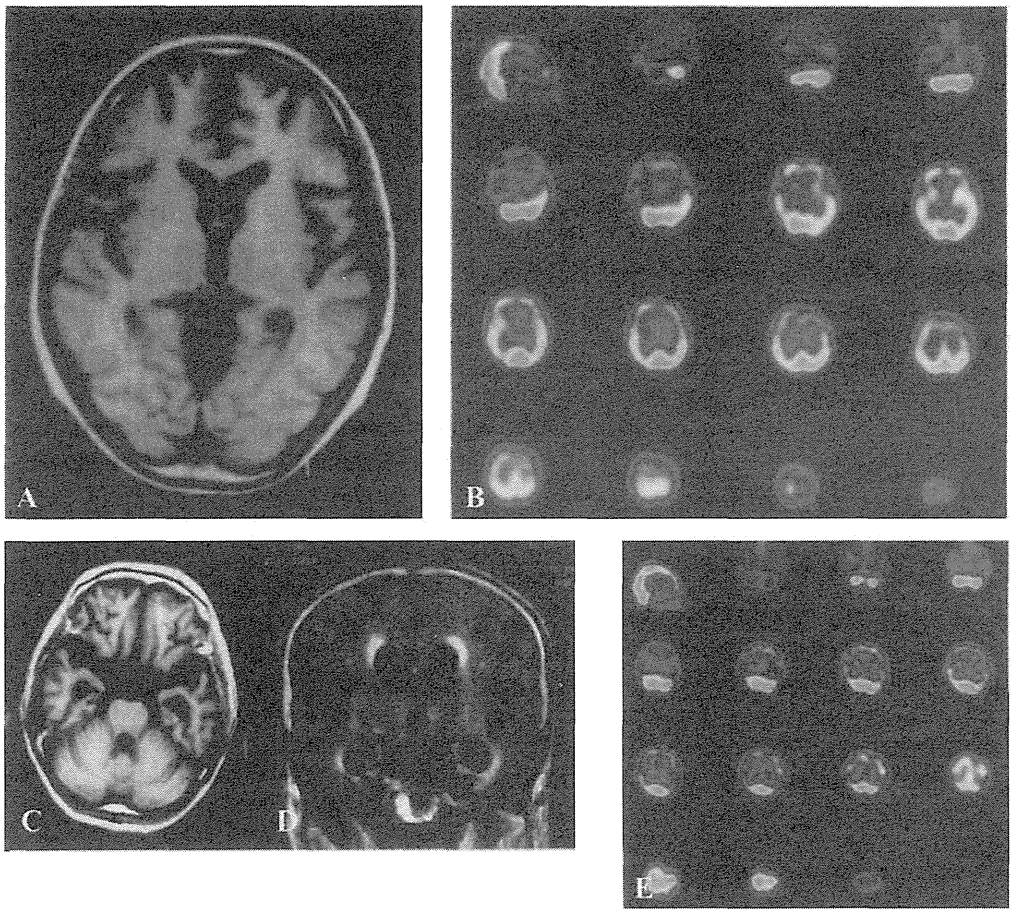


Fig. 3.3 Neuroimaging of Kii ALS and PDC. Kii ALS cases show no or mild atrophy of the frontal and temporal lobes on magnetic resonance imaging (a) with obvious decrease in cerebral blood flow of the frontal and temporal lobes on SPECT with a tracer technetium ($^{99m}$Tc) ethyl cysteinate dimer (b), while Kii PDC cases show marked atrophy of the frontal and temporal lobes with ventricular dilatation on magnetic resonance imaging (c) with marked loss in cerebral blood flow of the frontal and temporal lobes on SPECT (d). Note that the atrophy and decrease in cerebral blood flow are symmetrical.

reduction in cerebral blood flow on ($^{99m}$Tc) ethylcysteinate SPECT (Figure 3.3d). In the neuroimaging study, findings of the frontal and temporal lobes, exact symmetry in atrophy, and hypometabolism were characteristic of Kii ALS/PDC, in contrast to the marked asymmetry in the frontotemporal dementias, including behavioural variant, semantic dementia, and progressive non-fluent dementia (23).

## Neuropathological findings

Typical ALS pathology combined with neurofibrillary degeneration of mild to moderate degrees in the neurones of the brainstem and cortex of the temporal and frontal lobes was reported in pure Kii ALS cases from the Kozagawa focus (4,10,24). We reported similar findings in pure ALS cases from the Hohara focus and the first neuropathological findings of Kii PDC in 2001 (17). More than 12 autopsy cases have been studied to date (18).

