Lhermitte-Duclos disease with neurofibrillary tangles in heterotopic cerebral grey matter

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Abstract

We report a 46-year-old patient with Lhermitte-Duclos disease (LDD) who underwent a successful surgery but died of other causes four years later. The autopsy revealed Lhermitte-Duclos disease asymmetrically affecting both cerebellar hemispheres. The subcortical white matter of both cerebral hemispheres contained several foci of grey matter heterotopia. Only heterotopic neurons contained tau-positive neurofibrillary tangles (NFT), displaying characteristic ultrastructural features of paired helical filaments (PHF). Neither senile plaques nor NFTs were found in other areas of the central nervous system. The brain also showed other developmental abnormalities such as megalencephaly, numerous foci of meningeal glial heterotopia and multifocal telangiectasia. Although some of these findings were previously described in LDD, this is the first case of this disease with NFTs selectively accumulating in the neuronal heterotopic tissue.

Key words: *Lhermitte-Duclos disease, grey matter heterotopia, neurofibrillary tangles, PTEN, macrocephaly.*

Introduction

Lhermitte-Duclos disease (LDD), a disorder first described by French physicians Lhermitte and Duclos in 1920 [25], is a benign, slow growing dysplastic gangliocytoma of the cerebellum, characterized by replacement of the granule cell layer by abnormal granule and Purkinje like cells. The most frequent presenting signs and symptoms are megalocephaly, increased intracranial pressure, nausea, hydrocephalus, ataxia, gait abnormalities, and intermittent headaches, all of which are attributed to the mass effect [6,11,25]. Many cases are associated with a mutation in the phosphatase and tensin homolog or *PTEN* gene which is also involved in numerous otherwise unrelated central nervous system abnormalities, namely Cowden syndrome [1,6,11], autism spectrum disorder [18], cerebral cortical dysplasia [11,30] and Bannayan-Riley-Ruvalcaba syndrome [30]. The presence of cortical heterotopia has been reported in a small number of LDD cases [3,5,17,32]. We describe a unique case of LDD with cerebral cortical heterotopic grey matter containing neurofibrillary tangles.

Clinical history and autopsy findings

This 42-year-old male with a large head and partial left ocular paresis since childhood, presented with headaches, neck pain, ataxia and signs of raised

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intracranial pressure. Magnetic resonance imaging (MRI) scan showed left cerebellar mass and obstructive hydrocephalus. A ventriculo-peritoneal shunt was inserted. A posterior fossa craniectomy was carried out and multiple biopsies of the diffusely enlarged left cerebellar hemisphere were performed. Postoperatively the patient made a good recovery and returned to full employment until his sudden death from myocardial infarction and right parietal "stroke" four years later. He had no other significant past medical history. He was a university graduate and worked as an accountant. Post-mortem examination confirmed massive myocardial infarction with endocardial thrombi and systemic atherosclerosis.

The brain weighed 2,220 grams after formalin fixation. The combined weight of the cerebellum and brainstem was 250 grams. The left cerebellar hemisphere was enlarged and measured approximately 6.5 × 5.5 × 7.0 cm. The right (grossly normal) hemisphere measured 5.0 × 4 × 5.0 cm. The lateral and inferior aspects of the affected hemisphere displayed coarse, broad, firm and more numerous gyri, measuring from 5 to 10 mm in width. The brainstem was normal.

Coronal sections of the cerebral hemispheres confirmed a recent haemorrhagic infarct in the posterior parietal area. Both frontal lobes contained subcortical foci of heterotopic grey matter, each measuring approximately 2 cm in the largest diameter. Similar, smaller heterotopic nests were also present in the temporal and parietal lobes on each side.

