Neuropathological phenotype of a distinct form of lissencephaly associated with mutations in TUBA1A

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Lissencephalies are congenital malformations responsible for epilepsy and mental retardation in children. A number of distinct lissencephaly syndromes have been characterized, according to the aspect and the topography of the cortical malformation, the involvement of other cerebral structures and the identified genetic defect. A mutation in TUBA1A, coding for alpha 1 tubulin, was recently identified in a mutant mouse associated with a behavioural disorder and a disturbance of the laminar cytoarchitectony of the isocortex and the hippocampus. Mutations of TUBA1A were subsequently found in children with mental retardation and brain malformations showing a wide spectrum of severities. Here we describe four fetuses with TUBA1A mutations and a prenatal diagnosis of major cerebral dysgeneses leading to a termination of pregnancy due to the severity of the prognosis. The study of these fetuses at 23, 25, 26 and 35 gestational weeks shows that mutations of TUBA1A are associated with a neuropathological phenotypic spectrum which consistently encompasses five brain structures, including the neocortex, hippocampus, corpus callosum, cerebellum and brainstem. Less constantly, abnormalities were also identified in basal ganglia, olfactory bulbs and germinal zones. At the microscopical level, migration abnormalities are suggested by abnormal cortical and hippocampal lamination, and heterotopic neurons in the cortex, cerebellum and brainstem. There are also numerous neuronal differentiation defects, such as the presence of immature, randomly oriented neurons and abnormal axon tracts and fascicles. Thus, the TUBA1A phenotype is distinct from LIS1, DCX, RELN and ARX lissencephalies. Compared with the phenotypes of children mutated for TUBA1A, these prenatally diagnosed fetal cases occur at the severe end of the TUBA1A lissencephaly spectrum. This study emphasizes the importance of neuropathological examinations in cases of lissencephaly for improving our knowledge of the distinct pathogenetic and pathophysiological mechanisms.

Keywords: TUBA1A; lissencephaly; abnormal corpus callosum; abnormal hippocampus; brainstem and cerebellum hypoplasia

Abbreviations: GFAP = glial fibrillary acidic protein; PBA = protein blocking agent


Introduction

Lissencephalies are congenital malformations affecting cortical gyration and lamination, associated with severe epilepsy and mental retardation in children. Lissencephaly is a descriptive term that refers to a ‘smooth brain’ and emphasizes the most characteristic feature of these malformations, an abnormal gyral pattern of the cortex involving absent, or a reduced number of crude gyri (Harding, 1996). At the microscopic level, lissencephalies are distinct disorders (Francis et al., 2006). In the traditional neuropathological literature, two types of lissencephaly have been described: type I or ‘classical’ lissencephaly and type II or ‘cobblestone’ lissencephaly. Our knowledge of the lissencephaly syndromes is expanding rapidly with the generalized use of MRI, the recent advances of molecular biology, and the study of spontaneous or induced mutant mouse models of human cortical malformations (Walsh, 1999). Indeed, in recent years, an increasing number of new distinct
lissencephaly syndromes have been characterized, according to the aspect and the topography of the cortical malformation, the involvement of other cerebral structures and the identified genetic defect.

*LI*1 and doublecortin (DCX) mutations are responsible for classical lissencephaly characterized by a typical disturbance of the laminar architecture of the cortex (Reiner et al., 1993; des Portes et al., 1998, Gleeson et al., 1998). Classical lissencephaly due to mutations of *LI*1 (or deletions in 17p13.3) shows typical MRI and neuropathological features associating complete agyria or agyria/pachygyria with more severe lesions in the posterior areas of the cortex, an absence of the Sylvian fissure and a thickened cortex. Mutations of DCX, located on chromosome X (in Xq22.3), are responsible for a different phenotype in males and females (Pinard et al., 1994). Males have a severe form of lissencephaly or X-LIS, similar but not identical to LI1 cases, often with a predominance of lesions in the anterior areas (Pilz et al., 1998). Females, in contrast, harbour a less severe malformation referred to as subcortical band heterotopia or double cortex’. In addition, the corpus callosum is abnormally thin, largely absent or abnormally short and thick in these cases and the hippocampus is also affected (Kappeler et al., 2007). Neuropathological studies for classical lissencephaly have shown a thick four-layered cortex instead of the normal six layers. This abnormal cortex is made up of an external molecular layer (layer I), a layer of pyramidal cells resembling the fifth and sixth layers of the normal neocortex (layer II), a sparse cellular layer (layer III) and a thick band of disorganized neurons of variable size (layer IV) extending into the white matter (Jellinger and Rett, 1976). Forman et al. (2005) confirmed by neuropathological studies that distinct subtypes of classical lissencephaly exist demonstrating that the lamination of the cortex in cases mutated for DCX is not identical to LI1 mutated cases. In addition, it has been demonstrated that subcortical band heterotopia can be observed in males with somatic mosaic mutations in DCX and in males and female with missense or somatic mosaic mutations in LI1 (Leventer et al., 2001; d’Agostino et al., 2002; Poolos et al., 2002; Sicca et al., 2003).

Homozygous Reelin (RELN) mutations have been identified in four cases of lissencephaly associated with severe cerebellar and pontine hypoplasia (Hong et al., 2000; Chang et al., 2007; Zaki et al., 2007). As yet, no neuropathological data, especially concerning the cortical cytoarchitecture, have been reported for such cases. ARX has also been found mutated in cases of X-linked lissencephaly associated with corpus callosal abnormalities and abnormal genitalia (Kitamura et al., 2002). Neuropathological studies show however, a quite different form of lissencephaly, associated with an absence of cortical interneurons (Bonneau et al., 2002).

In 2007, Keays et al. identified a mutation in *Tuba1a* in an ethyl–nitrosurea-induced mutant mouse, exhibiting hyperactivity and a disturbance of the laminar cytoarchitecture of the isocortex and the hippocampus. Neuroanatomical similarities between the *Tuba1a* mutant mouse and mutant mice deficient for *Dcx* and *Lis1* (Fleck et al., 2000; Corbo et al., 2002; Kappeler et al., 2007), led the authors to suggest that mutations in the human homolog of *Tuba1a*, might be responsible for human cortical malformations. Subsequently, heterozygous *de novo* mutations in *TUBA1A* were found in several children with mental retardation showing on MRI, malformations of the cortex, hippocampus, corpus callosum and cerebellum, with a wide spectrum of severities (Keays et al., 2007; Poirier et al., 2007).

