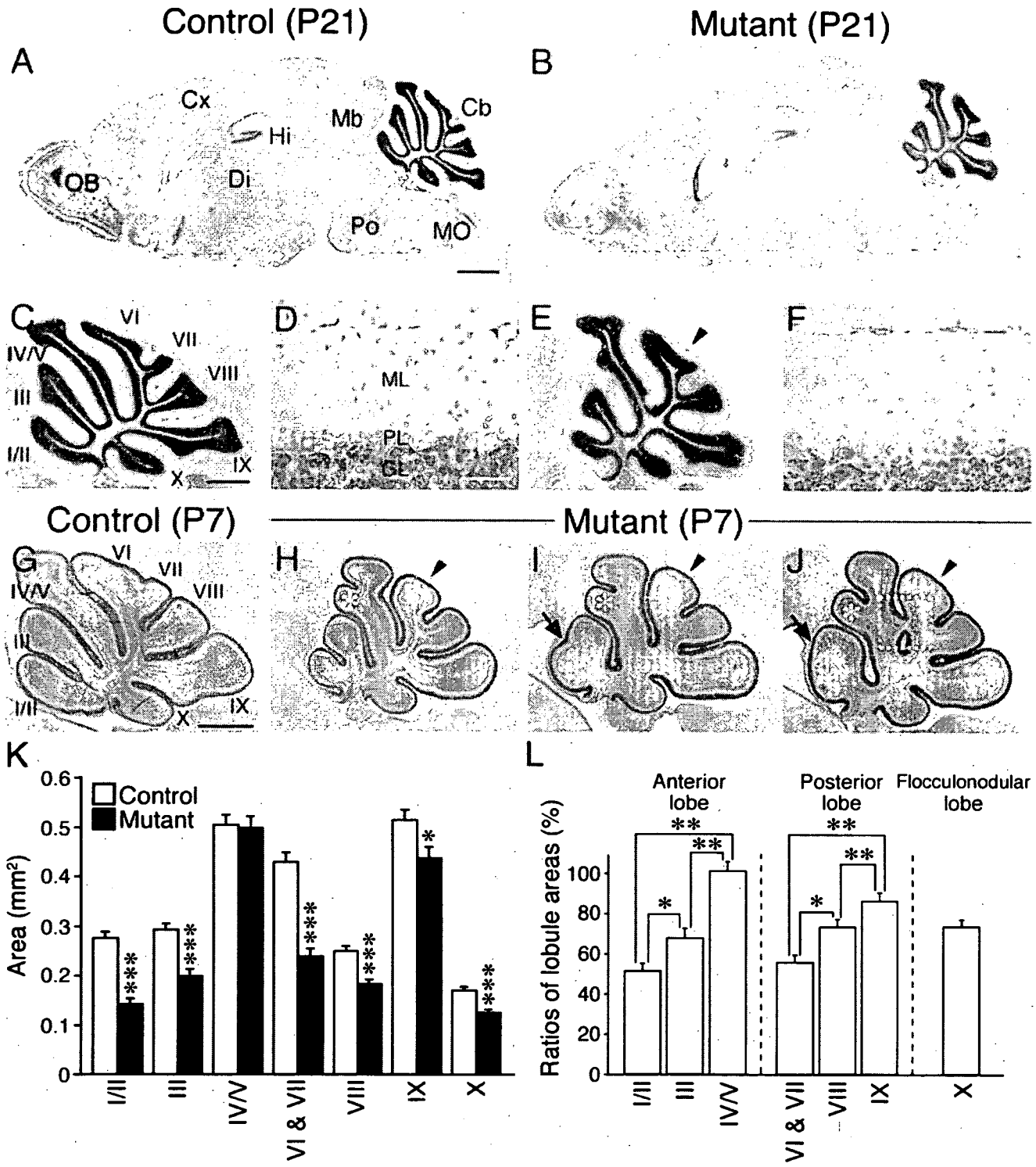


FIG. 3. Abnormal cerebellar foliation in neuron/glia-specific FAK mutant mice. (A–F) Hematoxylin staining of parasagittal paraffin sections through control (A, C and D) and mutant (B, E and F) brains at postnatal day (P)21. High-powered micrographs of the cerebellar cortex (D and F). The arrowhead in (E) indicates the absence of the intercrural fissure between the lobules VI and VII. (G–J) Cresyl violet staining of midsagittal cerebellar sections from control (G) and mutant (H–J) mice at P7. The arrowheads and arrows in (H–J) indicate the absences of the intercrural fissure between the lobules VI and VII, and the precentral fissure between the lobules I/II and III, respectively. Asterisks indicate the lobule-like protrusion in the lobule IV/V. A boxed region in (J) indicates the fusion of the lobules IV/V and VI. (K) Decrease in the area of cerebellar lobules in mutant mice at P7 ($n = 16$ for each). Data are expressed as mean ± SEM. $^{*}P < 0.05$, $^{***}P < 0.001$, Student's *t*-test. (L) Ratios of lobule areas between control and mutant mice at P7 ($n = 16$ for each). Data are expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, one-way ANOVA with *post hoc* Tukey–Kramer test. Cb, cerebellum; Cx, cerebral cortex; Di, diencephalon; GL, granular layer; Hi, hippocampus; I–X, cerebellar folium I–X; Mb, midbrain; ML, molecular layer; MO, medulla oblongata; OB, olfactory bulb; PL, PC layer; Po, pons. Scale bars: 1 mm (A); 200 μm (C and G); 50 μm (D).

increased in the white matter at P21, suggesting its late onset of glial expression, most likely, in oligodendrocytes. In mutant mice, the hybridization signals were markedly diminished throughout the brain, with residual low expression in the caudal cortex, deep layers of the cerebral cortex and hippocampus (Figs 1E and 2D–F). Gene ablation mediated by the nestin-Cre transgenic mice is incomplete in some regions, including the hippocampus (Imai *et al.*, 2006).

The FAK expression pattern was examined at P21 by immunohistochemistry. Strong FAK immunolabeling was detected in the cerebral cortex, hippocampus and thalamus in control mice (Fig. 1F). In mutant

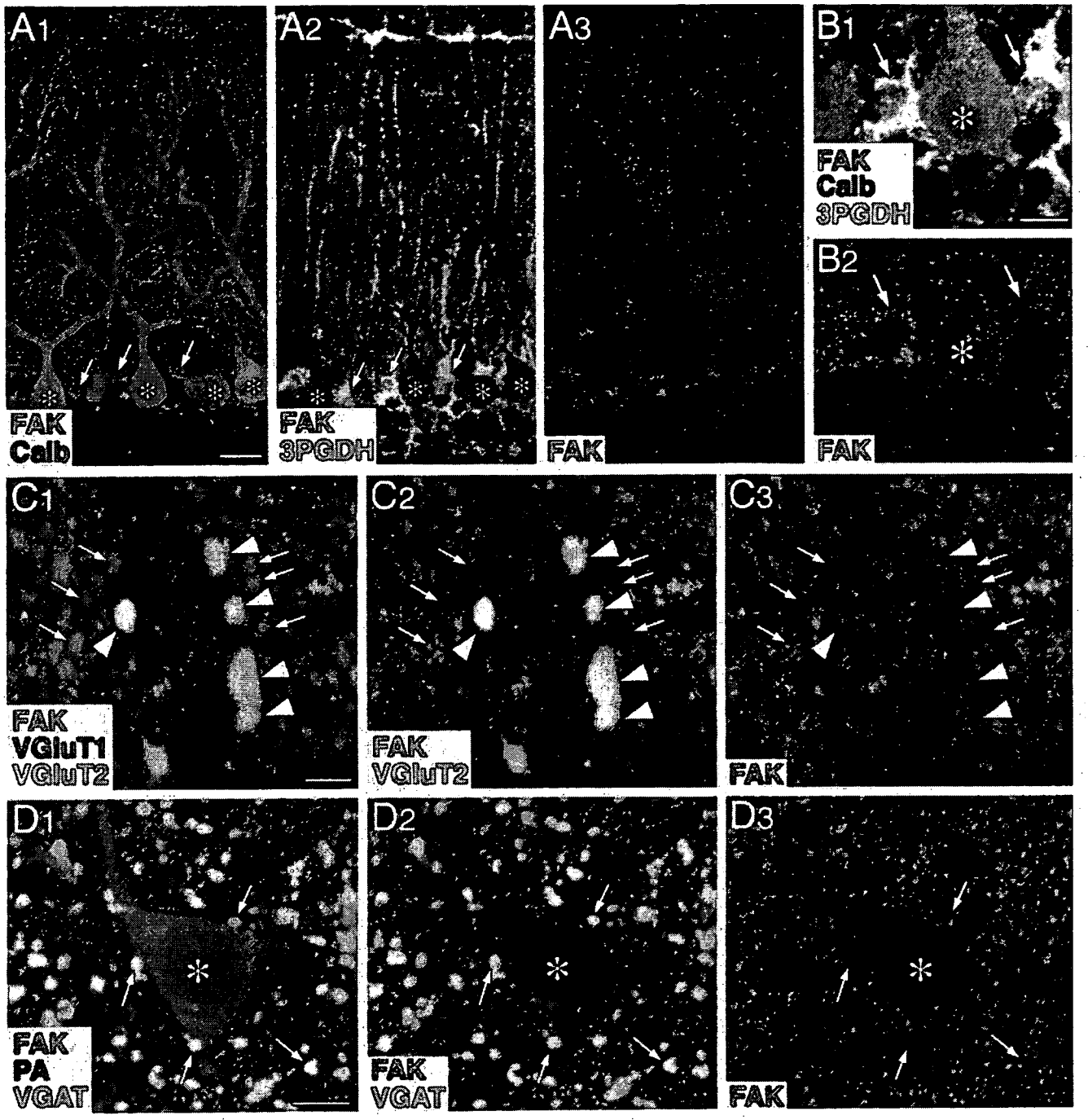


FIG. 5. Expression patterns of focal adhesion kinase (FAK) protein in the cerebellum. (A–D) Triple immunofluorescence for FAK (red) and cellular/terminal markers (blue or green) in control mice at P21. Markers include calbindin (Calb) for PCs (blue, A1 and B1), 3-phosphoglycerate dehydrogenase (3PGDH) for BG (green, A2 and B1), vesicular glutamate transporter (VGluT)1 for PF-terminals (blue, C1), VGluT2 for CF-terminals (green, C1 and C2), parvalbumin (PA) for inhibitory interneurons (blue, D1) and vesicular GABA transporter (VGAT) for terminals of inhibitory interneuron (green, D1 and D2). The arrows and asterisks in (A and B) indicate Bergmann cell bodies and PC bodies, respectively. The arrows and arrowheads in (C) indicate PF- and CF-terminals, respectively. The arrows and asterisks in (D) indicate inhibitory interneuron terminals and cell bodies, respectively. Scale bars: 20 μm (A1); 10 μm (B1 and D1); 5 μm (C1).

mice, FAK immunolabeling was almost diminished except in the caudal cortex, deep layers of the cerebral cortex and hippocampus (Fig. 1G). The FAK expression pattern was consistent with that of the *Fak* mRNA.