## Motor neurone degeneration and neurofibrillary degeneration

The neuropathological findings of 12 autopsy cases of ALS/PDC from seven different families (families A–G) in the Hohara focus are shown in Table 3.1. Macroscopically, the brains of pure ALS and ALSDC cases showed mild atrophy, while brains of PDC and PDC/ALS cases showed marked atrophy of the frontal and temporal lobes (Figure 3.4a).

**Table 3.1** Neuropathological findings of 12 ALS/PDC cases

| Case number according to their families (A–G) | Clinical diagnosis | Brain weight | Motor neurone disease pathology | | NFT | | | SP | LB |
|---|---|---|---|---|---|---|---|---|---|
| | | (g) | UMN | LMN | Bunina body | Mesial temporal | Brainstem | Spinal cord | | |
| A-1 | ALS | 1190 | 1+ | 3+ | – | 1+ | 1+ | – | – | – |
| B-1 | ALS | 1275 | 1+ | 3+ | 1+ | 3+ | 3+ | – | – | – |
| C-1 | ALS | 1140 | 1+ | 2+ | | 1+ | 1+ | – | – | – |
| D-1 | ALS | 1190 | 3+ | 3+ | 2+ | 1+ | 2+ | – | – | 2+ |
| E-1 | ALSDC | ND | | NA | NA | 3+ | 2+ | NA | 2+ | 2+ |
| B-2 | ALSDC | 1210 | 1+ | 3+ (XII) | NA | 3+ | 2+ | NA | – | 2+ |
| E-2 | ALSDC | 1320 | 1+ | NA | NA | 2+ | 2+ | NA | – | 1+ |
| E-3 | ALSDC | 1300 | – | 2+ | 2+ | 3+ | 3+ | 1+ | – | – |
| A-2 | PDC | 935 | 1+ | 2+ | | 3+ | 2+ | 1+ | – | – |
| F-1 | PDC | 1085 | 1+ | 2+ | | 3+ | 3+ | 1+ | – | – |
| E-4 | PDC/ALS | 960 | 3+ | 2+ | 1+ | 3+ | 3+ | 1+ | – | – |
| G-1 | PDC/ALS | 875 | 3+ | 1+ | 1+ | 3+ | 3+ | – | – | – |

ALS, amyotrophic lateral sclerosis; ALSDC, ALS-dementia complex; LB, Lewy body; LMN, lower motor neurone; NA, not available; ND, not described; NFT, neurofibrillary tangle; PDC, parkinsonism–dementia complex; PDC/ALS, PDC overlapping ALS; SP, senile plaque; UMN, upper motor neurone; 1+, mild; 2+, moderate; 3+, marked.