*TUBA1A* mutations have now been identified in four fetal cases, with a prenatal diagnosis of severe cerebral dysgenesis leading to a termination of pregnancy. We report detailed neuropathological data for these fetuses with the purpose of delineating the fetal phenotype associated with *TUBA1A* mutations. We compare this phenotype with other lissencephalies with known gene mutations in *LI*1, DCX, ARX and RELN, and with different cases of lissencephaly reported in the literature for which the molecular defects are unknown. Our study reveals a novel neuropathological phenotype associated with *TUBA1A* lissencephaly.

**Methods**

**Subjects with mutations**

*TUBA1A* missense mutations were identified in four fetuses presenting an ‘atypical lissencephaly with cerebellar and callosal anomalies’. In all four cases, the pregnancy was terminated for medical reasons at 23, 25, 26 and 35 weeks of gestation (GW), after a prenatal ultrasound diagnosis of severe brain dysgenesis, with additional MRI data in the 35 GW fetus. Cases 1 and 3 were identified prospectively and cases 2 and 4 were selected retrospectively. In six additional fetuses presenting ‘atypical lissencephaly with cerebellar and callosal anomalies’, TUBA1A was not found mutated. In these non-mutated cases, the cerebral phenotype was close to that of the mutated cases. The main difference was the presence, in each case, of several associated visceral and/or skeletal malformations. In all cases pregnancy termination was performed in accordance with French laws. Written consents for post-mortem and diagnostic studies were obtained from parents.

**Control subjects**

We studied at the same time, eight control subjects, consisting of two age-matched controls for each age group, selected among fetuses obtained after spontaneous abortion (2), *per partum* death (1) or after pregnancy termination for visceral malformations: incurable heart malformation (3), bilateral kidney agenesis (1) and diaphragmatic hernia (1). None of these had any detectable central nervous system malformation or lesion.

**Neuropathological procedures**

After removal from the skull, each brain was fixed in a 10% formaldehyde solution containing NaCl (9 g/l) and ZnSO4 (3 g/l) for variable times, according to the volume of the brain,
from 3 to 6 weeks. The brains were cut in a coronal plane and sections involving one or both hemispheres were embedded in paraffin. Paraffin blocks were cut at either 5 µm (brainstem and cerebellum) or 8–10 µm (hemispheres). Sections were stained with hemalun–phloxin and cresyl violet or cresyl violet—luxol fast blue (Klüver–Barrera) stains, according to standard methods.

**Immunohistochemical procedures**

Immunohistochemical procedures were performed in areas selected after histological study of mutated subjects and one control case for each age. Paraffin sections were immunostained for Glial Fibrillary Acidic Protein (GFAP) with a polyclonal antibody (1:200–1:400, Dako, USA), NFp70 (1/50, DAKO), NeuN (1/500, MAB377, CHEMICON), Reelin (1:1000, de Bergeyck et al., 1998), MAP2 (1:100, HM2, SIGMA), Calbindin (1:500; SWANT laboratories, Switzerland), Calretinin (1:1000, SWANT Laboratories, Switzerland), MIB1 (1:75, DAKO) and DCX (1/300, Nter, Francis et al., 1999). The Universal Immunostaining System Streptavidin-Peroxidase Kit (Coulter) was used to reveal the reaction. Slides were incubated in citrate buffer at 98°C, in a microwave for 30 min, rinsed in distilled water, incubated in a 3% H2O2 solution for 5 min and rinsed in distilled water. After washing with PBS, they were treated with Protein Blocking Agent (PBA) for 5 min (100 µl/slide) at room temperature, without rinsing. Then, they were incubated with primary antibodies, diluted as specified earlier, at room temperature, for 60 min, and washed with PBS two times for 5 min each. Next, they were incubated with biotinylated secondary antibody for 30 min at room temperature, washed with PBS two times for 5 min each and incubated in streptavidin–peroxidase complex for 45 min. After washing two times for 5 min in PBS, each slide was entirely covered with a freshly prepared DAB–Chromogen solution (100 µl/slide) for 5 min and finally rinsed with distilled water for 5 min. Haematoxylin was used to counterstain the brain sections.

All sections were examined under a light microscope (Eclipse 800 NIKON) and some were selected for photographic documentation.

**Genetical analysis**

The four cases studied here were found negative for mutations in LIS1, DCX and ARX genes. De novo heterozygote TUBA1A mutations were identified in each case. For each DNA sample, the complete TUBA1A coding sequence (accession number NM_006009) and splice sites were amplified in five independent PCR reactions from genomic DNA, as described in Poirier et al. (2007). PCR products were checked by 2% agarose gel electrophoresis before they were subjected either to DHPLC (by the WAVE nucleic-acid fragment analysis system Transgenomic, San Jose, CA) and/or direct sequencing using the BigDye dideoxy-terminator chemistry (Applied Biosystem, Foster City, CA), and ABI3700 DNA analyser (Applied Biosystem). For DHPLC analysis, melting profiles and resolution temperatures were predicted by the transgenomic WAVEMAKER software version 4.1. To further establish that the described variations are likely to be disease-causing mutations, the TUBA1A gene was analysed in the parents of fetuses 1, 3 and 4, as well as in the parents of five children with TUBA1A mutations (Poirier et al., 2007) and none of the identified changes was found in the parents, suggesting a de novo occurrence of these mutations. Maternity and paternity were confirmed by segregation analysis of 11 informative polymorphic markers, in order to consolidate these results. In addition, 360 control individuals were screened without identifying TUBA1A mutations.

**Results**

**Case I**

In a 29-year-old pregnant woman who already had one healthy child, ultrasound examination showed dilated ventricles in the fetal brain at 21 GW. The unrelated parents and their first daughter were healthy with no familial history of neurological disease or mental retardation. At 22 GW a further ultrasound examination was performed, showing triventricular dilation, agenesis of the corpus callosum, an abnormal orientation of the thalami, a hypoplastic cerebellum and a possible ocular abnormality, suggesting Walker Warburg syndrome. The karyotype was established to be 46 XY, 14pstk (a banal morphological variant). Based on the ultrasound findings, a pregnancy termination was performed at 23 GW. A foetopathological examination of this case showed hypoplastic external genital organs. Subsequent genetic testing revealed a heterozygous L286F mutation in the TUBA1A gene (Poirier et al., 2007).

The neuropathological examination showed biometric parameters of the brain less than the fifth percentile with a brain weight of 50 g, occipito-frontal diameters of 63 mm and the cerebellum with brainstem weighing 2.8 g. A macroscopical examination showed no olfactory sulci and bulbs and a smooth surface of the cerebral hemispheres without operculation of the Sylvian fissure (Fig. 1A and B). In addition, it was not possible to identify the calcaneus fissure and the parieto-occipital sulcus, normally present from the age of 18 GW (Fig. 1C). Sagittal sections further disclosed an agenesis of the corpus callosum (Fig. 1C). The brainstem and cerebellum were found to be hypoplastic and the fourth ventricle widely opened, suggesting partial ageneses of the cerebellar vermis.

