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Lissencephaly: expanded imaging and clinical classification

Nataliya Di Donato^{1,2}, Sara Chiari³, Ghayda M. Mirzaa^{2,4}, Kimberly Aldinger², Elena Parrini³, Carissa Olds², A. James Barkovich⁵, Renzo Guerrini^{3,6}, and William B. Dobyns^{2,4,7}

¹Institute for Clinical Genetics, TU Dresden, Dresden, Germany

²Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, WA, USA

³Paediatric Neurology and Neurogenetics Unit and Laboratories, A. Meyer Children's Hospital, Florence, Italy

⁴Department of Pediatrics (Genetics), University of Washington, Seattle, WA, USA

⁵Department of Radiology and Biomedical Imaging, University of California, San Francisco, CA, USA

⁶IRCCS Stella Maris Foundation, Pisa, Italy

⁷Department of Neurology, University of Washington; Seattle, WA, USA

Abstract

Lissencephaly ("smooth brain", LIS) is a malformation of cortical development associated with deficient neuronal migration and abnormal formation of cerebral convolutions or gyri. The LIS spectrum includes agyria, pachygyria, and subcortical band heterotopia. Our first classification of LIS and subcortical band heterotopia (SBH) was developed to distinguish between the first two genetic causes of LIS – LIS1 (PAFAH1B1) and DCX. However, progress in molecular genetics has led to identification of 19 LIS-associated genes, leaving the existing classification system insufficient to distinguish the increasingly diverse patterns of LIS.

To address this challenge, we reviewed clinical, imaging and molecular data on 188 patients with LIS-SBH ascertained during the last five years, and reviewed selected archival data on another ~1,400 patients. Using these data plus published reports, we constructed a new imaging based classification system with 21 recognizable patterns that reliably predict the most likely causative genes. These patterns do not correlate consistently with the clinical outcome, leading us to also develop a new scale useful for predicting clinical severity and outcome. Taken together, our work provides new tools that should prove useful for clinical management and genetic counselling of patients with LIS-SBH (imaging and severity based classifications), and guidance for prioritizing and interpreting genetic testing results (imaging based classification).

Keywords

Agyria; classification; lissencephaly; pachygyria; subcortical band heterotopia; tubulinopathy

INTRODUCTION

Lissencephaly (LIS, which subsumes the terms agyria and pachygyria), together with subcortical band heterotopia (SBH) comprises a spectrum of malformations of cortical development caused by insufficient neuronal migration. The key features of LIS are an abnormally thick cortex with reduced or absent formation of the cerebral convolutions, while SBH consists of abnormal bands of neurons beneath a normal cortex, although the cerebral gyri may be separated by unusually shallow sulci [Dobyns et al., 2012; Forman et al., 2005]. A few patients with lissencephaly have severe congenital microcephaly, a condition designated as microlissencephaly (MLIS). The multiple congenital anomaly syndromes with LIS include the Miller-Dieker and Baraitser-Winter cerebrofrontofacial syndromes, and X-linked lissencephaly with abnormal genitalia or XLAG [Dobyns and Barkovich 1999; Dobyns et al., 1983; Ramer et al., 1995].

Discovery of the genetic causes of LIS began in 1982 with discovery of deletion 17p13.3 in children with severe LIS and characteristic facial dysmorphism designated as Miller-Dieker syndrome [Dobyns et al., 1983; Stratton et al., 1984] and continued with discovery of the two most common causal genes – *LIS1* (aka *PAFA1H1*) and *DCX* – in 1993 and 1998 [des Portes et al., 1998; Gleeson et al., 1998; Reiner et al., 1993]. Subsequent work by many groups has to date identified 19 LIS-SBH associated genes including *LIS1*, many of which are microtubule structural (tubulin) or microtubule-associated proteins (MAPs), including: *ACTB*, *ACTG1*, *ARX*, *CDK5*, *CRADD*, *DCX*, *DYNC1H1*, *KIF2A*, *KIF5C*, *LIS1*, *NDE1*, *RELN*, *TUBA1A*, *TUBA8*, *TUBB*, *TUBB2B*, *TUBB3*, *TUBG1*, and *VLDLR* [Abdollahi et al., 2009; Boycott et al., 2009; Breuss et al., 2012; Di Donato et al., 2016a; Gleeson et al., 1998; Hong et al., 2000; Jaglin et al., 2009; Keays et al., 2007; Kitamura et al., 2002; Magen et al., 2015; Poirier et al., 2013a; Poirier et al., 2010; Reiner et al., 1993; Riviere et al., 2012; Willemsen et al., 2012]. We include *NDE1*, as the associated gyral malformation resembles tubulinopathy-associated dysgyria [Paciorkowski, 2013 PMID 23704059]. An abnormal gyral pattern has also been described with mutations of *RNU4ATAC*, but too few brain images have been published to allow us to define the malformation [Abdel-Salam et al., 2013].

Our early work on LIS defined a grading system for severity of the gyral malformation (grades 1–6) and recognized several subtypes based on the anterior-posterior gradient, which could be diffuse (no gradient), posterior predominant, or anterior predominant [Dobyns et al., 1992; Dobyns et al., 1999b; Kato and Dobyns 2003; Pilz et al., 1998]. This brain imaging-based system proved useful for separating *LIS1*- and *DCX*-linked LIS syndromes, but has not been sufficient to distinguish the increasingly diverse patterns of malformation seen with all of the novel genes.

Here, we report our experience with LIS patterns and syndromes in more than 1,500 affected individuals. For this study, we systematically reviewed brain MRI and molecular data from 188 LIS-SBH patients ascertained over the past 5 years in two research programs. Using our existing system, we could classify only about 70% of patients in the two cohorts, and even among these did not separate groups with different causal genes effectively. In many

patients, we found variant features that have not been used for classification. We therefore developed a new system that incorporates additional imaging and molecular data. We re-ordered the classification from least to most severe, as this order is more commonly used with other clinical grading systems. We also correlate these data with neuropathological data when available. Our goal was to develop an integrated classification system able to use the LIS-SBH imaging pattern to reliably predict the most likely causative genes.

MATERIALS AND METHODS

Subjects

The two senior authors (WBD, RG) have ascertained more than 1500 children with LIS or SBH over the past 30 years. From these two cohorts, we selected 129 patients referred predominantly from North America (Seattle cohort) and 59 from Europe (Florence cohort) between 2009 and 2015, when high resolution scans were available for most patients. The combined cohort includes 93 males, 90 females and 5 individuals of unknown sex with ages varying from 1 day to 40 years. When specific questions arose regarding rare LIS patterns, we selectively reviewed patients ascertained prior to 2009. Institutional review boards at the University of Chicago, Seattle Children's Hospital, and Meyer Children's Hospital, Florence approved this study.

Our cohort includes patients with malformations intermediate between LIS and polymicrogyria, the pattern we address below as tubulinopathy-associated dysgyria. We excluded patients with cobblestone cortical malformations, the malformation associated with Walker-Warburg syndrome, muscle-eye-brain disease and GPR56-associated dysgyria, as the pathological changes are different from LIS and other classifications have been proposed [Brun et al., 2017; Devisme et al., 2012]. The only exception is that we have included rare patients with severe tubulinopathies who have features overlapping with cobblestone malformations [Fallet-Bianco et al., 2014].

Clinical data

Clinical information and results of genetic testing were obtained from medical records submitted together with MRI scans by referring physicians or parents of the patients. Clinical data was available for all patients. We personally examined 23 of 129 (18%) patients from the Seattle cohort and all 59 from the Florence cohort. Additional molecular data were obtained from targeted sequencing done by various methods (Sanger sequencing, targeted panel and whole exome sequencing) over the last five years in the Dobyns or Guerrini labs. In all, molecular data was available for 70/129 (56%) patients in the Seattle cohort and 55/59 (93%) in the Florence cohort. To supplement these data, we also performed an extensive literature review, and summarized the clinical features attributed to the recently discovered genes associated with LIS.

Brain imaging

Brain magnetic resonance imaging (MRI) studies of all patients from the Seattle and Florence cohorts were available for review. The studies included T1- and T2-weighted sequences, and variable high-resolution (less than 2 mm) volumetric and other sequences

obtained in sagittal, axial and usually coronal planes. All were reviewed with particular attention to the severity (LIS grade) and gradient of the gyral malformation, cortical thickness and presence of associated brain malformations.

Agyria was defined as cortical regions with sulci >3 cm apart, pachygyria as abnormally wide gyri with sulci 1.5–3 cm apart, and subcortical band heterotopia (SBH) as longitudinal bands of gray matter located deep to the cerebral cortex and separated from it by a thin layer of normal appearing white matter. The gyral pattern is characterized by abnormally shallow sulci. The cerebral cortex in agyria and pachygyria was either very thick, measuring 10–20 mm (thick or classic LIS), or less often mildly thick measuring 5–10 mm (“thin” LIS, see results). We used the term “dysgyria” for a cortical appearance intermediate between LIS (pachygyria) and polymicrogyria consisting of mixed large and small gyri separated by shallow sulci with a smooth gray-white border.

