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Tau progression in single severe frontal traumatic brain injury in human brains

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ABSTRACT

The neuropathological features of chronic traumatic encephalopathy (CTE), caused by repeated traumatic brain injury (TBI), include abnormal accumulations of hyper-phosphorylated tau (p-tau) protein in neurons, neurites and astrocytes, considered to progress via neuronal circuits in brains. Some previous reports suggest that a single severe TBI (sTBI) can also induce CTE and p-tau accumulation, but it is not clear whether the pathology is the same as that of repetitive TBI (rTBI). Since prefrontal leucotomy could be regarded as a model of sTBI, in this study we evaluated two autopsied schizophrenia with this procedure. Histopathologically, gliosis and neuronal loss were found not only in the primary ablated prefrontal region, but also in secondary affected areas, i.e., cingulate gyrus, medial nucleus of the thalamus, and nucleus accumbens, which are connected to prefrontal areas. Accumulation of p-tau was mostly seen in neurons, neurites and glias around small blood vessels in the leucotomized prefrontal region. In addition, secondary regions showed some p-tau-positive neurons/glias, as well as many axonal spheroids. Regions of neuronal/glial p-tau pathology showed immunoreactivity to both 3R/4R tau antibodies. Immunoblot analyses of sarkosyl-insoluble tau from frozen brains showed an AD-type tau banding pattern with strong immunoreactivities. sTBI patients showed limited comorbidities, such as TDP-43, alpha-synuclein or AD pathology, whereas rTBI patients have high frequencies of them. The findings suggest that p-tau in the primary affected lesion might progress to connected regions via neuronal circuits over time, and a single severe axonal injury might lead to CTE pathology different from that caused by rTBI.

1. Introduction

Chronic traumatic encephalopathy (CTE) is a distinct neurodegenerative disease caused by repeated traumatic brain injury (TBI) associated with contact sports [1]. Recent immunohistochemical analysis showed that CTE brains contain abnormal accumulation of hyperphosphorylated tau protein (p-tau) [1]. Consensus panels have defined the neuropathological features of CTE as a patchy distribution of abnormal p-tau accumulations in neurons (neurofibrillary tangles), with astrocytes and cell processes distributed in irregular patterns, typically around small blood vessels at the depth of cortical sulci [1,2]. The pathological mechanism of CTE is considered to be as follows: head trauma induces focal axonal injury, microhemorrhage and gliosis in close proximity to the affected area, then abnormal p-tau accumulates in neurons, axons and dendrites in the perivascular regions due to axonal injury, breach of the blood-brain barrier, and neuroinflammation, and finally p-tau spreads throughout the brain [3]. The severity of CTE pathology might be related to age at death and duration of participation in contact sports [2]. Staging of CTE severity on the basis of NFT distribution has been attempted [2], but remains controversial, since there are discrepancies among pathological descriptions in various studies [4,5]. Although CTE has been widely considered to be a consequence of exposure to repetitive TBI (rTBI) [1–3], some reports suggest that a single severe TBI (sTBI) can also induce p-tau accumulation and CTE [4,6,7]. One recent report suggested that a prefrontally leucotomized brain can be considered as a model of a sTBI with severe axonal damage, leading to p-tau accumulation and CTE pathology [8]. Prefrontal leucotomy, which is a form of psychosurgery, was widely performed in

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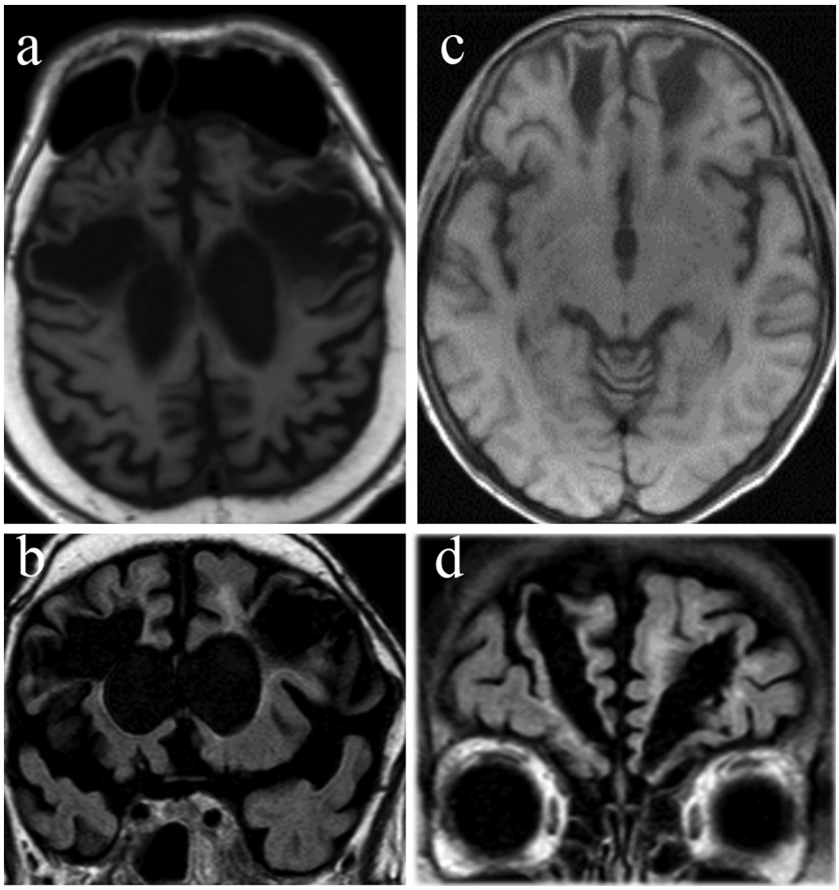