In coronal sections of the cerebral hemispheres, the ventricles were dilated and basal ganglia were dysmorphic (Fig. 2A–F). Histological examination showed a slightly thinner cortex than control, with poorly differentiated MAP2 positive pyramidal cells present abnormally in all layers (Supplementary Fig. 1A–D). Further immunolabeling showed the presence of normal RELN-positive Cajal–Retzius cells (data not shown). Abnormal large cells labelled with MAP2 were scattered in the white matter and additional abnormal bundles of fibres were present in periventricular regions (data not shown). However, immunostaining for vimentin and GFAP showed normal radial glial cells (data not shown).

The amygdala, barely identifiable, was only very crudely organized, compared with controls, (Fig. 2C and D) and the hippocampus could also not be clearly identified (Fig. 2F and H). The germinal zones in the area of the ganglionic eminence appeared voluminous (Fig. 2C–F). Immunohistochemical studies with MIB1 showed however,
an approximately equal density of proliferating cells compared with the control (Supplementary Fig. 1E and F). Histological examination further revealed the callosal agenesis (Fig. 2B) and the absence of the septum pellucidum and the hippocampal commissure. No Probst bundles were observed.

The striatum and pallidum were of normal size (Figs 2C, D, 3A and B), but appeared voluminous compared with the pallium. The pallidum had an abnormal aspect, showing highly disorganized NFp70-positive fibres (Fig. 3A and B). There was no identifiable internal capsule and the caudate nucleus and putamen were fused. The thalami were reduced in size and dysmorphic (Fig. 2E and F) and there were no hypothalamic structures. In medial sagittal sections, histological studies showed a global hypoplasia of the cerebellar vermis and confirmed a posterior vermian agenesis involving lobule X (Fig. 3C and D). The fourth ventricle was partially open at the posterior end because of the vermian defect. In addition, small nodules of heterotopic neurons were observed in the cerebellar white matter. In the midbrain, the tectum was normal, as were the red nucleus and the substantia nigra, however the corticospinal tract was disorganized, as it was in the hindbrain (Fig. 3E–H, black arrows). At the level of the latter, the cranial nerve nuclei could not be identified and the pontine nuclei were hypoplastic (Fig. 3G and H). At the lateral edges of the brainstem, bands of heterotopic cells were observed, most probably destined for the pontine and olivary nuclei. Clustered olivary heterotopias were also evident (Supplementary Fig. 2A and B, black arrows). Thus, a variety of brain structures were found to be affected in this TUBA1A case examined at 23GW.
Case 2

In a 33-year-old pregnant woman ultrasound examination of the fetus at 24 GW showed corpus callosal agenesis, a small cerebellum and an abnormal Sylvian fissure. The unrelated couple had no neurological or other pathological
familial history and a previous sibling was healthy. A pregnancy termination was performed at 25 GW. A neuropathological examination of this male fetus showed a brain weight of 96 g (fifth percentile for age). Subsequent genetic testing revealed a heterozygous I238V mutation in the TUBA1A gene.

A macroscopical examination showed an abnormally smooth brain without sulci usually present at this term (Fig. 1E and F), and the corpus callosum was absent (Fig. 1F). The pons was asymmetrical and hypoplastic on the left side with partial vermician agenesis. Histological examination confirmed the complete lack of sulci and gyri and the lack of the opercularization of the Sylvian fissure (Fig. 4A–D). The ventricles were abnormally enlarged because of the underdeveloped hemispheric mantle, which was reduced in thickness compared to age-matched controls (Fig. 4B and D). In particular, the neocortex was reduced in depth, there was no lamination and the neurons were immature with few differentiated pyramidal cells in layer V. Bilaterally, in the posterior parietal lobes, the neocortex showed a particular abnormality, characterized by the presence of glomerular structures made up of eosinophilic fibrillar material surrounded by immature cells. Such structures were present in deep parts of the cortex and also present in the subcortical white matter (Fig. 4F). The most striking abnormality was the presence of numerous abnormal fascicles either intercrossed or turned back on themselves, located in the deep white matter in the periventricular area (Fig. 4G and H). These abnormal fascicles were also observed in the anterior columns of the fornix and sometimes in the cortex and the subcortical white matter. In the left frontal pole, heterotopic nodules were located in the deep white matter (Fig. 4I). The white matter also contained numerous, abnormally oriented large pyramidal neurons labelled with MAP2 and NeuN (data not shown).

The hippocampus was hypoplastic, not folded correctly and oriented vertically (Fig. 5A and B). In addition, the corpus callosum and the septum pellucidum were absent (Fig. 5C–F). In anterior regions, at the level of the cingulate cortex, intercrossing fibres were observed with random orientations. In more posterior regions however, small Probst bundles were present, with fibres oriented in an anterior–posterior direction (Fig. 5D). The germinal zones were voluminous compared with an age-matched control (Fig. 5E–H) and there was also no recognizable anterior commissure (Fig. 5I–L). The basal ganglia were abnormal and asymmetrical (Fig. 5J and L) containing numerous abnormally oriented small fibre bundles which were particularly numerous in the pallidum. In addition, the internal capsule was identified only on one side and showed a fragmented aspect, with multiple small bundles of abnormally oriented fibres (Figs 4B, D, 5J and L, black arrows). Thalami were also hypoplastic and dysmorphic with a disorganized architecture (as shown in Fig. 4D). They were asymmetrical with on one side numerous

![Fig. 4](image-url) Coronal sections of the brain from case 2 (B, D, F, G–I) compared to an age-matched control (A, C and E). (A–D) A completely smooth surface of the hemispheres without an identifiable Sylvian fissure (B and D) and an absence of the corpus callosum (D) are observed in this case. The pallium is poorly developed compared to the age-matched control and ventricles are enlarged. Basal ganglia are asymmetrical, the internal capsule is evident but abnormal on one side (arrow) and absent on the other side. Thalami (th) are hypoplastic (D). (E and F) Histological sections of the posterior parietal cortex show a reduced thickness and the presence of unusual structures (F) consisting of rosettes of immature cells surrounding eosinophilic fibrillar material. These structures were also observed in the subcortical white matter. (G and H) In the periventricular white matter abnormal short fascicles (indicated by white arrows) were observed which were anarchically organized (boxed region in G is shown enlarged in H). (I) In the left frontal pole, nodules of heterotopic neurons were observed in the white matter. Scale bar = 1 mm (A–D) and 100 μm (E–I). Scale bar in F is equivalent for E.
The cerebellum was also hypoplastic and its lobulation was simplified and the dentate nuclei had an abnormal crudely organized aspect (Fig. 6A and B). In addition, there was a posterior agenesis of the vermis and nodules of heterotopic neurons were present in the white matter of cerebellar hemispheres, similar to case 1 (data not shown). Calbindin labelling also showed a reduced number of Purkinje cells compared to control (Fig. 6C and D, colour version Supplementary Fig. 3). At the level of the mesencephalon, the tectum and tegmentum were largely normal; however the ventral part of the brainstem was hypoplastic and flattened (Fig. 6E and F). The corticospinal tract was abnormal, asymmetrical and only present at the level of the mesencephalon. In this region, on one side, it was made up of abnormally oriented fascicles and on the other side, although small lateral fascicles were present, it was difficult to be certain that these belonged to the corticospinal tract (Fig. 6F, black arrow). At the level of the pons, its ventral part was flattened to the extent that the pontine nuclei and the corticospinal tract were almost completely absent (data not shown). In addition, numerous heterotopias were scattered throughout the tegmentum. In the medulla oblongata the corticospinal tract was absent and the inferior olivary nuclei were abnormal and fragmented (Fig. 6G and H), and voluminous olivary heterotopias were observed on each side (boxed region, Fig. 6H). Thus, case 2 resembles case 1 with numerous probable migration and differentiation abnormalities.

