Lissencephaly, Type I

Jeffrey A. Golden

CNR	cadherin-related receptor
DC	doublecortin
ILS	isolated lissencephaly sequence
LIS	lissenecephaly
MDS	Miller-Dieker syndrome
MRI	magnetic resonance imaging
PAF	platelet-activating factor
VZ	ventricular zone
WM	white matter
XLAG	X-linked lissencephaly with ambiguous genitalia

DEFINITION

Lissencephaly, derived from the Greek works "lissos" meaning smooth and "enkephalos" which means brain, is a descriptive term defining a class of human cerebral malformations characterized by the agyric surface of the brain. In fact, most cases of lissencephaly do not have a complete loss of gyri; often the most ventral and medial gyri are relatively spared along with other brain regions that are variable preserved depending on the specific genetic mutation.

The underlying defect in lissencephaly is a defect in cell migration. Cell migration has been implicated in many disorders (chapters 5, 6, 7, 9); however, lissencephaly, along with heterotopia, are the only disorders that are clearly pathogenetically proven to be cell migration defects. Resulting from a slowing or delay in cell migration and not an absolute loss of movement, a second hallmark of lissencephaly is a thickening of the cerebral cortex. In addition to the absence of gyri and the thickening of the cerebral cortex, brains from patients with lissencephaly may show a variety of associated malformation.

Lissencephaly is not a single malformation, but a descriptive term applied to many malformations with distinct genetic etiologies. This chapter will focus on the lissencephalies associated with a failure in normal cell migration as opposed to the next chapter that will examine the lissencephalies resulting from an apparent over migration of neurons (chapter 5). Heterotopia, also a migration disorder, will be addressed in chapter 7. The lissencephalies described in this chapter have also been referred to as type I lissencephaly or classical lissencephaly. For the purposes of this chapter these disorders will be defined as type I lissencephaly. As the genetic basis of lissencephaly continues to be delineated, these definitions will likely evolve to include specific genetic defects.

SYNONYMS AND HISTORICAL ANNOTATIONS

Lissencephaly, agyria and pachygyria have, at various times, been used to describe an overlapping spectrum of disorders. Pachygyria has generally been reserved for cases with patchy or focal malformations whereas agyria and lissencephaly are used to describe a diffuse malformation of the cerebral hemispheres. Recent studies indicate the same genetic mutation can give a spectrum of disorders ranging from localized lissencephaly or pachygyria to diffuse lissencephaly. An understanding of how the same mutation can give rise to distinct morphological anomalies is beginning to be understood. However, it is also important to recognize that this may not be the only explanation for pathological heterogeneity.

EPIDEMIOLOGY

Incidence and prevalence. The incidence of type I lissencephaly is difficult to estimate due to the paucity of reports. The best data comes from the Netherlands where 22 cases were identified in 11.7 million births giving an incidence of approximately 1:500 000 (18). As a result of diagnostic difficulties and subtleties in the classification, this incidence is believed to be an underestimate by as much as 10 fold.

Sex and age distribution. Type I lissencephaly is often cited to affect males and females equally; the presence of at least two X-linked forms suggests males are affected more frequently. The failure of epidemiologic studies to recognize this is likely a reflection of the overall low incidence and small numbers evaluated.

Risk Factors. There are no known risk factors for type I lissencephaly. All cases where the etiology is defined are clearly genetic in origin. Whether pachygyria has environmental, toxic, infectious or other associated risk factors remains to be established (see below).