Fig. 1. Imaging of the leucotomized schizophrenia patients. T1-weighted magnetic resonance images (MRI) of case 1 at age 76 (a, b) and case 2 at age 71 (c, d). MRI revealed cystic lesions around the anterior horn of the lateral ventricles as low intensity signals and enlarged anterior horn of the lateral ventricles. Atrophy is present in the corpus callosum.

the 1940's for the treatment of refractory mental illnesses, especially schizophrenia. In this surgery, white matter of the prefrontal lobe was focally cut [9], based on the idea that cutting the fiber connections to the prefrontal region would alter functional connections and intend to the improvement of psychiatric symptoms [10,11]. This surgery has been prohibited since the 1970's for ethical reasons, and because of the development of psychopharmacology for schizophrenia patients. The pathology of leucotomized patients has been reported previously [9,11–14], but we considered that more detailed immunohistochemical examinations might be worthwhile to throw light on the possible long-term consequences of sTBI.

The pathology underlying neurodegenerative diseases is associated with the formation of cytoplasmic inclusions. Pathological proteins such as abnormal p-tau have been considered to spread via neuronal circuits [2,3,15–18]. Many studies suggest that spreading of neuronal p-tau progresses at different rates in human brains [15–18]. Those results are consistent with the idea that pathological proteins spread from neuron to neuron via axonal transport in a hierarchical manner [19,20]. However, the structural nature of the progression of p-tau in CTE brains remains unclear.

Therefore, the aims of this study are to clarify the neuropathological features of CTE resulting from a single sTBI and to elucidate the distribution of abnormal p-tau accumulation in relation to the connectivity of affected fibers with axonal damage, based on autopsy studies of two patients with schizophrenia historically treated with prefrontal leucotomy, which we take as a model of single sTBI.

2. Methods

2.1. Neuropathological examination

Autopsy was performed with written consent from family members. The brains were fixed in 10% formalin and embedded in paraffin. Sections 10 μm thick were stained with hematoxylin-eosin (HE), Klüver-Barrera (K-B), Holzer, and Gallyas-Braak. Immunohistochemistry was performed for tau, AT8 (p-tau: pSer199 & pSer202, mouse monoclonal, 1:1000, Thermo Scientific), AP422 (p-tau: pSer422, rabbit polyclonal, 1:1000, made by M. Hasegawa), α-synuclein (pSyn#64, mouse monoclonal, 1:1000 Wako), TDP-43 (pSer409/410, rabbit polyclonal, 1:1000; made by M. Hasegawa), Aβ (E50: aa17–31, rabbit polyclonal, 1:1000, made by H. Akiyama), RD3 (directed to residues 209–224, 1:500, Millipore), RD4 (directed to residues 275–291, 1:500, Millipore) and anti-glial fibrillary acidic protein (GFAP, polyclonal, rabbit, 1:3100, DAKO). Primary antibody labelling was visualized using 0.2% 3,3′-diaminobenzidine in combination with an Envision Plus kit (Dako Japan, Tokyo), according to the manufacturer's instructions, as described previously [18].

2.2. Immunoblot analyses

Fresh frozen samples of the ablated frontal region, parahippocampal region, striatum and MD in which p-tau pathology was present were obtained from both cases. Frozen brain tissue (0.2–0.5 g) was homogenized in 20 volumes (v/w) of extraction buffer containing 10 mM Tris–HCl (pH 7.5), 0.8 M NaCl, 10% sucrose, 1 mM EGTA, 2% sarkosyl and incubated for 30 min at 37 °C. After centrifugation at 20,000 × *g* for 10 min at 25 °C, the supernatants were taken and ultracentrifuged at 100,000 × *g* for 20 min at 25 °C. The pellets were washed by ultracentrifugation with 0.5 mL of sterile saline, solubilized in SDS-sample buffer and subjected to 4–20% gradient polyacrylamide gel (Wako) SDS-PAGE as described (Taniguchi-Watanabe et al., 2016). pS396 (phospho-Ser396: Calbiochem, 1:2000, Calbiochem) was used for immunoblotting. Primary antibody labeling on the membranes was visualized with 3,3′-diaminobenzidine as a chromogen, in combination

with a Vectastain ABC kit® (Vector Lab., USA).