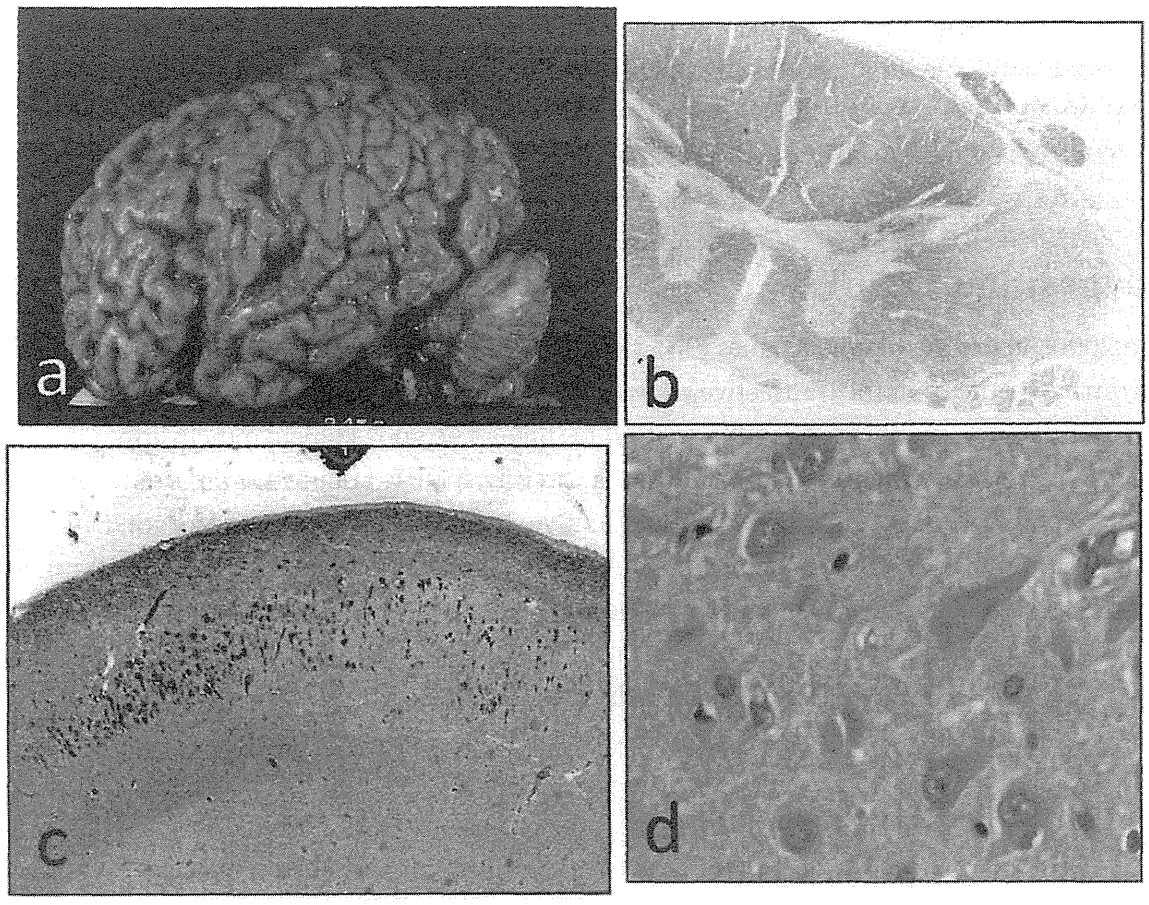


**Fig. 3.4** Neuropathological findings of Kii PDC. (a) Fresh unfixed brain shows marked atrophy of the frontal and temporal lobes, particularly in the poles. (b) The spinal cord shows degeneration of the lateral tracts and loss of anterior horn cells (Klüver–Barrera stain). (c) Many NFTs in the hippocampus demonstrated with Bielschowsky stain. (d) Basophilic NFTs and Hirano bodies in the hippocampus (haematoxylin–eosin stain). Reprinted from Shigeki Kuzuhara et al., *Annals of Neurology*, Wiley 2001.

Spinal cords and anterior spinal roots were thin in ALS, ALSDC, and PDC/ALS cases. Pure Kii ALS cases showed classical pathology of sporadic ALS, including pallor of the corticospinal tracts (Figure 3.4b), degeneration and loss of anterior horn cells, and Bunina bodies in the anterior horn cells. Similar degeneration of the upper and lower motor neurones was present in the PDC cases without obvious clinical signs of MND.

Neurofibrillary degeneration associated with no or few senile plaques was observed throughout the cerebral cortex, brainstem, and spinal cord. This was most prominent in cases of PDC, PDC/ALS, and ALSDC, and mildly to moderately evident in pure ALS cases. They were most numerous in the brainstem, hippocampus, parahippocampal gyrus, and cortex of the temporal and frontal lobes (Figure 3.4c). NFTs in pure ALS cases were quantitatively less in number than in PDC and other dementia–ALS/parkinsonism complex, but qualitatively the same in the distribution pattern (25). A similar overlap of the neuropathological findings of ALS and PDC, namely coexistence of selective motor system degeneration and widespread neurofibrillary degeneration, has also been reported in ALS/PDC of Guam (20). As observed with either silver staining or immunohistochemistry (amyloid β-protein), senile plaques were absent or very few in number. Haematoxylin and

eosin preparations of the hippocampus revealed numerous basophilic and eosinophilic NFTs, granulovacuolar degeneration and Hirano bodies (Figure 3.4d). Lewy body pathology was present in approximately one-third of cases examined on preparations with haematoxylin and eosin stain or α-synuclein immunohistochemistry (Table 3.1).

## Transactive response DNA-binding protein of 43 kDa deposition

In 2006, Neumann and colleagues (26) and Arai and colleagues (27) reported independently from each other that the transactive response DNA-binding protein of 43 kDa (TDP-43) was the primary component of the ubiquitinated inclusions and skein-like inclusion of frontotemporal lobar degeneration and ALS. Subsequently, TDP-43 deposition in brains of Guam PDC cases was reported (28). We subsequently demonstrated the deposition of TDP-43 in brains of both ALS and PDC cases of Kii ALS/PDC on Western blotting and immunohistochemistry (29). Using a polyclonal antibody against TDP-43 (ProteinTech Group, Chicago, IL, USA), we observed neuronal cytoplasmic inclusions within neurones of the dentate gyrus of the hippocampus (Figure 3.5a), and skein-like inclusions (Figure 3.5b), granular inclusions (Figure 3.5b), and round bodies (Figure 3.5c) in anterior horn cells. We also observed neuritic inclusions and glial inclusions in the spinal cord. Intranuclear type inclusions were not detected.