Material and methods

Large hemispherical blocks of the cerebellum and frontal lobes as well as an additional set of brain tissue samples removed from all representative areas of the left cerebral hemisphere, were formalin fixed and routinely processed for paraffin embedding and staining with hematoxylin and eosin (H&E), Luxol Fast blue combined with Periodic Acid Schiff (PAS & LFB) as well as Kluver-Barrera method, and Palmgren stain for axons. Selected sections of the hippocampus, amygdala and frontal cortex with heterotopic foci were immunostained for Tau protein (Tau, rabbit polyclonal antibodies, 1 : 200), neurofilaments (NF, monoclonal antibody, 1 : 400), glial fibrillary acidic protein (GFAP, polyclonal antibody, 1 : 500) and ubiquitin (UBQ, 1 : 300), all from DAKO as previously reported [20,28]. Glutaraldehyde fixed biopsy specimen from the cerebellar tumour was routinely

processed for plastic embedding and ultrastructural study as previously reported [28]. Small fragments of heterotopic grey matter tissue in the frontal lobe were carefully removed from the large paraffin block, deparaffinised, washed in several changes of distilled water, post-fixed in 2% glutaraldehyde for 24 hrs, and re-embedded in epoxy-resin. Routinely stained thin sections were examined in a Phillips 200 electron microscope as previously reported [28].

Results

Microscopically, the cerebellar folia showed striking "inverted" architecture with a thickened molecular layer markedly enriched in the myelinated axons (Fig. 1). Normal Purkinje and granule cells were replaced by dysplastic ganglionic cells of various sizes, often reminiscent of abnormal Purkinje cells (Fig. 2A). Intermediate zones between the normal and abnormal cerebellar tissues showed gradual transitions of a steadily increasing number of large dysplastic cells replacing the small granule cells. Dysplastic areas showed rich vascularization and numerous interstitial vacuoles. Subcortical white matter was markedly reduced in volume and displayed loss of axons and myelin. Scattered intraparenchymal and meningeal vessels contained dusty mural calcifications. The dentate nucleus was abnormal in shape but otherwise not remarkable. The right cerebellar hemisphere contained several, small subpial foci of gangliocytoma composed of dysplastic neurons as well as a disorganized network of
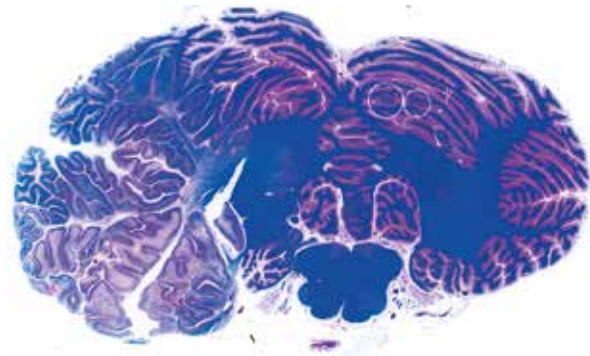