EMBRYOLOGY

Lissencephaly is generally agreed to be a defect in radial cell migration. During development newly born neurons must migrate from the inner most layer of the neural tube, where a cell exits the cell cycle (defining the birthdate) outwards toward the surface of the brain (Figure 1). The radial pathway of cell migration was first postulated by Ramon y Cajal in the 1890s and experimentally delineated in the 1960s and 1970s (38, 67, 68). Upon exiting from the cell cycle, the newly born neuroblast associates with specialized glia known as radial glial cells. Radial glia are bipolar cells with one short process extended to the adjacent ventricular surface and a second projecting to the pial surface (66). Recently radial glia have been shown to be neural stem cells in addition to their role as a scaffold for radial cell migration (58). A 2-way signaling process occurs between the migrating neuron and the radial glial fiber that permits the neuroblast to migrate and provides a signal to maintain the structure of the radial glial fiber (38). This process requires known receptors and ligands such as neuregulin and ErbB4 (2, 70), cell adhesion molecules (3, 36, 62), putative ligands with unknown receptors such as astrotactin (25, 76, 81), and extracellular matrix molecules (45) and their cell surface receptors (28). Blocking any of these components can slow down or prevent radial cell migration and thus are candidate pathways for the development of type I lissencephaly.

The cerebral cortex of higher vertebrates is organized into 6 layers or laminae. These layers are generated in an inside-to-outside sequence (6). The first cells to exit the ventricular zone (VZ) and populate the cortical plate (future cortex) segregate into 2 specialized cell types. An outer cell layer composed largely of Cajal-Retzius neurons and an inner layer composed of subplate neurons (Figure 1). Once the pre-plate is established, subsequent cells

migrate out of the VZ and settle between these 2 layers. The first cells to arrive will eventually reside in the deepest layer, layer 6. Later born cells will migrate past the existing cells to reside in progressively more superficial layers (Figure 1).

Cells migrating into the cortical plate must also stop at the appropriate location. This choice point is essential for normal cerebral cortical laminar development. Insights into the mechanisms governing how cells know when to stop have begun to be elucidated through the analysis of several mouse mutants. In particular, the characterization of the Reeler mutant mouse has provided the first insights into the process of laminar organization. The Reeler mouse was first identified as a postnatal behavioral defect (1) and the neuropathology has been extensively studied (8, 9). In Reeler mutant mice the cortical layering appears inverted (8, 9). In other words, the first cells of the definitive cortex to migrate out of the VZ end up residing in the superficial cortical plate and subsequent cells migrate to and stop in progressively deeper positions. This pattern is opposite of the normal inside to outside development of the cerebral cortex. The *Reelin* gene was subsequently cloned and shown to encode a putative extracellular matrix protein (16). Reelin is expressed by Cajal-Retzius cells and is found extracellularly in the molecular layer (layer 1) (16, 17, 60). These data suggest Reelin is required for the normal inside to outside positioning of cells as they migrate from the VZ (38, 51). This was the first component of a signaling pathway guiding cells to the correct location in the cerebral cortex. Confirming the relationship of radial cell migration to type I lissencephaly, mutations in the human *RELN* have now been identified in patients with lissencephaly and cerebellar hypoplasia (42).