By immunoblot analysis at P21, we found that the amount of FAK in whole-brain homogenates of mutant mice decreased to 18% of that in control mice (Fig. 1H; $n = 3$ for each; Student's t-test, $P < 0.001$).

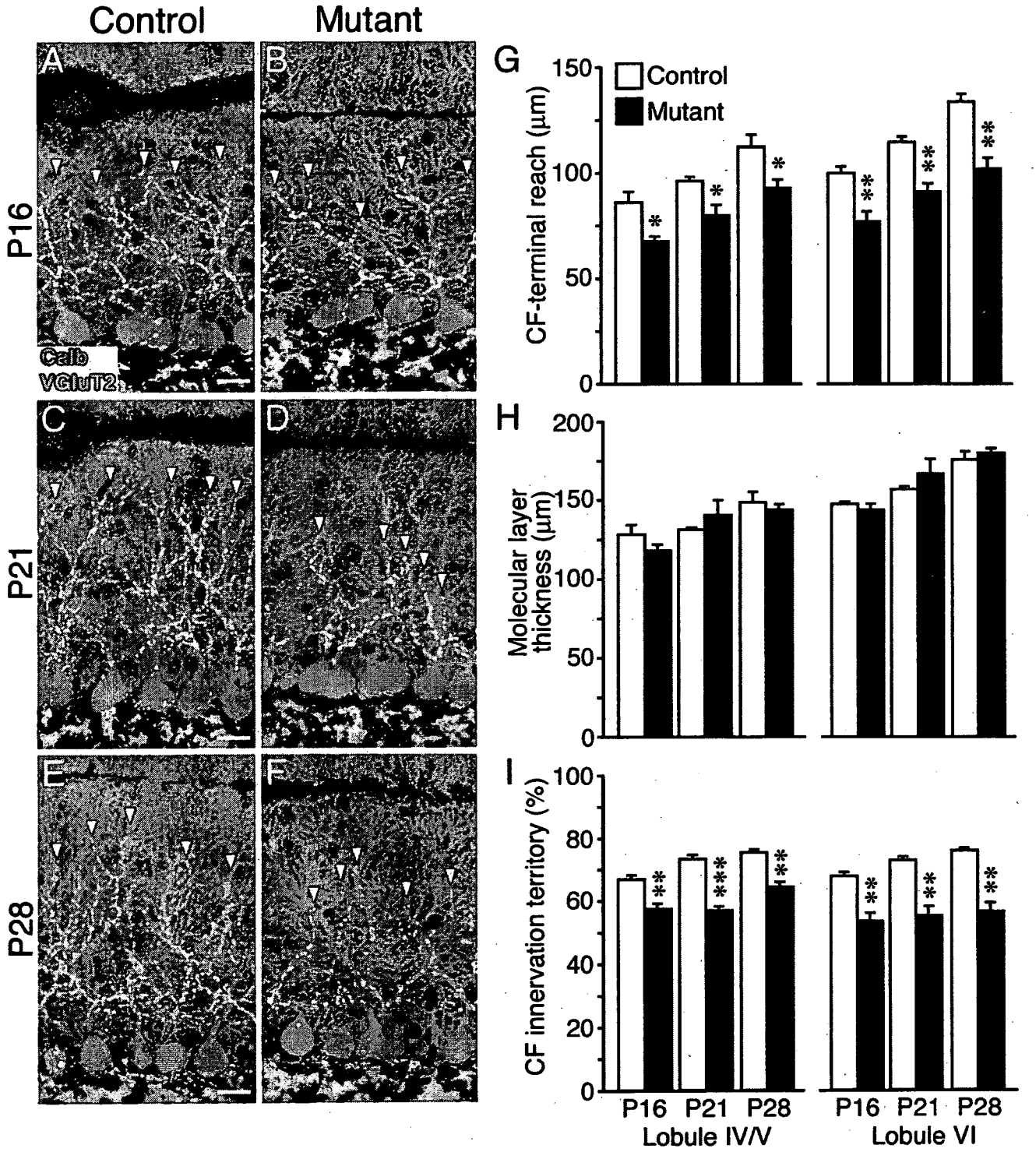


FIG. 7. Quantitative analysis of climbing fiber (CF) innervation territory in neuron/glia-specific FAK mutant mice. (A–F) Double-immunofluorescence for calbindin (Calb; red) and vesicular glutamate transporter (VGluT)2 (green) in the cerebellar molecular layer of control (A, C and E) and mutant (B, D and F) mice at postnatal day (P)16 (A and B), P21(C and D) and P28 (E and F). The arrowheads indicate the most distal tip of continuous VGluT2-positive terminals. (G–I) CF-terminal reach (G), molecular layer thickness (H) and CF innervation territory (I) in the lobules IV/V and VI in control (open bars) and mutant (closed bars) mice at P16, P21 and P28 ($n = 4$ for each). Data are expressed as mean ± SEM. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, two-way ANOVA with *post hoc* Student's t-test. Scale bars: 20 μm (A, C and E).

Impairment in cerebellar foliation

To investigate the effect of FAK ablation on the brain histoarchitecture, parasagittal sections at P21 were examined by hematoxylin or Cresyl violet staining. Histological findings on the telencephalon, diencephalon, midbrain, pons and medulla oblongata in neuron/glia-specific FAK mutant mice were comparable to those in control mice (Fig. 3A and B). However, histological abnormalities were reproducibly observed in terms of the foliation pattern of the cerebellum. In the cerebellar vermis, the intercrural fissure between lobules VI and VII was absent (Fig. 3C and E, arrowhead). Moreover, cerebellar lobules in mutant mice were generally smaller than those in control mice (Fig. 3C and E). In contrast to the affected foliation and size, the tri-laminar organization, i.e. the molecular layer, PC layer and granular layer, was normally differentiated in mutant mice (Fig. 3D and F).

At P7, there were morphological variations in the cerebellar vermis of mutant mice (Fig. 3G–J). In all the mutant mice examined ($n = 22$), the intercrural fissure was absent (arrowheads in Fig. 3H–J) and there was an additional lobule-like protrusion in lobule IV/V (asterisks in Fig. 3H–J). In four of 22 mice, the precentral fissure between lobules I/II and III was absent (arrows in Fig. 3I and J). In severely affected mice (three of 22), lobules IV/V and VI were found to be fused (box in Fig. 3J), in addition to the above abnormalities. The area of each lobule measured in the vermis decreased to various extents in mutant mice at P7 (Fig. 3K). Interestingly, rostral-to-caudal gradients were noted in terms of areal reduction with the orders being lobule I/II > lobule III > lobule IV/V in the anterior lobe, and lobules VI and VII > lobule VIII > lobule IX in the posterior lobe (Fig. 3L). These results indicate that the loss of FAK causes aberrant cerebellar foliation, although there are some variations among animals. Hereafter, we focused on the anatomical analyses of the cerebellum.

Distribution of FAK in the cerebellum

Previous studies showed that FAK immunoreactivity is distributed in the cerebellar cortex of rodent cerebella (Burgaya *et al.*, 1995;