Fig. 1. Whole mount of cerebellum and brainstem showing massive enlargement of the left cerebellar hemisphere. Broadened and abnormal folia show myelination of the molecular layer and loss of myelin in the underlying white matter. Two independent small foci of gangliocytoma are present in the contralateral cerebellar hemisphere (encircled) (LFB&PAS stain).
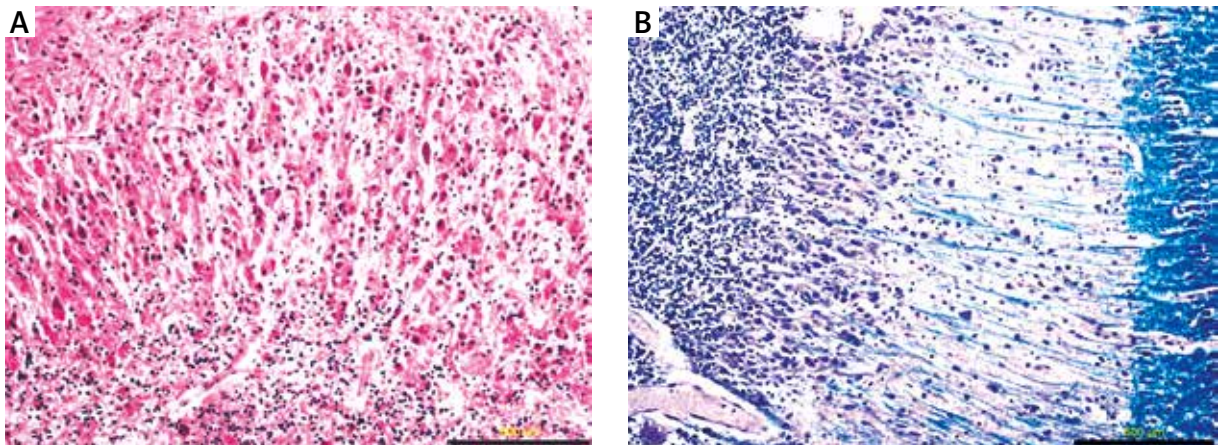
Fig. 2. Tumour in the left cerebellar hemisphere. Replacement of the molecular and Purkinje cell layers by dysplastic neurons (**A**). Contralateral hemisphere. Dysplastic ganglionic cells streaming from the Purkinje cells layer to the molecular layer. Granule cells are reduced in number. The broad band of subpial myelination of the molecular layer is on the right hand side.
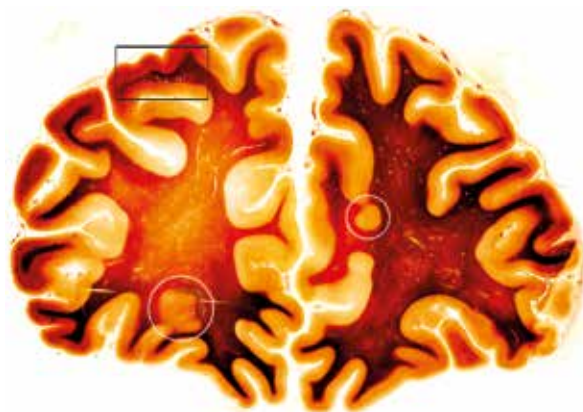

Fig. 3. Palmgren stain of the whole mount of frontal lobes. Subcortical foci of heterotopic grey matter in each hemisphere (encircled). Marked area in the left superior frontal gyrus contains capillary telangiectasia illustrated in Figure 6. The white matter of both lobes displays increased vascular markings due to a large number of telangiectatic capillaries.
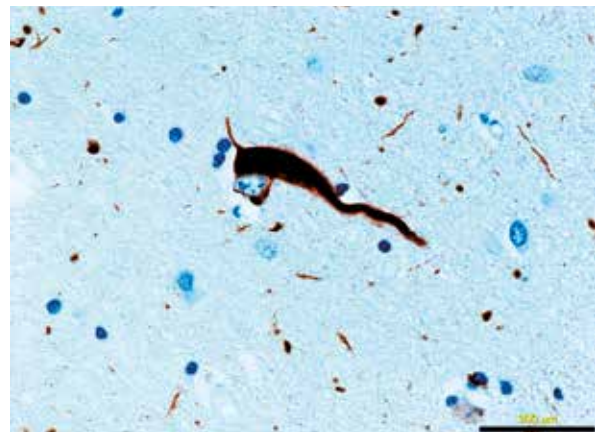

Fig. 4. Tau positive neurofibrillary tangle and multiple neuritic threads in the neuropil of heterotopic grey matter tissue from the frontal lobe.

myelinated axons and glia. Many folia showed dysplastic neurons overlying depleted granule cells and accompanied by dense myelination of the molecular layer (Fig. 2B), a change indistinguishable from that in the contralateral, main tumour mass.