3. Results

3.1. Clinical findings

3.1.1. Case 1

This male patient presented with continuous refractory delusions, catatonic behavior, and impulsive behaviors such as tearing his clothes and shouting incomprehensibly, and was diagnosed with schizophrenia at a young age. He was leucotomized to relieve persistent symptoms in his teens. He gradually developed negative symptoms, including diminished emotional expression and social dysfunction. In his 70's, he suffered physical comorbidities such as cerebral infarction, pneumonia, and ileus. Brain MRI at age 76 revealed cystic lesions around the anterior horn of the lateral ventricles as low intensity signals and enlarged anterior horn of the lateral ventricles (Fig. 1a,b). He had long been hospitalized due to intermittent psychomotor excitement prior to his death from myocardial infarction at the age of 76 years.

3.1.2. Case 2

This female Japanese patient presented with auditory hallucinations, persecutory delusions, disorganized thinking, and negative symptoms, and was diagnosed with schizophrenia in her late teens. She was repeatedly hospitalized due to exacerbation of the symptoms. In her twenties, she underwent orbito-ventromedial leucotomy but her symptoms were not improved. She entered remission for a while, but at age 70, she attempted suicide, leading to re-admission to our hospital. She often presented empty monologues and impulsive behaviors, despite various medications. At age 71, brain MRI revealed low intensity signals in the bilateral cortices and subcortices of the rectal, orbitofrontal, and superior frontal gyri (Fig. 1c,d). At age 72, her Positive and Negative Syndrome Scale (PANSS) score for schizophrenia was 136 (Positive score: 36, Negative score: 45, General Psychopathology score: 55), her Revised Hasegawa Dementia Scale (HDS-R) was 5, and her Mini-Mental State Examination (MMSE) score was 8, indicative of cognitive impairment. She died of a bacterial liver abscess at age 73.

3.2. Neuropathological findings

The brains weighed 937 g (case 1) and 1341 g (case 2) before fixation and each displayed a leucotomy incision scar on the dura of the bilateral frontal lobe. Convolutions and sulci around the scar were damaged more in Case 1 than in Case 2. Case 1 revealed severe congestion on the surface of the brain without atherosclerotic change. Coronal sections showed evident surgical incision scars with cysts, with a maximum diameter of 1.5 cm (case 1) and 2.5 cm (case 2), on the surface of the bilateral frontal lobe. Atrophy of caudate nucleus and medial part of the thalamus was observed, but no atrophy was apparent in other regions, including hippocampus, amygdala, brain stem, and spinal cord. Microscopically, a surgical incision scar was evident in the ventromedial part of the frontal lobe (Fig. 2a). Severe neuronal cell loss with gliosis was prominent in a magnified area of the ablated prefrontal area (shown by the rectangle in a) (Fig. 2b). Severe demyelination and gliosis next to the ablated prefrontal lesion were also observed (shown by the rectangle in b) (Fig. 2c). In this region, some neurons showed a balloon shape (Fig. 2d). In the medial nucleus of thalamus (MD), severe neuronal cell loss with gliosis was also prominent, with many axonal spheroids (Fig. 2e). Mild gliosis without neuronal cell loss was observed in the nucleus accumbens (NAcc) (Fig. 2f) and the cingulate gyrus (CG) (Fig. 2g). Slight gliosis was seen in the basal ganglia, including caudate nucleus, putamen, and subthalamic nucleus. Case 2 showed milder neuronal cell loss with gliosis than case 1. Immunohistochemically, GFAP immunoreactivity confirmed gliosis with massive astrocyte accumulation in each region (Supplementary Fig. 1a–d). P-tau immunostaining revealed considerable numbers of tau-positive neurons and glia in the frontal area (Fig. 3a–d), and small numbers in CG, MD, and NAcc, as secondary affected areas. Occasional perivascular accumulation of p-tau (ps422) aggregates was found (Fig. 3a). Prominent astrocytic tangles and neurites were present in the subcortical lesions (Fig. 3b). Astrocytic tangles (Fig. 3c) and NFTs (Fig. 3d) were seen, though less frequently, in the cortex. P-tau immunohistochemistry in case 2 revealed lower extents of p-tau accumulation in the neurons and glia in the frontal area and weaker but distinct immunoreactivity in the CG and MD (Fig. 3e) as secondary affected areas, in comparison with Case 1. Some RD3 pathology was detected in neurons (Fig. 3g) and astrocytes (Fig. 3h), while RD4 immunoreactivity was weaker (Fig. 3f).

