Role of cytoskeletal abnormalities in the neuropathology and pathophysiology of type I lissencephaly

Gaëlle Friocourt · Pascale Marcorelles · Pascale Saugier-Veber · Marie-Lise Quille · Stephane Marret · Annie Laquerrière

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Abstract Type I lissencephaly or agyria-pachygyria is a rare developmental disorder which results from a defect of neuronal migration. It is characterized by the absence of gyri and a thickening of the cerebral cortex and can be associated with other brain and visceral anomalies. Since the discovery of the first genetic cause (deletion of chromosome 17p13.3), six additional genes have been found to be responsible for agyria–pachygyria. In this review, we summarize the current knowledge concerning these genetic disorders including clinical, neuropathological and molecular results. Genetic alterations of LIS1, DCX, ARX, TUBA1A, VLDLR, RELN and more recently WDR62 genes cause migrational abnormalities along with more complex and subtle anomalies affecting cell proliferation and differentiation, i.e., neurite outgrowth, axonal pathfinding, axonal transport, connectivity and even myelination. The number and heterogeneity of clinical, neuropathological and radiological defects suggest that type I lissencephaly now includes several forms of cerebral malformations. In vitro experiments and mutant animal studies, along with neuropathological abnormalities in humans are of invaluable interest for the understanding of pathophysiological mechanisms, highlighting the central role of cytoskeletal dynamics required for a proper achievement of cell proliferation, neuronal migration and differentiation.

Keywords Agyria–pachygyria · Epilepsy · Neuronal migration · Microtubules · Nucleokinesis · Microtubule-associated proteins

Introduction

Neuronal migration disorders account for a substantial number of cortical malformations, including microcephaly, agyria–pachygyria, heterotopia and polymicrogyria.
Original classifications made through neuropathological and clinical descriptions are now supplemented by magnetic resonance nuclear imaging (MRI) and molecular genetics. A few years ago, Barkovich et al. [7] proposed a new classification of cortical malformations based on two additional factors, gene abnormality and timing of the earliest stage of development at which cortical development is affected, leading to the recognition of four major groups. The first group comprises cortical malformations resulting from abnormal neuronal and glial proliferation in the ventricular zone (VZ) and subventricular zone (SVZ) giving rise to abnormal brain size; in the second group, abnormal migration of post-mitotic neuroblasts results in lissencephaly and heterotopias; in the third group, abnormal cortical organization and/or late neuronal migration are responsible for polymicrogyria and schizencephaly; the fourth group is associated with either inherited errors of metabolism or diseases still to be classified.

The term “lissencephaly”, or agyria–pachgyria, was introduced by Owen in 1868 to distinguish the flat “lissencephalic” brains of lower mammalian species or fetuses from the folded “gyrencephalic” brains. This term was then applied to human malformed brains lacking or having a substantial reduction in the number of cortical convolutions by homology to the brain of inferior vertebrates [55]. Though agyria is characterized by a total absence of gyri and sulci, most patients rather present with pachgyria, a more patchy malformation in which the medial and ventral gyri are relatively spared, along with other brain regions, depending on the type of gene and mutation involved [70].

Lissencephaly

Lissencephaly, whose overall incidence is estimated around 1.2/100,000 births, has traditionally been separated into two categories: type I (MIM#607432) or classical type, also named agyria–pachgyria, and type II or cobblestone lissencephaly (MIM#236670). The latter is related, in more than 60% of cases, to a defect of O-glycosylation of extracellular matrix proteins resulting in a defect of the integrity of the pial surface and subsequent overmigration of cortical neurons [16]. In classical lissencephaly, the pia is intact but the normal six-layered organization of the cortex is severely impaired. A few other pathologically distinct forms of lissencephaly have been described, but the genes or the cellular mechanisms involved remain to be identified [46, 52, 99]. Depending on associated defects such as cerebellar hypoplasia, corpus callosum, basal ganglia or white matter defects, classical lissencephaly resulting from mutations in L1 (also called PAFAH1B1, platelet-activating factor acetylhydrolase isofrom1B, alpha subunit; MIM#6001545) or DCX (Doublecortin; MIM#300121) is easily differentiated from lissencephaly-variants due to mutations in ARX (Ar-isteless-related homeobox gene; MIM# #300382), TUBA1A (Tubulin alpha1A; MIM#602529), WDR62 (WD repeat domain 62), RELN (Reelin; MIM#600514) or VLDLR (very low density lipoprotein receptor; MIM#224050) [12, 17, 37, 65, 85, 97, 101, 137]. Although most forms of lissencephaly have a genetic origin, lissencephalic-like syndromes may also result from environmental insults such as hypoxic–ischemic damage, fetal alcohol syndrome, cocaine intoxication during pregnancy or fetal cytomegalovirus infection, as well as from maternal diseases, in particular, maternal diabetes and phenylketonuria [72, 73].

Clinical presentation

Clinical presentation of lissencephalic syndromes may vary depending on the severity and the molecular basis of the agyria–pachgyria. The most severe syndromes, prenatally detected using ultrasound performed during the second trimester of the pregnancy, show an absence of primary sulci, and are confirmed by antenatal cerebral MRI performed from 30 weeks of gestation.

Most patients present with hypotonia or severe neurological distress at birth. In classical lissencephaly due to L1 or DCX mutations, seizures occur in 75% of infants and develop during the first year of life [35, 110]. In the Miller–Dicker syndrome (MDS), clinical symptoms at birth also include facial dysmorphism and visceral abnormalities. Other specific phenotypes are observed exclusively in males, as for example, in the severe X-linked lissencephaly associated with abnormal genitalia (XLAG) due to severe ARX mutations [95, 101]. This form of lissencephaly is typically characterized by respiratory distress at birth, neonatal-onset intractable epilepsy, severe hypotonia from birth, hypothalamic dysfunction with deficient temperature regulation, and chronic diarrhea. External genitalia are ambiguous or underdeveloped. Adducted thumbs may also be present. All patients display craniofacial dysmorphism including congenital microcephaly, psychomotor delay and most of them die before the age of 18 months [42, 95, 101, 126].