Evaluation algorithm

We first separated SBH from LIS because of the relatively normal 6-layered cortical architecture seen in classic SBH as well as the strong association of SBH with mosaicism, leaving rare patients with mixed LIS-SBH (frontal pachygyria and posterior bands) within the LIS group, as most known mutations have been germline. We then separated patients by gradient, initially into diffuse (no visible gradient), posterior-predominant, and anterior-predominant groups. The severity gradient was determined based on the gyral width, with gyri typically >5 mm wider over the more severely affected regions. The increased gyral width typically correlates with thicker cortex, although this may not be apparent with lower resolution images. Patients with rare perisylvian-predominant pachygyria were combined with the posterior group. We also defined a small temporal-predominant group that included only boys with severe *ARX* mutations.

Finally, we evaluated the remaining imaging features that include cortical appearance and non-cortical malformations, which have previously been used in supplementary classification systems only [Barkovich et al., 2012; Cushion et al., 2013; Jissendi-Tchofo et al., 2009; Kato and Dobyns 2003; Ross et al., 2001]. Imaging criteria are summarized in Table I, while Figures 1–3 demonstrate the typical morphological patterns. We found cerebellar hypoplasia (CBLH) with a dysplastic foliar pattern to be the easiest to assess and most frequent malformation associated with LIS. Other non-cortical malformations such as basal ganglia dysgenesis, agenesis of the corpus callosum, enlarged tectum and brainstem hypoplasia were frequently associated with cerebellar hypoplasia. We reviewed only one patient who had dysplastic basal ganglia and tectal hyperplasia with normal cerebellum.

RESULTS

We reviewed brain MRI studies on all 188 children in this study, as well as selected children enrolled prior to 2005 to confirm rare subtypes, and catalogued all malformations observed. We first separated LIS and SBH based on appearance and association of SBH with mosaicism, and also separated children with birth head circumference below –4 SD into a separate cohort with “microlissencephaly”, most of whom also had severe cerebellar hypoplasia. We next organized patterns into (1) severe malformations with no apparent

gradient that include only diffuse agyria and diffuse SBH, and (2) those with diffuse or partial LIS or SBH with a clear gradient, which could be more severe frontally (A>P) or more severe posteriorly (P>A). The most common pattern overall proved to be a diffuse malformation of intermediate severity with frontal pachygyria and posterior agyria, previously associated with deletions and mutations of the *LIS1* gene. Many other patients had diffuse or partial pachygyria, but always with an anterior to posterior gradient. Stated another way, we encountered no patients with diffuse pachygyria whose scan lacked a clear gradient. With partial pachygyria, the remainder of the brain appeared normal, or rarely transitioned to partial SBH and then to a normal gyral pattern. Patients with a clear gradient of LIS or SBH were next separated into anterior (A>P) and posterior predominant (P>A) groups, plus a small temporal predominant (temporal>P>A) LIS group. These steps repeat the logic of our existing classification, but with simpler severity grades. We distinguished 7 patterns but noted several large and heterogeneous groups (Supplementary Table 1).

New criteria—To divide these into smaller and more homogenous groups, we selected two additional imaging features that varied significantly within several of these groups – cortical or band thickness, and presence or absence of non-cortical malformations. Our stepwise analysis using these features is shown in Supplementary Tables I–II, while the known LIS and SBH genes associated with each of the new subgroups are shown in Supplementary Table III.

Cortical thickness is a novel classification criterion for LIS that distinguishes thick (10–20 mm), thin (5–10 mm; here “thin” refers to relatively thin compared to other forms of LIS), and variable (thick in some areas, thinner in others) cortex. Our data shows that posterior-predominant forms of LIS are more common than anterior-predominant or diffuse forms, and that thick cortex (also called “classic” LIS) is much more common than variable or thin cortex among LIS subtypes (Supplementary Table I). Similarly, we separated SBH into those with thick (>7 mm) and thin (1–7 mm) bands.

The reliability of the cortical measurement depends strongly on the imaging quality. For newborns and neonates younger than 3 months, we recommend use of heavily T2-weighted images, preferably with 3D (volumetric) acquisition, and use images in the axial plane for measurements. 3D images can be re-oriented if necessary to assure that measurements of thickness are made perpendicular to the cortex. The cortical thickness cannot be measured reliably on scans done between 3 and 24 months of age, due to ongoing myelination that causes blurring of the cortical-white matter junction. For older children and adults, the cortex can be measured using T1-weighted images giving the best cortical-white matter contrast (for example gradient echo or inversion recovery). 3D images are preferable if possible.

We also introduce or re-introduce [Kloss et al., 2002] the term *dysgyria* to refer to any abnormal gyral and sulcal pattern, modified as needed. The term “*dysgyria not otherwise specified*” is intended to encompass any cortical malformation that does not fit into one of the well-defined categories of malformations of cortical development such as LIS, SBH, cobblestone malformation, focal cortical dysplasia, or polymicrogyria. For example, several of the original reports of cortical malformations with tubulin genes (*TUBB2B*, *TUBB*,

TUBB3) described “polymicrogyria” rather than LIS [Breuss et al., 2012; Jaglin et al., 2009; Poirier et al., 2010]. But our experience and published reports since then have shown that the brain-imaging features are intermediate between LIS (pachygyria) and polymicrogyria, consisting of mixed large and small gyri separated by shallow sulci, a smooth gray-white border and little infolding [Cushion et al., 2013; Mutch et al., 2015; Oegema et al., 2015]. We will refer to this pattern, as suggested by Mutch et al, as *tubulinopathy-related dysgyria*.

The presence or absence of cerebellar hypoplasia (usually diffuse or vermis predominant), as the most frequent of the non-cortical malformations seen with LIS-SBH, is the final new criterion used to separate different forms of LIS. Small cerebellar vermis and in particular small and dysplastic anterior vermis has recently been described as a consistent feature in patients with tubulin and MAP gene mutations [Mutch et al., 2015]. Other non-cortical malformations such as basal ganglia dysgenesis, agenesis of the corpus callosum, enlarged tectum and brainstem hypoplasia were frequently associated with cerebellar hypoplasia, but rarely seen without cerebellar hypoplasia.

Clinical application of the new LIS-SBH classification system

Brain imaging—Data regarding LIS-SBH patterns seen in the Seattle and Florence cohorts, and the causative genes associated with each pattern are summarized in Table II. This table was designed for clinicians to take the LIS-SBH imaging pattern and reliably predict the most likely causative genes. However, the same imaging patterns are not as useful in predicting clinical severity, such as the intellectual disability and epilepsy.

Clinical severity—The LIS-SBH severity grade generally correlates with clinical outcome, with severe LIS (agyria) more severe than intermediate LIS (pachygyria), and both more severe than SBH. But when other imaging criteria are added, correlation with the clinical severity becomes less consistent, making clinical management more difficult. To address this need, we have correlated the major imaging patterns with the typical developmental outcome based on published reports and our extensive clinical experience, and adapted this to fit with the new classification scheme (Table III). Each of three clinical severity grades – mild (1), moderate (2) and severe (3) – includes three to four morphological subtypes that have a different impact on the neurological status and mental development. Exceptions may occur with these subtypes, most frequently with severe epilepsy, which often lowers overall function.

Microlissencephaly (MLIS)

We first classified patients with severe congenital microcephaly (defined as birth OFC more than 3 SD below the mean, or when not available earliest OFC more than 4 SD below the mean) and LIS – designated as “microlissencephaly” (MLIS) – separately as the phenotypes were always very severe and brain imaging different from thick LIS [Ashwal et al., 2009; Barkovich et al., 1998; Dobyns and Barkovich 1999]. The Dobyns cohort included only 5 patients with MLIS (5/129, 3.9%), although our entire database includes many more (61/1422, 4%). The first and most common type consists of MLIS with cerebellar hypoplasia. Several patients have had mutations of tubulin or related microtubule associated genes, such as TUBA1A and NDE1, so this group was combined with the tubulinopathy

subgroup of lissencephaly with cerebellar hypoplasia [Alkuraya et al., 2011; Paciorkowski et al., 2013] [Bahi-Buisson et al., 2014].

Several other rare MLIS syndromes are represented in our complete cohort, but not in the smaller 5-year cohorts. The best examples include MLIS with primordial dwarfism [Juric-Sekhar et al., 2011; Klinge et al., 2002], and the rare Barth MLIS syndrome [Barth et al., 1982; Klinge et al., 2002]. We intentionally excluded other forms of severe congenital microcephaly associated with other malformations of cortical development, primarily polymicrogyria, as seen for example in patients with biallelic mutations in *WDR62* or *KATNB1*.