In both cases, the hippocampus, mammillary body, amygdala, cerebellum, and brainstem showed no remarkable histopathologic changes or immunopositivity. NFT/Aβ stages around the hippocampal region and other regions were mild, Braak & Braak NFT stage I (case 1)/II (case 2) and Aβ stage A [15]. No other aging-related proteins, including α-synuclein and TDP-43, were noted (Supplementary Fig. 1e,f). The severity and distribution of pathology in the two cases are shown in Table 1.

3.3. Immunoblot analyses

The results of immunoblot analyses of samples from the two patients are shown in Fig. 4. In the frontal cortex, striatum, MD and parahippocampal regions, AD-type triplet tau bands of 60, 64 and 68 kDa with C-terminal fragments were detected with anti-pTau antibody (pS396), suggesting that both 3R and 4R tau isoforms are accumulated in sTBI cases, as in AD. The amount of insoluble tau in the parahippocampal regions was smaller than that in the ablated frontal regions in these patients (F2 > T in case 1, F1, F3 > T in case 2). Since we recognized patchy distribution of p-tau pathology around the frontal regions neuropathologically, we analyzed several tissues from these regions, and observed intensity differences in some samples of the frontal regions even from the same individual.

4. Discussion

Pathological abnormal proteins are considered to spread via axonal pathways to other regions, and the patterns of the propagation are highly organized, being constrained by the extraordinarily complex topology of the underlying neural architecture [19]. Magnetic resonance diffusion tensor imaging (DTI), which can detect alterations in white matter fiber tract integrity, has revealed atrophy with connectivity to the frontal region in patients over 40 years after leucotomy [21,22]. Indeed, DTI images of brains from patients including our two cases have revealed fractional anisotrophy (FA), with lower FA in the corpus callosum, CG, MD, NAcc, internal capsule, and brainstem, in comparison with brains from non-leucotomized patients with schizophrenia and healthy controls [23].

As for neuronal circuits, the orbital and medial prefrontal cortex (PFC) has strong connections with MD [24], forming a part of the basal ganglia-thalamocortical pathways. The limbic loop is a neuronal circuit in which the PFC projects to the ventral striatum (composed of striatum and NAcc). In turn, the ventral striatum projects to the globus pallidus, which projects to the MD [25,26]. The CG has a strong projection from the dorsolateral PFC [26]. These structures of the basal ganglia constitute a network with the PFC or CG, and there are strong connections between the PFC and MD. Previous neuropathological studies of patients with leucotomy, summarized in Table 2, indicate that neuronal cell loss and gliosis are more common in MD [12–14]. These studies also suggest that secondary neurodegeneration may occur in the diffuse white matter fiber tract, especially in the frontopontine fibers, superior and inferior longitudinal fasciculus, uncinate fasciculus, CG, and olfactory tract, which had not been cut directly [11,27]. Thus, sTBI cases in this study appears to induce neurodegeneration in a wide range of connected regions. This is in accordance with the neuropathological