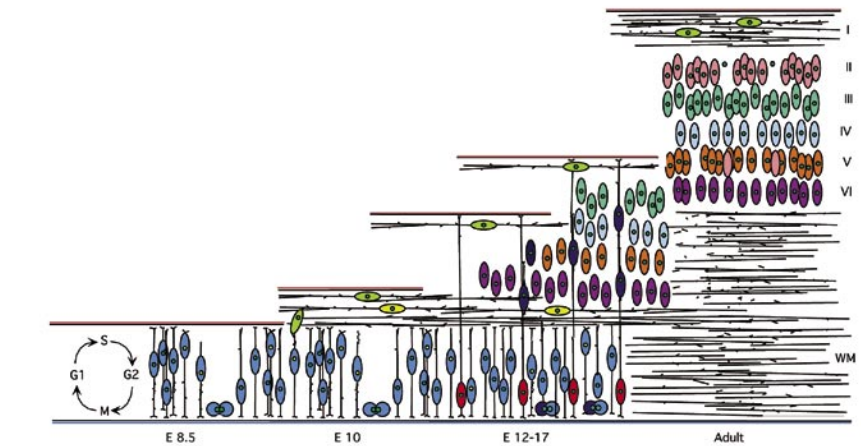

Figure 1. *Schematic outline of early mouse cortical development.* At E 8.5 the wall of the neural tube consists of a pseudostratified neuroepithelium (band of blue cells). Cell processes maintain contact with both the inner (bottom of figure) and outer (top of figure) surfaces of the neural tube. However, the nucleus migrates according to the cell cycle with mitoses (M phase) occurring at the ventricular surface and S-phase at the pial surface. At E10, the first cells delaminate from the germinal neuroepithelium, which defines the VZ, and form the preplate. The preplate is composed of Cajal-Retzius neurons (light green cells) and subplate neurons (yellow cells). During the ensuing days those VZ cells which exit the cell cycle become neuroblasts (dark blue cells) and migrate from the VZ along radial glia (red cells in the VZ) to populate the definitive cortical plate. The neuroblasts that become layers II-VI split the preplate, leaving the subplate neurons adjacent to the VZ while the Cajal-Retzius cells remain in contact with the pial surface. Cells accumulate from inside-to-out beginning with layer 6 (purple cells) and ending with layer II neurons (pink cells).

GENETICS

Sporadic, autosomal dominant, autosomal recessive, and X-linked inheritance of type I lissencephaly are all well defined. To date 4 genes have been causally linked. The first to be identified was *LIS1* on chromosome 17p. *LIS1* is deleted in all patients with the Miller-Dieker syndrome (MDS) (see signs and symptoms below for more on the MDS) and many patients with isolated lissencephaly sequence. The more severally affected MDS patients are likely a reflection of larger deletions that include other genes. Recent evidence suggests one of the critical genes for the more severe neurologic and neuropathologic abnormalities in patients with the MDS is the loss of *14-3-3e*, located telomeric to *LIS1* (11, 46, 79). The *LIS1* transcript encodes a 410 amino acid protein known as LIS1, or PAFAH1B1 (see below).

The second gene to be identified was *XLIS*, which encodes doublecortin (DCX) (19, 31). DCX is a microtubule associated protein, however its exact function remains uncertain (32). *XLIS* maps to Xq22.3-23 and mutations result in lissencephaly in males. Females heterozygous for XLIS show subcortical band heterotopia (33), the presence of the normal overlying cortex and the subcortical band of cortical neurons presumably reflecting 2 populations of migratory neurons that are distinguished by normal Lyonization (X chromosome inactivation). Those cells randomly inactivating the X chromosome with the mutant allele will only express the normal allele and should migrate normally to form the cortex. Cells inactivating the normal allele will only express the mutant allele and will not migrate normally, thus residing in the subcortical region.

The third gene to be identified was *RELN* (42). RELN is required for normal migration into appropriate layers. Reeler mice show an inverted cortex and severe migrational anomalies of the cerebellum (8, 35). An autosomal recessive form of lissencephaly with cerebellar hypoplasia characterizes patients with *RELN* mutations. Lymphedema and neuromuscular problems are seen in some patients (42).

The most recently identified gene is the transcription factor *ARX* (49). Mutations in this gene, which is also located on the X chromosome, result in lissencephaly and ambiguous genitalia in males. The spectrum of disorders in females is more heterogeneous but does not appear to include lissencephaly.

CLINICAL FEATURES

Signs and symptoms. The clinical presentation of all type I lissencephaly syndromes show considerable overlap. Most patients exhibit moderate to severe developmental delay and neuromotor impairment. Patients with isolated type I lissencephaly most commonly have profound

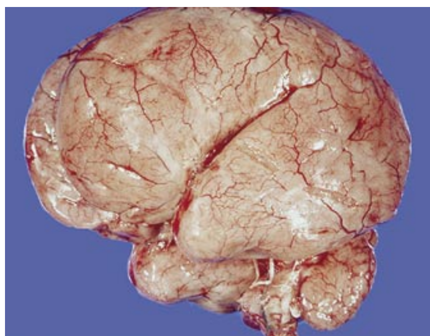

Figure 2. *External view of brain with lissencephaly.* Note the complete absence of gyri and sulci on the surface.

mental retardation and seizures that are often intractable. Infantile spasms may be present, however they are most common with mutations in *ARX* (73, 77). Patients with isolated lissencephaly may be less severely affected than those with the MDS. This is likely due to deletions involving *14-3-3e* in addition to *LIS1* (79).