Lissencephalies with cerebellar hypoplasia (LCH) encompass heterogeneous disorders which have been related to mutations in L1, DCX, RELN and TUBA1A. In the LCH due to RELN or VLDLR mutations, phenotypic features, besides the classical clinical symptoms, include myopia, nystagmus and lymphoedema [17, 18, 24, 85]. More recently reported, WDR62- and TUBA1A-patients present with congenital microcephaly, neurological disabilities and epilepsy. Additional features such as strabismus and facial diplegia with transient oropharyngoglossal dysfunction responsible for neonatal feeding.
problems and later drooling have been reported for TUBA1A-patients [5, 104, 121].

The prognosis of lissencephaly is extremely poor since most patients present with post-natal failure to thrive, feeding problems and repeated aspiration pneumonias which are responsible for a shortened lifespan. Living patients present profound mental retardation, spastic quadriplegia and epilepsy. Status epilepticus may occur in the first post-natal days or later on, but may also be identified during pregnancy. Drug-resistant epilepsy comprises atypical absences, drop attacks, myoclonic, tonic and tonic-clonic seizures, partial epilepsy or West syndrome [44, 75].

Imaging studies

Apart from the absence of cortical circonvolutions, classical lissencephaly is characterized by increased cortical thickness (between 10 and 20 mm instead of 4 mm from birth). In order to help in determining the most likely genetic cause, Dobyns and Truwit have developed a classification considering both the severity of the anomalies and their anterior (a) or posterior (p) predominance: the spectrum varies from complete or near complete agyria (grades 1 and 2) to pachygyria (grade 4) and SCLH (grade 6). Intermediate grades consist of mixed agyria–pachygyria (grade 3) and mixed pachygyria–SCLH (grade 5) [40, 43]. As an example, LIS1 mutations usually give rise to agyria or pachygyria (grades 2–4 of severity), the parietal and occipital lobes being the most severely affected areas (p > a gradient) [21, 43, 131, 143]. On the contrary, lissencephaly due to DCX mutations is more severe in the frontal cortex (a > p gradient) [44, 109, 131]. However, rare males with DCX mutations have also been described with SCLH which is then more severe in anterior parts, and appears as an undulating band of gray matter in the frontal lobes beneath a nearly normal cortex [41]. The variant form of lissencephaly resulting from ARX loss-of-function mutations is characterized by a moderate increase in thickness of the cortex (up to 10 mm) with a p > a gradient associated with constant corpus callosum agenesis, severe congenital or post-natal microcephaly, olfactory hypoplasia and diffusely hypoplastic and dysplastic basal ganglia with cysts and abnormal white matter signals, contrasting with normal infratentorial structures. Ventriculomegaly is mild to moderate with enlarged trigone and posterior horns of the lateral ventricles [9, 42, 95, 101, 118, 126]. TUBA1A mutations are responsible for gyral malformations ranging from pachygyria (p > a gradient) to subtle SCLH. Other features include severe microcephaly, perisylvian pachygyria, dysgenesis of the corpus callosum, disorganization of the hippocampus, absence or hypoplasia of the anterior limb of the internal capsule, characteristic fullness of the striatum and colpocephaly, brainstem and cerebellar hypoplasia which mainly involves the inferior vermis. Very recently, nine patients with mutations in the WDR62 gene have been described. They all have extreme microcephaly, pachygyria, varying degrees of cortical thickening, a loss of gray–white matter junction and hypoplasia of the corpus callosum [12].

Ross and colleagues [142] have proposed a classification of LCH into four different subgroups depending on the severity of cortical, cerebellar and brainstem defects. The first group (LCHA) corresponds to rare mutations in LIS1 (3/4) or DCX (1/4) with a thick cortex and a mild hypoplastic but normally organized cerebellum. The second group (LCHB) is characterized by severe cerebellar hypoplasia and pachygyria with a thin cortex and anterior predominance of deficits and results from mutations in RELN. The degree of cortical malformation varies from agyria to simplification of the gyral pattern and from near normal cortical thickness to marked thickening of the cortical gray matter. The cerebellum is small with no foliation and hypoplasia of the cerebellar hemispheres as well as of the inferior vermis. These abnormalities are usually associated with hippocampal dysplasia and hypoplastic brainstem [17, 18, 24, 85, 175]. LCHC includes microcephaly, agyria–pachygyria with severe cerebellar hypoplasia, cleft lip and palate, and a high mortality rate during the perinatal period. The last group, LCHD, corresponds to severe microcephaly, thick cortex, grades 1–2 agyria, diffuse cerebellar hypoplasia of both hemispheres and vermis as well as mild defects of the brainstem. Some patients with TUBA1A mutations belong to this group [5].

Nevertheless, irrespective of the type of lissencephaly (classical or variant) observed, some supratentorial and infratentorial abnormalities remain below the limits of resolution of MRI and are only detected by careful post-mortem neuropathological examination (Fig. 1).

Neuropathology of type I lissencephaly

Lissencephaly associated with LIS1 mutations and Miller–Dieker syndrome

The first detailed neuropathological description was reported in 1956 by Crome who introduced the notion of a “four-layered” cortex. Since then, several cases have been reported [34, 39, 52, 55, 70, 78, 90, 107, 139, 168].

On gross examination, the brain weight is either normal or reduced. In the most severe forms, the cerebral hemispheres are smooth, with poorly defined central and Sylvian fissures (Fig. 2a). When present, the gyri are broad, small in number and coarse, with a failure or a delayed
operculization of the Sylvian fissure. In less severe cases, primary temporal and occipital fissures may be observed and the hippocampal area, as well as the adjacent cortex, may have a normal gyration. The cerebellum is usually normal, rarely hypoplastic. On coronal sections, the cortical ribbon is thicker than in normal brain (up to 2 cm in width) and poorly delineated from the white matter which is markedly reduced, leading to an inverted proportion between the intermediate zone or white matter and the cortical ribbon (Fig. 2b). In MDS, the internal capsule, the claustrum, the corpus callosum and the cingular gyrus are not identified [39, 124, 168, 169].