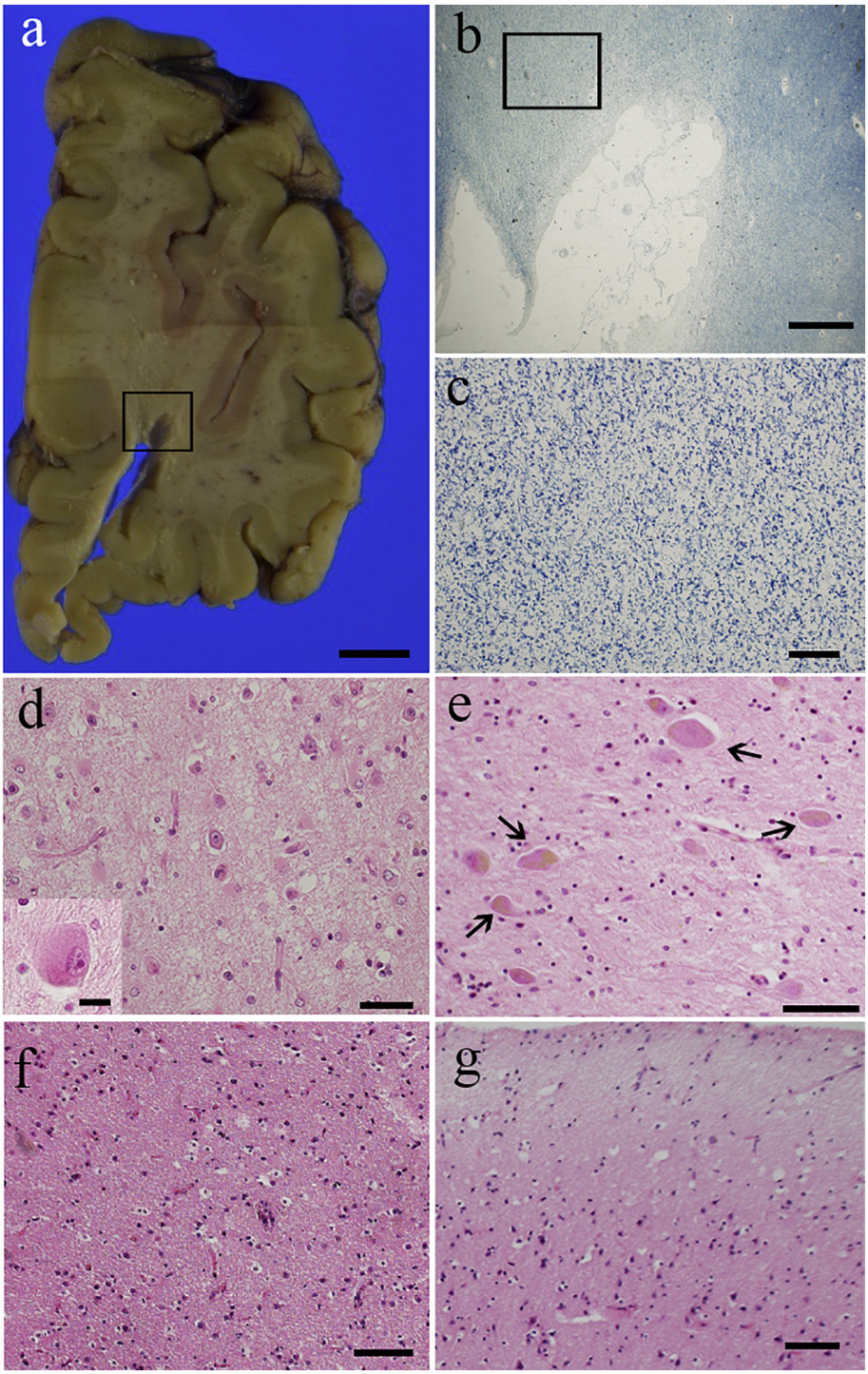

Fig. 2. General neuropathological findings in patients.
Macroscopic photograph of the left frontal lobe showing a surgical incision scar in the ventromedial region (a). Severe tissue loss with gliosis in the magnified area of the ablated prefrontal area (shown by the rectangle in A) (Kluver-Barrera stain) (b). Severe demyelination and gliosis next to the ablated prefrontal lesion (shown by the rectangle in B) (Kluver-Barrera stain) (c). Neuropathological change in the medial nucleus of the thalamus (d, e). Severe neuronal cell loss with gliosis; some neurons are ballooned (d), axonal spheroids (black arrows) (e). Mild neuronal cell loss with gliosis in the nucleus accumbens (f) and the cingulate gyrus (g). (Hematoxylin-eosin stain) (d-g). case 2 (a, b, c) and case 1 (d, e, f, g). Scale bars, 1 cm (a), 200 μm (b) 100 μm (c,f,g), 50 μm (d,e), 10 μm (within the framework of d).

findings showing severe axonal damage with many axonal spheroids in affected secondary areas and ballooned neurons in the primary frontal cortex. There is also p-tau accumulation in some regions. In our two cases, regions with degeneration coincided with those showing lower fractional FA. Thus, the findings overall suggest that single sTBI can induce focal axonal injury and gliosis near the primary/secondary affected area, as well as abnormal p-tau accumulation in neurons, axons and dendrites due to axonal injury with neuroinflammation.

Immunohistochemical analysis in our study supports the presence of six brain tau isoforms similar to those found in AD. Indeed, the morphologies of tau filaments in CTE were recently reported, and all six brain tau isoforms assemble into filaments in CTE, as well as in AD, though the conformation of the β-helix region in CTE is different from that in AD [28]. This region creates a hydrophobic cavity that is absent in tau filaments in AD, and the filaments in CTE have distinct protofilament interfaces, compared to those of AD. It was suggested that the conformers of filamentous tau might define distinct neurodegenerative diseases [15–18,28].