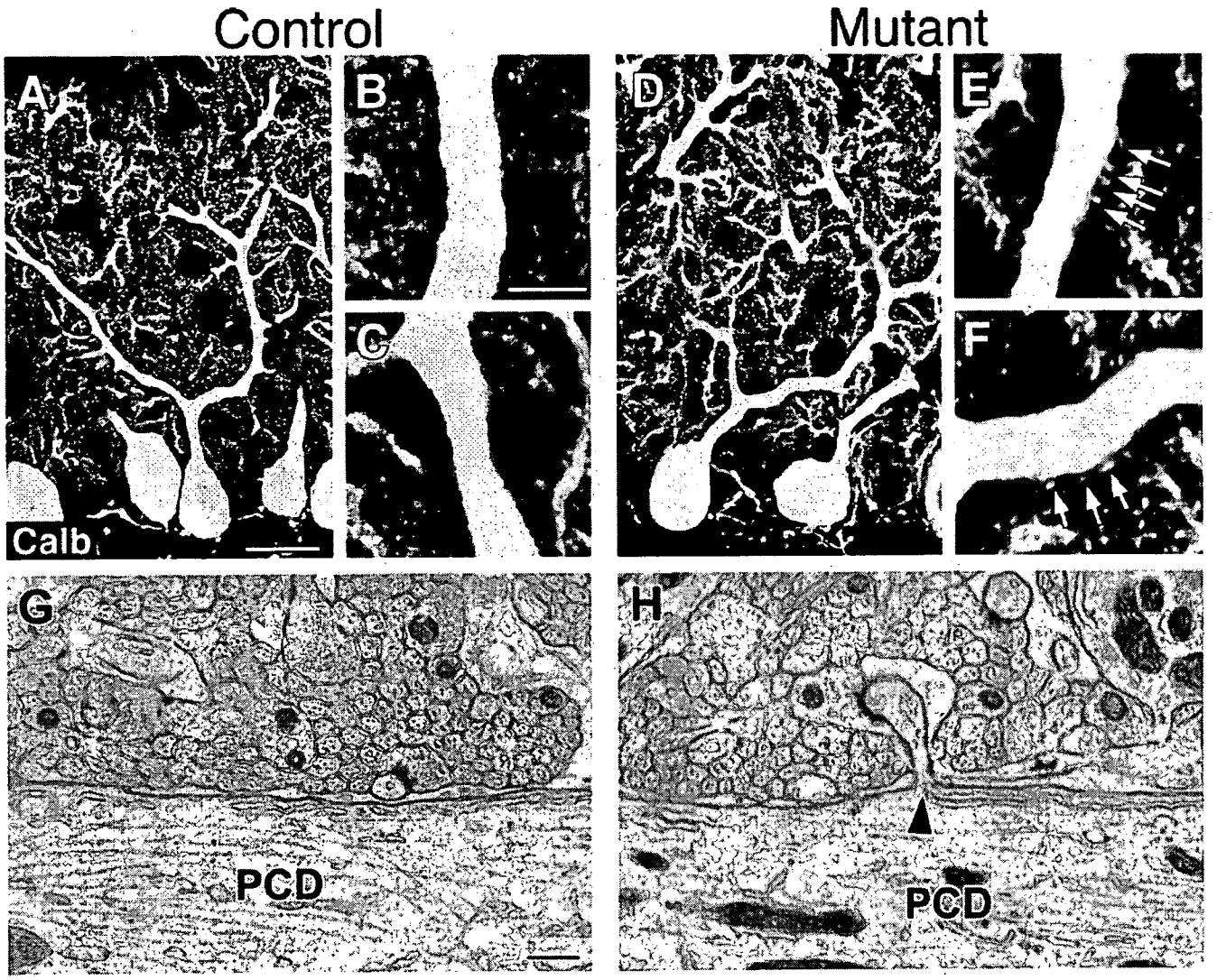
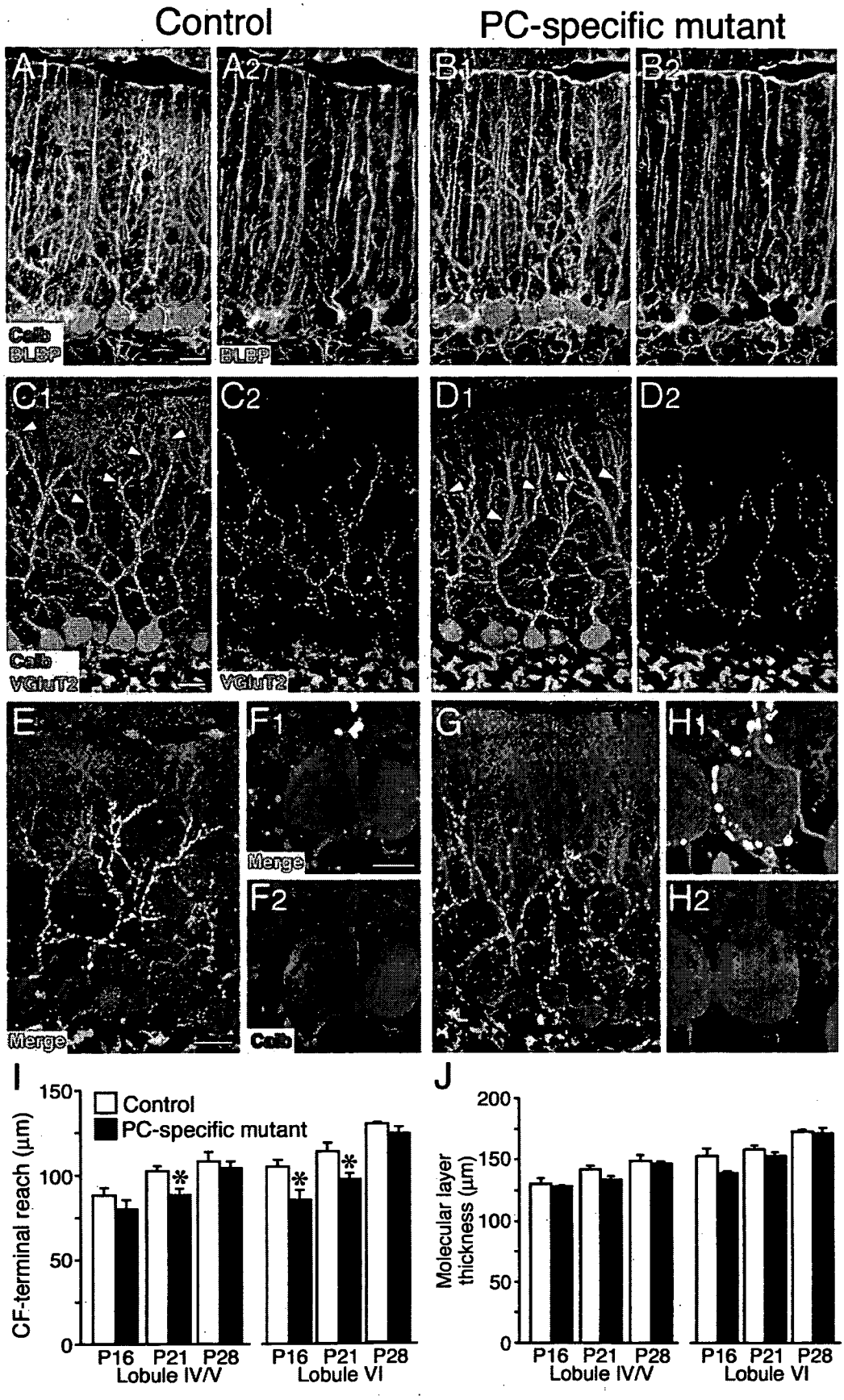


FIG. 9. Light and electron microscopic analyses of proximal shaft dendrites of Purkinje cells (PCs) in neuron/glia-specific FAK mutant mice. (A–F) Immunofluorescence for calbindin (Calb) in control (A–C) and mutant (D–F) mice. The arrows indicate spine-like protrusions from proximal shaft dendrites in mutant mice. (G and H) Electron micrographs of proximal shaft dendrites of PCs in control (G) and mutant (H) mice. The arrowheads indicate the neck of ectopic spines protruding from a shaft dendrite of PC. PCD, PC dendrite. Scale bars: 20 μm (A); 10 μm (B); 500 nm (G).



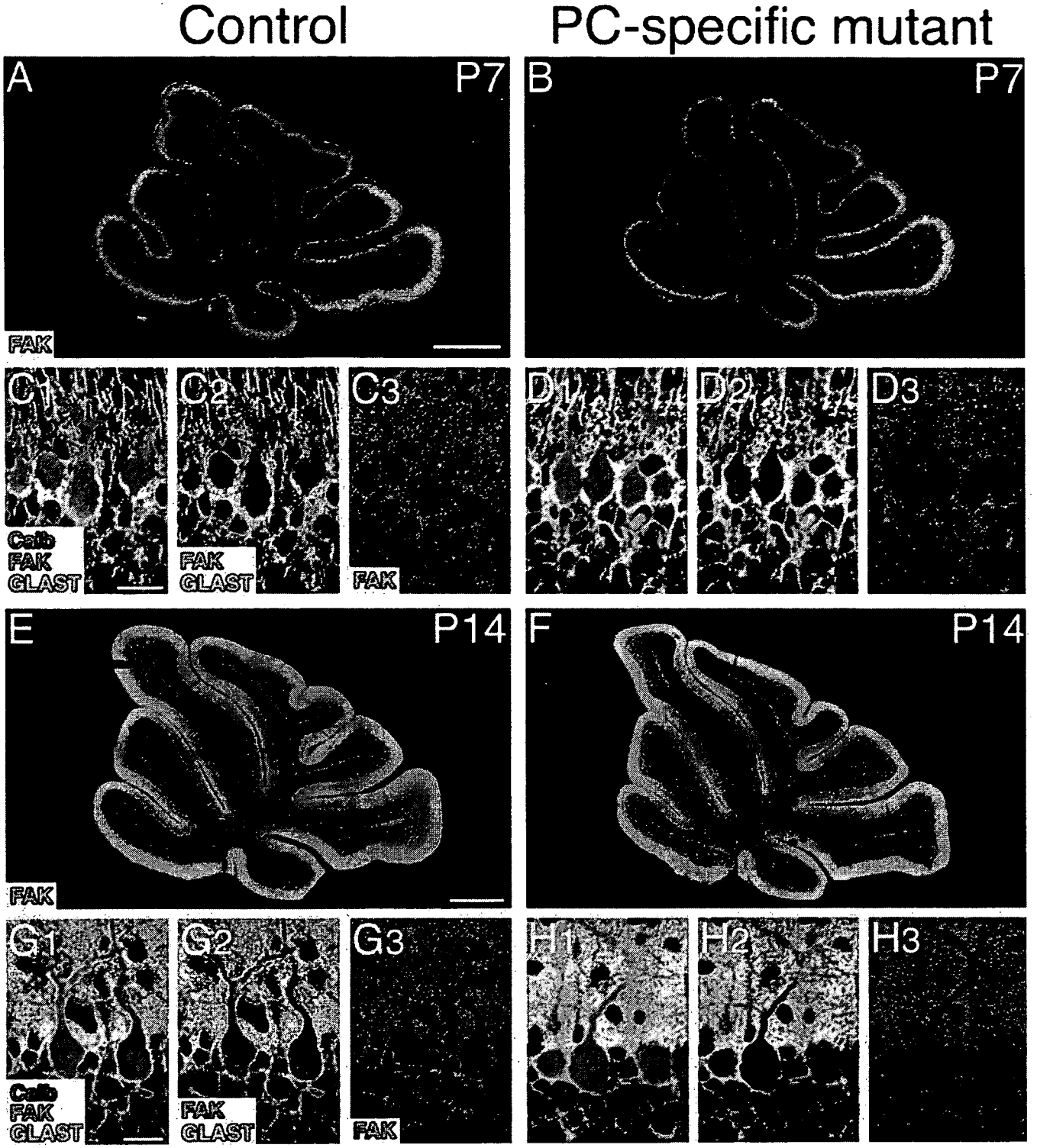


FIG. 12. PC-specific FAK ablation in PC-specific FAK mutant mice at P7 and P14. (A, B, E and F) Low-power immunofluorescence for FAK in control (A and E) and PC-specific mutant (B and F) cerebella at P7 (A and B) and P14 (E and F). Note no apparent interlobular differences in FAK expression in control and PC-specific mutant mice. (C, D, G and H) Triple-immunofluorescence for FAK (red) and cellular markers (blue and green) in the cerebellar cortex of control (C and G) and PC-specific mutant (D and H) mice at P7 (C and D) and P14 (G and H). Markers include calbindin for PCs (blue) and GLAST for BG (green). Note PC-specific ablation of FAK in PC-specific mutant mice at P7 and P14. Scale bars: 200 μm (A and E); 20 μm (C1 and G1).