On microscopic examination, the cortical plate exhibits the characteristic four-layered pattern (Fig. 3a). The molecular layer I contains Cajal–Retzius cells located close to the pia. Layer II is composed of densely packed pyramidal neurons which are mainly observed in its upper part, and underlined by an irregular sheet of granular cells (Fig. 3b). Layer III consists of scattered fusiform, rounded or multipolar neuronal elements. Layer IV is particularly poorly demarcated from the underlying white matter and composed of pleomorphic neuronal cells, with misoriented pyramidal neurons. The reduced white matter contains multiple arrested post-mitotic neuroblasts. The remaining subependymal cell layer is also poorly demarcated from the deep white matter and sometimes contains periventricular heterotopias. In children of over 1 year of age, additional sublayers consisting of sheets of myelinated fibers with a radial arrangement and crossing the deeper layers may be recognized, in particular, in layers II and III [30, 52, 55, 70, 123].
The internal capsule is histologically observed, hypoplastic and irregular in shape. The septum which constantly contains aggregates of microcalcifications in MDS contains microcalcifications in 20% of cases of microdeletions but never in \( \text{LIS1} \)-mutated brains [39, 107, 122, 124]. The cytoarchitecture of the different hippocampal fields appears to be generally preserved but the dentate gyrus is occasionally distorted or thickened and composed of densely packed granular cells, conversely to the pyramidal cell layer where neuronal density is often decreased (Fig. 4a).
The deep gray nuclei are not dysmorphic in shape and location, even if decreased neuronal density is noted in the striatum and the thalamus. Intense gliosis is dispersed throughout the white matter, and radial glia persists later in the posterior regions.

Histological examination of infratentorial structures reveals diffuse decreased neuronal density of several brainstem nuclei at the mesencephalic and pontine levels. Cortico-spinal tracts may be absent or fragmented and hypoplastic. In the medulla, the inferior olivary complex is particularly hypoplastic or dysplastic (Fig. 4e), with multiple heterotopic supernumerary foci of olivary neurons located in and between the dorsal nuclei and fascicles. The pyramids and their decussation are most often observed. In the cerebellum, multiple foci of Purkinje cells are disseminated in the deep white matter extending toward the middle...
cerebellar peduncles. Poorly convoluted and slightly fragmented dentate nuclei are often aberrantly located in the vicinity of the ventricular surface [55, 78, 107, 114].

Lissencephaly associated with DCX mutations in males

In contrary to LIS1, morphological descriptions of DCX syndrome are rare. The first neuropathological case to be reported was interpreted as a four-layered cortex similar to the LIS1-mutated cortex with infratentorial anomalies consisting of inferior olivary nuclei pachygyria [8]. In hemizygous DCX-mutated males, the brain surface is agyric or pachygyric, the anterior areas being more severely affected, with hypoplasia of the frontal lobes (Fig. 2c). On coronal sections, the corpus callosum displays various aspects described as thick, thin or absent, with a thickened
septum pellucidum [8, 169]. The caudate nuclei are normal in shape and volume. Shortly before birth, several cysts of germinolysis occupy the walls of the lateral ventricles (Fig. 2d). Basal ganglia, internal capsule and claustrum are hardly discernible. The cortical ribbon is thick and poorly demarcated from the intermediate zone (IZ). The cerebellum and the brainstem are macroscopically normal.

On microscopic examination, the cortex displays various anomalies. It has first been described as a vague four-layered cortex coexisting with a band of ectopic neurons of diverse sizes and shapes organized in columns or clusters lying in a reduced rim of white matter, or as two-layered [8, 52, 141]. In fact, apart from layer I, all other layers show a different pattern from LIS1 cortex [52] with an ill-defined but six-layered cortex [114, 169]. Layer II consists of a thin band containing an admixture of pyramidal and granular neurons. Layer III is poorly delimited and paucicellular, made up of granule and immature neurons, and contains more pyramidal neurons than in LIS1-mutated cortex (Fig. 3e). The remaining layers IV, V, and VI are located within the white matter. In the superficial white matter, layer IV forms a thick ribbon of scattered, sometimes clustered pyramidal cells and immature neurons. Layer V is located in the intermediate zone and composed of large nests of arrested migrating neurons, arranged in a radially and columnar pattern [52, 114]. These cells seem to emerge from the layer VI which is formed by the juxtaposition of numerous nodules of varying size with clear-cut boundaries and widely distributed through the deep white matter and the striatum (Fig. 3d). Thalamic and hypothalamic nuclei are diffusely invaded by haphazardly dispersed heterotopic neurons, resulting in the formation of an indistinct cellular mass. The internal capsule is disrupted and displaced by the nodular heterotopies, resulting in the formation of coarse and irregular fascicles separated by nodules and columns (Fig. 3e). The hippocampal formation is hypoplastic (Fig. 4b). At last, as in LIS1-mutated brains, a prominent reactive astrocytic gliosis is observed in the cortex and white matter of the cerebral hemispheres, as well as in the basal ganglia [8, 114].

In the lower brainstem, the inferior olivary complex is pachygyric (Fig. 4f), and conversely to LIS1, ectopic olivary nuclei are infrequently observed. Bilateral and symmetric corticospinal tracts are identified. In the cerebellum, the dentate nuclei are hypoplastic. The main cerebellar anomaly consists in some cases in the presence of dispersed heterotopic Purkinje cells throughout the cerebellar white matter, or arranged in nodules of variable size [8, 52, 114, 169].