DISCUSSION

The first classification system for LIS and SBH was developed after the first two genetic causes of LIS were identified, and considered only LIS with a thick (10–20 mm) cortex, and smooth cortical surface and gray-white border [Dobyns et al., 1999b; Kato and Dobyns 2003; Pilz et al., 1998]. Later reports described several variant types of LIS associated with mildly thick cortex or with non-cortical malformations such as cerebellar hypoplasia, but these were not systematically integrated into the classification system. Based on detailed reviews of 188 patients with LIS-SBH and archival data on another ~1,400 patients, we have revised the system to take advantage of two additional imaging features, especially cortical thickness and non-cortical malformations, and advances in molecular genetics to separate the cohort into (so far) 21 brain imaging-driven patterns (Table II). This system also corresponds well to the neuropathological features.

Lissencephaly patterns

Common patterns—As expected, a large majority of patients (147/188, 78%) had classic (thick) LIS corresponding to several now well-known patterns and syndromes. The single most common pattern (55/188, 29.2%) was partial agyria-pachygyria that was most severe posteriorly, almost exclusively caused by severe mutations of *LIS1* including whole gene and intragenic deletions, as well as truncating point mutations. We also confirmed that the *TUBA1A*:p.R402C mutation is a rare cause of this common pattern [Kumar et al., 2010]. The next most common patterns were posterior predominant pachygyria (44/183, 24%; this group includes some with posterior mild pachygyria and a few with posterior normal gyral pattern), tubulinopathy-related dysgyria (21/188, 11%), and diffuse agyria with or without cerebellar hypoplasia (23/188, 12.2%). Anterior predominant LIS is much less frequent than posterior predominant LIS. The cumulative frequency of all anterior types was much lower (23/188, 12.2%) than posterior types even excluding TUB-dysgyria [de Rijk-van Andel et al., 1990; Dobyns et al., 1999b; Haverfield et al., 2009].

Rare patterns—Most patients left unclassified by the old system fell into one of two groups characterized by either thin LIS (i.e. only mildly thick cortex) or LIS with severe congenital microcephaly defined as head circumference below –3 standard deviations at birth, or below –4 standard deviations postnatally [Dobyns 2002]. Our new expanded classification subdivides **thin LIS** (defined as between 5 and 10 mm thickness) into a very severe phenotype consisting of thin LIS with diffuse agyria always accompanied by CBLH,

and a relatively mild phenotype with thin undulating pachygyria. The latter is further divided into anterior predominant thin LIS with severe cerebellar hypoplasia, anterior predominant thin LIS with normal cerebellum, and temporal predominant thin LIS with agenesis of the corpus callosum (ACC) and abnormal white matter (Figure 4).

Microlissencephaly includes a heterogeneous group of rare syndromes with very variable features. MLIS with cerebellar hypoplasia and other non-cortical malformations represents the most severe end of the tubulinopathy spectrum. Among those tested, several have had mutations of *TUBA1A* or *TUBB2B* [Fallet-Bianco et al., 2014]. Biallelic *NDE1* mutations cause severe congenital microcephaly with cortical malformation resembling a tubulinopathy-associated dysgyria, although the cortex is not completely agyric. This specific phenotype may be best described as “microdysgyria” [Paciorkowski et al., 2013]. The same may be true for biallelic mutations of *RNU4ATAC* [Abdel-Salam et al., 2013].

The next group includes a few patients with microcephalic osteodysplastic primordial dwarfism type 1 (MOPD1), a syndrome that we view as heterogeneous. The frequency of MLIS in patients with MOPD1 is not known, but several reports describe anterior-predominant LIS with thin 7–8 mm cortex and ACC [Abdel-Salam et al., 2013; Juric-Sekhar et al., 2011; Klinge et al., 2002; Meinecke and Passarge 1991; Ozawa et al., 2005]. Similarly, at least one patient with Meier-Gorlin syndrome and mutation of *ORC1* had a cortical malformation [Bicknell et al., 2011], although normal development is seen in most patients.

Finally, a few reports show an even more severe form of MLIS consisting of diffuse agyria with 4–8 mm cortex, enlarged extra-axial space surrounding the cerebrum, and severe brainstem and cerebellar hypoplasia, which we designate as the Barth microlissencephaly syndrome [Barth et al., 1982; Kroon et al., 1996; Miyata et al., 2004]. While very rare, we have reviewed data on another 9 patients from our entire database. This is a provisional classification for MLIS that may need further subdivisions as more knowledge is gained.

Neuropathology

The neuropathological features of classic or thick LIS are well known, and include a thick and poorly organized 4-layered cortex with (1) molecular, (2) superficial cellular (pyramidal cell), (3) cell sparse, and (4) deep cellular layers [Crome 1956; Jellinger and Rett 1976]. Several descriptions of the brain in Miller-Dieker syndrome due to deletion 17p13.3 and LIS1 match this pattern [Miller 1963; Sasaki et al., 2012]. However, pathological descriptions are available for only ~8 of 19 LIS-associated genes, and only two reports provide detailed descriptions of LIS in a series of patients with known genetic causes [Fallet-Bianco et al., 2014; Forman et al., 2005]. Data from these and other reports of single subjects or small series are summarized in Table II [Fallet-Bianco et al., 2014; Forman et al., 2005; Magen et al., 2015]. No pathological reports are available for LIS associated with mutations of *CRADD*, *DYNC1H1*, *KIF5C*, *KIF2A*, *NDE1*, *RNU4ATAC*, *RELN* or *VLDLR*.

Analysis of the available data suggests a spectrum of cortical malformations associated with mutations of tubulins and tubulin-associated proteins including *TUBA1A*, other tubulins and *LIS1* that includes 2-layered LIS, 4-layered LIS with a posterior gradient, and

tubulinopathy-associated dysgyria. Specifically, brains from patients with severe mutations of *TUBA1A* and less often *TUBB2B* have 2-layered LIS [Fallet-Bianco et al., 2014]. Brains from patients with mutations of *LIS1* have 4-layered thick LIS with a posterior more severe than anterior gradient, relatively thick superficial cellular layer (layer 2) and blurred cortical-white matter margin, designated LIS-4LP [Forman et al., 2005]. Reports of the brain with thick LIS due to *TUBA1A* mutations are likely the same [Fallet-Bianco et al., 2014]. Brains from patients with less severe mutations of *TUBA1A* or *TUBB2B*, or most mutations of *TUBB* and *TUBB3* show distinct features intermediate between thick LIS and polymicrogyria, which we designate tubulinopathy-associated dysgyria [Fallet-Bianco et al., 2014]. The neuropathological and imaging features in these three conditions overlap, suggesting a spectrum related to centrosomal and microtubule dysfunction.

Remarkably, the neuropathological features of thick LIS associated with *DCX* mutations are different consisting of thick 4-layered LIS with an anterior more severe than posterior gradient, and a superficial cellular layer (layer 2) that appears relatively thin compared to *LIS1* brains, and contains only a few pyramidal neurons. At the cortical-white matter margin, the cortex transitions to multiple small nodules of subcortical heterotopia that differ markedly from *LIS1* brains, a pattern designated LIS-4LA [Forman et al., 2005]. The same LIS-4LA pattern was seen in patient 1 in this report (a.k.a. patient LP90-050), a girl with Baraitser-Winter cerebrofrontofacial syndrome later shown to have a mutation in *ACTB* [Forman et al., 2005; Riviere et al., 2012]. The shared pathological pattern supports our classification of *DCX*-associated and *ACTB*-associated LIS in the same group.

Genotype-phenotype analysis—Use of the new criteria separated patients into 21 groups with robust **genotype-phenotype correlations** as shown in Table II. While a few genes are seen in multiple groups, several correlations are obvious:

1. Almost all LIS with a posterior gradient are associated with mutations in *LIS1* or tubulin genes including *TUBG1*.
2. Mutations of *DCX* and actin isoforms (*ACTB*, *ACTG1*) account for almost all anterior predominant LIS with thick cortex (classic LIS).
3. The only genes associated with both anterior- and posterior-predominant LIS were microtubule-associated motor proteins (*DYNC1H1* and *KIF5C*).
4. The most severe type of LIS, diffuse agyria, has been associated with mutations of only five genes: *DCX* (almost exclusively in males), *LIS1* (most often *LIS1* plus *YWHAE* deletion), *TUBA1A*, *TUBB2B* (rare), and *CDK5* (based on a single homozygous mutation).
5. The large group of 55 patients with posterior thick (classic) LIS can be separated into mixed agyria-pachygyria (N=37) and complete or partial pachygyria (N=18). The former was seen almost exclusively with *LIS1* deletions or mutations plus the *TUBA1A* p.R402C mutation, while the latter was associated with several tubulin or tubulin associated genes (*LIS1*, *DYNC1H1*, *TUBA1A*, *TUBG1*). Patients with the *TUBA1A* p.R402H mutation had diffuse agyria with mild cerebellar vermis hypoplasia [Kumar et al., 2010]. A few other mutations in

TUBA1A or TUBB2B may be associated with thick posterior-predominant pachygyria based on fetal pathology [Fallet-Bianco 2014 PMID 25059107].