FAK mutant mice than in the neuron/glia-specific FAK mutant mice. Considering the presence of FAK in CF-terminals, it is thus possible that FAK in CF-terminals may also promote growth and extension of CF innervation to some extent. In addition to its function as a protein tyrosine kinase, FAK is a large adaptor protein carrying binding sites for many proteins, including phosphatidylinositol 3-kinase, Src, p130Cas, RhoGTPases regulators (Graf, Trio, p190RhoGEF), ASAP1 and cytoskeletal proteins such as paxillin (Hildebrand *et al.*, 1996; Medley *et al.*, 2003; Parsons, 2003; Zhai *et al.*, 2003). Many of these proteins regulate the activities of the Rho family of GTPases, which in turn control axonal dynamics via cytoskeletal regulation (Billuart *et al.*, 2001). In cultured *Xenopus* spinal neurons, impaired FAK activity causes a considerable decrease in the rate of neurite outgrowth. Furthermore, impaired FAK activity leads to impaired growth of Rohon–Beard sensory neurons *in vivo* (Robles & Gomez, 2006). Alternatively, it is possible that FAK in CFs may regulate neurotransmitter release, thereby helping CFs extend toward PC dendrites, because FAK interacts with amphiphysin, and the disruption of their interaction causes a defected endocytosis (Messina *et al.*, 2003). Rico *et al.* (2004) reported that the PC-specific ablation of FAK results in increased axon branching and synapse formation by PC axons in the deep cerebellar nuclei. Thus, FAK in PCs appears to play differential roles in CF synapses on the PC proximal dendrites and axon terminal synapses in the deep cerebellar nuclei. Whether FAK-mediated signaling is differential depending on synaptic type and developmental stage should be addressed in the future.

In conclusion, FAK in neurons and glia plays important roles in the development of the cerebellar histoarchitecture, cytoarchitecture and synaptic circuits.

Acknowledgements

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Abbreviations

3PGDH, 3-phosphoglycerate dehydrogenase; BG, Bergmann glia; BLBP, brain lipid-binding proteins; CF, climbing fiber; DTR, dextran Texas red; ES, embryonic stem; FAK, focal adhesion kinase; frt, Flp recognition target; GABA, γ-aminobutyric acid; neo, neomycin phosphotransferase; P, postnatal day; PC, Purkinje cell; PF, parallel fiber; VGAT, vesicular γ-aminobutyric acid transporter; VGluT, vesicular glutamate transporter.

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INTERNAL MEDICINE

ORIGINAL ARTICLE

Neuropathological Studies of Patients with Possible Non-Herpetic Acute Limbic Encephalitis and So-called Acute Juvenile Female Non-Herpetic Encephalitis

Koichi Okamoto[1], Tsuneo Yamazaki[1], Haruhiko Banno[2], Gen Sobue[2], Mari Yoshida[3] and Masamitsu Takatama[4]

Abstract

We report three rare autopsied cases; one was non-herpetic acute limbic encephalitis (NHALE) and two were so-called acute juvenile female non-herpetic encephalitis (AJFNHE). In NHALE, neuronal loss with gliosis and microglia/macrophage infiltrations were mainly seen in the CA1 areas in the hippocampus. However, there were no apparent anoxic neuronal changes in the remaining neurons in the CA1, and astrocyte proliferations and microglia/macrophage infiltrations were also observed in the claustrum, while these were mildly present in the basal ganglia. In AJFNHE, pathological findings differed from those of NHALE with regard of the absence of limited pathology in the limbic system, microglia/macrophages widely infiltrated the brain including the hippocampal areas and mild lymphocytic infiltrations were observed in the subarachnoid spaces as well as in the parenchyma. The pathomechanism of NHALE and AJFNHE is obscure and an autoimmune theory is proposed, however we must collect and examine many autopsied cases in order to clarify the pathomechanism.

Key words: non-herpetic acute limbic encephalitis, acute juvenile female non-herpetic encephalitis, hippocampal sclerosis

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Introduction

Many diseases affect the limbic system, and limbic encephalitis (LE) is usually classified into paraneoplastic LE, LE by viral infections, LE associated with autoimmune disease such as LE with antibody against voltage-gated potassium channels, and LE of unknown etiology (1-6). Non-herpetic acute limbic encephalitis (NHALE) is regarded as a new subgroup of LE (7-9). Patients with NHALE differ from those with herpes simplex encephalitis in terms of the lack of evidence of herpes simplex virus (HSV) and showed magnetic resonance imaging (MRI) findings localized to the limbic system such as bilateral hippocampi and amygdalae (7, 8, 10, 11). However, similar patients with so-called acute juvenile female non-herpetic LE (AJFNHLE) without abnormal MRI findings in the limbic systems have also been reported mainly in Japan (12, 13). The relationship between NHALE and AJFNHLE are equivocal because autopsied patients have very rarely been reported. Here, we describe three autopsied cases consisting of probable one NHALE and two AJFNHLE. For comparison, we also studied 10 autopsied cases of hippocampal sclerosis mainly caused by anoxia.

Clinical Findings

Case 1

Four days after fever onset in September 1985, a 43-year-old Japanese woman developed grand mal seizures, which expanded to status epilepticus and the patient was trans-

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ferred to the Geriatric Research Institute and Hospital. At the admission, she showed status epilepticus and several anticonvulsants were not effective and she was controlled under respirator. CSF examinations showed cells 16/mm3, protein 73 mg/dl, glucose 100 mg/dl. EEG showed periodic sharp waves. Brain CT 15 days after the onset showed low densities in the bilateral medial regions of the temporal lobes, however MRI could not be examined at that time. Viral titers in CSF were unremarkable including herpes simplex virus. She died 28 days after the onset.

Case 2

Maeda et al (14) previously reported this patient in a Japanese language journal in 1974, and we reexamined the case pathologically. Three days after common cold-like symptoms in March 1970, a 32-year-old Japanese woman developed confusion, abnormal behavior and automatism. Ten days after the onset, she refused to eat and showed urinary incontinence, forced laughing, tic-like involuntary movement and high fever, and was transferred to our hospital 13 days after onset. Her consciousness was drowsy, then myoclonus and grand mal seizures appeared 17 days after the onset. Status epilepticus and decerebrate posture persisted for 10 days. On admission to Gunma University Hospital, CSF examinations showed cells 67/mm3, protein 25 mg/dl, glucose 75 mg/dl. Virus titers were not examined. EEG showed diffuse high delta activities with 5-6 c/s sporadic theta waves in the parietal regions. She died 26 days after the onset.

Case 3

Eleven days after fever onset and perioral eruptions in September 2003, a 27-year-old Japanese woman developed visual hallucinations and depressive state, and was admitted to the Department of Neurology, Nagoya University Hospital. She showed a moderately high fever, intermittent grand mal seizure without apparent motor palsy. Laboratory data were as follows. Serum CK 2,234 IU/l, TSH 18.09 μU/ml, fT3 2.45 pg/ml, fT4 0.84 ng/dl, anti-thyroid peroxidase antibody 97.36 U/ml and anti-thyroglobulin antibody 14.42 U/ml. Serum autoantibody against alpha-enolase was negative. CSF examinations showed cells 14 /mm3, protein 24 mg/dl, glucose 66 mg/dl. Viral titers in CSF were unremarkable including herpes simplex virus. MRI studies were unremarkable. Pelvic CT was also unremarkable. Steroid pulse therapy was not effective. Generalized seizures were continued, and pancytopenia, septic shock were added. She died of multiple organ failure 50 days after the onset.

Materials and Methods

We examined the brains of the three patients described above and 13 brains of control patients from the Geriatrics Research Institute and Hospital. Ten controls showing hippocampal sclerosis were selected from among 320 serial autopsies files, and patient ages ranged from 54 to 90 years, and survival durations ranged from 17 days to 10 months after acute respiratory failure. And another 3 cases without pathologic cerebral changes including hippocampus were also examined. In all cases, the autopsies were performed in accordance with established procedures and the samples were used in this study after obtaining informed consent from the family of each patient.

Brains were fixed in 4% paraformaldehyde in phosphate-buffered solution (PBS) (pH 7.4) and multiple sections including the hippocampus were embedded in paraffin. Five micrometer thick sections were examined by H-E and K-B staining, and were also immunostained, which was carried out using a polyclonal rabbit anti-GFAP antibody (1 : 1,000, Dako, Denmark), monoclonal mouse anti-phosphorylated neurofilament (SM1 31) (1 : 10,000, Sternberger, USA), monoclonal mouse anti-synaptophysin antibody (1 : 200, Chemicon, USA), polyclonal rabbit anti-herpes simplex virus type 1 (HSV-1) antibody (1 : 800, Dako, Denmark), monoclonal mouse anti-human CD68 antibody (1 : 200, Dako, Denmark). CD68 antibody labels macrophages and other members of monoclonal phagocytes. For enhancement, autoclave treatment for 5 minutes was performed for synaptophysin and CD68. Sections were blocked in normal serum for 30 minutes at room temperature, then labeled with the first antibody at 4℃ overnight, washed in PBS for 30 minutes, incubated with the second antibody provided by Histofine SAB-PO kit (Nichrei, Japan), washed in PBS for 30 minutes, and finally visualized by the avidin-biotonperoxidase method.