XLAG syndrome

The XLAG syndrome has been the first human disorder in which a failure in tangential migration of GABAergic interneurons has been demonstrated. Since the first neuropathological descriptions based on three families, only four additional cases have been reported so far in the literature [15, 52, 118, 128]. In all reported cases, the brain is smaller than in age-matched controls. The external surface is agyric in the occipital and parietal lobes, and pachygyric in the more anterior regions. Olfactory bulbs are absent most of the time. On coronal sections, the cortical ribbon is better delineated from the white matter than in LIS1- and DCX-mutated brains (Fig. 2e). The white matter, which is still not myelinated after 1 year of age, is atrophic. The internal capsule, fornices and corpus callosum are absent. Several diencephalic structures are not identified. The hippocampi are dysplastic and hypoplastic. The lateral ventricles are laterally bordered by thick Probst bundles. The third ventricle is enlarged without narrowing of the aqueduct of Sylvius. The boundaries of the striatum and basal ganglia are not apparent. Interhemispheric or occipital cysts may also be present. No infratentorial lesions are detected on macroscopic examination.

Histological analysis reveals in all cases the characteristic pattern of a three-layered cortex with a global decrease in neuron number affecting massively interneurons (Fig. 3f). The cortical plate is almost entirely composed of pyramidal cells (Fig. 3g). The molecular layer contains a few Cajal–Retzius cells lying under the pia, as well as foci of heterotopic small neurons. Leptomeningeal glioneuronal heterotopias may also be identified. Layer II is composed of numerous densely packed pyramidal neurons, sometimes forming nodules protruding into the molecular layer. Layer III, whose thickness increases in the posterior lobes, is irregular in width and less cellular, and contains small-to-medium-sized neurons, with among them randomly dispersed pyramidal cells of various sizes. The underlying atrophic white matter is diffusely gliotic and contains heterotopic neuronal clusters especially in the frontal lobes, as well as vessels circumscribed by enlarged Virchow–Robin spaces. In the diencephalon, striatal and thalamic structures are disorganized. The caudate nuclei are markedly reduced in size and cellularity, and so are the putamen, the pallidum and the thalami. Most hypothalamic nuclei are not identified and their absence has been correlated with some clinical hallmarks of the disease [118]. The hippocampus is rudimentary, especially the dentate gyrus (Fig. 4f).

Histological examination of the brainstem and cerebellum reveals several abnormalities. The cerebral peduncles and substantia nigra are dislocated, the corticospinal tracts are atrophic and fragmented from the cerebral peduncle level to the lower medulla where their decussation is generally not visualized (Fig. 4g). However, the cerebellum displays no lesion.
Lissencephaly associated with \textit{TUBA1A} mutations

Mutations in \textit{TUBA1A} cause a wide spectrum of brain malformations ranging from laminar heterotopia or perisylvian pachygyria to agyria resembling the classical lissencephaly. At the present time, neuropathological descriptions are based on five fetal observations in which prenatal ultrasound and MRI examination revealed severe cortical dysgenesis from the second trimester of the pregnancy \cite{[47, 108, 133]}. Neuropathological examination showed lesions which, similar to children, involve five brain structures including the neocortex, the hippocampus, the corpus callosum, the brainstem and the cerebellum.

On macroscopic examination, the brain and infratentorial weights are below the 5th percentile. The brain surface is either agyric or pachygyric, with a postero-anterior gradient of severity. Sylvian fissures are missing or are extremely short and vertically oriented (Fig. 2f). The olfactory tracts are usually absent (Fig. 2g). The cerebellum is also hypoplastic and complete or partial agenesis of the vermis may be found. In one of the cases, the inferior part of the cerebellar hemispheres exhibited a micronodular pattern, whereas the cerebellar folia were normal in its superior part \cite{[108]}. On coronal sections, ventricular dilatation is diffuse, and there is an absence of demarcation between gray and white matter (Fig. 2h). The corpus callosum is described as completely or partially absent, thick, or thin. Probst bundles are infrequently observed. Basal ganglia are abnormally oriented.

Histological examination of the cerebral hemispheres reveals a quasi absence of lamination forming a two-layered cortex (Fig. 3h). Layer I contains scarce and misplaced Cajal–Retzius cells, and is underlined by a single band of neurons extending from the inferior limit of the marginal zone to the periventricular zone. Nevertheless, histological examination makes it possible in almost all cases to distinguish a roughly ordered six-layered cortical plate in the less affected areas, including a layer II with a poor density of immature, often misoriented or inverted pyramidal neurons, and a paucicellular layer III consisting of scattered granular cells. The deepest layers consist of numerous and haphazardly dispersed immature neuronal cells filling the IZ and SVZ, occasionally with a nodular or columnar arrangement. In the hippocampus, the dentate gyrus is absent, hypoplastic or discontinuous and not properly folded. The pyramidal cell layer of the Ammons’ horn is thick or hypoplastic (Fig. 4d). In the parietal and occipital cortices, cortical lamination is more apparent. Basal ganglia are hypo- and dysplastic, sometimes asymmetric and most often fragmented, in particular, the pallidum. Thalamic nuclei appear prominent and displaced, and contain an indistinct mass of neurons. Caudate and hypothalamic nuclei are dramatically reduced in size and shape. The internal capsule is hypoplastic and fragmented with aberrantly directed fascicles, or not identified. Anterior commissure, fornices and septum pellucidum may be missing. In the mesencephalon, the pons and the medulla, neurons are most often scattered in the neuropil, with hardly any organization. Neuronal density of the pontine nuclei is globally decreased. Most of the brainstem nuclei are indiscernible. Corticospinal tracts are hypoplastic and aberrantly placed. In the medulla, olivary nuclei are agyric and sometimes reduced to small foci of immature neuronal cells (Fig. 4h), the vast majority of them being arrested in the dorsal part of the medulla and of the pons, as well as in the inferior cerebellar peduncles. In the cerebellum, dentate nuclei are also pachygryic and fragmented, forming scattered and small nests of neurons in the white matter. In the cerebellar cortex, Purkinje cells are reduced in number, some of them being arrested in the cerebellar white matter, arranged in small clusters or in streaks intermingled with hypoplastic deep cerebellar nuclei \cite{[108]}.