Patients with type I lissencephaly may also exhibit extra CNS abnormalities. Patients with isolated lissencephaly typically have acquired microcephaly and mild dysmorphic facial features. In contrast, patients with the MDS have bitemporal hollowing, hypertelorism, frontal bossing, a short-upturned nose, a small jaw and a prominent upper lip with a thin vermillion border. Other anomalies occasionally associated with the MDS include clinodactyly, polydactyly, cryptorchidism, sacral dimples and congenital heart defects. Patients with *ARX* mutations exhibit microphalus (and thus ambiguous genitalia) and small adrenal glands giving rise to the X-linked lissencephaly with ambiguous genitalia (XLAG) syndrome. *RELN* mutations result in congenital lymphedema in a subset of patients.

Imaging. MRI is the modality of choice for evaluating the lissencephalic brain for structural anomalies. The loss of sulci and gyri over the surface of the brain combined with the thickened cerebral cortex (1-2 cm in contrast to the normal 0.3-0.5 cm) is characteristic of type I lissencephaly. In addition, heterotopia, subcortical bands, hydrocephalus, and posterior fossa anomalies can be evaluated and may be helpful in guiding genetic testing. Furthermore, the pattern of the structural anomalies of the brain may assist in guiding genetic testing. A loss of posterior gyri

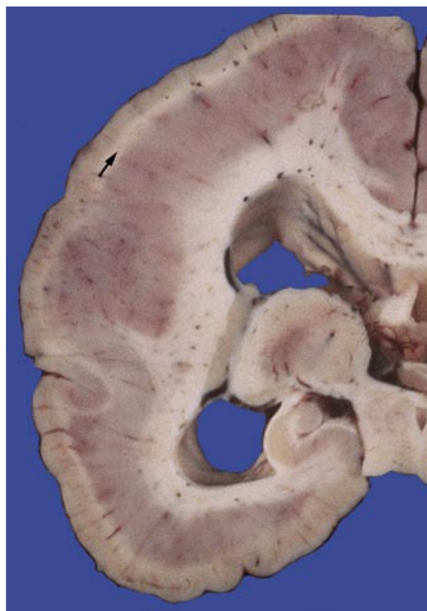

Figure 3. *Hemisection of brain from patient with lissencephaly.* The cerebral cortical grey matter is markedly thickened and the white matter is diminished. Myelination is layer 3 can be seen (arrow). There is mild sparing of the temporal lobe where some evidence of gyral formation is present and the hippocampus is preserved.

(occipital) with relative sparing anteriorly (frontal lobes; a posterior to anterior gradient) favors a *LIS1* mutation, whereas an anterior to posterior gradient (more severe in the frontal lobes) suggests a mutation in DCX (37). An anterior to posterior gradient with severe cerebellar hypoplasia would favor a *RELN* mutation. Grading of the lissencephaly is generally based on the MRI (21).

Laboratory findings. There are no specific laboratory findings associated with type I lissencephaly. The 2 families that have been described with mutations in *RELN* had an absolute or near complete loss of REELIN in their serum suggesting this as a possible diagnostic test for patients that fit the phenotype of a *RELN* mutation.