The clinical/neuropathological entity of CTE has not yet been fully defined [5,29]. No consensus has been reached on staging the severity of CTE pathology [29] and the pathophysiological mechanisms of p-tau accumulation and progression in CTE are still unknown. In primary affected regions, p-tau pathology is produced by conformationally abnormal protein or peptide assemblies, modulated by genetic risk

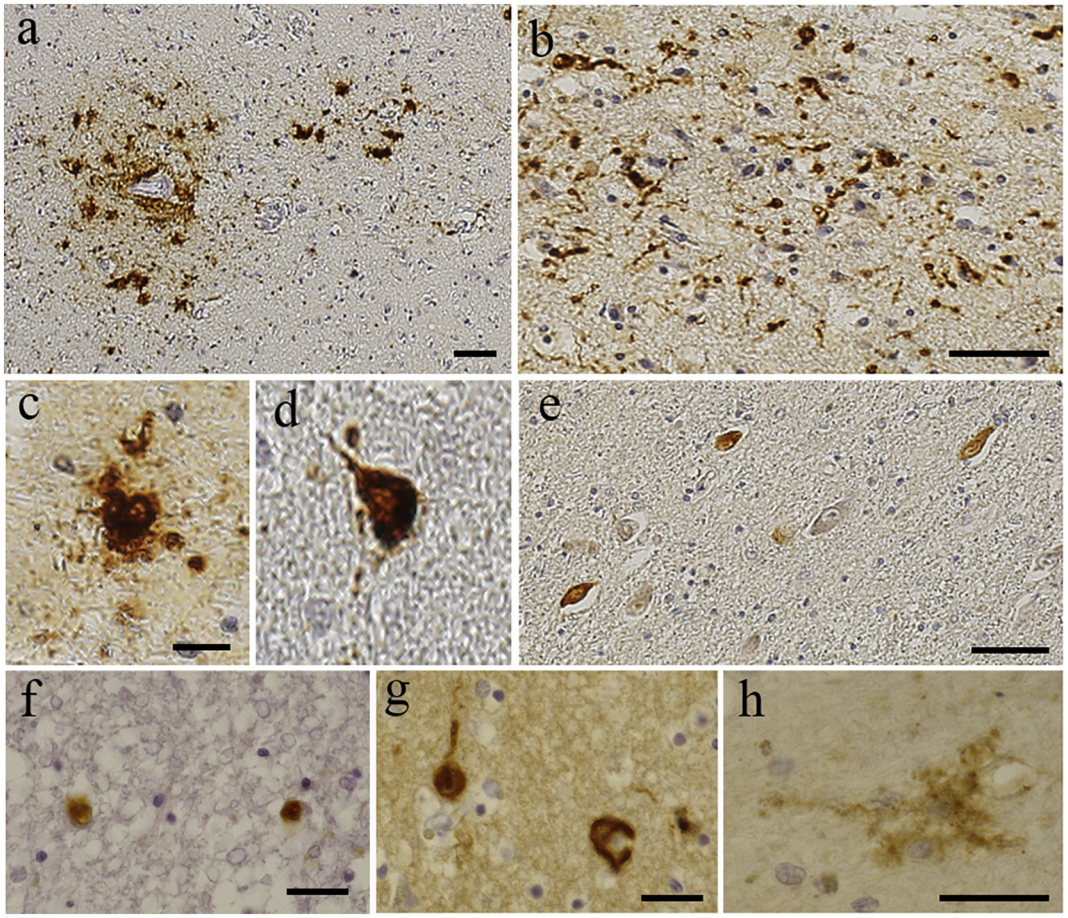

Fig. 3. p-Tau pathology in sTBI patients.
Tau accumulation was observed in the ablated frontal lobe in case 1 (a-d, f-h) and in the thalamus in case 2 (e). Perivascular accumulation of p-tau (ps422) aggregates in astrocytes (a). p-tau-positive astrocytes and neurites are found in the subcortical lesions (b). Astrocytic tangle in the cortex (c). NFT in the cortex (d). Neuronal p-tau accumulation is also seen in the thalamus (e). RD3-positive glias(f) and RD4-positive neurons (g) are present in the affected frontal cortex. Astrocytic tangles with RD4 (h) are more prominent than those with RD3. Scale bars, 50 μm (a, b, e), 25 μm (c, d, f, g, h).

factors, environmental factors and so on. Over several decades, many different self-propagating "strains" of tau aggregates might be formed in the primary affected lesion and be replicated in cells, leading to distinct neuropathological lesions [30–32]. In accordance with this hypothesis, finally, they might progress to secondary regions with connecting pathways to the primary lesion [32,33]. Also, the p-tau distribution is not consistent with the affected regions of rTBI, as described in the CTE diagnostic criteria [1,2]. Those findings support the idea that p-tau progresses as an anterograde/retrograde reaction in neuronal circuits secondary to old trauma, suggesting that the processes of the p-tau formation after TBI might drive progression over many years [5]. In fact, previous reports suggested that patients with moderate/severe sTBI did not show NFTs within months after trauma [7,32], but exhibited massive p-tau pathology beyond 1 year [34,35]. The mechanism remains unclear, but severe and massive sections in leucotomy might induce irreversible neurodegeneration.