To summarize, type I lissencephaly which constitutes a primary defect in neuronal migration during development and which has traditionally been considered as a disorder of radial migration also results from non-radial migration impairment (Fig. 5). Nevertheless, genes implicated in type I lissencephaly are not only crucial for appropriate migration toward the cortex but also for other developmental events including cell proliferation, neuronal differentiation, axonal pathfinding and transport, as well as connectivity and myelination. Irrespective of the gene involved, neuropathological features argue for more complex underlying pathogenic mechanisms and are summarized in Table 1.

\subsection*{Molecular basis of type I lissencephalies}

\textbf{LIS1}

\textit{LIS1}, located at 17p13.3, consists of 11 exons and encodes an atypical microtubule-associated protein (MAP) characterized by the presence of 7 WD40 repeat domains \cite{[144]}. \textit{LIS1} haploinsufficiency resulting either from intragenic mutations or deletions encompassing \textit{LIS1} alone generally causes isolated lissencephaly and more rarely SCLH or LCH \cite{[22, 111, 113, 132, 143, 153, 166]}. Larger deletions encompassing \textit{LIS1} and the neighboring genes are responsible for MDS \cite{[19, 42]}. Interestingly, large duplications encompassing the region of the MDS are responsible for mild to moderate mental retardation, hypotonia and dysmorphism but without lissencephaly \cite{[140]}.

To date, 70 intragenic heterozygous mutations of the \textit{LIS1} gene have been described \cite{[22, 143, 166]}. They usually occur de novo and familial recurrences are rare with an
autosomal dominant mode of inheritance [22]. The majority of mutations are truncating mutations (84%) whereas missense mutations distributed along the whole coding sequence account for 16% of the mutations only (Fig. 6). No evident genotype–phenotype correlations have been found: neither the nature (missense/truncating) nor the position of the mutation is correlated with the clinical severity, the degree of agyria or cortical thickness [143, 166]. Clinical presentation only correlates with the degree of agyria and cortical thickening. Intragenic LIS1 mutations or genomic rearrangements are identified in 85–87% of the patients affected with classical lissencephaly with a p gradient and therefore constitute the most frequent cause of this form of lissencephaly [80, 117]. However, somatic mosaicism which has been estimated as high as 14% might hamper the detection of LIS1 mutations [166].

LIS1 was initially identified as the non-catalytic subunit of platelet-activating factor acetylhydrolase (PAF-AH), a heterotrimeric enzyme that inactivates the lipid messenger PAF [79]. In addition, Lis1 was shown to be associated with microtubules [144] and is present in regions of high microtubule density in cells, especially at the centrosome and microtubule-organizing centre (MTOC) [48, 155]. Several studies in different organisms have revealed that Lis1 is part of a conserved evolutionary pathway that regulates cytoplasmic dynein, the main cytoplasmic microtubule-minus end-directed motor protein, involved in several cellular processes such as vesicle transport, nuclear migration and organization of the mitotic spindle (see for reviews [76, 167, 172]) (Fig. 7).

DCX

The DCX gene, which encodes Doublecortin, was identified in Xq22.3 [37, 65, 156]. Hemizygous DCX mutations in males lead to classical lissencephaly whereas heterozygous mutations in females lead to SCLH [36], which is probably due to random inactivation of one of the X chromosomes resulting in two genetically distinct populations of cells that segregate in a cell-autonomous fashion. Hence, affected females display a mosaic phenotype that is intermediate in severity characterized by epilepsy and mild to moderate intellectual disability, usually correlating with the thickness of the band and the degree of pachygyria [67, 74, 111]. The DCX gene contains seven exons and mutations have been described in approximately 38% of males with classical lissencephaly and 85% in females with SCLH [3, 37, 65, 68, 109, 115, 132, 145]. In contrast to LIS1, missense mutations of DCX are more common than truncating mutations, since they represent nearly 80% of the mutations [109].

The Doublecortin protein is characterized by the presence of two evolutionary conserved tandem repeats which define a new family of MAP and constitute microtubule-binding modules [53, 66]. Each of these two domains binds to tubulin
### Table 1: Morphological lesions in supra- and infratentorial structures in the different types of classical lissencephaly resulting from tangential versus radial migration and from abnormalities of differentiation, including axonal pathfinding, connectivity and myelination