In summary, selective degeneration of the upper and lower motor neurones consistent with ALS, widely distributed NFTs throughout the central nervous system, and TDP-43-immunoreactive inclusions in the neurones of the brain and spinal cord were the core neuropathological changes common to all clinical phenotypes of ALS/PDC. However, the severity of each individual pathological change varied among the different clinical phenotypes.

## Pathogenesis

### Environmental factors

Yase reported low calcium and magnesium and high aluminium and manganese in water taken from wells, rivers, and soil in the high-incidence ALS foci in the Kii Peninsula and Guam (30). On the basis of these observations, he proposed the 'metal-induced calcified neurone degeneration hypothesis' that chronic hypocalcaemia and hypomagnesaemia, which had been produced by long-term intake of excess aluminium and too low calcium levels in drinking water and food might have caused secondary hyperparathyroidism and induced motor neurone degeneration. Experimental studies in rodents and primates, which had been fed with diet and water containing low calcium and/or high aluminium, succeeded in producing widespread neuronal degeneration of the central nervous system, but failed to produce selective motor neurone degeneration of ALS and NFTs (31). In the 1990s, Iwami and colleagues re-examined the levels of minerals in the drinking water and food in Hohara and neighbouring control areas, and compared the data with the incidence rates of ALS (32). They found excess manganese intake from food coupled with low