Case 3

During the fourth pregnancy of a 40-year-old woman, an ultrasound examination of the fetus at 22 GW showed bilateral ventricular dilatation, with an absent corpus callosum. At 24 GW, a temporal porencephaly was noted, and at 26 GW abnormal gyration was suspected. The unrelated couple had three previous healthy siblings and the familial history was unremarkable. A pregnancy termination was performed at 26 GW. A post-mortem examination of this male foetus showed normal biometric parameters with no visceral or skeletal abnormalities. The placenta was normal. Subsequent genetic testing revealed a heterozygous P263T mutation in the TUBA1A gene (Poirier et al., 2007).
A neuropathological examination showed a brain weight of 100 g, an occipito-frontal diameter of 70 mm and a bi-parietal diameter of 62 mm, each at the fifth percentile for age. Cerebellar biometric parameters on the other hand were clearly below the fifth percentile for age, with a cerebellar weight of 3.6 g.

A macroscopical examination showed a smooth brain surface with an abnormal Sylvian fissure, showing no operculization (Fig. 1H). Central, precentral and postcentral sulci and the frontal superior sulcus, normally present at this age, were absent. The olfactory sulci and bulbs were also absent. Examination of medial sagittal sections confirmed the absent corpus callosum and the absence of sulci, particularly the calcarine fissure and the parieto-occipital sulcus (Fig. 1I). Examination of coronal sections showed enlarged ventricles with small cavities in the germinal zone of the frontal and temporal lobes. Histological examination showed an abnormal cortical plate without gyri and sulci and lacking normal lamination, with however an apparently normal cell density (Fig. 7B, Supplementary Fig. 4). The superficial layers of the cortex were strikingly abnormal: the subpial granular layer was particularly thin and discontinuous (Supplementary Fig. 4C and D); the underlying molecular layer was irregular and contained abnormal, intercrossing fibre bundles shown labelled with DCX (Fig. 7C and D, colour Supplementary Fig. 5), with scattered normal Cajal–Retzius cells (Supplementary Fig. 4A and B). In frontal and temporal cortex, layer II showed an irregular folded aspect, distinct from polymicrogyria (Fig. 7D). Immunohistochemistry for MAP2, which, at this age, normally specifically labels differentiated pyramidal neurons in layer V, in this case, labelled small poorly differentiated neurons throughout the cortex (Supplementary Fig. 4C–F). NeuN, a neuronal marker, which, at this age, would normally label all neurons of the cortex except the last formed neurons of layer II, in this case, labelled only sparse neurons throughout the cortex (Supplementary Fig. 4G and H). In addition, numerous small, randomly oriented MAP2-positive neurons were labelled in both subcortical and periventricular white matter (Supplementary Fig. 4I and J). These combined data suggest both neuronal migration and differentiation defects.

The hippocampus was abnormal in this case, with little evidence that the dentate gyrus had begun to form and the pyramidal layer of Ammon’s horn was hypoplastic and disorganized compared with controls (Fig. 7E–H). The amygdala nuclei were also hypoplastic and the external capsule was absent (data not shown). In addition, cavities lined with macrophages were observed both in the white matter, in the germinal matrix and subventricular zone of the frontal lobe and in the sublenticular region in the temporal lobe (Fig. 7B). Concerning the absent corpus callosum, no Probst bundles were identified, suggesting that callosal neurons were either not differentiated or absent (Fig. 7B). The anterior and hippocampal commissures were also absent. The basal ganglia showed a disorganized architecture and the internal capsule was hypoplastic, with fibres either absent or oriented haphazardly (Fig. 8A–D).
The brainstem and cerebellum were hypoplastic and the cerebellar surface of this case was abnormally smooth without lobules (Fig. 9A and B). Cerebellar peduncles were severely hypoplastic (Fig. 9B). The neocerebellar cortex showed an extensive delay of maturation, made up three layers, instead of five layers normally seen at this age, resembling therefore a cerebellar cortex of 19–20 GW (Fig. 9C and D). The corticospinal tract was however, also severely hypoplastic in this case, probably due to the severe cortical abnormalities (Fig. 9I and J, black arrow). Thus case 3 shows a number of similarities with cases 1 and 2, although with certain distinguishing features, such as the abnormalities in the superficial areas of the neocortex, the cavities in the

**Fig. 7** Coronal sections showing the neocortex and hippocampus of case 3 (B, D, F and H) compared with control (A, C, E and G). (A and B) Case 3 had a smooth cortex without a Sylvian fissure (SylF), agenesis of the corpus callosum (without Probst bundles), enlarged germinal zones (gz) containing cystic cavities and abnormal basal ganglia with an absent internal capsule (arrows). (C and D) Abnormal cortical plate with discontinuous subpial granular layer, the presence of abnormal short fascicles in the molecular layer (shown by DCX labelling) and a folded layer 2, which could be distinguished from polymicrogyria since there was no apparent fusion of the molecular layer (D and data not shown). (E and F) Abnormally shaped and oriented hippocampus with a poorly differentiated dentate gyrus (F) indicated by arrows in both subject and control. (G and H) Disorganized pyramidal neurons in the Ammon’s horn compared with the control (boxed regions in E and F are shown enlarged in respectively G and H). Scale bars, A, B = 1 mm; E, F = 1 mm and C, D = 100 µm; G, H = 100 µm.