Pathological Findings

Case 1

Brain weight was 1,190 g, and macroscopic findings were unremarkable. Microscopically, there were no lymphocyte infiltrations in the meninges or brain parenchyma, and there were no infarcts or demyelination either. Neurons in the CA 1 (15) were markedly lost, and astrocytic gliosis, spongiosis (Fig. 1), however, there were no anoxic changes in the remaining neurons (Fig. 2), and binucleated astrocytes were rarely seen (Fig. 2). Hippocampal granular neurons were also lost with astrocyte proliferations. There were no neuronophagia or perivascular lymphocytic infiltrations in the hippocampal areas. CD68 immunostaining showed increased microglia/macrophages in the hippocampal areas. HSV-1 immunostaining was negative, and synaptophysin were relatively well preserved. Astrocyte proliferations and microglia/macrophage infiltrations were not apparent in the cerebrum (Fig. 3A), however those changes were clearly present in the claustrum (Fig. 3B, 3C) and mildly in the basal ganglia.

There was no tumor in the general organs including ovary.

Case 2

Brain weight was 1,200 g and the only macroscopically

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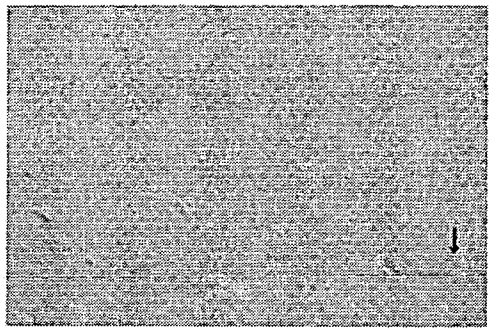

Figure 1. Low magnification of hippocampal CA1 area in Case 1. Neuronal loss with astrocyte proliferations and spongiosis were apparent in CA1. Perivascular lymphocytic infiltrations were not observed. Hematoxylin and Eosin staining, ×40.

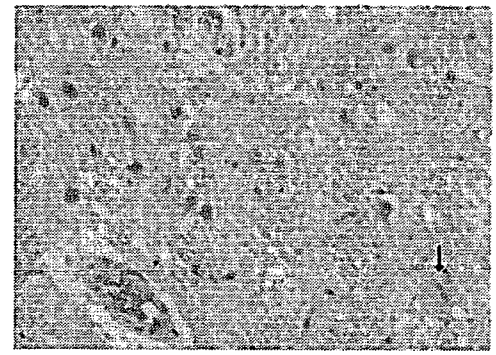

Figure 2. High magnification of right low corner of Figure 1. There were few anoxic changes in the remaining neurons, and binucleated astrocytes were rarely seen (binucleated astrocyte shown by the arrows was the same in Figs. 1 & 2). Hematoxylin and Eosin staining, ×200.

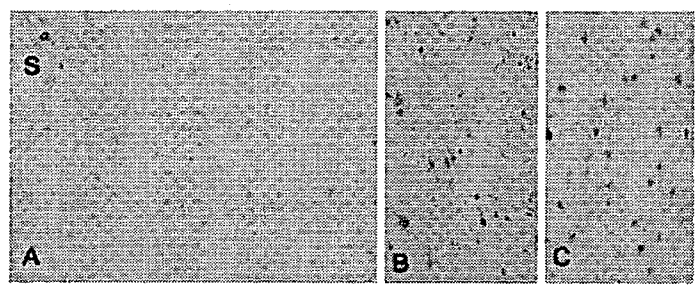

Figure 3. Insular cortex and claustrum in a same section of Case 1. There were a few CD68-positive microglia/macrophages in the insular cortex (A). However, CD68-positive microglia/macrophages (B) and GFAP-positive astrocytes (C) were abundant in the claustrum. S, subarachnoid space; A, ×100; B, ×200; C, ×200.

abnormal finding was brain swelling. There was no necrosis or bleeding. Mild lymphocytic infiltrations were observed in the subarachnoid spaces throughout in the cortices, brain stem and cerebellum (Fig. 4A, 4B). In the parenchyma, perivascular lymphocytic infiltrations were also seen in the superficial layers of the cortices (Fig. 4A), in the basal ganglia and in the Ammon's horns (Fig. 4B). In the Ammon's horns, neurons were relatively well preserved and there was no gliosis but limited neuronophagia was seen in the CA1 area (Fig. 4C). Microglia/macrophage infiltrations were apparent (Fig. 4D); however, there was no gliosis in those areas. Hippocampal granular neurons were well preserved. Diffuse microglia/macrophage infiltrations were observed throughout in the cerebral cortices. HSV-1 immunostaining was negative. Bilateral soybean-sized cysts were seen in the ovary, however histological examinations did not show teratoma.

Case 3

Brain weight was 1,276 g and the macroscopic findings were unremarkable. Histologically, the brain showed slight edematous and many small pericapillary bleeding, however, there was no necrosis, vasculitis or intranuclear inclusion. Mild lymphocytic infiltrations were seen around the small vessels in the cortices (Fig. 5A) and in the subarachnoid spaces. Lymphocytic infiltrations were somewhat predominant in the frontal lobe, however mild lymphocytic infiltrations were also seen in the basal ganglia, brain stem and cerebellum. Microglia/macrophages diffusely infiltrated the cerebral cortices (Fig. 5B). Neurons in the hippocampal areas were well preserved (Fig. 5C), and microglia/macrophages were diffusely infiltrated in the hippocampal areas (Fig. 5D) without gliosis. HSV-1 immunostaining was negative.

Hippocampal sclerosis

In our 10 patients with hippocampal sclerosis, many remaining neurons in CA1 areas showed anoxic features such as eosinophilic atrophic changes in the earlier stages, and marked neuronal loss with gliosis in the advanced stages.

Discussion

Because many previously reported cases of NHALE have shown a rather favorable prognosis, only a few autopsied patients have been reported. Mochizuki et al (8) reported a

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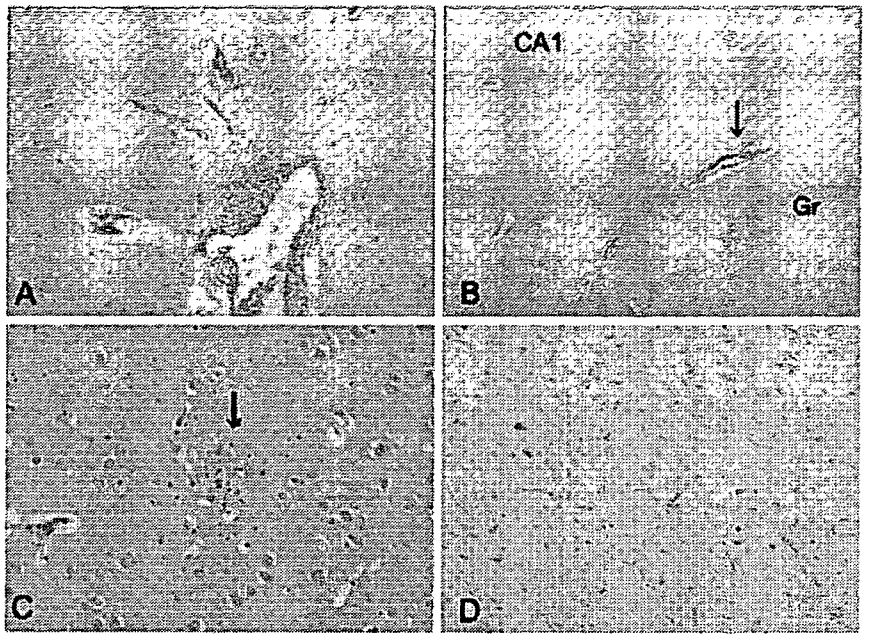

Figure 4. Lymphocytic infiltrations were seen in the subarachnoid spaces and in the perivascular spaces of the superficial cortices (A) and in the hippocampal areas (arrow, B) in Case 2. A few neuronophagia were seen in the CA1 area (arrow), and rod-shaped CD68-postive cells were abundant (D), but there were few GFAP-positive astrocytes (not shown). C and D were almost same areas in serial sections. Gr: granular cell layer. A, Hematoxylin and Eosin staining×100; B, ×40; C, ×200; D, Hematoxylin and Eosin staining×200.

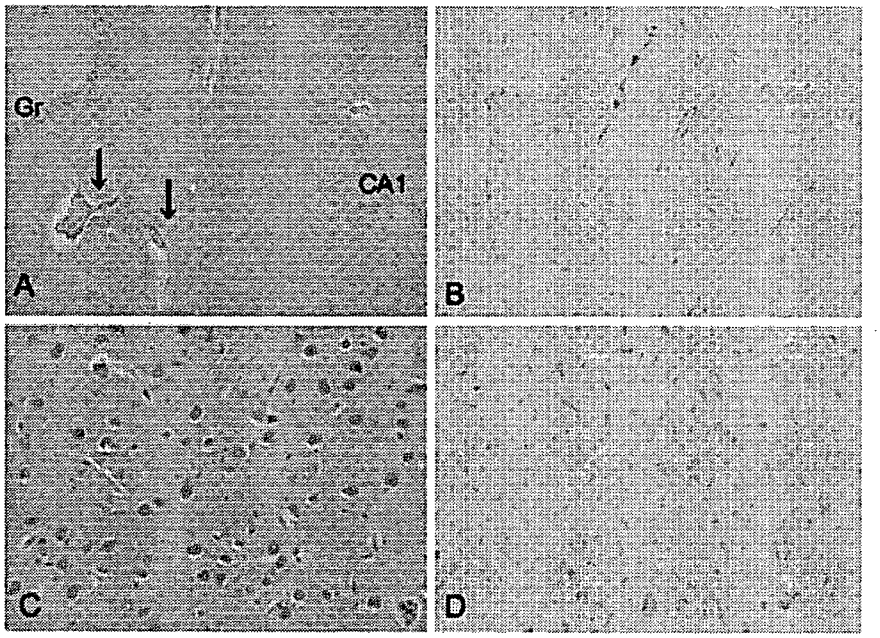

Figure 5. Perivascular lymphocytic infiltrations were seen in the molecular layers of the hippocampus (arrows, A), and CD68 positive microglia/macrophage were increased in the cortex (B) in Case 3. Neurons were well preserved in CA1 (C) with abundant CD68-positive cells (D). C and D were almost same areas in serial sections. Gr, granular cell layer; A, Hematoxylin and Eosin staining×40 ; B, ×200; C, Hematoxylin and Eosin staining×200; D, ×200.