Our findings here indicate that the distribution of p-tau pathology in sTBI differs from that in rTBI, as described in reported diagnostic criteria [1], since p-tau pathology in sTBI is not distributed in sulcus. Previous studies of sTBI associated with single attack head trauma, unilateral severe infarction or hemorrhage have found p-tau pathology in basal ganglia, such as putamen, globus pallidus [6], thalamus [36], and nucleus basalis of Meynert [37,38], with sparse or no NFTs in the limbic regions [6]. As far as we know, no previous report describes abnormal tau accumulation after neurosurgical sections for epilepsy or brain tumor. Interestingly, an sTBI rat model induced by needlestick injury in the cerebral cortex showed accumulation of p-tau, in addition to Abeta and APP [39]. The evidence suggests that damage to the brain, especially hemorrhagic damage, can lead to p-tau pathology.

Furthermore, our sTBI cases showed limited comorbidities, such as TDP-43, α-synuclein or AD pathology (massive NFTs in limbic regions and Aβ pathology), whereas rTBI patients have high frequencies of these comorbidities [2,34,40]. We have to consider relatively low age at death in both patients, however, sTBI might not accelerate multiple pathologies, in contrast to rTBI. Those data suggest that the pathological processes induced in sTBI are distinct from those in rTBI [5,41], at least in part. CTE has recently been described in settings with no history of TBI, so the nature of the pathology that we call CTE needs to be better defined [29].

Although no increased incidence of AD pathology has been reported in elderly patients with schizophrenia [42], care might be needed in administering antipsychotic drugs in schizophrenia patients [43]. Many previous reports suggest that schizophrenia is a neurodevelopmental disorder, and it might be related to maturity of the nerve system. However, in most regions of schizophrenia brains, neuronal loss is not common, and several reports have concluded that dementia in elderly schizophrenic patients shows no pathological evidence indicative of any known neurodegenerative disorder, including specific tau pathology [44–46]. Thus, we consider that the neuropathological changes may represent the natural pathological process of sTBI in the brains of these patients, as in healthy controls.

As a limitation of this study, it must be emphasized that the number

Table 1
Distribution and severity of neurodegenerative changes/tau pathology in patients.

	Case 1		Case 2	
Brain weight (g)	937		1341	
Cortical atrophy	F		F	
Brain regions	Neuronal loss/gliosis	Tau pathology	Neuronal loss/gliosis	Tau pathology
Frontal cortex	+ +	+ +	+ +	+ +
Cingulate gyrus	+	+	+	+
Temporal cortex	−	−	−	−
Hippocampus	−	+	−	+
Amygdala	−	−	−	−
Corpus callosum	+	−	+	−
Medial nucleus of the thalamus	+ +	+	+	+
Nucleus accumbens	− +	+	− +	+
Caudate nucleus	+	−	− +	−
Putamen	+	−	+	−
Globus pallidus	−	−	−	−
Subthalamic nucleus	− +	−	+	−
Nucleus basalis of Mynert	−	−	−	−
Cerebellar dentate nucleus	−	−	−	−
Red nucleus	−	−	−	−
Substantia nigra	−	−	−	−
Locus coeruleus	−	−	−	−
Pontine nucleus	−	−	−	−
Dorsal vagal nucleus	−	−	−	−
Hypoglossal nucleus	−	−	−	−
Inferior olivary nucleus	−	−	−	−
Spinal cord	−	−	−	−
Braak NFT stage	II		I	
Braak Aβ stage	A		A	

F, frontal lobe; T, temporal lobe; NFT, neurofibrillary tangle; SP, senile plaque.

of cases investigated was small, and we did not examine APOE or other AD pathology-related genes. [47].

To conclude, patients with sTBI show p-tau accumulation in the primary affected frontal lesion that progresses to connected regions via neuronal circuits over time, but, although the p-tau pathology resembles that in CTE, the mechanism of progression might be different from that in diagnostic criteria. However, we should note that p-tau accumulation and spread in those patients involves a very specific setting, and further studies are needed to confirm the putative difference between sTBI and rTBI, and to clarify in detail the pathophysiology of CTE.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jns.2019.116495.

Ethics approval and consent to participate

The patient in one case and the next of kin in the other case had provided written consent for autopsy and postmortem analyses for research purposes. This study was approved by the ethics committee at the Tokyo Metropolitan Matsuzawa Hospital, and was performed in accordance with the ethical standards outlined in the 1964 Declaration of Helsinki and its later amendments.

Consent for publication

Details that might disclose the identity of the participants in this study were omitted.