MACROSCOPY

The external surface of the brain from patients with lissencephaly reveals a marked paucity of gyri and sulci (Figure 2). The abnormality need not include the entire brain and as described above relative sparing of the frontal or occipital gyri and sulci usually reflects mutations in distinct genes. Gross sectioning of the brain reveals a markedly thickened cerebral cortex and loss of the underlying white matter (Figure 3). A transition to a more normal thickness of the cortex is seen in areas that are grossly spared. Transition areas can include areas that have a subcortical band heterotopia phenotype. Periventricular heterotopia and white matter heterotopia may also be observed. In older individuals a white matter band can be seen in the superficial cortex corresponding to layer 3. The ventricles are usually generous in size. The cerebellum may be hypoplastic in some cases, again corresponding to the genetic mutation in some patients. Inferior olivary dysplasias may be present in the medulla of many cases, including those with a *LIS1* mutation.

Pachygyria is a localized thickening of the cortex, which in most other ways resembles lissencephaly. It can involve a single gyrus or a greater region of one or both hemispheres. When extensive and bilateral it can be difficult to distinguish from lissencephaly, although multifocal pachygyria is usually not bilaterally symmetric.

HISTOPATHOLOGY

The cerebral cortex of type I lissencephaly is classically described as a 4-layered cortex replacing the normal 6-layered ribbon (Figure 4). The outermost layer is the molecular layer, which contains Cajal-Retzius neurons in most cases (layer 1). Layer 2 is a band of primarily medium to large pyramidal neurons that may be correctly oriented but more often show variable degrees of disorganization. The third layer has a decreased density of neurons and contains numerous axons, which in children older than one to 2 years is myelinated. This layer can be seen by neuroimaging. Finally, layer 4 is composed of a broad band of disorganized neurons (59). Layer 4 can be of variable thickness but always contains a mixed population of small and medium sized neurons that have no clear organization or orientation. The white matter, which is severely reduced in volume, occasionally contains individual neurons or collections of neurons forming heterotopia. This description corresponds to the areas of classic lissencephaly. Sampling other cortical regions give a variety of pathological features ranging from a normal cortex, through areas where an

apparently normal 6 layer cortex exists but a subcortical band heterotopia is present below the cortex separate by a swath of white matter. Finally, a disorganized and thickened cortex that does not strictly coincide with the classic 4-layer or normal 6-layered cortex frequently exists (47). Inferior olivary nucleus heterotopia and simplification of the inferior olivary nucleus, cerebellar cortical dysplasias, and corticospinal anomalies may also be present (26, 59).

Pachygyria histologically resembles lissencephaly but is more localized, involving only a single gyri upto a lobe. It can also be multifocal. Pachygyria characteristically shows a loss of the grey-white junction along with the thickening of the cortex and the border with normal cortex is difficult to identify. This is in distinction to polymicrogyria (chapter 6) where the grey-white junction and the border with adjacent normal cortex are usually well defined.