**Fig. 8** Basal ganglia of case 3 (B and D) compared with an age-matched control (A and C). (A and B) At the level of the head of the caudate nucleus, no internal capsule was identified (arrow in control). The caudate nucleus (ca) and putamen (pu) were therefore joined in one structure. (C and D) Labelling with NFp70 at the level of lenticulate nucleus shows the quasi-absence of the internal capsule (ic in control C), some abnormally organized fibres were however observed more dorsally (thin arrow in D). Scale bar shown in B, A–D = 500 µm.
germinal zone and the severity of the lesions in rhombencephalic structures.

**Case 4**

Ultrasound fetal examination at 32 GW in a 30-year-old pregnant woman showed abnormal gyration of the brain suggesting lissencephaly. This was the first pregnancy of an unrelated couple, with unremarkable familial history. Ultrasound data were subsequently confirmed by prenatal MRI. The pregnancy was terminated at 35 GW. Foeto-pathological examinations showed a male fetus with facial dysmophia and camptodactyly. There were no visceral abnormalities. Subsequent genetic testing revealed a heterozygous R402C mutation in the **TUBA1A** gene (Poirier et al., 2007).

A neuropathological examination showed a brain weight of 256 g, occipito-frontal diameters of 92 and 94 mm and a biparietal diameter of 85 mm. The cerebellum weighed 17 g. The brain weight was therefore at the fifth percentile, whereas the cerebellar biometric parameters were at the 25th percentile.

A macroscopical examination showed a completely smooth brain surface with an abnormal Sylvian fissure (Fig. 1K). Olfactory sulci and bulbs were present in this case. The cerebellar vermis was hypoplastic and the fourth ventricle was abnormally open (Fig. 1L). The lissencephaly was evident in coronal sections of the cerebral hemispheres. However, unlike the other cases, the cortical ribbon was thickened and the white matter was reduced (Fig. 10A–D). Ventricular cavities were not enlarged. The corpus callosum, columns of the fornix and septum pellucidum were abnormally thick (Figs 10C, D, 11C and D) and the hippocampus was dysmorphic (Figs 10B, 11A and B). Histological examination showed severe neuronal migration abnormalities, most probably responsible for the thickened cortex. In the molecular layer, well-differentiated Cajal–Retzius cells were present. The subpial granular layer was however, absent (Fig. 10E and F). Under the molecular layer, there was a thin layer of large pyramidal neurons likely to correspond to those normally present in layers 5 and 6 and under this a layer less dense in cells. Below this, there was a thick layer of neurons, containing different sized cells; some were small, whereas others had an aspect of large pyramidal cells. In the deepest part of the cerebral mantle, heterotopic neurons were observed, with a radial columnar organization in the white matter (Fig. 10G). Near the ventricular surface, voluminous heterotopic nodules were observed (Fig. 10H), although the heterotopic lesions were asymmetrical. Immunohistochemical studies showed RELN-positive Cajal–Retzius cells and a normal diffuse labelling of the rest of the molecular layer. The density and distribution of DCX-labelled cells in the cortex appeared similar between case 4 and the control (Supplementary Fig. 7A and B). Heterotopic neurons in the white matter were strongly labelled with DCX (Supplementary Fig. 7C),

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**Fig. 9** Brainstem and cerebellum of case 3 (B, D, F, H and J) compared with an age-matched control (A, C, E, G and I). (A and B) Case 3 exhibited a smooth hypoplastic cerebellum without lobules and severely reduced cerebellar peduncles. The brainstem was also abnormally developed with flattened ventral parts due to a severe hypoplasia of the pontine nuclei and the corticospinal tract (arrows in subject and control). (C and D) The cerebellar cortex was immature with three layers compared with the five-layered cortex of the control. (E and F) Immunohistochemical analyses showed abnormal labelling of the entire external granular layer with anti-NeuN (F), compared to the control when only the deepest part is labelled. (G and H) Calbindin labelling showed a paucity of labelled Purkinje cells (H). (I and J) At the level of the medulla oblongata the corticospinal tract was absent (black arrow) and the olivary nuclei were abnormally small and malformed. Scale bars: A, B = 1 mm and C, D = 100 μm; E, F = 100 μm; G, H = 100 μm and I, J = 100 μm.
and in addition by calbindin and calretinin. The latter two markers also labelled interneurons in the cortical plate, as in the control (data not shown). The cortex in the temporal and occipital lobes differed from the frontal lobes since the cortical plate, although immature, was a similar thickness to the control (Fig. 10B).

The hippocampus was hypoplastic and abnormally shaped, in particular the Ammon’s horn (Fig. 11A and B). The dentate gyrus could not be identified in one of the hemispheres, and in the other it was present, however, abnormally shaped and discontinuous. The germinative zones in this case unlike cases 1–3 were a similar size compared with control (Fig. 11C–F). Interestingly, the corpus callosum was abnormally thick, as previously observed macroscopically (Fig. 11C and D) and therefore different from cases 1 to 3. The basal ganglia were also histologically normal unlike the three previously described cases (Fig. 11E and F).

In medial sagittal sections of the cerebellar vermis and the brainstem, the vermis was hypoplastic and rotated posteriorly (Fig. 12A and B). Dentate nuclei were hypoplastic and disorganized (Fig. 12C and D). In the cerebellar cortex, Purkinje cells were well-differentiated and numerous, although heterotopic Purkinje cells were observed at different levels in the internal granular layer (Supplementary Fig. 8A and B). Multiple and numerous nodular heterotopias were observed in the posterior part of the cerebellar hemispheres (Fig. 12E), which were strongly labelled with calbindin (Supplementary Fig. 8C). In the brainstem, the pontine nuclei were moderately hypoplastic and, in the medulla oblongata, olivary nuclei were not identified, however bilateral, voluminous olivary heterotopias were observed (Fig. 12F and G). Corticospinal tracts were present but abnormally shaped, flattened and spread out ventrally and laterally (Fig. 12G, black arrows). Thus, case 4, although showing globally a number of the same affected brain structures as cases 1–3, appears in a number of ways to be distinct from the other three cases.

Discussion

When performing the neuropathological study of these cases, our goal was to define a cerebral phenotype associated with TUBA1A mutations and to compare it to that of lissencephalies due to mutations in LIS1, DCX, RELN and ARX genes. The first case we studied was initially diagnosed as atypical lissencephaly with hippocampal, callosal and cerebellar abnormalities which could thus have resembled RELN cases (Hong et al., 2000). However, an immunohistochemical

Fig. 10 Coronal sections of hemispheres (B, D, F and H) of case 4, compared with control (A, C and E). (A–D) The cortex was completely agyric and abnormally thick without a Sylvian fissure (SyF). The corpus callosum (arrow) was also abnormally thick (D). The ventricles were not enlarged in this case. Note the normal basal ganglia (B). (E and F) In the neocortex, the subpial granular layer, marked with an arrow in the control, was absent, although the molecular layer contained well-differentiated Cajal–Retzius cells. The thickened cortical plate showed an abnormal lamination and the cell density appeared lower than in the control. There was no clear boundary between grey and white matter suggesting that numerous cells had stopped their migration prematurely (B). and in addition by calbindin and calretinin. The latter two markers also labelled interneurons in the cortical plate, as in the control (data not shown). The cortex in the temporal and occipital lobes differed from the frontal lobes since the cortical plate, although immature, was a similar thickness to the control (Fig. 10B).