| Brain structure | Gene | Radial (R)/tangential (T) migration abnormalities | Differentiation deficits |
|----------------|------|-----------------------------------------------|------------------------|
| Cortex         | LIS1 | Transient external subpial layer persistence (T) | Immature cortical neurons |
|                |      | Absent lamination, four-layered cortex, absence of external granular cell layer (R) | |
|                |      | CR+ interneuron decrease in the upper layers (R/T) | |
|                |      | Heterotopic CR+ interneurons in layer IV, IZ and VZ (T) | |
|                | DCX  | Scattered Cajal–Retzius cells in layer I (T) | |
|                |      | Two- or four-layered or ill-defined six-layered cortex (R/T) | |
|                | ARX  | Three-layered cortex, pyramidal type exclusively (T) | |
|                |      | Absence of CR+ interneurons (T) | |
|                |      | Absence of layers II and IV (R/T) | |
|                |      | Rare dispersed Cajal–Retzius cells in layer I (T) | |
|                | TUBA1A | Scattered Cajal–Retzius cells in layer I (T) | Immature cortical neurons |
|                |      | Two-layered cortex or unlayered (R/T) | |
|                |      | Absence of layer II (R/T) | |
|                |      | CR+ interneuron decrease in the cortex (R/T) | |
|                |      | Misoriented neurons (R) | |
| Hippocampus    | LIS1 | Hypoplastic (R/T) | |
|                | DCX  | Disorganized, dysplastic (R/T) | |
|                | ARX  | Rudimentary, dysplastic (R/T) | |
|                | TUBA1A | Hypoplastic and dysplastic (R/T) | |
| IZ/WM          | LIS1 | Persistent radial glia (R) | Internal capsule hypoplasia |
|                |      | Multiple arrested neuronal cells (R) | Corpus callosum and septum pellucidum abnormalities |
|                |      | Periventricular heterotopias (R/T) | Immature neurons |
|                |      | Arrested CR+ and CB+ interneurons (T) | |
|                |      | Premature disappearance of EGL (T) | |
|                | DCX  | Layers IV, V and VI in the deep WM (R) | Dysmorphic corpus callosum |
|                |      | Columnar and nodular periventricular heterotopias (R/T) | Immature neurons- disorganized dendritic network |
|                |      | CR+ and CB+ interneurons in the IZ/WM and SVZ (T) | |
|                | ARX  | Dispersed neurons or in clusters in the IZ/WM (R) | Absent olfactory bulbs |
|                |      | Rare CR+ cells in the IZ/SVZ (T) | Agenesis of the corpus callosum |
|                | TUBA1A | Heterotopic pyramidal neurons and interneurons (dispersed or in clusters) (R/T) | Heterotopic immature neurons in the IZ |
|                |      | Persistent radial glia (R) | Absent olfactory tracts and bulbs |
| Basal ganglia   | LIS1 | Decreased neuronal density in the striatum, pallidum and thalami (R/T) | Agenesia of the anterior commissure |
|                |      | CR+ and CB+ interneuron decrease in the striatum (T) | Thin optic nerves and chiasm |
|                | DCX  | Fragmentation of the striatum by nodular and columnar heterotopias (R/T) | Dysplastic corpus callosum |
|                | ARX  | Absent diencephalic nuclei, quasi absence of CR+ and CB+ interneurons in the striatum (R/T) | Absence, hypoplasia or fragmentation of the internal capsule. Immature neurons in the cortex |
|                | TUBA1A | Hypoplastic and dysplastic hypothalamic nuclei and basal ganglia (R/T) |
but both repeats are necessary for microtubule polymerization and stabilization [25, 86, 119, 120]. Mutations are clustered in the two microtubule-binding domains and most of them have been shown to impair microtubule polymerization both in vitro and in vivo [145, 159] (Fig. 6). Missense mutations in the N-terminal microtubule-binding domain have been suggested to cause a more severe lissencephaly phenotype compared with those located in the C-terminal domain [109], probably due to their different properties: the N-terminal domain of DCX binds only to assembled microtubules, whereas the C-terminal domain binds to both microtubules and unpolymerized tubulin [100].

Intragenic deletions encompassing one or more DCX exons have also been identified [80, 117]. Therefore, the screening for partial deletions and duplications is necessary to complete sequencing analysis to ensure an accurate molecular diagnosis of this condition. In addition, a high rate of mosaicism has been reported [3, 68], which complicates the genetic counseling in affected families, and a significant recurrence risk exists even if the mother is not a carrier.

ARX

The ARX gene, located at Xp22.13, was identified in 2002 as a novel homeobox transcription factor with striking homology to the Drosophila gene aristaless. In addition to a prd-type homeodomain, ARX contains four polyalanine tracts, an octapeptide and a C-terminal aristaless domain. Mutations in ARX were first identified in non-syndromic X-linked mental retardation (XLMR) [11] and in X-linked West syndrome [95, 157]. Since then, more than 60 distinct mutations have been described and broadened the spectrum of phenotypes, ranging from severe developmental abnormalities of the brain to syndromic and non-syndromic forms of XLMR with intrafamilial and interfamilial variability (reviewed in [61, 64, 151]) (Fig. 6). The resulting phenotypes can be divided into two groups. The first group includes XLAG [42, 101, 126], hydranencephaly with abnormal genitalia (MIM#300215) and Proud syndrome (MIM#300004), characterized by XLMR, agenesis of the corpus callosum and abnormal genitalia [95]. The second group gathers together phenotypes with mental retardation, epilepsy and dystonia without cerebral or visceral malformations (reviewed in [61, 64, 151]). Phenotype/genotype correlation studies have suggested that truncating mutations or missense mutations within the homeobox DNA-binding domain lead to the more severe phenotypes whereas missense mutations outside the homeobox or expansions/deletions of polyalanine tracts lead to phenotypes without any malformations (Fig. 6) [95, 150].

Arx knock-out mice display a phenotype very similar to the human XLAG [101]. Studies of the role of Arx in these mice as well as in different other models have indicated that it is essential for important developmental processes such as regionalization, proliferation, cell differentiation and neuronal migration (see for review [58]).

TUBA1A

In 2007, Keays et al. [97] reported the identification of a mouse mutant with abnormal laminar architecture of the
Fig. 6 Summary of the mutations identified in DCX, LIS1, TUBA1A and ARX: for each gene, mutations are shown on a schematic representation of the protein, except for LIS1 which represents the gene since a number of mutations are localized outside the exons. Exonic mutations are indicated at protein level in order to observe the predicted consequences of the mutations on the protein and intronic mutations are indicated at nucleotidic level as no protein effect can be predicted. For ARX, mutations figured above the protein are responsible for malformation phenotypes (group 1, see text) whereas mutations shown below are responsible for mental retardation with or without epilepsy (group 2). The accession numbers used for the nomenclature are: for DCX cDNA, NM_178152.1; for LIS1 cDNA, NM_000430.3; for TUBA1 cDNA, NM_006009.2; for ARX cDNA, NM_139058.2.
hippocampus and cerebral cortex resulting from impaired radial neuronal migration. The causative mutation was identified in the guanosine triphosphate (GTP) binding pocket of one of the α-tubulin gene (tuba1a). The similarity of anatomical and behavioral phenotypes between these mice and the Lis1 [51] and Dcx knockout mice [29, 94] led these authors to screen a cohort of patients with brain anomalies and identified several heterozygous de novo mutations in the TUBA1A gene (previously called TUBA3), located at 12q13.12 [5, 97, 133]. All the 38 TUBA1A mutations detected so far are de novo heterozygous missense mutations (Fig. 6). Four recurrent mutations were described: c.790C>T (p.R264C), c.1204C>T (p.R402H), c.1205G>A (p.R402C) and c.1265G>A (p.R422H). Interestingly, three mutations affect the same amino acid, the arginine in position 402: c.1204C>T (p.R402H), c.1205G>A (p.R402C) and c.1205G>T (p.R422H) [5, 97, 104, 121, 133]. Genotype–phenotype correlation studies reveal that p.R402C causes a phenotype similar to LIS1 deletions or intragenic mutations, whereas p.R402H resembles MDS. In contrast, the effects of the other mutations differ dramatically from the LIS1-associated phenotype, with more variable severity, a > p lissencephaly and hypoplasia of the corpus callosum and cerebellum, and in some cases LCHc or d [104]. TUBA1A mutations account for 1–4% of lissencephaly and are therefore considered as a rare cause of lissencephaly [104, 121].