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59-year-old woman with disturbance of consciousness, uncontrolled generalized seizures, and abnormal MRI signals in the bilateral medial temporal lobe and along the lateral part of the putamen. She died 12 days after onset. Autopsy examination demonstrated scattered foci consisting of neuronal loss, neuronophagia and some perivascular lymphocytic infiltrations in the hippocampus and amygdala. However, there was no hemorrhagic necrosis in the brain and HSV was also immunohistologically negative. They suggested that their patient showed neuropathological changes of NHALE as a possible new clinicopathological entity. Another similar patient was reported in an abstract form. Briefly, Maki et al (16) reported a 53-year-old woman who died 36 days after the onset of illness and showed abnormal MRI findings in the hippocampus and amygdala. She developed generalized seizures and status epileptics and finally multiple organ failure. Autopsy disclosed marked neuronal loss and gliosis mainly in the CA1 areas and amygdala without lymphocytic infiltrations and necrosis in the brain.

Our Case 1 is similar to the two patients described above with regard to clinical features and pathological findings mainly limited to the hippocampal areas. Classical hippocampal sclerosis in which neuronal loss is most severe in CA1 accompanied by gliosis may be induced by many causes, such as epilepsy, stroke, cardiopulmonary arrest, encephalitis and neurodegenerative diseases (17-19). In our Case 1 and that reported by Maki et al (16), the pathology was similar to hippocampal sclerosis without inflammatory changes, however the pathomechanism remains obscure. One possibility is that the two patients showed more prolonged courses than the case of Mochizuki et al (8), so the inflammations might be subsided. The second possibility is that the hippocampal lesions were caused by severe seizures. Misumi et al (20) reported a 30-year-old man with sudden onset seizure showing abnormal MRI signal in the right medial temporal lobe, and brain biopsy showed edema without specific abnormalities and they suggested that secondary brain edema induced by seizure must be considered. Seizure-induced transient brain edema is not rare in the temporal lobe, and these findings may reflect transient cytotoxic and vasogenic edema induced by seizure (21-24). The majority of NHALE patients showed severe generalized seizures or status epilepticus, so we must carefully consider this possibility when abnormal MRI findings are seen in the medial regions of the temporal lobe. In our 10 patients with hippocampal sclerosis, many remaining neurons in CA1 areas showed anoxic features such as eosinophilic atrophic changes. However, the remaining neurons in CA1 of our Case 1 did not show such eosinophilic atrophic changes, therefore the hippocampal changes may not be simply caused by anoxia. More studies are needed to consider the pathogenesis of the hippocampal lesions.

Claustrum frequently showed abnormal MRI findings in NHALE cases (11, our cases: data not shown), and astrocytic proliferations and microglia/macrophage infiltrations observed in the claustrum in our Case 1 may correlate with those abnormal MRI findings.

Kamei (12) proposed a new clinical entity named acute juvenile female non-herpetic encephalitis (AJFNHE), and the characteristics of AJFNHE were defined as follows: 1) a clinical profile of encephalitis with psychosis, disturbance of consciousness, and/or convulsion, 2) progression to coma and status epilepticus, 3) a prolonged clinical course, 4) a relatively good long-term outcome despite a severe clinical course in the acute stage, 5) a predilection for juvenile females, 6) a lack of abnormal intensity on cranial MRI, 7) negative data for HSV infection. Clinically, our Cases 2 and 3 were almost consistent with AJFNHE criteria, however no MRI was done in Case 2. Case 3 showed hypothyroid laboratory data with positive anti-thyroid peroxidase and anti-thyroglobulin antibodies, therefore we must differentiate Hashimoto's encephalopathy. Hashimoto's encephalopathy has been recognized as rare clinical entities and characterized by progressive or fluctuating neurological symptoms, and response to corticosteroid treatment is universally excellent (25, 26). Postmortem examination demonstrated mild perivascular lymphocytic infiltration throughout the brain and leptomeninges plus diffuse gliosis of gray matter in the cortex and basal ganglia, and to a lesser extent, the parenchymal white matter (25). Recently, Fujii et al (27) reported that autoantibodies against the amino terminal of alpha-enolase are a useful diagnostic marker for Hashimoto's encephalopathy. Clinical courses with untreatable status epilepticus, the lack of a steroid therapy and the absence of autoantibody against alpha-enolase may be different from those in Hashimoto's encephalopathy.

Our Cases 2 and 3 differed from Case 1 with regard to the absence of limited pathology in the limbic system, microglia/macrophages widely infiltrated the brain including the hippocampal areas and mild lymphocytic infiltrations were observed in the subarachnoid spaces and in the parenchyma. HSV infections were ruled out because of the lack of hemorrhagic necrosis, intranuclear inclusions and negative HSV on the immunohistological study. These mild inflammatory changes with diffuse microglia/macrophages activation in the brain might be the main pathological findings in our Cases 2 and 3, and the pathological findings suggest the mild viral infectious or postinfectious state in the CNS. Relationship between NHALE and AJFNHE is obscure, however both diseases seem to be different in some points. Especially, NHALE showed more limited pathology in the limbic system, whereas AJFNHE showed widespread pathology with microglia/macrophage activation. N-methyl-D-aspartate glutamate receptor epsilon 2 (GluR ε2) is frequently found in the serum and CSF in both disorders, suggesting an autoimmune mechanism (10, 28). Recently, Dalmau et al (3) reported paraneoplastic anti-N-methy-D-aspartate (NMDA) receptor encephalitis associated ovarian teratoma. Tumor resection and immunotherapy resulted in improvement or full recovery of eight of nine patients. Two of three patients without tumor resection died of neurological deterioration. Two autopsies showed extensive microgliosis, rare T-cell in-

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filtrates, and neuronal degeneration predominantly involving, but not restricted to the hippocampus. Similar extensive microgliosis were also seen in our Cases 2 and 3. We have to collect and examine many autopsied patients to order to clarify the pathomechanism. More recently, Iizuka et al (29) reported that 4 Japanese women diagnosed with AJFNHE showed positive against antibodies to NR1/NR2 heteromers of NMDA receptor in serum or CSF, and their findings indicate that majorities of AJFNHE in Japan may anti-NMDA receptor encephalitis.

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CONSENSUS PAPER

Neuropathologic diagnostic and nosologic criteria for frontotemporal lobar degeneration: consensus of the Consortium for Frontotemporal Lobar Degeneration

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Abstract The aim of this study was to improve the neuropathologic recognition and provide criteria for the pathological diagnosis in the neurodegenerative diseases grouped as frontotemporal lobar degeneration (FTLD); revised criteria are proposed. Recent advances in molecular genetics, biochemistry, and neuropathology of FTLD prompted the Midwest Consortium for Frontotemporal Lobar Degeneration and experts at other centers to review and revise the existing neuropathologic diagnostic criteria for FTLD. The proposed criteria for FTLD are based on existing criteria, which include the tauopathies [FTLD with Pick bodies, corticobasal degeneration, progressive supranuclear palsy, sporadic multiple system tauopathy with dementia, argyrophilic grain disease, neurofibrillary tangle dementia, and

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FTD with *microtubule-associated tau* (*MAPT*) gene mutation, also called FTD with parkinsonism linked to chromosome 17 (FTDP-17)]. The proposed criteria take into account new disease entities and include the novel molecular pathology, TDP-43 proteinopathy, now recognized to be the most frequent histological finding in FTLD. TDP-43 is a major component of the pathologic inclusions of most sporadic and familial cases of FTLD with ubiquitin-positive, tau-negative inclusions (FTLD-U) with or without motor neuron disease (MND). Molecular genetic studies of familial cases of FTLD-U have shown that mutations in the *progranulin* (*PGRN*) gene are a major genetic cause of FTLD-U. Mutations in *valosin-containing protein* (*VCP*) gene are present in rare familial forms of FTD, and some families with FTD and/or MND have been linked to chromosome 9p, and both are types of FTLD-U. Thus, familial TDP-43 proteinopathy is associated with defects in multiple genes, and molecular genetics is required in these cases to correctly identify the causative gene defect. In addition to genetic heterogeneity amongst the TDP-43 proteinopathies, there is also neuropathologic heterogeneity and there is a close relationship between genotype and FTLD-U subtype. In addition to these recent significant advances in the neuropathology of FTLD-U, novel FTLD entities have been further characterized, including neuronal intermediate filament inclusion disease. The proposed criteria incorporate up-to-date neuropathology of FTLD in the light of recent immunohistochemical, biochemical, and genetic advances. These criteria will be of value to the practicing neuropathologist and provide a foundation for clinical, clinico-pathologic, mechanistic studies and in vivo models of pathogenesis of FTLD.