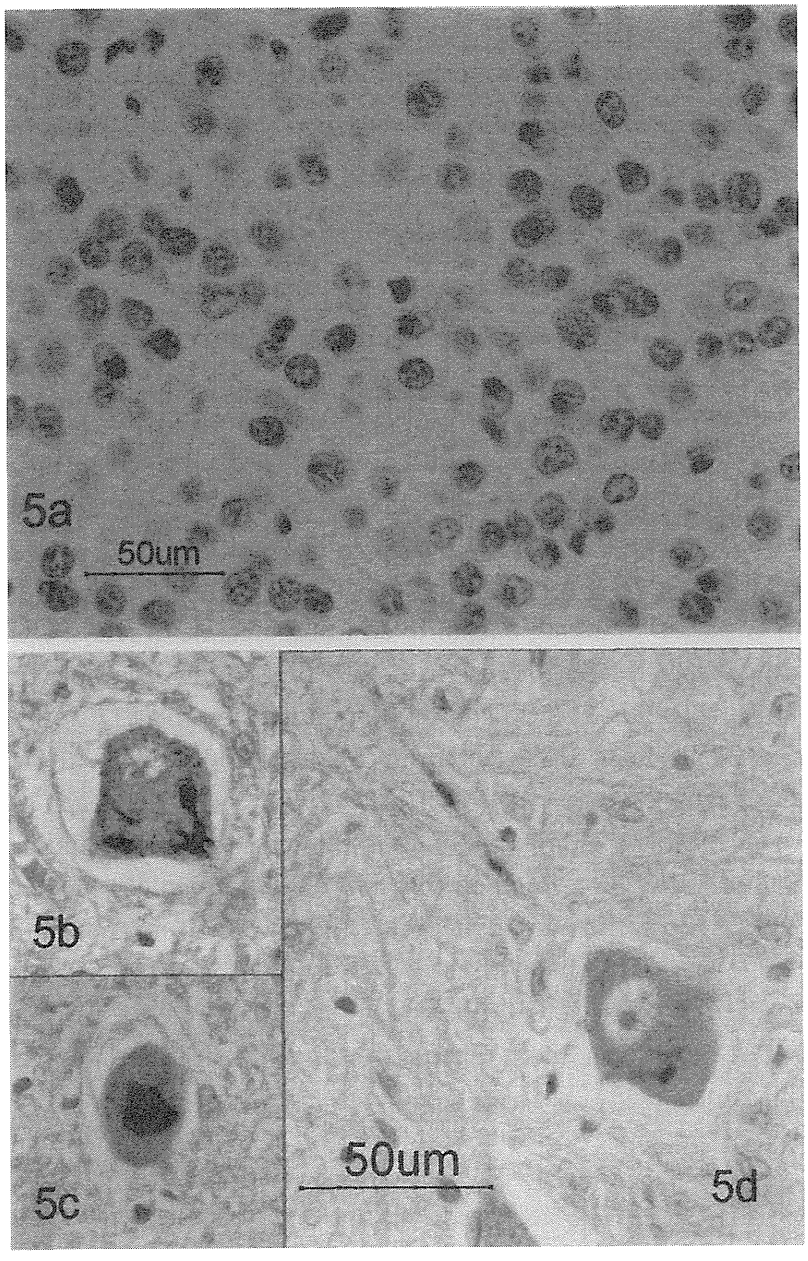


Fig. 3.5 Transactive response DNA-binding protein 43 (TDP-43) immunohistochemistry. A polyclonal antibody against TDP-43 (ProteinTech Group, Chicago, IL, USA) immunolabels many cytoplasmic inclusions in neurones of the dentate gyrus of the hippocampus, with the nuclei of the nerve cells containing inclusions unlabelled (a). TDP-43 immunoreactive skein-like inclusions (b), round inclusions (c), and granular inclusions (d) were observed in the anterior horn cells of the spinal cord.

magnesium in drinking water, and concluded that these findings could account for the high incidence rates of ALS in this focus. We examined serum contents of minerals such as calcium, phosphate, magnesium, and aluminium together with serum levels of alkaline phosphatase and parathyroid hormone in residents in Hohara and neighbouring control areas. We observed that all mineral levels as well as alkaline phosphatase and parathyroid hormone levels were within normal limits, with no significant differences between Hohara and control regions (unpublished data). These findings suggested that

the *in vivo* metabolism of calcium and other minerals could be modulated normally even if the levels of minerals and metals in the drinking water and food were far higher or lower than average. Of note, Ahlskog and colleagues reported normal levels of calcium, aluminium, potassium, and parathyroid hormone in the serum of patients with ALS/PDC in Guam (33).

In the Guam focus of ALS and ALS/PDC, toxins derived from cycad, including β-*N*-methylamino l-alanine, were suspected to have caused neurodegeneration and produced ALS/PDC (34,35) (see also Garruto, Chapter 1). Subsequent research failed to convincingly establish a causal link with cycad consumption and ALS/PDC. However, in recent years, Cox and colleagues have proposed a new cycad hypothesis in which β-*N*-methylamino l-alanine was originally biosynthesized by cyanobacteria in cycad roots and then biomagnified in the body of cycad-eating fruit bats (36,37). They postulated that Chamorro people had ingested high levels of toxins by eating the bats, and developed ALS/PDC decades later. Disappearance of fruit bat populations by overhunting resulted in cessation of eating bats and led to the decline of ALS/PDC decades later. Borenstein and colleagues subsequently reported that eating cycad in young adulthood was a risk factor for dementia, mild cognitive impairment, and PDC on Guam, but that there were no associations with the outcomes for consumption of fruit bats or exposure to cycad used as a tropical medicine (38). In the 1980s, cycad had been suspected as the culprit of Kii ALS, but people in Japan, including the Kii Peninsula, had never consumed cycad as daily food although a small amount of cycad flour might have been taken by a few people as an ingredient of herb medicine (39,40). Our survey on medical history revealed that none of the patients with ALS/PDC in the Hohara focus had taken cycad or bats before onset of the disease. While exposure to cycad may be in part related to the pathogenesis of ALS/PDC in Guam, there is thus no evidence that it played a pathogenic role in the Kii Peninsula.

## Genetic studies

In the Hohara focus, approximately 70% of patients with ALS/PDC had a positive family history of ALS/PDC in the 1960s and approximately 80% in the 1990s (unpublished data). In some families, the disease occurred vertically in several generations or horizontally in siblings, suggestive of either an autosomal-dominant or autosomal-recessive transmission. Consanguineous marriage was frequent in the 1960s in Hohara (4). We confirmed more than 10 patients who had developed ALS/PDC many years (24–51 years) after they had left the village, and most of them had affected relative(s) of the first or second degree (unpublished data). A high frequency of positive family history in the residents in the high-incidence ALS focus and development of ALS/PDC after long latent periods from migration from the focus suggested genetic abnormalities in the pathogenesis of Kii ALS/PDC.

Gene abnormalities, which had been previously established as the cause or risk of familial or sporadic ALS, dementia, familial Parkinson's disease, and parkinsonism were analysed, but all of them were negative (Table 3.2) (17,41). Gene analyses were performed on

**Table 3.2** Causative and risk gene analysis

| Neurodegenerative disease | Causal and risk genes |
|---|---|
| Alzheimer's disease | ApoE-ε4 |
| Familial ALS/motor neurone disease | SOD1, TDP-43, FUS |
| Spinal muscular atrophy | SMN |
| Frontotemporal dementia/lobar degeneration | MAPT (exons and introns), PPGN, TDP-43, GSK3 |
| Familial parkinsonism/Parkinson's disease | SNCA, LRRK2 (dardarin), PRKN, DJ-1, PINK1, ATP13A2, MAPT |
| Progressive supranuclear palsy | MAPT intron polymorphism in dinucleotide repeats |
| Guam ALS/PDC | TRPM7 |