6. Finally, thin LIS demonstrated the strongest genotype-phenotype correlation. Thin LIS with diffuse agyria and CBLH was associated with mutations in *TUBA1A*, *TUBB2B*, and a very recently reported single mutation in *CDK5* [Magen et al., 2015]. Anterior predominant thin LIS with severe CBLH is an autosomal recessive condition and strongly associated with mutations in *RELN* and *VLDLR*. In contrast, biallelic *RELN* and *VLDLR* mutations have never been seen in patients with anterior thin LIS and normal cerebellum. Mutations of a single gene – *CRADD* – have been found in the latter group. An additional diagnostic clue for *CRADD* mutations is mild megalencephaly [Di Donato et al., 2016a]. Lastly, temporal-predominant thin LIS with ACC and abnormal white matter is caused by mutations in *ARX*. This is one of the few syndromic forms of LIS, causing X-linked LIS with abnormal genitalia or XLAG [Berry-Kravis and Israel 1994; Dobyns et al., 1999a; Kato et al., 2004; Kitamura et al., 2002].

Non-cortical malformations—As previously reported, almost all patients with mutations of tubulin (excluding the *TUBA1A* p.R402C and p.R402H mutations) or tubulin motor genes had multiple non-cortical malformations including basal ganglia dysplasia, partial agenesis of the corpus callosum, enlarged tectum, brainstem hypoplasia, and cerebellar hypoplasia. Patients with mutations of *RELN* or *VLDLR* had severe cerebellar and, usually, hippocampal hypoplasia and dysplasia, and patients with mutations of *ARX* had severe, usually complete agenesis of the corpus callosum often associated with hypoplastic basal ganglia.

Subcortical band heterotopia

We perform a separate analysis of SBH as the brain imaging appearance is different from all types of LIS (Figure 5), and the genetic basis differs as well. We found similar correlations, except that only two causative genes known to date. Anterior predominant partial SBH with typically thin bands is caused by mutations of *DCX* in essentially all patients. Diffuse thick and thin SBH are also caused by mutations in *DCX*, although at least 20% have negative testing for *DCX* and remain unexplained. Posterior partial SBH has been associated with mutations of *LIS1* in about 30% of patients, with predominantly mosaic mutations. Short SBH are sometimes seen between regions of frontal pachygyria and normal posterior cortex in patients with BWCFF syndrome due to mutations of *ACTB* or *ACTG1* [Di Donato et al., 2016b].

Clinical severity

To supplement our expanded imaging classification, we have correlated the more common imaging patterns with typical clinical outcome (Table III). The major medical problems influencing the developmental prognosis and lowering the life expectancy are feeding difficulties that include gastro-esophageal reflux and aspiration, epilepsy of many different types, and pneumonia. 35–85% of children with classic (thick) LIS develop infantile spasms [Guerrini and Dobyns 2014]. Seizures are often intractable and many patients have the

classic electrophysiological signs of Lennox-Gastaut syndrome. Cognitive development often slows with the onset of seizures [Guerrini and Dobyns 2014].

Differences in cohorts

Overall, we found similar frequencies of different types of LIS and SBH between the Seattle and Florence cohorts (Table II). The small differences include a higher frequency of more severe subtypes, such as diffuse agyria with cerebellar hypoplasia and MLIS, in the Seattle cohort, and a higher frequency of less severe subtypes, such as partial and thin SBH, in the Florence cohort. These differences are most likely due to differences in ascertainment. About 80% of patients in the Seattle cohort were referred from other centers for review of an abnormal brain imaging study, leading to a referral bias of more severe malformations. In contrast, the Department of Pediatric Neurology at Meyer Children Hospital of Florence is a large referral center for European children with epilepsy. Therefore, the absence of patients with clinically more severe LIS in the Florence cohort is likely related to the early lethality of these patients, before they could be admitted for the specialized epilepsy care, and the severity of the associated neurodevelopmental disabilities. The Florence cohort also has fewer patients with thin undulating lissencephaly, probably because these patients have lower frequency of epilepsy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Abdel-Salam GM, Abdel-Hamid MS, Hassan NA, Issa MY, Effat L, Ismail S, Aglan MS, Zaki MS. Further delineation of the clinical spectrum in RNU4ATAC related microcephalic osteodysplastic primordial dwarfism type I. *Am J Med Genet.* 2013; A161A:1875–1881.
- Abdollahi MR, Morrison E, Sirey T, Molnar Z, Hayward BE, Carr IM, Springell K, Woods CG, Ahmed M, Hattin L, Corry P, Pilz DT, Stoodley N, Crow Y, Taylor GR, Bonthron DT, Sheridan E. Mutation of the variant alpha-tubulin TUBA8 results in polymicrogyria with optic nerve hypoplasia. *Am J Hum Genet.* 2009; 85:737–744. [PubMed: 19896110]
- Alkuraya FS, Cai X, Emery C, Mochida GH, Al-Dosari MS, Felie JM, Hill RS, Barry BJ, Partlow JN, Gascon GG, Kentab A, Jan M, Shaheen R, Feng Y, Walsh CA. Human mutations in NDE1 cause extreme microcephaly with lissencephaly [corrected]. *Am J Hum Genet.* 2011; 88:536–547. [PubMed: 21529751]
- Ashwal S, Michelson D, Plawner L, Dobyns WB. Quality Standards Subcommittee of the American Academy of N, the Practice Committee of the Child Neurology S. Practice parameter: Evaluation of the child with microcephaly (an evidence-based review): report of the Quality Standards

- Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. *Neurology*. 2009; 73:887–897. [PubMed: 19752457]
- Bahi-Buisson N, Poirier K, Fourniol F, Saillour Y, Valence S, Lebrun N, Hully M, Bianco CF, Boddaert N, Elie C, Lascelles K, Souville I, Beldjord C, Chelly J. The wide spectrum of tubulinopathies: what are the key features for the diagnosis? *Brain : a journal of neurology*. 2014; 137:1676–1700. [PubMed: 24860126]
- Barkovich AJ, Ferriero DM, Barr RM, Gressens P, Dobyns WB, Truwit CL, Evrard P. Microlissencephaly: a heterogeneous malformation of cortical development. *Neuropediatrics*. 1998; 29:113–119. [PubMed: 9706619]
- Barkovich AJ, Guerrini R, Kuzniecky RI, Jackson GD, Dobyns WB. A developmental and genetic classification for malformations of cortical development: update 2012. *Brain*. 2012; 135:1348–1369. [PubMed: 22427329]
- Barth PG, Mullaart R, Stam FC, Slooff JL. Familial lissencephaly with extreme neopallial hypoplasia. *Brain Dev*. 1982; 4:145–151. [PubMed: 7091570]
- Berry-Kravis E, Israel J. X-linked pachygyria and agenesis of the corpus callosum: evidence for an X chromosome lissencephaly locus. *Ann Neurol*. 1994; 36:229–233. [PubMed: 8053659]
- Bicknell LS, Bongers EM, Leitch A, Brown S, Schoots J, Harley ME, Aftimos S, Al-Aama JY, Bober M, Brown PA, van Bokhoven H, Dean J, Edrees AY, Feingold M, Fryer A, Hoefsloot LH, Kau N, Knoers NV, Mackenzie J, Opitz JM, Sarda P, Ross A, Temple IK, Toutain A, Wise CA, Wright M, Jackson AP. Mutations in the pre-replication complex cause Meier-Gorlin syndrome. *Nat Genet*. 2011; 43:356–359. [PubMed: 21358632]
- Boycott KM, Bonnemann C, Herz J, Neuert S, Beaulieu C, Scott JN, Venkatasubramanian A, Parboosingh JS. Mutations in VLDLR as a cause for autosomal recessive cerebellar ataxia with mental retardation (dysequilibrium syndrome). *J Child Neurol*. 2009; 24:1310–1315. [PubMed: 19332571]
- Breuss M, Heng JI, Poirier K, Tian G, Jaglin XH, Qu Z, Braun A, Gstrein T, Ngo L, Haas M, Bahi-Buisson N, Moutard ML, Passemard S, Verloes A, Gressens P, Xie Y, Robson KJ, Rani DS, Thangaraj K, Clausen T, Chelly J, Cowan NJ, Keays DA. Mutations in the beta-tubulin gene TUBB5 cause microcephaly with structural brain abnormalities. *Cell Rep*. 2012; 2:1554–1562. [PubMed: 23246003]
- Brun BN, Mockler SR, Laubscher KM, Stephan CM, Wallace AM, Collison JA, Zimmerman MB, Dobyns WB, Mathews KD. Comparison of brain MRI findings with language and motor function in the dystroglycanopathies. *Neurology*. 2017; 88:623–629. [PubMed: 28087826]
- Cardoso C, Leventer RJ, Matsumoto N, Kuc JA, Ramocki MB, Mewborn SK, Dudlicek LL, May LF, Mills PL, Das S, Pilz DT, Dobyns WB, Ledbetter DH. The location and type of mutation predict malformation severity in isolated lissencephaly caused by abnormalities within the LIS1 gene. *Hum Mol Genet*. 2000; 9:3019–3028. [PubMed: 11115846]
- Cavallin M, Hubert L, Cantagrel V, Munnich A, Boddaert N, Vincent-Delorme C, Cuvellier JC, Masson C, Besmond C, Bahi-Buisson N. Recurrent KIF5C mutation leading to frontal pachygyria without microcephaly. *Neurogenetics*. 2015
- Crome L. Pachygyria. *J Pathol Bacteriol*. 1956; 71:335–352. [PubMed: 13398879]
- Cushion TD, Dobyns WB, Mullins JG, Stoodley N, Chung SK, Fry AE, Hehr U, Gunny R, Aylsworth AS, Prabhakar P, Uyanik G, Rankin J, Rees MI, Pilz DT. Overlapping cortical malformations and mutations in TUBB2B and TUBA1A. *Brain : a journal of neurology*. 2013; 136:536–548. [PubMed: 23361065]
- D'Agostino MD, Bastos A, Piras C, Bernasconi A, Grisar T, Tsur VG, Snipes J, Juhasz C, Chugani H, Guerrini R, Cross H, Andermann E, Dubeau F, Montes J, Olivier A, Andermann F. Posterior quadrant dysplasia or hemi-hemimegalencephaly: a characteristic brain malformation. *Neurology*. 2004; 62:2214–2220. [PubMed: 15210885]
- de Rijk-van Andel JF, Arts WF, Barth PG, Loonen MC. Diagnostic features and clinical signs of 21 patients with lissencephaly type 1. *Dev Med Child Neurol*. 1990; 32:707–717. [PubMed: 2210085]
- des Portes V, Francis F, Pinard JM, Desguerre I, Moutard ML, Snoeck I, Meiners LC, Capron F, Cusmai R, Ricci S, Motte J, Echenne B, Ponsot G, Dulac O, Chelly J, Beldjord C. doublecortin is