The heterotopic grey matter of the frontal lobes (Fig. 3) contained scattered neurons with neurofibrillary tangles (NFT) as well as neuritic threads, both strongly positive for Tau (Fig. 4) and UBQ. Electron microscopic examination revealed tight collections of paired helical filaments (PHF) in the perikaryon and myelinated axons in the background (Fig 5A). They measured approximately 20 nm and displayed periodic constrictions (Fig. 5B). However, the diameters deviated upwards and downwards of these values, most likely due to suboptimal processing of the tissue. In addition, there was an almost equal number of long straight 15 nm tubules without constrictions (Fig. 5B). The neuropil of the heterotopic foci as well as the adjacent white matter displayed a moderate degree of gliosis. The cortex overlying heterotopia did not demonstrate architectural abnormalities. However, cortical disorganization with marked gliosis was present in the left superior and middle
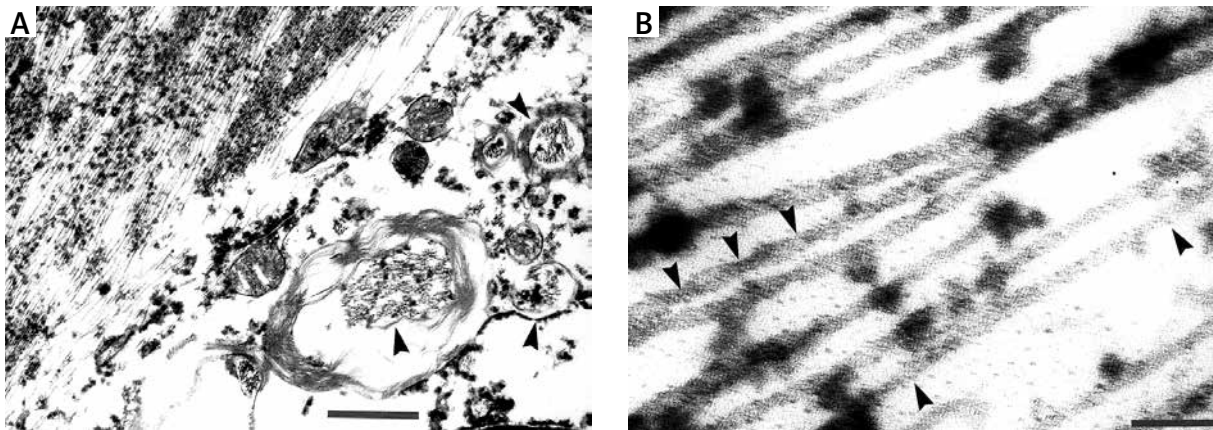
Fig. 5. Low power electron micrographs showing accumulation of paired helical filaments in neurofibrillary tangle. Arrows point to myelinated axons filled with PHFs. Bar = 250 nm (**A**). High power view of neurofibrillary tangle showing twisted filaments (three arrows pointing downwards) and straight filaments (two arrows). Bar = 65 nm (**B**).
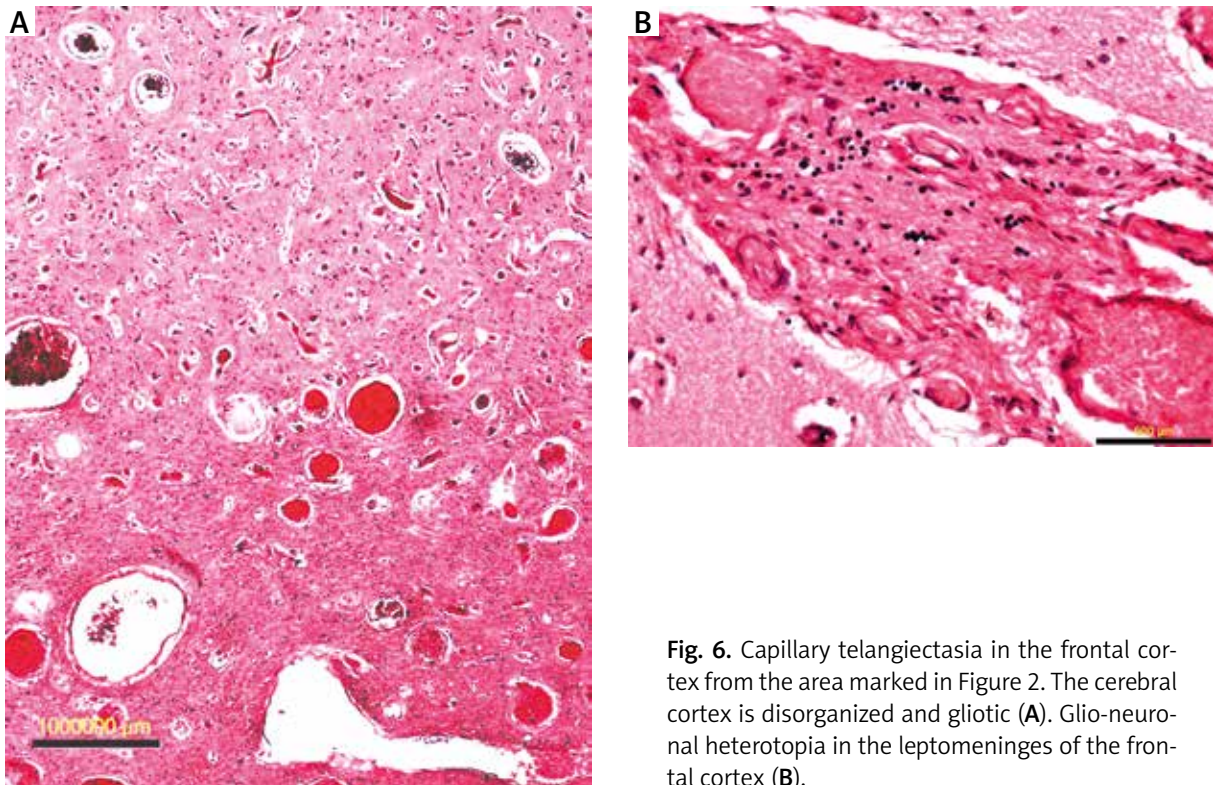