Keywords Frontotemporal dementia · Semantic dementia · Progressive non-fluent aphasia · Frontotemporal lobar degeneration · Motor neuron disease · Tauopathy · Ubiquitin · TDP-43 proteinopathy · Progranulin · Valosin-containing protein · Charged multivesicular body protein 2B · Neuronal intermediate filament inclusion disease · Neuropathologic diagnosis

Introduction

In this paper, we follow the convention that FTLD is an umbrella term that groups several different neurodegenerative diseases characterized by predominant destruction of the frontal and temporal lobes. After Alzheimer disease (AD) and dementia with Lewy bodies (DLB), frontotemporal lobar degeneration (FTLD) is the third most common neurodegenerative cause of dementia in industrialized countries [59, 60, 69]. Most commonly, patients with FTLD present with frontotemporal dementia (FTD), a change in personal and social conduct, often associated with disinhibition, with gradual and progressive changes in language [53]. Other patients falling under the diagnostic umbrella of FTLD may

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present with early and progressive changes in language function, and two syndromes have been recognized: semantic dementia (SD) and primary progressive non-fluent aphasia (PNFA) [40, 43, 53, 65, 67, 78]. In later stages of these particular syndromes, both behavioral and language dysfunction may be present. A proportion of patients with FTLD present with or develop parkinsonism as part of their disease process. Clinical amyotrophic lateral sclerosis/motor neuron disease (ALS/MND) may also be found in a proportion of patients with FTLD, especially those with FTD, indicating a spectrum of clinical phenotypes that relate to common neuropathologic lesions [3, 61, 69].

FTD, SD, or PNFA refer to the main clinical syndromes linked to the FTLD group. Typically, at least in the early course of the disease, patients with FTD do not have an amnestic syndrome, which distinguishes them clinically from AD [46, 53], but there are exceptions [29]. Although no pre-symptomatic biomarkers have been identified, at least in sporadic cases, clinical assessment, neuropsychology, and neuroimaging may help to distinguish FTD and the related disorders of SD and PNFA from other neurodegenerative causes of dementia [12, 43, 46]. The diagnosis of FTD, SD, or PNFA may only be considered when other potential causes of dementia including other nervous system diseases (e.g., small and large vessel disease), systemic conditions (e.g., hypothyroidism), tumors, and substance abuse have been excluded.

The *apolipoprotein E* (*APOE*) gene 4 allele is a major risk factor for AD, though this is not the case in most association studies of FTLD (but see ref. [71]), and none of the autosomal dominant mutations in genes associated with some familial cases of AD [*amyloid precursor protein* (*APP*), *presenilin 1* (*PS1*) and *presenilin 2* (*PS2*)] acts as a risk factor for FTLD.

Recent developments in the molecular pathology and genetics of FTLD now dictate that a minimal panel of pathological investigations is required for correct diagnosis in this group of diseases. Standardization of nomenclature and approach will facilitate better understanding of clinico-pathologic correlations, provide insights into pathogenesis, and guide the construction and validation of in vivo models.

Neuropathologic evaluation

With the exception of those cases in which a gene defect has been identified, examination of the brain and neuropathology are essential in order to determine the disease entity underlying FTLD. Even in those cases that have been genetically characterized, it is not uncommon to find coexisting neurodegenerative disease and other pathology, which may have contributed to the clinical picture to a varying degree. The neuropathology of the brain, either on autopsy or, rarely, on biopsy, remains the "gold standard" for determining the neuropathologic diagnosis. Although most cases seen by a neuropathologist are likely to be cases of advanced disease, there is an increasing awareness that the molecular pathology of all neurodegenerative disease is present often several years prior to the onset of clinical symptoms, and this knowledge will inform the neuropathologist of pre-clinical FTLD in an otherwise cognitively and behaviorally normal subject.

Macroscopy

Examination of the brain of a patient with FTLD typically shows symmetrical focal atrophy of the frontal or temporal lobes, or both. In some patients there is asymmetry of atrophy, typically reflected in perisylvian loss on one side of the brain. Macroscopic atrophy of the basal ganglia and loss of pigmentation from the substantia nigra are seen in a proportion of cases. This focal atrophy is, not infrequently, the most dramatic in all of neuropathology. Conversely, in some individuals, for example, those who die at an earlier stage, the brain is unremarkable. The pattern of atrophy may assist in staging disease severity [11, 39, 41].

Microscopy

In most forms of FTLD, examination of the cerebral cortex with H&E staining shows microvacuolation and neuronal loss. In many cases, this is most evident around layer II of the affected cortical regions. In advanced cases, there is transcortical microvacuolation and neuronal loss. Swollen cortical neurons may be seen and highlighted with immunostaining for alpha B-crystallin; however, they are not specific for any disease subtype. White matter myelin loss and astrocytic gliosis may be seen. There may be significant neuronal loss from the basal ganglia and substantia nigra in some cases.

Specific diagnosis of disease within the broad group of FTLD now requires immunohistochemistry (IHC) to determine the molecular pathology, morphology, and distribution of lesions in the neuraxis, and thereby identify the neurodegenerative disease. In the routine microscopic evaluation of the brain of a patient with FTLD, other neurodegenerative diseases may be identified, most commonly AD [18], DLB [52], and, rarely, prion disease [33] and hereditary diffuse leukoencephalopathy with axonal spheroids [76].

Although some neurodegenerative diseases can be readily identified using conventional staining techniques (e.g., modified Bielschowsky and Gallyas silver impregnations and thioflavine-S for AD pathology [8, 18, 54]), more sensitive and reliable IHC techniques are now preferred. IHC methods are more consistent and dependable than are silver impregnation techniques, they have greater inter-rater reliability, as shown by the BrainNet Europe Consortium study [1], and IHC results can suggest or identify underlying molecular pathology.

For example, antibodies raised against epitopes of tau readily label the neurofibrillary tangles, neuritic plaques, and neuropil threads of AD; anti- -amyloid antibodies detect diffuse and compact -amyloid deposits and cerebral amyloid angiopathy [10]; while anti- -synuclein antibodies label Lewy bodies and Lewy neurites, the signature lesions of DLB [10, 52]. Neuropathologic staging schemes have been developed using tau, -amyloid and -synuclein IHC, and IHC is now replacing conventional stains in the neuropathologic diagnostic criteria for AD and DLB [10, 18, 52]. Prion IHC may be used reliably to detect or exclude prion disease in most cases [33]. Proteins targeted for degradation are ubiquitinated and several hallmark inclusions in neurodegenerative disease either in neurons or glia or both are detected by ubiquitin IHC [17, 19, 47, 48]. There is also age-related accumulation of ubiquitinated material in the brain [23], which can make detection of certain ubiquitin-related pathologies difficult. Until recently, ubiquitin IHC was the only marker for certain neuronal inclusions seen in FTLD and ALS/MND that contained neither tau nor -synuclein epitopes. P62 (sequestosome-1) IHC has recently been highlighted as an alternative method to detect a range of ubiquitin-immunoreactive structures in neurodegenerative diseases including ALS/MND, and FTLD. Like ubiquitin IHC, a range of pathological and age-related abnormalities are detected, but an advantage over anti-ubiquitin IHC appears to be that there is better contrast in the detection of intracellular pathology. More recently, TDP-43 has been identified as a major component of the inclusions of FTLD with ubiquitin-positive, tau- and -synuclein-negative inclusions (FTLD-U) [3, 61], formerly called FTLD with MND-type inclusions, but without MND [53]. This protein now defines a novel class of neurodegenerative diseases collectively called TDP-43 proteinopathies [16], and TDP-43 IHC may be used to characterize a majority of FTLD-Us, but not all [16].

Although IHC is essential for determining the underlying molecular pathology of the majority of neurodegenerative diseases, other techniques may be available in dementia research centers and complement the routine neuropathologic diagnosis. The reliable and robust detection of abnormally aggregated proteins either within neurons or glia or both, or in the neuropil, is necessary for neuropathologic diagnosis. However, the density and distribution of abnormal protein aggregates, as identified by IHC, do not always correlate well with clinical symptoms. Other markers, such as synaptic and neuronal loss in affected brain areas, may correlate better with cognitive impairment and motor dysfunction. Thus, stereologic methods that assess synaptic and neuronal loss in an unbiased manner may be useful in clinico-pathologic studies in the dementia research center, but are not necessary, or usually feasible, for routine neuropathologic diagnosis.

Biochemistry is also useful, but not essential for diagnosis. Methods of fractionating brain homogenates may be used to rationally classify the tauopathies in a research setting [13]. In the adult brain, there are normally six isoforms of the microtubule-associated protein tau (MAPT): three isoforms with 0, 1, or 2 inserts contain three microtubule-binding repeats (3R tau) and three isoforms, also with 0, 1, or 2 inserts, contain four microtubule-binding repeats (4R tau) [28]. The tauopathies have a biochemical signature: tau protein in these disorders is relatively insoluble and these insoluble species can be detected by biochemical fractionation methods. The insoluble fractions may be further characterized according to the pattern of tau isoforms. For example, in AD, all six isoforms are abnormally hyperphosphorylated and migrate as three major bands and one minor band when visualized by immunoblotting. Treatment with the enzyme alkaline phosphatase removes phosphate groups, and the tau isoforms appear as six bands (3R and 4R tau). This biochemical signature may be used to distinguish AD from the FTLD tauopathies [13]. Thus, brain tissue from patients with FTLD where Pick bodies are present is characterized biochemically by predominantly 3R tau, while CBD, PSP, argyrophilic grain disease (AGD), and sporadic multiple system tauopathy with dementia (MSTD) are predominantly 4R tauopathies [13, 74], and neurofibrillary tangle dementia (NTD), also called tangle predominant form of senile dementia, has inclusions containing a mixture of 3R and 4R tau [34, 35]. FTLD with *MAPT* mutation, of which more than 40 have been described, is biochemically heterogeneous with different mutations being associated with 3R, 4R, or 3R and 4R tauopathy [15]. Monoclonal antibodies, which discriminate between 3R and 4R tau [22] are now commercially available; so, the molecular classification of tauopathies by isoform type may be easily undertaken in the histology laboratory that does not have access to biochemistry.