- the major gene causing X-linked subcortical laminar heterotopia (SCLH). *Hum Mol Genet.* 1998; 7:1063–1070. [PubMed: 9618162]
- Devisme L, Bouchet C, Gonzales M, Alanio E, Bazin A, Bessieres B, Bigi N, Blanchet P, Bonneau D, Bonnieres M, Bucourt M, Carles D, Clarisse B, Delahaye S, Fallet-Bianco C, Figarella-Branger D, Gaillard D, Gasser B, Delezoide AL, Guimiot F, Joubert M, Laurent N, Laquerriere A, Liprandi A, Loget P, Marcorelles P, Martinovic J, Menez F, Patrier S, Pelluard F, Perez MJ, Rouleau C, Triau S, Attie-Bitach T, Vuillaumier-Barrot S, Seta N, Encha-Razavi F. Cobblestone lissencephaly: neuropathological subtypes and correlations with genes of dystroglycanopathies. *Brain.* 2012; 135:469–482. [PubMed: 22323514]
- Di Donato N, Jean YY, Maga AM, Krewson BD, Shupp AB, Avrutsky MI, Roy A, Collins S, Olds C, Willert RA, Czaja AM, Johnson R, Stover JA, Gottlieb S, Bartholdi D, Rauch A, Goldstein A, Boyd-Kyle V, Aldinger KA, Mirzaa GM, Nissen A, Brigatti KW, Puffenberger EG, Millen KJ, Strauss KA, Dobyns WB, Troy CM, Jinks RN. Mutations in CRADD Result in Reduced Caspase-2-Mediated Neuronal Apoptosis and Cause Megalencephaly with a Rare Lissencephaly Variant. *Am J Hum Genet.* 2016a; 99:1117–1129. [PubMed: 27773430]
- Di Donato N, Kuechler A, Vergano S, Heinritz W, Bodurtha J, Merchant SR, Brenningstall G, Ladda R, Sell S, Altmuller J, Bogershausen N, Timms AE, Hackmann K, Schrock E, Collins S, Olds C, Rump A, Dobyns WB. Update on the ACTG1-associated Baraitser-Winter cerebrofrontofacial syndrome. *Am J Med Genet.* 2016b; A170:2644–2651.
- Dobyns WB. Primary microcephaly: new approaches for an old disorder. *Am J Med Genet.* 2002; 112:315–317. [PubMed: 12376930]
- Dobyns WB. The clinical patterns and molecular genetics of lissencephaly and subcortical band heterotopia. *Epilepsia.* 2010; 51(Suppl 1):5–9. [PubMed: 20331703]
- Dobyns WB, Barkovich AJ. Microcephaly with Simplified Gyral Pattern (Oligogyric Microcephaly) and Microlissencephaly: Reply. *Neuropediatrics.* 1999; 30:104–106.
- Dobyns WB, Berry-Kravis E, Havernick NJ, Holden KR, Viskochil D. X-linked lissencephaly with absent corpus callosum and ambiguous genitalia. *Am J Med Genet.* 1999a; 86:331–337. [PubMed: 10494089]
- Dobyns WB, Curry CJ, Hoyme HE, Turlington L, Ledbetter DH. Clinical and molecular diagnosis of Miller-Dieker syndrome. *Am J Hum Genet.* 1991; 48:584–594. [PubMed: 1671808]
- Dobyns WB, Elias ER, Newlin AC, Pagon RA, Ledbetter DH. Causal heterogeneity in isolated lissencephaly. *Neurology.* 1992; 42:1375–1388. [PubMed: 1620349]
- Dobyns WB., Guerrini R., Leventer, RJ. Malformations of Cortical Development. In: Swaiman, KF.Ashwal, S.Ferriero, DM., Schor, NF., editors. *Swaiman's Pediatric Neurology: Principles and Practice.* 5. Edinburgh: Elsevier Saunders; 2012. p. 202-231.
- Dobyns WB, Stratton RF, Parke JT, Greenberg F, Nussbaum RL, Ledbetter DH. Miller-Dieker syndrome: lissencephaly and monosomy 17p. *J Pediatr.* 1983; 102:552–558. [PubMed: 6834189]
- Dobyns WB, Truwit CL, Ross ME, Matsumoto N, Pilz DT, Ledbetter DH, Gleeson JG, Walsh CA, Barkovich AJ. Differences in the gyral pattern distinguish chromosome 17-linked and X-linked lissencephaly. *Neurology.* 1999b; 53:270–277. [PubMed: 10430413]
- Fallet-Bianco C, Laquerriere A, Poirier K, Razavi F, Guimiot F, Dias P, Loeuillet L, Lascelles K, Beldjord C, Carion N, Toussaint A, Revencu N, Addor MC, Lhermitte B, Gonzales M, Martinovich J, Bessieres B, Marcy-Bonniere M, Jossic F, Marcorelles P, Loget P, Chelly J, Bahi-Buisson N. Mutations in tubulin genes are frequent causes of various foetal malformations of cortical development including microlissencephaly. *Acta Neuropathol Commun.* 2014; 2:69. [PubMed: 25059107]
- Forman MS, Squier W, Dobyns WB, Golden JA. Genotypically defined lissencephalies show distinct pathologies. *J Neuropathol Exp Neurol.* 2005; 64:847–857. [PubMed: 16215456]
- Gleeson JG, Allen KM, Fox JW, Lamperti ED, Berkovic S, Scheffer I, Cooper EC, Dobyns WB, Minnerath SR, Ross ME, Walsh CA. Doublecortin, a brain-specific gene mutated in human X-linked lissencephaly and double cortex syndrome, encodes a putative signaling protein. *Cell.* 1998; 92:63–72. [PubMed: 9489700]
- Guerrini R, Dobyns WB. Malformations of cortical development: clinical features and genetic causes. *Lancet Neurol.* 2014; doi: 10.1016/S1474-4422(1014)70040-70047