Fig. 6. Capillary telangiectasia in the frontal cortex from the area marked in Figure 2. The cerebral cortex is disorganized and gliotic (**A**). Glio-neuronal heterotopia in the leptomeninges of the frontal cortex (**B**).

frontal gyri involved by capillary telangiectasia (Fig. 6A). An increased number of telangiectatic capillaries was noticeable in the white matter of both cerebral and cerebellar hemispheres. Meningeal nests of heterotopic, glio-neuronal and glial tissue were very frequent in all cerebral cortical areas (Fig. 6B) as well as in the leptomeninges overlying the non-tumorous cerebellar folia. There was no evidence of NFTs, amyloid angiopathy or senile plaques in any other part of the brain, including hippocampal formations. Reexamination of the surgical specimen of the tumour biopsy revealed no PHFs in the neuronal or glial cells. The tumour tissue was not available for additional molecular or immunohistochemical testing.

Discussion

Lhermitte-Duclos disease is characterized by thickened cerebellar folia due to replacement of the granule cell layer by enlarged neurons of various sizes, most often reminiscent of Purkinje cells [10,25]. Other key features include the absence or reduction in numbers of Purkinje and granule cells, and myelination of the molecular layer, creating appearance of inverted folia. The lack or very low proliferative activity indicates that LDD may represent a malformation rather than the tumour [25]. However, progression to malignant and other benign tumours such as anaplastic ganglioglioma [36] and DNET [24] has been reported in a few cases. The new tumour is usually discovered many years after the initial LDD diagnosis [24,36].

Most cases of LDD present unilaterally, with no preference of side. However, bilateral LDD has been reported in five patients [5,7,32,34,39]. Four cases exhibited large bilateral lesions [7,32,34,39], the other had foci of LDD-like changes such as inverse myelination and dysplastic changes in the contralateral hemisphere [5]. Our case was similar to the latter, displaying several small nests of gangliocytic tumour and multifocal aberrant myelination of the molecular layer in the contralateral hemisphere.