Functional studies of some of these mutations have suggested that several of them lead to defective interactions in proteins involved in the microtubule network, including CLIP-170, which is usually localized at the plus end of growing microtubules. Lis1, Dcx and their protein partners play a major role in nucleokinesis by coupling the centrosome with the nucleus. Lis1 and Dcx are also found at the extremities of neuronal processes where they potentially regulate their stabilization as well as axonal elongation and cell adhesion.

WDR62

Very recently, five recessive missense and truncating mutations have been described in the WDR62 gene in nine patients from different consanguineous marriages, using a whole-exome sequencing approach. These mutations result in microcephaly, pachygyria with cortical thickening and hypoplasia of the corpus callosum [12]. WDR62 has been shown to be enriched in neural progenitors of the VZ and SVZ of mouse and human fetal brain [12]. Although the function of this gene is not yet known, it has been shown to localize in the nucleus, but not at the centrosome like other known microcephaly genes, and interacts with
c-Jun N-terminal kinase (JNK), thus potentially playing a role in the mitogen-activated protein kinase (MAPK) signaling cascade [12, 171]. More studies are required to assess the frequency of WDR62 mutations in cortical malformations and fully understand its role in corticogenesis.

**RELN and VLDLR**

To date, only two splice-site mutations in the RELN gene have been identified in two consanguineous families with an autosomal recessive form of lissencephaly with cerebellar hypoplasia and defects of the hippocampus and brainstem [24, 85]. The human phenotype is reminiscent of what is observed in homozygous reeler mice, which display ataxia, cerebellar hypoplasia with a decreased number of Purkinje cells, inverted layering of the cortex and abnormal axonal connectivity due to abnormal neuronal migration (see for review [33]). An additional consanguineous pedigree has been reported with an homozygous apparently balanced reciprocal translocation, t(7;12)(q22;p13) disrupting the RELN gene at 7q22.1 [175]. As only three consanguineous families have been described, the clinical phenotype might be wider, and to our knowledge, no neuropathological description is presently available. Patients display hypotonia, nystagmus, severe mental retardation and generalized epilepsy [85].

Reelin encodes a large glycoprotein that controls cell–cell interactions critical for cell positioning in the brain [31, 83, 127]. Secreted by Cajal–Retzius cells, Reelin acts on migrating cortical neurons by binding to Vldlr and Apoer2 (apolipoprotein E receptor 2) receptors [31, 32, 82, 163], and triggers off the recruitment of a cytoplasmic adaptor molecule, mDab1, to the receptors and its phosphorylation by non-receptor tyrosine kinases of the Src-family: Src and Fyn [4, 14, 92, 105] (Fig. 8). Mutations in mDab1 exhibit a phenotype very similar to the reeler one, further supporting the notion that these proteins participate in the same pathway [87, 149, 170]. More recently, a homozygous deletion encompassing VLDLR has been reported in affected individuals with non-progressive ataxia, mental retardation, inferior cerebellar hypoplasia and mild gyral simplification in the Hutterite population [17, 18]. As VLDLR is part of the Reelin signaling pathway, it is not surprising that these two genes result in similar phenotypes.

**Alterations of cytoskeletal dynamics in type I lissencephaly**

Defects of neuron generation and/or cell proliferation

In most cases of type I lissencephaly, the brain is of small size, suggesting some defects in neuron generation and/or cell proliferation. Accordingly, Lis1 has been found to be required for interkinetic nuclear migration and cell division of cortical VZ neuroblasts [164], through the regulation of the mitotic spindle orientation which determines the cleavage plane [154, 174]. In addition, mutant mice for Lis1 or Ndel1 show a thinner cortex [50, 84] and human neural precursors from a fetus with MDS demonstrated diminished rates of cell proliferation and increased cell death [148]. As the timing of the last division affects the final destination of a cortical neuron, it is thus possible that the altered distribution of neurons observed in human lissencephaly might also result from a defect in cell division [130].

Similarly, in utero electroporation in mice to knockdown or overexpress Arx in the developing cortex show that Arx is important to maintain progenitor proliferation [60]. These results are in agreement with the microcephaly observed in the cortex of Arx knockout mice [101] and human patients with XLAG [42, 95, 101, 126].

Unlike Lis1, Arx or Wdr62, Dcx and Tuba1a proteins are described as specifically expressed in post-mitotic migrating and differentiating neurons during development [26, 53, 66, 69]. Regarding Tuba1a, it is conceivable that the microcephaly phenotype may be the result of cell death either due to abnormal neuronal migration or due to a failure of differentiation, and, in particular, of synaptogenesis and synapse remodeling, responsible for a defect of connectivity between cells. Otherwise, a study has recently described spindle orientation abnormalities similar to Lis1 mutants in radial glial cells of Dcx knock-out mice, which, in turn, lead to moderate proliferation defects [135], suggesting that Dcx may play a critical role in neurogenesis through unknown mechanisms.

Alteration of cytoskeletal dynamics results in defects of neuronal migration

Consistent with an important role for Lis1 in neuronal migration, mice with decreased levels of Lis1 exhibit dose-dependent disorganization of cortical layers, hippocampus, cerebellum and olfactory bulbs [20, 62, 84]. In vitro analyses of heterozygous cerebellar granule neurons show slower migration and display a lengthening of the distance between the centrosome and the nucleus, suggesting that Lis1 is required for nuclear movement by coupling the nucleus with the centrosome [13, 84, 158]. Similarly, cell-autonomous reductions of Lis1, Ndel1 or dynein by RNAi also affect neuronal migration in vivo by affecting soma translocation [152, 164, 165]. A few studies have also reported that Lis1 plays a role in cortical interneuron migration in both mouse and human [114, 116, 123, 129].