- Guerrini R, Filippi T. Neuronal migration disorders, genetics, and epileptogenesis. *J Child Neurol*. 2005; 20:287–299. [PubMed: 15921228]
- Haverfield EV, Whited AJ, Petras KS, Dobyns WB, Das S. Intragenic deletions and duplications of the LIS1 and DCX genes: a major disease-causing mechanism in lissencephaly and subcortical band heterotopia. *Eur J Hum Genet*. 2009; 17:911–918. [PubMed: 19050731]
- Hong SE, Shugart YY, Huang DT, Shahwan SA, Grant PE, Hourihane JO, Martin ND, Walsh CA. Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. *Nat Genet*. 2000; 26:93–96. [PubMed: 10973257]
- Jaglin XH, Poirier K, Saillour Y, Buhler E, Tian G, Bahi-Buisson N, Fallet-Bianco C, Phan-Dinh-Tuy F, Kong XP, Bomont P, Castelnau-Ptakhine L, Odent S, Loget P, Kossorotoff M, Snoeck I, Plessis G, Parent P, Beldjord C, Cardoso C, Represa A, Flint J, Keays DA, Cowan NJ, Chelly J. Mutations in the beta-tubulin gene TUBB2B result in asymmetrical polymicrogyria. *Nat Genet*. 2009; 41:746–752. [PubMed: 19465910]
- Jamuar SS, Lam AT, Kircher M, D’Gama AM, Wang J, Barry BJ, Zhang X, Hill RS, Partlow JN, Rozzo A, Servattalab S, Mehta BK, Topcu M, Amrom D, Andermann E, Dan B, Parrini E, Guerrini R, Scheffer IE, Berkovic SF, Leventer RJ, Shen Y, Wu BL, Barkovich AJ, Sahin M, Chang BS, Bamshad M, Nickerson DA, Shendure J, Poduri A, Yu TW, Walsh CA. Somatic Mutations in Cerebral Cortical Malformations. *N Engl J Med*. 2014; 371:733–743. [PubMed: 25140959]
- Jellinger K, Rett A. Agyria-pachygyria (lissencephaly syndrome). *Neuropadiatrie*. 1976; 7:66–91. [PubMed: 988918]
- Jissendi-Tchofo P, Kara S, Barkovich AJ. Midbrain-hindbrain involvement in lissencephalies. *Neurology*. 2009; 72:410–418. [PubMed: 19020296]
- Juric-Sekhar G, Kapur RP, Glass IA, Murray ML, Parnell SE, Hevner RF. Neuronal migration disorders in microcephalic osteodysplastic primordial dwarfism type I/III. *Acta Neuropathol*. 2011; 121:545–554. [PubMed: 20857301]
- Kato M, Das S, Petras K, Kitamura K, Morohashi K, Abuelo DN, Barr M, Bonneau D, Brady AF, Carpenter NJ, Cipero KL, Frisone F, Fukuda T, Guerrini R, Iida E, Itoh M, Lewanda AF, Nanba Y, Oka A, Proud VK, Saugier-Verber P, Schelley SL, Selicorni A, Shaner R, Silengo M, Stewart F, Sugiyama N, Toyama J, Toutain A, Vargas AL, Yanazawa M, Zackai EH, Dobyns WB. Mutations of ARX are associated with striking pleiotropy and consistent genotype-phenotype correlation. *Hum Mutat*. 2004; 23:147–159. [PubMed: 14722918]
- Kato M, Dobyns WB. Lissencephaly and the molecular basis of neuronal migration. *Human molecular genetics*. 2003; 12(Spec No 1):R89–96. [PubMed: 12668601]
- Keays DA, Tian G, Poirier K, Huang GJ, Siebold C, Cleak J, Oliver PL, Fray M, Harvey RJ, Molnar Z, Pinon MC, Dear N, Valdar W, Brown SD, Davies KE, Rawlins JN, Cowan NJ, Nolan P, Chelly J, Flint J. Mutations in alpha-tubulin cause abnormal neuronal migration in mice and lissencephaly in humans. *Cell*. 2007; 128:45–57. [PubMed: 17218254]
- Kitamura K, Yanazawa M, Sugiyama N, Miura H, Iizuka-Kogo A, Kusaka M, Omichi K, Suzuki R, Kato-Fukui Y, Kamiirisa K, Matsuo M, Kamijo S, Kasahara M, Yoshioka H, Ogata T, Fukuda T, Kondo I, Kato M, Dobyns WB, Yokoyama M, Morohashi K. Mutation of ARX causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. *Nat Genet*. 2002; 32:359–369. [PubMed: 12379852]
- Klinge L, Schaper J, Wiczorek D, Voit T. Microlyssencephaly in microcephalic osteodysplastic primordial dwarfism: a case report and review of the literature. *Neuropediatrics*. 2002; 33:309–313. [PubMed: 12571786]
- Kloss S, Pieper T, Pannek H, Holthausen H, Tuxhorn I. Epilepsy surgery in children with focal cortical dysplasia (FCD): results of long-term seizure outcome. *Neuropediatrics*. 2002; 33:21–26. [PubMed: 11930272]
- Kroon AA, Smit BJ, Barth PG, Hennekam RC. Lissencephaly with extreme cerebral and cerebellar hypoplasia. A magnetic resonance imaging study. *Neuropediatrics*. 1996; 27:273–276. [PubMed: 8971750]
- Kumar RA, Pilz DT, Babatz TD, Cushion TD, Harvey K, Topf M, Yates L, Robb S, Uyanik G, Mancini GM, Rees MI, Harvey RJ, Dobyns WB. TUBA1A mutations cause wide spectrum

- lissencephaly (smooth brain) and suggest that multiple neuronal migration pathways converge on alpha tubulins. *Human molecular genetics*. 2010; 19:2817–2827. [PubMed: 20466733]
- Leventer RJ, Cardoso C, Ledbetter DH, Dobyns WB. LIS1 missense mutations cause milder lissencephaly phenotypes including a child with normal IQ. *Neurology*. 2001; 57:416–422. [PubMed: 11502906]
- Leventer RJ, Pilz DT, Matsumoto N, Ledbetter DH, Dobyns WB. Lissencephaly and subcortical band heterotopia: molecular basis and diagnosis. *Mol Med Today*. 2000; 6:277–284. [PubMed: 10859564]
- Magen D, Ofir A, Berger L, Goldsher D, Eran A, Katib N, Nijem Y, Vlodavsky E, Tzur S, Behar DM, Fellig Y, Mandel H. Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with a loss-of-function mutation in CDK5. *Hum Genet*. 2015; 134:305–314. [PubMed: 25560765]
- Matsumoto N, Leventer RJ, Kuc JA, Mewborn SK, Dudliceck LL, Ramocki MB, Pilz DT, Mills PL, Das S, Ross ME, Ledbetter DH, Dobyns WB. Mutation analysis of the DCX gene and genotype/phenotype correlation in subcortical band heterotopia. *Eur J Hum Genet*. 2001; 9:5–12. [PubMed: 11175293]
- Meinecke P, Passarge E. Microcephalic osteodysplastic primordial dwarfism type I/III in sibs. *J Med Genet*. 1991; 28:795–800. [PubMed: 1770539]
- Miller JQ. Lissencephaly in 2 Siblings. *Neurology*. 1963; 13:841–850. [PubMed: 14066999]
- Miyata H, Chute DJ, Fink J, Villablanca P, Vinters HV. Lissencephaly with agenesis of corpus callosum and rudimentary dysplastic cerebellum: a subtype of lissencephaly with cerebellar hypoplasia. *Acta Neuropathol*. 2004; 107:69–81. [PubMed: 14566414]
- Mutch CA, Poduri A, Sahin M, Barry B, Walsh CA, Barkovich AJ. Disorders of Microtubule Function in Neurons: Imaging Correlates. *AJNR Am J Neuroradiol*. 2015
- Oegema R, Cushion TD, Phelps IG, Chung SK, Dempsey JC, Collins S, Mullins JG, Dudding T, Gill H, Green AJ, Dobyns WB, Ishak GE, Rees MI, Doherty D. Recognizable cerebellar dysplasia associated with mutations in multiple tubulin genes. *Hum Mol Genet*. 2015
- Ozawa H, Takayama C, Nishida A, Nagai T, Nishimura G, Higurashi M. Pachygyria in a girl with microcephalic osteodysplastic primordial short stature type II. *Brain Dev*. 2005; 27:237–240. [PubMed: 15737708]
- Paciorkowski AR, Keppler-Noreuil K, Robinson L, Sullivan C, Sajan S, Christian SL, Bukshpun P, Gabriel SB, Gleeson JG, Sherr EH, Dobyns WB. Deletion 16p13.11 uncovers NDE1 mutations on the non-deleted homolog and extends the spectrum of severe microcephaly to include fetal brain disruption. *Am J Med Genet*. 2013; A161A:1523–1530.
- Pilz DT, Matsumoto N, Minnerath S, Mills P, Gleeson JG, Allen KM, Walsh CA, Barkovich AJ, Dobyns WB, Ledbetter DH, Ross ME. LIS1 and XLIS (DCX) mutations cause most classical lissencephaly, but different patterns of malformation. *Hum Mol Genet*. 1998; 7:2029–2037. [PubMed: 9817918]
- Poirier K, Lebrun N, Broix L, Tian G, Saillour Y, Boscheron C, Parrini E, Valence S, Pierre BS, Oger M, Lacombe D, Genevieve D, Fontana E, Darra F, Cances C, Barth M, Bonneau D, Bernadina BD, N’Guyen S, Gitiaux C, Parent P, des Portes V, Pedespan JM, Legrez V, Castelnau-Ptakine L, Nitschke P, Hieu T, Masson C, Zelenika D, Andrieux A, Francis F, Guerrini R, Cowan NJ, Bahi-Buisson N, Chelly J. Mutations in TUBG1, DYNC1H1, KIF5C and KIF2A cause malformations of cortical development and microcephaly. *Nat Genet*. 2013a; 45:639–647. [PubMed: 23603762]
- Poirier K, Lebrun N, Broix L, Tian G, Saillour Y, Boscheron C, Parrini E, Valence S, Pierre BS, Oger M, Lacombe D, Genevieve D, Fontana E, Darra F, Cances C, Barth M, Bonneau D, Bernadina BD, N’Guyen S, Gitiaux C, Parent P, des Portes V, Pedespan JM, Legrez V, Castelnau-Ptakine L, Nitschke P, Hieu T, Masson C, Zelenika D, Andrieux A, Francis F, Guerrini R, Cowan NJ, Bahi-Buisson N, Chelly J. Mutations in TUBG1, DYNC1H1, KIF5C and KIF2A cause malformations of cortical development and microcephaly. *Nat Genet*. 2013b
- Poirier K, Saillour Y, Bahi-Buisson N, Jaglin XH, Fallet-Bianco C, Nabbout R, Castelnau-Ptakine L, Roubertie A, Attie-Bitach T, Desguerre I, Genevieve D, Barnerias C, Keren B, Lebrun N, Boddaert N, Encha-Razavi F, Chelly J. Mutations in the neuronal α -tubulin subunit TUBB3 result in malformation of cortical development and neuronal migration defects. *Hum Mol Genet*. 2010; 19:4462–4473. [PubMed: 20829227]