Lhermitte-Duclos disease is often associated with macrocephaly and other developmental abnormalities such as subcortical grey matter heterotopia, meningeal glial heterotopia and vascular malformations [25]. Macrocephaly is found in approximately half of LDD cases and is usually present in other PTEN-related conditions such as Bannayan-Ruvalcaba-Riley syndrome [14,25]. However, this number may be higher as Mary Ambler suggested that isolated macrocephaly might be a sign of subclinical LDD [3]. Leptomeningeal glial nests result from an over-migration of glia beyond the pia limitans to the subarachnoid space [8,12]. Experimental studies have revealed that these heterotopia occur after damage to the pial basal lamina [23]. Physical injury to the pial basal lamina during the development, or the dysfunctions as well as deficiencies of proteins comprising the basal lamina, such as laminin, can result in an over-migration of glia [23]. To our best knowledge, there is no known link between LDD or PTEN mutations and any of these conditions. However, PTEN mutations are associated with a high rate of vascular malformations [37] and several studies demonstrated cavernous and venous angiomas [22] as well

as other developmental vascular abnormalities in LDD [2,27,41]. An association of this mutation with LDD may explain striking hypervascularisation of gangliocytomas observed in one large study [1], and possible contribution to the widespread presence of telangiectasias in our patient. Multifocal, clinically silent cerebral subcortical grey matter heterotopia is the most unique finding among the constellation of brain abnormalities in our patient. Cortical heterotopia is a malformation caused by an arrest in neuronal migration from the ventricular zone in the developing brain [4,38]. Heterotopia are often associated with malformations in the overlying cortex that may display dyslamination and the presence of abnormal neurons [38]. Lhermitte-Duclos disease and other diseases with heterotopic grey matter or other forms of aberrant neuron migration, share many features such as formation of dysplastic and hypertrophic neurons [19,21], as well as predisposition to megalencephaly in carriers [31].

The presence of Tau-positive neuritic threads and neurofibrillary tangles (NFT) with the ultrastructural PHF characteristics has not been reported in association with LDD. Paired helical filaments in this patient were present only in the heterotopic neurons and not detected in the neoplastic cells or glia in the biopsy tissue, or any other part of the post-mortem brain. Although tau-positive neurons have been found in other forms of cortical dysplasia [16], tau immunoreactivity does not infer presence of NFTs or PHFs [9]. Hyperphosphorylated tau NFTs are typically associated with Alzheimer's disease [40]. They may also develop at any age in other unrelated conditions such as subacute sclerosing panencephalitis, ALS, certain heavy metal poisoning, dementia pugilistica, Down syndrome, tuberous sclerosis [15,40] and gangliogliomas [26]. It is unknown if the etiological factors responsible for the given disease are also responsible for the production of PHF or whether the environment of chronically diseased brain predisposes to NFT formation [40]. It has been suggested that the presence of NFT-like inclusions in neurons in cortical dysplasia could be secondary to pre-existing cytoskeletal abnormalities [16]. Sen *et al.* [33] reported absence of NFTs in paediatric cases with cortical dysplasia, while older patients with similar cortical anomalies had classical NFTs. Therefore, it is very plausible that abnormal neurons in dysplastic foci are much more prone to degeneration and premature aging than histologically normal neurons [33].

Lhermitte-Duclos disease is genetically linked to a mutation in PTEN, which acts as a tumour suppressor protein for diverse pathways. The defective function of PTEN can cause excessive mTOR activation that can lead to cell proliferation, hypertrophy, and improper migration [19]. Disrupting PTEN in the cerebellum and cortex of murine models created pathology resembling human LDD and cortical dysplasia, respectively [19,21]. This model had all the features of LDD with hypertrophic neurons, loss of Purkinje cells, and symptoms of increased intracranial pressure [19]. The development of NFT in conjunction with LDD can also be traced to the PTEN pathway. However, the mechanisms that cause dysplastic neurons to accumulate NFTs are uncertain [29]. A study by Griffen *et al.* [13] has revealed a negative correlation between PTEN levels and the severity of tau tangles in AD brains. It has been subsequently confirmed that PTEN accumulates in NFTs, most likely as a consequence of deregulation of downstream signalling and nuclear dysfunction of PTEN in AD neurons [35]. Unfortunately, this is an archival case from the period when molecular studies for detection of this mutation were unavailable and possible relation between occurrence of NFT and PTEN gene mutation in our patient remains unknown.

Disclosure

Authors report no conflict of interest.

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