ApoE-ε4, apolipoprotein E4 gene; ATP13A2, ATPase type 13A2 gene; DJ-1, oncogene and causative gene of PARK7; FUS/TLS, fused in sarcoma/translated in liposarcoma; GSK3B, glycogen synthase kinase-3β gene; LRRK2 (dardarin), leucine-rich repeat kinase 2 gene; MAPT, tau gene; PGRN, progranulin gene; PINK1, PTEN-induced putative kinase 1 gene; PRKN, parkin gene; SMN, survival motor neurone gene; SNCA, α-synuclein gene; SOD1, Cu/Zn superoxidase dismutase-1 gene; TDP-43, transactive response (TAR) DNA-binding protein of 43 kDa gene; TRPM7, transient receptor potential melastatin 7 gene; UCH-L1, ubiquitin carboxy-terminal hydrolase L1.

apolipoprotein E4 genotype (apoe-ε4) phenotype as well as for mutations in the amyloid precursor protein gene, Cu/Zn superoxidase dismutase-1, *TDP-43* gene, survival motor neurone gene, progranulin, microtubule-associated protein *tau*, glycogen synthase kinase-3β, α-synuclein, leucine-rich repeat kinase 2 (dardarin), parkin, DJ-1, PTEN-induced putative kinase 1, and ATPase type 13a2. All were negative.

Hermosura *et al.* have recently discovered a nuclear mutation in the calcium/magnesium membrane ion channel transient receptor potential melastatin 7 (*TRPM7*) gene in a subset of patients with Guam ALS/PDC (42). They speculated that the defective channel might have caused abnormal metabolism of these metals and induced neuronal degeneration resulting in ALS/PDC in Guam, as the channel is a bifunctional protein involved in homeostatic regulation of intracellular calcium and magnesium and trace metal concentration. In patients with Kii ALS/PDC, however, no mutation was found in the *TRPM7* gene (43). Further studies to identify the susceptible loci or genes of Kii ALS/PDC are ongoing.

## Epidemiology

Kimura, Yase and their colleagues started a continuous epidemiological survey and enrolment of patients with ALS in Hohara and Kozagawa in the 1960s, including a retrospective survey of the 1940s (2,3). The diagnoses were confirmed based on neurological examination and information from patients' families, medical records, and charts from hospitals, clinics, and nursing homes. The survey was discontinued in the late 1980s when no new cases had been identified over the course of several years (15). In the early 1990s, however, we restarted the survey after having observed new patients with ALS from Hohara, and included other neurodegenerative disorders (17).

## Changing patterns and incidence rates of the Kii amyotrophic lateral sclerosis/parkinsonism–dementia complex in Hohara (1950–2004)

Eighty-four patients were enrolled in the survey between 1950 and 2004 (Table 3.3). This included 40 cases of pure ALS, 14 ALSDC, 22 PDC, and eight PDC/ALS (44). The 5-year-average annual incidence rates per 100 000 population age-adjusted to the Japanese population in 1985 were calculated for pure ALS cases versus PDC and other dementia–ALS/parkinsonism complex (Figure 3.6). The annual incidence rate of pure ALS per 100 000 sharply declined from 107 in the 1950s to 20 in the 1980s, while that of PDC and other dementia–ALS/parkinsonism complex sharply arose from 20 before 1980 to 60 after 1980, mainly due to increase of PDC and PDC/ALS.

The cause(s) of the marked declines of pure ALS and increases of PDC and other dementia–ALS/parkinsonism complex remains unsolved. Using the urinary 8-hydroxydeoxyguanosine (8-OHDG)/creatinine ratio as a marker of oxidative stress, we examined 11 patients with Kii ALS/PDC and eight normal controls and observed an elevation of the mean level of urinary 8-OHDG/creatinine ratio of patients with Kii ALS/PDC in comparison with that of control subjects (45). This suggests that oxidative stress might be related to pathogenesis of Kii ALS/PDC and that changes of environmental factors related to oxidative stress may have influenced the change in phenotypic patterns of Kii ALS/PDC.