- Ramer JC, Lin AE, Dobyns WB, Winter R, Ayme S, Pallotta R, Ladda RL. Previously apparently undescribed syndrome: shallow orbits, ptosis, coloboma, trigonocephaly, gyral malformations, and mental and growth retardation. *Am J Med Genet.* 1995; 57:403–409. [PubMed: 7545868]
- Reiner O, Carrozzo R, Shen Y, Wehnert M, Faustinella F, Dobyns WB, Caskey CT, Ledbetter DH. Isolation of a Miller-Dieker lissencephaly gene containing G protein beta-subunit-like repeats. *Nature.* 1993; 364:717–721. [PubMed: 8355785]
- Riviere JB, van Bon BW, Hoischen A, Kholmanskikh SS, O’Roak BJ, Gilissen C, Gijsen S, Sullivan CT, Christian SL, Abdul-Rahman OA, Atkin JF, Chassaing N, Drouin-Garraud V, Fry AE, Fryns JP, Gripp KW, Kempers M, Kleefstra T, Mancini GM, Nowaczyk MJ, van Ravenswaaij-Arts CM, Roscioli T, Marble M, Rosenfeld JA, Siu VM, de Vries BB, Shendure J, Verloes A, Veltman JA, Brunner HG, Ross ME, Pilz DT, Dobyns WB. De novo mutations in the actin genes ACTB and ACTG1 cause Baraitser-Winter syndrome. *Nat Genet.* 2012; 44:440–444. S441–442. [PubMed: 22366783]
- Ross ME, Swanson K, Dobyns WB. Lissencephaly with cerebellar hypoplasia (LCH): a heterogeneous group of cortical malformations. *Neuropediatrics.* 2001; 32:256–263. [PubMed: 11748497]
- Sasaki A, Shioda K, Homma T, Fukatsu R, Koide H. A 14-year-old girl with lissencephaly and craniofacial dysmorphism. *Neuropathology.* 2012; 32:675–678. [PubMed: 22414170]
- Spencer-Smith M, Leventer R, Jacobs R, Luca CD, Anderson V. Neuropsychological profile of children with subcortical band heterotopia. *Dev Med Child Neurol.* 2009; 51:909–916. [PubMed: 19416314]
- Stratton RF, Dobyns WB, Airhart SD, Ledbetter DH. New chromosomal syndrome: Miller-Dieker syndrome and monosomy 17p13. *Hum Genet.* 1984; 67:193–200. [PubMed: 6745939]
- Willemsen MH, Ba W, Wissink-Lindhout WM, de Brouwer AP, Haas SA, Bienek M, Hu H, Vissers LE, van Bokhoven H, Kalscheuer V, Nadif Kasri N, Kleefstra T. Involvement of the kinesin family members KIF4A and KIF5C in intellectual disability and synaptic function. *J Med Genet.* 2014; 51:487–494. [PubMed: 24812067]
- Willemsen MH, Vissers LE, Willemsen MA, van Bon BW, Kroes T, de Ligt J, de Vries BB, Schoots J, Lugtenberg D, Hamel BC, van Bokhoven H, Brunner HG, Veltman JA, Kleefstra T. Mutations in DYNC1H1 cause severe intellectual disability with neuronal migration defects. *J Med Genet.* 2012; 49:179–183. [PubMed: 22368300]

Figure 1. Brain imaging patterns in four patients with lissencephaly (LIS) or LIS with cerebellar hypoplasia (LCH) showing diffuse forms

(A–C) thin LCH with diffuse agyria, thin cortex, absent corpus callosum, dysplastic basal ganglia, thin brainstem, thick tectum and severe cerebellar hypoplasia; (D–F) thick LCH with the same features except for a thick cortex; (G–I) diffuse agyria without other features as seen in Miller–Dieker syndrome; (J–L) severe microcephaly, diffuse agyria, thin cortex, absent corpus callosum, remarkably severe brainstem hypoplasia, possibly enlarged tectum, and severe hypoplasia or absent cerebellum as seen in Barth microlissencephaly syndrome. Black arrow point to an enlarged tectum (A, D), while white asterisks denote cerebellar hypoplasia (A, D) or absence (J). Different gyral patterns are shown for the right hemispheres only with triple white arrows marking agyria.

These images were selected from subjects LR12-176 (A–C), LR09-393 (D–F), LR12-311 (G–I), LR01-051 (J–L).

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Figure 2. Brain imaging patterns in patients with posterior predominant forms
 (A–C) Frontal pachygyria and posterior agyria as seen in most patients with LIS1 deletions or mutations; (D–F) complete pachygyria more severe posteriorly without other features; (G–I) complete pachygyria more severe posteriorly with short corpus callosum and mild cerebellar hypoplasia; and (J–L) diffuse tubulinopathy-associated dysgyria with absent corpus callosum, mildly dysplastic basal ganglia, thin dysplastic brainstem, moderate cerebellar hypoplasia, and mildly enlarged posterior fossa or “mega-cisterna magna” (mcm), white asterisk denotes cerebellar hypoplasia. Different gyral patterns are shown for the right hemispheres with agyria (triple white arrows), pachygyria (double white arrows) and dysgyria (single white arrows) marked.

These images were selected from subjects LR14-023 (A–C), LR12-381 (D–F), LR12-162 (G–I), and LR09-250 (J–L).

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Figure 3. Brain imaging patterns in three patients with lissencephaly (LIS) showing diffuse and anterior predominant forms

(A–C) Diffuse agyria and mild cerebellar vermis hypoplasia as seen with severe *DCX* mutations in males; (D–F) anterior pachygyria as seen with mild *DCX* mutations in males or with mutations of *ACTB* or *ACTG1*; (G–I) anterior pachygyria that transitions to posterior subcortical band heterotopia as seen with mutations of *ACTB* or *ACTG1*, rarely *DCX*; (J–L) equivalent normal images at 18 months.

White line points out agenesis of the corpus callosum (G), while white asterisk shows cerebellar hypoplasia (D). Different gyral patterns are shown for the right hemispheres only with agyria (triple white arrows) and pachygyria (double white arrows) marked.

These images were selected from subjects LR03-352 (A–C), LR00-092 (D–F), LR13-123 (G–I), LR13-006 (J–L),

Figure 4. Brain imaging patterns in three patients with rare lissencephaly forms

(A–C) anterior predominant “thin” LIS and severe nearly afoliar cerebellar hypoplasia as seen with *RELN* mutations; (D–F) rare anterior predominant “thin” LIS with no other features typical for *CRADD* mutations; (G–I) temporal and posterior predominant “thin” LIS with absent corpus callosum and dysplastic basal ganglia as seen with severe *ARX* mutations in males; and (J–L) equivalent normal images at 7 weeks.

White line denotes agenesis of the corpus callosum (G), while white asterisks denotes cerebellar hypoplasia (A). Different gyral patterns are shown for the right hemispheres only with “thin” LIS marked with long single white arrows.

These images were selected from subjects LR14-063 (A–C), LR13-378 (D–F), LR00-185 (G–I), AND LR16-043 (J–L).

Figure 5. Brain imaging patterns in four patients with subcortical band heterotopia (SBH) (A–C) diffuse thick, (D–F) diffuse thin, (G–I) frontal thin and (J–L) posterior thin bands. T1-weighted midline sagittal (A, D, G, J) images are normal, except for mild cerebellar vermis hypoplasia in the second row (D). Axial T2-weighted (B–C, H–I) or three-dimensional (E–F, K–L) images through low or high lateral ventricles show the bands (white arrows) as well as simplified gyri with shallow sulci and a thin layer of white matter between the cortex and bands. These images were selected from patients LR10-053 (A–C), LR01-042 (D–F), LR16-044 (G–I), and LR11-302 (J–L).