The role of Dcx in radial neuronal migration has also been extensively studied in both mouse and rat models.
Tangential migration defects have also been reported more recently for olfactory bulb interneurons migrating from the SVZ to the olfactory bulb through the rostral migratory stream, and cortical interneurons which migrate from the medial ganglionic eminences. The role of Dcx in neuronal migration is not very well understood yet. Dcx is supposed to function with Lis1 and dynein to mediate nucleus–centrosome coupling. Overexpression of Dcx or Lis1 increases migration rates and Dcx overexpression can rescue the coupling defect caused by reduced levels of Lis1 or by dynein inhibition. Accordingly, both physical and genetic interactions between Lis1 and Dcx have been reported.

In the developing cortex, Arx is expressed in tangentially migrating interneurons emanating from the GE, but not in radially migrating neurons. The strong degree of colocalization between Arx and GABA as well as the absence of interneurons documented in the cortex of XLAG patients and in Arx mutant mice have led to the idea that XLAG syndrome was the result of defective tangential migration. In addition, Arx expression in cortical progenitors has been shown to be critical for radial migration. RNAi-mediated inactivation of Arx in cortical progenitors results in decreased motility of radially migrating neurons, with an accumulation of round cells in the SVZ/IZ, with very few or no processes. Similar cell morphology defects have been reported following inactivation or gain-of-function of proteins which interact with the cytoskeleton or are involved in neuronal polarity, suggesting that Arx may play a role in cell morphology through the cytoskeleton regulation.

Descriptions of the phenotype of the mutant mice or human adult and fetus cases with TUBA1A mutations strongly suggest that, similar to LIS1 and DCX, this gene is critical for neuronal radial and tangential migrations. Similarly, the role of TUBB2B in neuronal migration has been demonstrated by both neuropathological observations of neuronal heterotopias in the hemispheric and cerebellar white matter and the arrest of migrating neurons in the IZ following RNAi-mediated inactivation of tubb2b.
The commonly accepted explanation for the inverted cortex in reeler mice is that in the absence of Reelin, the newly arrived neurons fail to penetrate the preplate. As a result, the preplate is not split into the marginal zone and the subplate and newly born neurons cannot bypass previously deposited neurons and thus accumulate in an outside-in manner (see for review [18, 138]). Several studies have shown that, in particular, mDab1 is necessary for neurons to cross previously deposited neuronal layers and that, in turn, mDab1 should be down-regulated in neurons to properly terminate their migration and allow their siblings to bypass them [49, 77]. In addition to ApoER2 and VLDLR, Reelin has also been found to bind to α3β1-integrin [45] and protocadherins [147]. The relative importance and interactions of these Reelin receptors are currently unknown. α3β1-Integrin has been shown to be involved in the interaction between the migrating neuron and radial glia, and binding of Reelin induces detachment of neurons which stop migrating [45]. All available data have recently led to the “detach and go” model, in which Reelin may stimulate both the detachment from radial glia and the translocation of the cell soma to the top of the developing cortical plate [28].

Alteration of the cytoskeleton results in defects of neuronal differentiation

Several studies have demonstrated a role for dynein in the regulation of intracellular transport, suggesting that Lis1 may participate in the retrograde transport of vesicles and organelles from neuronal processes toward the nucleus. Atypical swellings have been reported in axons of Drosophila Lis1-deficient neurons [112] and in Purkinje cell dendritic trees of a fetus with MDS [114] suggestive of axonal transport abnormalities. More recently, it has been proposed that the Lis1/Ndel/dynein complex also influences the transport of plus-end-directed microtubules (anterograde transport) to the periphery of the migrating neuron, hence aiding the extension of the leading process [173] (Fig. 7). Similarly, Dcx has been reported to promote neuronal intracellular transport [38], probably by providing stable tracks for the plus-end-directed kinesins [120] and may be involved in clathrin-vesicular trafficking [56]. It is not yet clear whether all these functions are related to neuronal migration and axonal growth but it is coherent to think that defective vesicle transport during development may result in impaired membrane expansion at the leading process.

Most cases of type I lissencephaly display corpus callosum defects, which may result from impaired axonal elongation due to abnormal cytoskeletal dynamics. For example, Lis1 is necessary to stabilize polymerized microtubules in new branches [71] and in Lis1 knock-out mice, tangentially migrating cells exhibit a longer leading process and a reduced number of branches [123]. In addition, Dcx is enriched at the extremities of neuronal processes [54, 57, 63, 146], where microtubules play important roles in regulating process elongation and movement direction (Fig. 7). Similar to Lis1, a role in leading process stabilization or organization through the formation of stable microtubules has been described for Dcx [10, 38, 59, 94, 103, 158]. Descriptions of the phenotype of the mutant mice [97, 101] or human cases with ARX or TUBA1A mutations also strongly suggest that, similar to LIS1 and DCX, these two genes are involved in multiple important developmental processes including neuronal differentiation, axonal growth, protein transport and synaptogenesis.

Finally, several recent studies have reported abnormal network activity and synaptogenesis in the hippocampus and cortex of Dcx and Lis1 mutant mice, thus providing useful models to study seizures and the altered cognitive function in type I lissencephaly [2, 51, 91, 98, 106, 125].

Concluding remarks

All the genes which are implicated in classical lissencephalies have been shown to regulate both radial and tangential migration as well as cell proliferation and differentiation. Their products mediate a wide range of cellular functions including signal transduction, cell adhesion and motility but somehow or other they seem to regulate actin cytoskeleton or microtubule dynamics (Fig. 7). The observation that genes encoding two MAPs (LIS1 and DCX) and at least three tubulin isoforms (TUBA1A, TUBB2B, TUBB3) are responsible for lissencephaly/pachygyria disorders emphasizes the role of microtubule dynamics in the pathophysiology of type I lissencephaly.

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