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I, II and III indicate the molecular layer, the layer of pyramidal neurons and a third layer less dense in cells, situated above a fourth thick heterotopic layer (not shown). (G and H) Clusters of heterotopic neurons were observed in the deep white matter, either radially organized (G) or in the form of nodules close to the ventricles (H). Scale bars A, B = 2 mm and C, D = 2 mm and E, F = 200 μm; G, H = 200 μm.
study showed a normal expression of RELN in Cajal–Retzius cells in all cases. The recent cloning of TUBA1A (Keays et al., 2007; Poirier et al., 2007), subsequently found mutated in each of these cases, has allowed us to better define the neuropathological phenotype specifically associated with mutations in this gene.

Our histological study included three second-trimester fetuses (cases 1–3) and one-third-trimester fetus (case 4), and demonstrates that mutations in TUBA1A can result in different neuropathological phenotypes. However, five consistent developmental anomalies are observed in: (i) cerebral gyration, (ii) the corpus callosum, (iii) the hippocampus, (iv) the cerebellum and (v) the brainstem, with various degrees of severity. The gyration abnormalities were constantly severe and diffuse involving a complete agyria usually without an identifiable Sylvian fissure. The subpial granular

Fig. 11 Hippocampus, corpus callosum and basal ganglia of case 4 (B, D and F) compared to an age-matched control (A, C and E). (A and B) The hippocampus was hypoplastic and malformed, as shown in these anterior sections. The dentate gyrus (arrow in the control) was absent in this hemisphere (approximate expected position marked by an asterisk) and the pyramidal layer appeared fragmented (thick black arrow in B). (C and D) The corpus callosum (cc), trigonal columns (tc) and septum were abnormally thick compared to the control. The germinal zone was, however, similar in size compared to the control (arrows). (E and F) No differences were observed in the basal ganglia. Scale bar = 1 mm (A–F).

layer was consistently abnormal, discontinuous and of reduced volume in three cases and absent in one case. In cases 1–3, the cortical plate appeared thinner than in control although with a similar cell density, was immature for age

Fig. 12 Cerebellum and brainstem of case 4 (B, D, E and G) compared to control (A, C and F). (A and B) The cerebellar vermis was hypoplastic, abnormally shaped and rotated posteriorly. In addition, the posterior part of the vermis was abnormally positioned under the inferior part of the vermis. Fp = fissure prima. (C and D) The dentate nuclei were hypoplastic and fragmented. (E) In the posterior part of the cerebellar hemispheres, nodular heterotopias (h) were present. (F and G) In the medulla oblongata, olivary nuclei were absent (black arrowhead in F), instead olivary heterotopia were present (white arrowheads) and the corticospinal tracts were present but abnormally shaped (thin black arrows), spread out laterally and ventrally. Scale bars A, B = 1 mm; C, D = 1 mm; F, G = 1 mm.
and showed an absence of normal laminar organization. In case 2, the parietal cortex showed small rounded glomerular structures, frequently observed in alobar holoprosencephaly (Mizugushi and Morimatsu, 1989) and of unknown pathogenesis. In addition, in cases 1–3, numerous abnormal neurons, strongly labelled with MAP2 were scattered in the reduced white matter. Dystrophic axonal tracts with aberrant pathways were also observed in the periventricular white matter suggesting abnormal axonal guidance. Case 3 also showed abnormal intercrossing fibre bundles strongly labelled with NFp70 and DCX in the molecular layer. In these cases, the lateral and third ventricles were also enlarged most probably due to defective growth of the hemispheric mantle.

In case 4, the pattern was completely different: the cortex was abnormally thick and reminiscent of the four-layered cortex of classical lissencephaly. A four-layered pattern results from an arrest of migration occurring between the 11th and 14th weeks, and would be observed as soon as 20 GW. So the distinct cortical abnormality observed in this 35 GW case, and not in the cases analysed at 23, 25 and 26 GW, is, most probably, not explained by developmental stage alone. Both superficial neurons and deep heterotopic cortical cells could be labelled with either NeuN, MAP2, DCX, calbindin and calretinin, suggesting that migration abnormalities involved projection as well as interneurons. The ventricles were at most only slightly enlarged compared with the previous cases. The white matter was poorly developed and the periventricular areas contained many heterotopic immature neurons, not labelled with neuronal markers, which are rarely observed in classical lissencephaly. Except for the corpus callosum, few abnormal tracts were identified in this case: unlike the first three cases, the corticospinal tract at the level of the internal capsule was normal. Because the internal capsule can be well identified as early as 18 GW in control subjects, the presence of the normal tract in this case is unlikely to be related to the later developmental age. An abnormal pattern of the corticospinal tract at the level of the medulla still suggests though that axonal guidance was abnormal as in the other cases, but only at a distinct level. Indeed, the combined cortical abnormalities of cases 1–4 suggest a disturbance of migration, neuronal differentiation and axonal guidance, although these abnormalities are not identical in each case.

The cerebellum and brainstem were consistently affected in all cases with a variable spectrum of severities. In the three youngest cases, the entire cerebellum was globally hypoplastic with a simplified lobulation, an immature cortex, abnormally fragmented dentate nuclei, and in two cases, a vermian agenesis. The brainstem was also globally hypoplastic. In case 4, the vermis was notably the most affected structure. Pontine nuclei, olivary nuclei and corticospinal tracts were consistently hypoplastic in all four cases. The presence of heterotopic cells in the cerebellar hemispheres and olivary heterotopia in the medulla in all cases, suggests a diffuse disorder of neuronal migration.

Other structures were found to be less regularly involved, including the subcortical germinal zones (3/4), the basal ganglia (3/4) and the olfactory bulbs (2/4). The germinal zones were abnormally voluminous in cases 1–3, perhaps suggesting delayed proliferation and/or migration. A potential reduction of interneurons in the cortices of these cases, shown by preliminary calretinin labellings (data not shown), might confirm this. In case 4 however, the germinal zones were greatly diminished, as in the control, which might suggest that a potential retarded proliferation had been overcome by this stage. Alternatively, this difference might be related to the distinct phenotype of case 4 compared to cases 1–3. Similarly, the basal ganglia in cases 1–3 showed unusual features mainly due to the abnormal internal capsule, whereas in case 4 the internal capsule and basal ganglia were normal. As mentioned earlier, in control cases the internal capsule can be readily identified at 18 GW, thus these combined data suggest a completely different pathogenic process in case 4.