Table I

Imaging criteria used for LIS-SBH classification

<ul style="list-style-type: none"> • Gradient of gyral malformation <ul style="list-style-type: none"> – Diffuse – Anterior more severe than posterior (A>P) – Posterior more severe than anterior (P>A) – Temporal more severe than posterior and P>A • Grade of gyral malformation <ul style="list-style-type: none"> – SBH partial – SBH diffuse – LIS partial pachygyria – LIS diffuse pachygyria – LIS agyria-pachygyria – LIS diffuse agyria • Cortical thickness and appearance <ul style="list-style-type: none"> – Simplified gyration overlying SBH – Thin undulating – Thin variable dysgyria – Thin with enlarged lateral ventricles and thin mantle – Thick classic • Non-cortical brain malformations <ul style="list-style-type: none"> – Basal ganglia dysgenesis – Complete or partial agenesis (dysgenesis) of the corpus callosum – Tectal hyperplasia – Brainstem hypoplasia and dysgenesis – Cerebellar hypoplasia (either diffuse or vermis-predominate)

Table II

Frequencies and genetic causes of different lissencephaly types (N=188)

Lissencephaly types based on imaging	Gradient				Pathology ¹	Dobyns n=129	Guerrini n=59	Genes (in both cohorts)	Additional genes from the literature ²
	DIF	PA	AP	TL					
Lissencephaly with cerebellar hypoplasia , Agyria diffuse (thick or thin cortex)	+				2L	7 / 5.4%	0	TUBA1A, TUBB2B, CDK5	TUBA1A (p.R402H)
MLIS with cerebellar hypoplasia (TUB features)	++				2L *	3 / 2.3%	0	TUBA1A	TUBB2B, TUBB3, NDE1 ³
Classic thick lissencephaly									
Agyria diffuse	++				4L	8 / 6.2%	3 / 5%	LIS1-YWHAE, DCX	
Agyria-pachygyria p>a (mixed)		++			4LP	37 / 28.7%	18 / 31%	LIS1 TUBA1A (p.R402C)	
Pachygyria p>a (complete or partial)		++			4LP	15 / 11.6%	9 / 15%	LIS1, DYNC1H1, TUBG1	KIF5C
Pachygyria p>a with non-cortical malformations ⁴		+			-	7 / 5.4%	3 / 5%	TUBA1A, TUBB2B, DYNC1H1	KIF2A
Agyria-pachygyria a>p (mixed)			+		4LA				DCX
Pachygyria a>p (complete or partial)			+		4LA	8 / 6.2%	1 / 1.5%	DCX, ACTB, DYNC1H1	ACTG1, KIF5C
Pachygyria a>p with non-cortical malformations			+		-	0	0		KIF5C
Pachygyria-band a>p (complete or partial)			+		4LA	2 / 1.6%	0	ACTB, ACTG1	DCX
Tubulinopathy-related dysgyria									
Dysgyria p>a (complete or partial)		++			4L, 2L	14 / 10.9%	7 / 12%	TUBA1A, TUBB2B	DYNC1H1, TUBB, TUBB3, TUBA8
Dysgyria a>p (complete or partial)			+		-	0	0		KIF5C
Subcortical band heterotopia									
SBH diffuse thick (band >5 mm)	++				4LA	10 / 7.8%	8 / 14%	DCX	
SBH diffuse thin (band <5 mm)	+				-	1 / 0.8%	3 / 5%	DCX	
SBH partial p>a (band thick or thin)		+			-	3 / 2.3%	4 / 7%	LIS1, LIS1-YWHAE mosaic	
SBH partial a>p (band thick or thin)			+		-	0	1 / 1.5%		DCX
Thin undulating lissencephaly									
Pachygyria a>p thin with cerebellar hypoplasia ⁵			++		-	2 / 1.6%	1 / 1.5%	RELN	VLDLR
Pachygyria a>p thin with normal cerebellum			++		-	8 / 6.2%	1 / 1.5%	None	CRADD

Lissencephaly types based on imaging	Gradient			Pathology ¹	Dobyns n=129	Guerrini n=59	Genes (in both cohorts)	Additional genes from the literature ²
	DIF	PA	AP					
Pachygyria >p>a thin with ACC, abnormal WM				3L	2 / 1.6%	0	ARX	
Microlissencephaly (MLIS) other than TUBs						0		
MLIS with MOPD1	+			3L **	2 / 1.6%	0		RNU4ATAC ²
MLIS Barth type ⁶	+			4L ***	0	0		None

ACC – agenesis of the corpus callosum; a>p - anterior predominant gradient (AP); DIF – diffuse lissencephaly; MOPD1 - microcephalic osteodysplastic primordial dwarfism type I; p>a - posterior predominant gradient, incl. perisylvian lissencephaly (PA); >p>a - temporal predominant gradient (TL); TUB – tubulinopathy; WM – white matter; 2L – two-layered LIS, 3L – three-layered, 4L–four layered, 4LP – four-layered posterior, 4LA – four layered anterior.

¹Pathological data as discussed in [Forman et al., 2005], [Fallet-Bianco et al., 2014], [Magen et al., 2015]

²References by gene: ACTG1 [Riviere et al., 2012], CDK5 [Magen et al. 2015] CRADD [Di Donato et al., 2016a], DCX [Matsumoto et al., 2001; Pilz et al., 1998], DYNC1H1 [Januar et al., 2014; Poirier et al., 2013b; Willemssen et al., 2012], KIF2A [Poirier et al., 2013b], KIF5C [Cavallin et al., 2015; Januar et al., 2014; Poirier et al., 2013b; Willemssen et al., 2014], NDE1 [Paciorkowski et al., 2013], RNU4ATAC [Abdel-Salam et al., 2013], TUBA8 [Abdollahi et al., 2009], TUBB [TUBB [Breuss et al., 2012], TUBB3 [Fallet-Bianco et al., 2014], TUBB2B [Fallet-Bianco et al., 2014], VLDLR [Boycott et al., 2009]

³From limited data, the brain imaging phenotypes with NDE1 and RNU4ATAC mutations appear to be intermediate between lissencephaly and a severe simplified gyral pattern, possibly similar to some tubulinopathy scans.

⁴Non-cortical malformations include agenesis of severe hypoplasia of the corpus callosum, dysplastic basal ganglia, tectal hyperplasia, cerebellar hypoplasia with or without folial dysplasia (as described by Oegema et al., 2015)

⁵We have not observed posterior gradient in thin undulating lissencephaly in own cohorts. No patients with posterior predominant thin undulating lissencephaly have been reported in the literature to the best of our knowledge.

⁶Barth type microlissencephaly is a diffuse agyria with severe brain stem hypoplasia and rudimentary cerebellum [Barth et al., 1982; Kroon et al., 1996].

* 4-layered LIS was described in two fetuses, however, without providing an exact head measurement (severe microcephaly < –3 SD, or borderline ~ –2SD) [Fallet-Bianco et al., 2014]

** Pathology demonstrated a 3-layered LIS, significantly different from the pattern associated with ARX. Leptomeninges showed extensive glioneuronal heterotopia. Supperificial layers appeared hyperconvoluted, resembling polymicrogyria. The 1st molecular layer were sparse, in contrast to the hypercellular molecular layer seen with ARX mutations. [Juric-Sekhar et al., 2011]

*** Pathology showed a 4-layered LIS with neuronal cells seen also in the leptomeningeal space, with additional brain stem changes, cerebellar hypoplasia and a hyperplastic choroid plexus.[Barth et al., 1982]

Table III

Clinical severity of lissencephaly

Grade	Subtypes ¹	Imaging pattern	Clinical outcome
1. Mild	1-1	Partial SBH a>p or p>a	Borderline to moderate ID, seizures of variable severity, survival into adulthood expected [D'Agostino et al., 2004; Di Donato et al., 2016a; Guerrini and Dobyns 2014; Guerrini and Filippi 2005; Leventer et al., 2001]
	1-2	Diffuse thin SBH (<10mm)	
	1-3	Partial pachygyria ² a>p or p>a	
	1-4	Isolated "thin" or undulating lissencephaly	
2. Moderate	2-1	Diffuse thick SBH (>10mm)	Moderate to severe ID, severe language impairment, seizures often poorly controlled, life expectancy may be reduced although many survive to adulthood [Bahi-Buisson et al., 2014; Cardoso et al., 2000; de Rijk-van Andel et al., 1990; Dobyns 2010; Dobyns et al., 1992; Guerrini and Dobyns 2014; Leventer et al., 2000; Spencer-Smith et al., 2009]
	2-2	Mixed pachygyria-SBH	
	2-3	Diffuse pachygyria a>p or p>a	
3. Severe	3-1	Mixed pachygyria-agyria	Profound ID, poorly controlled seizures, short survival typical with mortality rate ~50% by 10 years with normal cerebellum, and much higher with cerebellar hypoplasia [Bahi-Buisson et al., 2014; Barth et al., 1982; de Rijk-van Andel et al., 1990; Dobyns et al., 1991; Dobyns et al., 1992; Ross et al., 2001]
	3-2	Diffuse agyria	
	3-3	Agyria with cerebellar hypoplasia	

¹ Primary groups based on severity of intellectual disability and epilepsy, subtypes based on imaging characteristics.