Availability of data and materials

All data and material are fully available without restriction.

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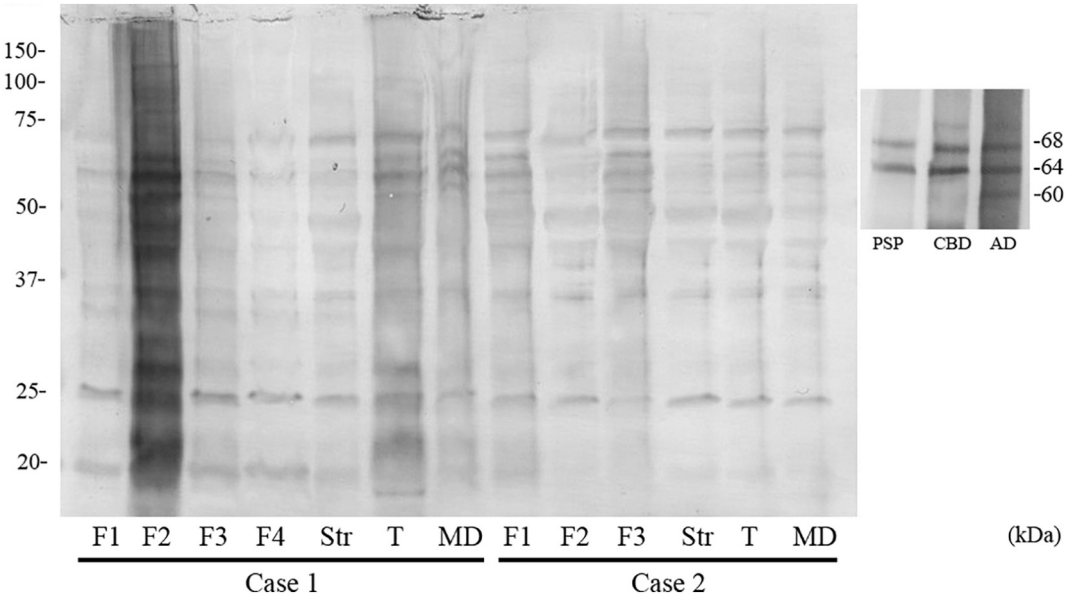

Fig. 4. Immunoblot analyses of sarkosyl-insoluble tau.
The sarkosyl-insoluble fractions of the ablated frontal regions (F1-4), striatum (Str), parahippocampal region (T), and medial nucleus of the thalamus (MD) were analyzed by immunoblotting. An anti-phospho-tau antibody, pS396 was used for detection. F2 in case 1 show strong immunoreactivities, while other regions present weak but distinct bands similar to those in AD.

Table 2
Previous studies of leucotomized patients as a model of severe single traumatic brain injury.

Authors	Ablated regions	Regions with neuronal loss/gliosis[a]	Other pathological findings
Greenwood et al. [8]	Prefrontal regions	F, MD	Arteriosclerosis
Yokoi et al. [40]	Prefrontal regions	Diffuse neuronal loss/gliosis, especially in F, MD, FPFs, SLF, ILF, UF, CG, OT	Arteriosclerosis
Mizushima et al. [23]	Prefrontal regions	Diffuse neuronal loss/gliosis, especially in F, MD, FPFs, SLF, ILF, UF, CG, OT	Arteriosclerosis
Tsuchiya et al. [37]	Medial orbital gyrus and rectal gyrus	F, MD, UF, AT, FPFs	Arteriosclerosis
Pakkenberg et al. [27,28]	Prefrontal regions	F, MD	–
Arai et al. [1]	Prefrontal regions	Thal, Severe diffuse demyelination in white matter of F	–
Shively et al. [31]	Prefrontal regions	Severe white matter damage with dense astrogliosis in F	p-tau immunoreactivity in neurons, astrocytes and neurites in perivascular regions in white matter/cortical sulcal depths

[a] F, ablated frontal regions; MD, medial nucleus of thalamus; FPFs, frontopontine fibers; SLF, superior longitudinal fasciculus, ILF, inferior longitudinal fasciculus; UF, uncinate fasciculus; CG, cingulate gyrus; OT, olfactory tract; AT, anterior temporal lobe Thal, thalamus.

Authors' contributions

YO drafted the initial manuscript and examined the clinical findings with IK. IK carried out the microscopic observation with YO, supervised the design and coordination of the study and worked up the manuscript. IK, KO and KN organized the brain archives including clinical information and performed neuropathological analyses. KW conceived of the study and participated in the initial design with YO. KI, HA and MH participated in the design of the study, gave advice on immunohistochemical examinations and helped to draft the manuscript. MH conducted the sample preparation and immunoblotting. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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