In cases 1 and 4, additional features were associated with the brain malformations. In case 1, hypoplastic external genitalia may be related to the absent hypothalamus, a feature observed only in this case. Case 4 showed a phenotype clearly distinct from that of the first three cases, including dysmorphic facial features and camptodactyly. These could be due to the more advanced developmental stage of this case, or to a particularity of the type of TUBA1A mutation, as discussed later.

The combined neuropathological features suggest that the critical pathogenetic mechanisms are a disturbance of neuronal migration and an impaired neuronal differentiation and axonal guidance (Table 1). In fitting with such defects, Tuba1a, like Dcx, seems predominantly expressed in immature post-mitotic neurons and not proliferating cells during mouse development (Francis et al., 1999; Gleeson et al., 1999; Gloster et al., 1999; Coksaygan et al., 2006; Poirier et al., 2007). Migration defects are suggested by abnormal cortical and hippocampal lamination; cerebellar heterotopia; fragmented dentate nuclei and olivary heterotopia. In addition, the periventricular columns and nodules of heterotopic cortical neurons observed in case 4 strongly suggest severe migration defects. Cases 1–3 also clearly show neuronal differentiation and axonal guidance abnormalities. Thus, abnormal fascicles and axon tracts are observed in layer I and the white matter, respectively; the internal capsule is absent; the corpus callosum is abnormal; and brainstem and corticospinal tracts are hypoplastic. On the other hand, case 4 seems to show mainly neuronal migration defects, although the corpus callosum and corticospinal tract are notably also abnormal.

The cerebral biometry of each of these fetal cases was at the fifth percentile compared with a previous study of neonates whose biometry was less than the third percentile (Poirier et al., 2007). suggesting that microcephaly appears after birth in these cases, when synaptogenesis is active. However, we cannot exclude an associated defect of
proliferation and/or neuronal death. Infants and children with TUBA1A mutations display a wide spectrum of phenotypes with different degrees of severity, particularly concerning cortical gyration and lamination (Poirier et al., 2007; Bahi-Buisson et al., in press). These range from mild to severe forms of perisylvian pachygyria, and agyria at the most severe end of the spectrum.

In our neuropathological study of fetal cases, two second-trimester fetuses showed a more severe posterior than anterior gradient of lissencephaly. In two out of eight patients (Patients 3 and 8) described by Poirier et al. (2007), MRI clearly showed a more severe posterior than anterior gradient of lissencephaly. In our neuropathological study of fetal cases, two second-trimester fetuses showed a more severe posterior than anterior gradient of lissencephaly. In two out of eight patients (Patients 3 and 8) described by Poirier et al. (2007), MRI clearly showed a more severe posterior than anterior gradient of lissencephaly.

The differences observed in case 4, which are unlikely to be explained by developmental stage alone, could be due to the particular TUBA1A mutation or to genetic background. The R402 amino acid residue, mutated to a cysteine in this case, is present in the extreme C terminal part of TUBA1A (Fig. 13) and may be involved in alpha–beta tubulin heterodimer formation and/or interactions between microtubules and other interacting proteins (Keays et al., 2007; Poirier et al., 2007). Previous studies have also described TUBA1A with proliferation and/or neuronal death. Infants and children with TUBA1A mutations display a wide spectrum of phenotypes with different degrees of severity, particularly concerning cortical gyration and lamination (Poirier et al., 2007; Bahi-Buisson et al., in press). These range from mild to severe forms of perisylvian pachygyria, and agyria at the most severe end of the spectrum.

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Table 1: Migration and differentiation abnormalities in the four cases

<table>
<thead>
<tr>
<th>Cases</th>
<th>Abnormalities of migration</th>
<th>Abnormalities of differentiation and axonal guidance</th>
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<tbody>
<tr>
<td>Case 1</td>
<td>Abnormal lamination: two-layered cortex</td>
<td>Abnormally thin cortical plate, immature neurons</td>
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<tr>
<td></td>
<td>Usual layers of hippocampus not identified</td>
<td>Absence of differentiated hippocampus</td>
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<tr>
<td></td>
<td>Heterotopia in cerebellar hemispheres</td>
<td>Abnormal short fascicles in the layer I</td>
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<tr>
<td></td>
<td>Fragmented dentate nuclei</td>
<td>Abnormal axonal tracts in the white matter</td>
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<td></td>
<td>Olivary heterotopia</td>
<td>Internal capsule absent</td>
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<td>Case 2</td>
<td>Abnormal lamination: two-layered cortex</td>
<td>Callosal agenesis without Probst bundle</td>
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<td></td>
<td>Disorganized hippocampal cortex</td>
<td>Immature cerebellar cortex</td>
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<tr>
<td></td>
<td>Heterotopic cells in the hemispheric white matter</td>
<td>Hypoplastic brainstem and cortico spinal tracts</td>
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<td></td>
<td>Heterotopia in cerebellar hemispheres</td>
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<td>Fragmented dentate nuclei</td>
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<td></td>
<td>Olivary heterotopia</td>
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<tr>
<td>Case 3</td>
<td>Abnormal lamination: two-layered cortex</td>
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<tr>
<td></td>
<td>Disorganized hippocampal cortex</td>
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<tr>
<td></td>
<td>Increased NeuN neurons in EGL, decreased NeuN neurons in IGL</td>
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<td></td>
<td>Fragmented dentate nuclei</td>
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<td></td>
<td>Olivary heterotopia</td>
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<tr>
<td>Case 4</td>
<td>Abnormal lamination: four-layered cortex</td>
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<tr>
<td></td>
<td>Disorganized hippocampal cortex</td>
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<td></td>
<td>Periventricular columns and nodules of heterotopic neurons</td>
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<td>Heterotopic Purkinje cells</td>
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<td></td>
<td>Olivary heterotopia</td>
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Fig. 13: Mutations identified in the TUBA1A gene. The structure of the protein is represented schematically according to Nogales et al. (1998). Mutations identified in living patients are indicated above the gene (from Keays et al., 2007; Poirier et al., 2007; Bahi-Buisson et al., in press), and the mutations identified in fetuses below. Most patients exhibit either perisylvian (R264C, L397P, R422C, G436R) or posterior (R422H, S419L) pachygyria with the exception of the patient with the I188L mutation who exhibits subcortical band heterotopia, and the patient with the R402H mutation who has a posterior agyria (Bahi-Buisson et al., in press). N ter = N terminal domain, C ter = C terminal domain, p = protein.