For the 50 years after World War II, both the natural and socio-cultural environments and lifestyle of residents in Hohara dramatically westernized, as in other areas in Japan. Drinking water from the well was changed to tap water supplied from the reservoir or

**Table 3.3** Number of new cases of ALS, PSC, PDC/ALS, and ALSDC, during each 5 year period

| Years | Population of the beginning year | Pure ALS | PDC and other ALS/PDC | | | |
|---|---|---|---|---|---|---|
| | | | | PDC | PDC/ALS | ALSDC |
| 1950–54 | 2681 | 9 | 1 | 0 | 0 | 1 |
| 1955–59 | 2163 | 4 | 2 | 1 | 0 | 1 |
| 1960–64 | 2185 | 6 | 0 | 0 | 0 | 0 |
| 1965–69 | 2125 | 3 | 2 | 0 | 1 | 1 |
| 1970–74 | 2160 | 3 | 2 | 0 | 1 | 1 |
| 1975–79 | 1816 | 4 | 0 | 0 | 0 | 0 |
| 1980–84 | 1745 | 2 | 2 | 0 | 0 | 2 |
| 1985–89 | 1684 | 1 | 2 | 0 | 0 | 2 |
| 1990–94 | 1642 | 4 | 10 | 7 | 2 | 1 |
| 1995–99 | 1543 | 3 | 15 | 11 | 1 | 3 |
| 2000–04 | 1441 | 1 | 8 | 3 | 3 | 2 |
| | | 40 | 44 | 22 | 8 | 14 |

ALS, amyotrophic lateral sclerosis; ALSDC, ALS–dementia complex; PDC, parkinsonism–dementia complex; PDC/ALS, PDC overlapping ALS.

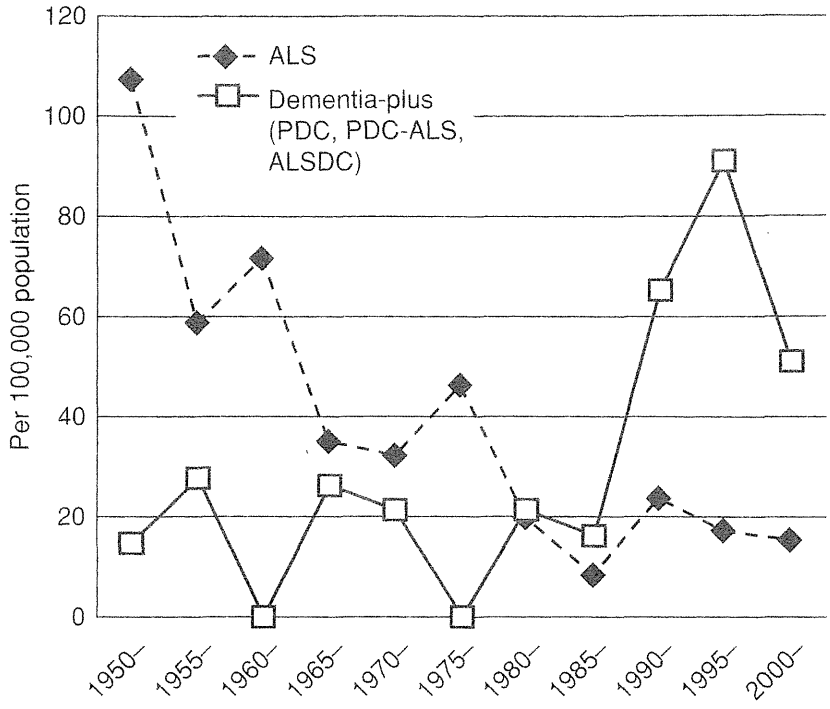


**Fig. 3.6** Five-year average incidence rates of Kii ALS/PDC (age-adjusted to the Japanese population in 1985). The incidence of pure ALS sharply declined during 1950 and 1980, while the incidence of PDC and other dementia–ALS/parkinsonism complex jumped up in 1990s.

mineral water bought in supermarkets and food was changed from traditional Japanese style to Western style. Another dramatic change was an increase in the elderly population, as the average life expectancy of Japanese people rose from 58.0 years in men and 61.5 years in women in 1950, to 77.7 years in men and 84.6 in women in 2000 (46). We have postulated that a combination of changes in diet, water source, and lifestyle and rapid progression of ageing of population in these 50 years may have modulated the pathogenesis of Kii ALS/PDC and resulted in the decrease in pure ALS and increase in PDC and other dementia–ALS/parkinsonism complex affecting the elderly.