FTLD with ubiquitin-positive, tau-negative inclusions (FTLD-U), also known as FTLD with MND-type inclusions or MND inclusion dementia, is the most common underlying pathology in FTLD with and without clinical MND [45, 73]. TAR DNA-binding protein 43 (TDP-43), a nuclear protein implicated in exon skipping and transcription regulation, was recently identified as a major protein component of the ubiquitin-immunoreactive inclusions characteristic of sporadic and familial FTLD-U, with and without clinical MND, as well as in sporadic ALS [3, 16, 21, 61]. Biochemistry in these disorders shows TDP-43 to be abnormally phosphorylated, ubiquitinated and cleaved to generate C-terminal fragments, and is recovered only from areas with ubiquitin-immunoreactive inclusions including hippocampus, neocortex, and spinal cord [61]. The neuropathology of these conditions is characterized by ubiquitin- and TDP-43-positive neuronal cytoplasmic inclusions (NCIs), neuronal intranuclear inclusions (NIIs), dystrophic neurites (DNs), and glial cytoplasmic inclusions (GCIs) that are negative for tau, -synuclein, -amyloid, neuronal

intermediate filaments, and expanded polyglutamines [21, 61]. The variability in the morphologic types of neuronal inclusions, their distribution, density, and immunohistochemical profile has led to the proposed classification of FTLD-U into four pathologic subtypes [16, 63, 66]. Recently, mutations in the *progranulin* (*PGRN*) gene [4, 20, 57], the molecular genetic basis of non-tau familial FTD linked to chromosome 17, were discovered. The neuropathology in these cases is FTLD-U with ubiquitin-positive neurites, NCIs and, most characteristically, NIIs [4, 50] (but see ref. [37]). However, NIIs can be seen in other FTLD-U cases where *PGRN* mutations are not found [16] and therefore such NIIs cannot be considered pathognomonic for *PGRN* or other (i.e., *valosin-containing protein VCP*) mutations associated with FTLD. As demonstrated by IHC and biochemistry, the ubiquitinated pathologic protein in these cases is not progranulin, but TDP-43 [4, 57]. Pathologic TDP-43 is detected biochemically in both affected gray and white matter, suggesting that both glial and neuronal pathology may contribute to the pathogenesis of FTLD-U caused by *PGRN* mutations [61].

Frontotemporal lobar degeneration with *VCP* gene mutation, also called inclusion body myopathy associated with Paget's disease of bone and frontotemporal dementia (IBMPFD), is a rare autosomal dominant disorder caused by mutations in the *VCP* gene [77]. VCP, a member of the AAA-ATPase gene super family (ATPase associated with diverse cellular activities), has multiple cellular functions including acting as a molecular chaperone in endoplasmic reticulum-associated protein degradation, stress response, programmed cell death, and interactions with the ubiquitin-proteasome system. The neuropathology in FTLD with *VCP* mutation is a unique subtype of FTLD-U and is characterized by numerous NIIs and relatively few NCIs and DNs [26]; the ubiquitinated inclusions are not primarily composed of the mutated protein (VCP), but rather TDP-43 [26, 63].

Frontotemporal lobar degeneration with *charged multivesicular body protein 2B* (*CHMP2B*) gene mutation is the cause of FTD linked to chromosome 3 in a large Danish pedigree [68]. Human CHMP2B is a component of the endosomal secretory complex, which becomes dysregulated by the gene defects. Recent studies have revealed ubiquitin-positive, but TDP-43 negative, NCIs in the frontal neocortex and hippocampus, so that this disease is an FTLD-U, but not a TDP-43, proteinopathy [16].

A genetic locus on chromosome 9p for familial FTD-MND has been described [56]. In one family, candidate gene sequencing revealed the presence of a putative disease segregating stop codon mutation (Q342X) in the *intraflagellar transport protein 74* (*IFT74*) gene [55]. IFT74 is a protein that localizes to the intracellular vesicle compartment and is a component of the intraflagellar transport system responsible for vesicular transport of material synthesized within the cell body into and along dendrites and axons. Neuropathology in a single case with the *IFT74* mutation was reported as showing all the stigmata of FTLD-U (ubiquitinated NCIs, DNs, and NIIs) and TDP-43 proteinopathy similar to that seen in other reported families with FTD, with or without MND linked to chromosome 9p [16]. Nonetheless, it remains to be established in other families and patients that *IFT74* is indeed a true locus for FTLD.

Neuropathologic classification of FTLDs

Following the principles of the previous consensus criteria for the neuropathologic diagnosis of FTLD [53], and the consensus criteria for the postmortem diagnosis of AD [18] and DLB [52], we acknowledge that only probabilistic statements can be made as to the causal relationship between the neuropathology and the clinical phenotype. Just as the constellation of clinical symptoms associated with FTD, SD, or PNFA do not predict reliably the underlying causative neurodegenerative disease, the presence of the neuropathology of FTLD does not predict with certainty one or other of the clinical phenotypes associated with FTLD, or even if the subject was demented. Small series of cases are inadequate to reliably and robustly determine clinico-pathologic correlations with any one form of FTLD. Multi-center collaborations are beginning to address this challenge [25], and it is only by pooling relatively rare cases from several research centers that reliable clinico-pathologic correlations are likely to emerge.

The neuropathologic criteria proposed here (Table 1) are an evolution of the 2001 criteria proposed by McKhann et al. [53], and take into account more recent descriptions of novel disease entities [6, 14], the discovery of causative gene defects (*PGRN*, *VCP*, *CHMP2B*) and linkage to chromosome 9p [4, 5, 20, 55, 56, 57, 68, 75, 77], and the novel (TDP-43) proteinopathy, which is present in most cases of FTLD-U with or without MND [3, 16, 21, 61]. The neuropathologic diagnosis of FTLD requires the exclusion of other neurodegenerative and systemic diseases, tumors, and drugs of abuse, which may cause a clinical FTLD phenotype. The proposed rational classification of neurodegenerative diseases associated with a clinical FTLD phenotype comprises seven distinct neurohistological types, and is based on the underlying molecular pathology as far as it is known.

Algorithm for the neuropathologic diagnosis of FTLD

The proposed criteria for the neuropathologic diagnosis and nosology of FTLD builds on, and extends, the existing criteria to include neuropathologic assessment using disease-specific antibodies, biochemistry, and molecular genetics to

Table 1 Comparison between the present proposed criteria and McKhann et al. [3] neuropathologic diagnostic criteria for FTLD

Present criteria	McKhann et al. criteria
1. Tauopathy (with associated neuron loss and gliosis) and insoluble tau with a predominance of 3R tau, the most likely diagnoses are:	1. When the predominant neuropathological abnormalities are tau-positive inclusions (with associated neuron loss and gliosis) and insoluble tau has a predominance of tau with three microtubule-binding repeats, the most likely diagnoses are:
FTLD with Pick bodies	(a) Pick disease
FTLD with *MAPT* mutation	(b) Frontotemporal dementia with parkinsonism linked to chromosome 17
	(c) Other as yet unidentified familial and sporadic frontotemporal disorders
2. Tauopathy (with associated neuron loss and gliosis) and insoluble tau with a predominance of 4R tau, the most likely diagnoses are:	2. When the predominant neuropathological abnormalities are tau-positive inclusions (with associated neuron loss and gliosis) and insoluble tau has a predominance of four microtubule-binding repeats, the most likely diagnoses are:
Corticobasal degeneration	(a) Corticobasal degeneration
Progressive supranuclear palsy	(b) Progressive supranuclear palsy
Argyrophilic grain disease	(c) Frontotemporal dementia with parkinsonism linked to chromosome 17
Sporadic multiple system tauopathy with dementia	(d) Other as yet unidentified familial and sporadic frontotemporal disorders
FTLD with *MAPT* mutation	
3. Tauopathy (with associated neuron loss and gliosis) and insoluble tau, with a predominance of 3R and 4R tau, the most likely diagnoses are:	3. When the predominant neuropathological abnormalities are tau-positive inclusions (with associated neuron loss and gliosis) and insoluble tau has a predominance of three and four microtubule-binding repeats, the most likely diagnoses are:
Neurofibrillary tangle dementia	(a) Neurofibrillary tangle dementia
FTLD with *MAPT* mutation	(b) Frontotemporal dementia with parkinsonism linked to chromosome 17
	(c) Other as yet unidentified familial and sporadic frontotemporal disorders
4. Frontotemporal neuronal loss and gliosis without tau- or ubiquitin/P62-positive inclusions, the most likely diagnosis is:	4. When the predominant neuropathological abnormalities are frontotemporal neuronal loss and gliosis without tau- or ubiquitin-positive inclusions and without detectable amounts of insoluble tau, the most likely diagnoses are:
FTLD (also known as dementia lacking distinctive histologic features)	(a) Frontotemporal lobar degeneration (also known as dementia lacking distinct histopathological features)
	(b) Other as yet unidentified familial and sporadic frontotemporal disorders
5. TDP-43 proteinopathy with associated neuronal loss and ubiquitin-positive/P62-positive, tau-negative inclusions, with MND or without MND but with MND-type inclusions, the most likely diagnoses are:	5. When the predominant neuropathological abnormalities are frontotemporal neuronal loss and gliosis with ubiquitin-positive, tau-negative inclusions and without detectable amounts of insoluble tau, with MND or without MND but with MND-type inclusions, the most likely diagnoses are:
FTLD-U with MND (FTLD-U types 1–3)	(a) Frontotemporal lobar degeneration with MND
FTLD-U but without MND (FTLD-U types 1–3)	(b) Frontotemporal lobar degeneration with MND-type inclusions but without MND, or
FTLD-U with *PGRN* mutation (FTLD-U type 3)	(c) Other as yet unidentified familial and sporadic frontotemporal disorders.
FTLD-U with *VCP* mutation (FTLD-U type 4)	
FTLD-U linked to chromosome 9p (FTLD-U type 2)	
Other as yet unidentified TDP-43 proteinopathies	