An 11-year-old patient with a R402H mutation, showing microcephaly, severe agyria and ventricular dilatation, mild cerebellar vermis and brainstem hypoplasy, a thin corpus callosum and dysgenesis of the internal capsule (Bahi-Buisson et al., in press). These abnormalities, at the resolution of MRI, at least partially resemble case 4 studied here. On the other hand, with these few cases, it is not yet possible to say that mutation of the R402 residue...
specifically gives rise to the particular features observed in case 4 in our neuropathological study. Furthermore, with only four cases studied here, no clear correlations can be made between the type of mutation and the phenotype.

Neuropathological findings in TUBA1A mutated cases were compared with those observed in other classical lissencephalies. Lissencephaly in boys with an absent corpus callosum, combined with genital abnormalities is often lissencephalies. Lissencephaly in boys with an absent corpus callosum was reported (Kato and Dobyns, 2005). Case 1 examined here shows similar abnormalities. However, neuropathological examinations of ARX cases have previously shown a slightly thickened, three-layered cortex containing exclusively pyramidal neurons (Bonneau et al., 2002). Our preliminary studies using calretinin and calbindin in TUBA1A cases suggest that there are at least certain interneurons in the cortex, although these may be reduced in number, as in other lissencephalies (Pancost et al., 2005; Francis et al., 2006). Above all, the cerebellum is largely unaffected in ARX cases and indeed, ARX is not expressed in the cerebellum (Bienvenu et al., 2002), and thus, TUBA1A cases are distinct from ARX cases.

While the abnormalities of cortical cytoarchitecture observed in cases 1–3 were different from classical lissencephaly due to DCX and LIS1 mutations, the cortical abnormalities of case 4 were characterized by a thick cortex with a typical four-layered pattern and reduced white matter. Mild involvement of the cerebellum has been described in certain cases of LIS1 and DCX lissencephaly, as well as a thin corpus callosum and/or an abnormal rostrum and splenium, and olivary heterotopia are constantly observed in the medulla (Barkovich et al., 1991; Pilz et al., 1998; Dobyns et al., 1999; Golden, 2001; Uyanik et al., 2007). However, the severe cerebellar and brainstem abnormalities such as those consistently observed in TUBA1A lissencephaly are not usually associated with LIS1 and DCX cases (Forman et al., 2005; our unpublished data). We have analysed eight cases of classical lissencephaly associated with LIS1 and DCX mutations and have never observed diffuse anomalies of migration in the cerebellum, such as that observed in case 4. Nevertheless, in 2001, Ross et al. identified a distinct form of lissencephaly known as ‘lissencephaly with cerebellar hypoplasia’ or LCH (Ross et al., 2001) and they described six distinct LCH subtypes based on imaging data. The first subgroup, LCHa, characterized by the association of lissencephaly and midline cerebellar hypoplasia involving mainly the vermis includes cases with LIS1 and DCX mutations. Such cases showed on imaging, a phenotype close to that of our case 4, however the corpus callosum was described as normal or mildly hypoplastic. So once again, TUBA1A cases can be distinguished from LIS1 and DCX cases with cerebellar hypoplasia, by the combination of affected brain structures.

One further subgroup associated with mutations in RELN, also shows many of the same affected brain structures, except for the corpus callosum, described as normal, and the autosomal recessive inheritance pattern (Hourihane et al., 1993; Hong et al., 2000). Thus, TUBA1A mutations can, in each case, be distinguished from DCX, LIS1, ARX and RELN mutated cases.

Mutated genes have not yet been identified for other LCH cases that include both sporadic and familial autosomal recessive forms (Al Shawan et al., 1996; Kroon et al., 1996; Farah et al., 1997; Kushner et al., 1999; Kato et al., 1999; Miyata et al., 2004; Sztriha et al., 2005, Forman et al., 2005). However certain of these, characterized by a pachygyria and a severe brainstem and cerebellar hypoplasia (Al Shawan et al., 1995; Farah et al., 1997; Kato et al., 1999) are also likely to be mutated for RELN. Other cases (Kroon et al., 1996; Sztriha et al., 2005) are probably ‘microlissencephaly’, a completely different entity characterized by severe fetal microcephaly with an absence of sulci and gyri, and severe hypoplasia of both the cerebral and cerebellum (Dobyns and Truwit, 1995).

Neuropathological studies have in fact been performed in only four LCH cases (Kerner et al., 1999; Miyata et al., 2004) which can therefore be compared more accurately with TUBA1A lissencephaly. In the two sibs reported by Kerner et al. (1999), and one further case reported by Forman et al. (2005), the lissencephaly was characterized by a two-layered cortex made up of a normal molecular layer and a single thick disorganized layer with randomly oriented neurons, and the presence of abnormal heterotopic neurons in the white matter. In one case an absent corpus callosum was reported (Kerner et al., 1999) and each case showed posterior fossa pathology. These cases thus resemble cases 1–3 described here. The occurrence in two sibs suggests an autosomal recessive mode of inheritance; however, germ cell mosaicism of an autosomal dominant trait cannot be excluded. Miyata et al. (2004) reported a sporadic case of lissencephaly with agenesis of the corpus callosum and a rudimentary dysplastic cerebellum in a more detailed neuropathological study. Numerous features of this 7-day-old case are similar to those observed in cases 1–3. Indeed, the lissencephaly was associated with an agenesis of the corpus callosum without Probst bundles, an abnormal hippocampus and basal ganglia, a hypoplastic brainstem and cerebellum. RELN was normally expressed in Cajal–Retzius cells and Ki67 immunohistochemistry showed a high-labelling index in the subventricular germinal zone. This case also showed a unique feature described as peri-ventricular undulating cortical ribbon-like structures, suggesting an abnormality of neuronal migration of precocious onset. Thus, although these four cases are not strictly identical, our neuropathological study allows us to suggest that certain could belong to the spectrum of TUBA1A-lissencephaly cases.

In summary, we describe here the neuropathological phenotype of a new type of lissencephaly associated with TUBA1A mutations. The study of four cases reveals a large neuropathological spectrum in which five features are regularly observed. The constant association of lissencephaly, with severe anomalies of the corpus callosum,
the hippocampus, the cerebellum and the brainstem, consistent with combined defects in neuronal migration, differentiation and axonal guidance, remains a distinguishing feature. So, although these anomalies are not specific and though some cases resemble superficially either LIS1 and DCX, or RELN lissencephalies, the particular combination of the involved cerebral structures and their neuropathological features makes this phenotype distinct from that of other lissencephalies associated with LIS1, DCX, RELN and ARX mutations. Neuropathological studies were thus of paramount importance for a precise description of the TUBA1A mutations, for improving our understanding of the pathogenesis of lissencephaly and for directing the molecular studies towards an accurate diagnosis.

Supplementary material
Supplementary material is available at Brain online.

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