## Comparison with the epidemiological trends of amyotrophic lateral sclerosis/parkinsonism–dementia complex in Guam

In Guam, the occurrence of ALS rapidly declined after World War II, and high incidence of ALS disappeared by the early 1980s (14) (see also Garruto, Chapter 1). Incidence rates of PDC also declined but still remain common (47). Similarly, in the Kii ALS/PDC focus, the incidence rates of pure ALS rapidly declined for these 50 years, while PDC and other dementia–ALS/parkinsonism complex rates sharply increased after 1980 (Figure 3.5). Recent epidemiological studies in Guam ALS/PDC have, however, indicated high prevalence rates of late-onset dementia or 'Mariana dementia' among elderly Chamorro people (48). While the clinical features of the Mariana dementia resemble those of Alzheimer's disease, it is not yet known whether the neuropathological characteristics are those of Alzheimer's disease or those of the ALS/PDC spectrum. A similar survey on dementia-only cases in late life has not been done in the Kii Peninsula ALS foci.

ALS/PDC in the Kii Peninsula and ALS/PDC in Guam seem quite akin to each other clinically and neuropathologically, but quite different from each other not only in their racial and ethnic backgrounds but also in environmental factors such as food, drinking water, culture, lifestyle, and others. It is also very intriguing that there are similar changes in the incidence rates and clinical phenotypes in both Guam and the Kii Peninsula, although the causes remain unknown for either. As discussed by Garruto and Yanagihara, the western Pacific hyperendemic foci of ALS/PDC, represent natural experimental models of chronic neurodegenerative disease that occur in different cultures, different ecological zones, and among genetically diverse human groups (49). In these hyperendemic areas, some hitherto unknown environmental factor(s) may exist that interact with susceptible gene(s) of the indigenous people, giving rise to both a tauopathy and a TDP-43 proteinopathy in the central nervous system, which clinically manifests ALS, parkinsonism, and dementia (Figure 3.7).

In summary, we have reviewed recent findings of current concepts in Kii ALS/PDC. This complex is composed of three core features (i.e. ALS, dementia, and parkinsonism). The clinical features of ALS were identical to those of classical sporadic ALS while the dementia was characterized by deficits of frontal functions that differed from both Alzheimer's disease and the frontotemporal dementias. Rigid akinetic parkinsonism unresponsive to levodopa was present in many, while parkinsonism tremor in a few. Regardless of clinical phenotype, all patients showed both ALS pathology and NFT pathology of varying degrees. TDP-43 immunohistochemistry revealed positive cytoplasmic inclusions in neurones of the dentate nucleus of the hippocampus, and skein-like inclusions and other inclusions in the spinal anterior horn cells in all clinical phenotypes. Lewy bodies were seen in approximately one-third of autopsy cases. Senile plaques were absent or few. Although the genetic basis of this disorder remains unknown, epidemiological studies suggest that environmental

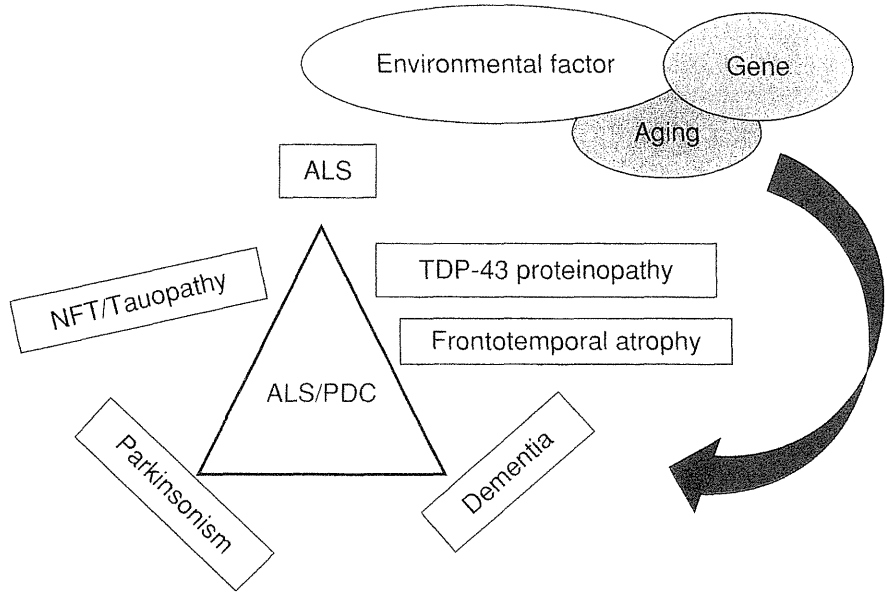


**Fig. 3.7** A scheme of the gene–environmental interaction hypothesis in the hyperendemic ALS/PDC foci in the western Pacific isolated islands.

factors interacting with susceptible gene(s), combined with ageing of the population, have contributed to a change in phenotypic patterns of Kii ALS/PDC. Further studies are necessary to clarify the pathogenesis and cause of this hyperendemic neurodegenerative disease.

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