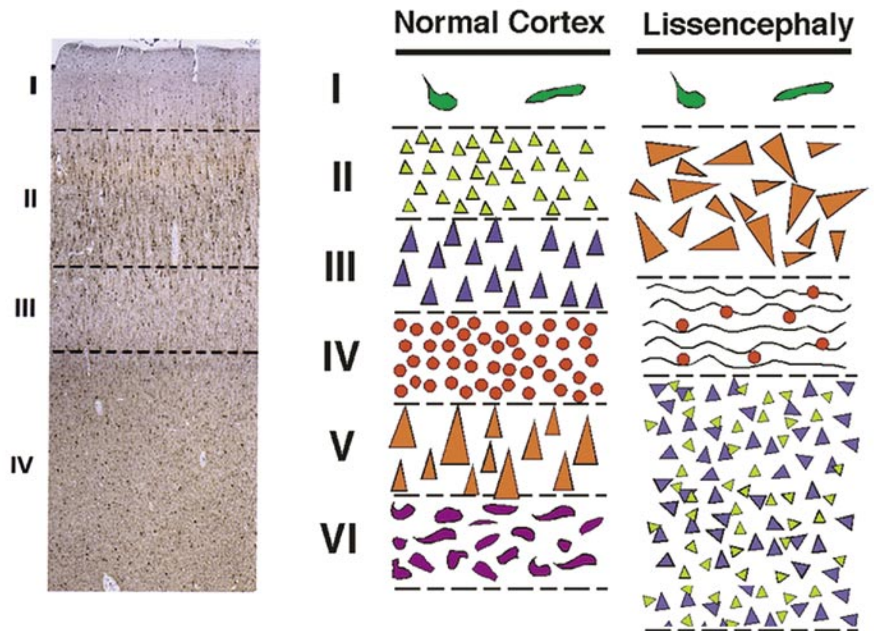

Figure 4. Neurofilament stained section from patient with lissencephaly and schematic of normal cortical laminar orginazation and that found in classical lissencephaly. Neurofilament staining highlights the few cells in layer 1, the numerous large and disorganized pyramidal neurons found in layer II, the relatively hypocellular layer III, which becomes myelinated (Figure 3), and the thick layer IV composed of medium and small neurons. The schematic highlights the normal organization of the cortex in contrast to that found in classical lissencephaly. Note the apparent reversal of layers with the exception of Cajal-Retzius neurons in layer I (green).

IMMUNOHISTOCHEMISTRY AND ULTRASTRUCTURAL FINDINGS

Immunohistochemistry with neurofilament antibodies is often helpful in defining the cortical architecture. Anti-RELN antibodies can be used to localize Cajal-Retzius neurons. Other studies are generally non-contributory, including Ultrastructural studies.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of lissencephaly is limited. When bilaterally symmetric and involving the entire brain the primary consideration is which gene to study first. Features such as the pattern of the lissencephaly by imaging or gross pathology, sex, and coexisting malformations all guide molecular genetic investigations. In less severe cases the differential diagnosis may include pachygyria, and this can be difficult to distinguish from polymicrogyria (chapter 6) and cortical dysplasia (chapter 9). Although radiographically distinguishable by MRI in some cases, this is not always true and only pathology can definitively separate out these entities in many cases.

EXPERIMENTAL MODELS AND PATHOGENESIS

The genes mutated in several human disorders of neuronal migration also provided a basis for linking the cytoskeleton to neuronal migration. The human *LIS1* gene was positionally cloned from patients with MDS and isolated lissencephaly sequence (ILS) both associated with type I lissencephaly (classical-LIS). It was found to encode the 45-kD enzymatic β-subunit of platelet-activating factor (PAF) acetylhydrolase, PAFAH1B1 (42-44). PAFAH1B1 homozygous mutant mice die at the trophoblast stage (41). Heterozygote mice are grossly normal, but show a dose dependent histopathological disorganization of cerebral cortical lamination, cerebellar cortical defects and disruptions of the pyramidal cell layer in the hippocampus (41). In vitro analysis of heterozygous cerebellar granule neurons show slowed migration but no alteration of neurite dynamics (5, 41). This reduced rate of migration has been hypothesized to be basis for the observed pathologic phenotype.

The available data do not distinguish between a direct role for PAFAH in transducing guidance signals to the cytoskeletal machinery versus a loss of component of the migration apparatus. However, the yeast homolog of the human *LIS1* gene, *NudF* (85) has provided some insight into the possible function of LIS1. Proper nuclear migration in the *Aspergillus nidulans* depends on NudF (85). NudF associates with NudC in yeast (55). NudC is a tyrosine kinase, and its binding partner, NudA, is a cytoplasmic dynein (85). The human homolog of NudC is highly conserved in higher vertebrates and in conjunction with the *LIS1* gene product regulates dynein activity during mitosis in vertebrate cells (29, 83). These data suggest that classical-LIS, due to *LIS1* mutations, is caused by a loss of dynein function, which is required for nuclear translocation. The inhibition or slowing of nuclear movement during cell migration may, in turn, retard the rate of cell migration.

Further evidence to support this hypothesis came from 4 laboratories reporting that LIS1 also interacts with NUDEL and mNudE (24, 48, 56, 72). This interaction again parallels that found in *Aspergillus nidulans* where NudF inter-

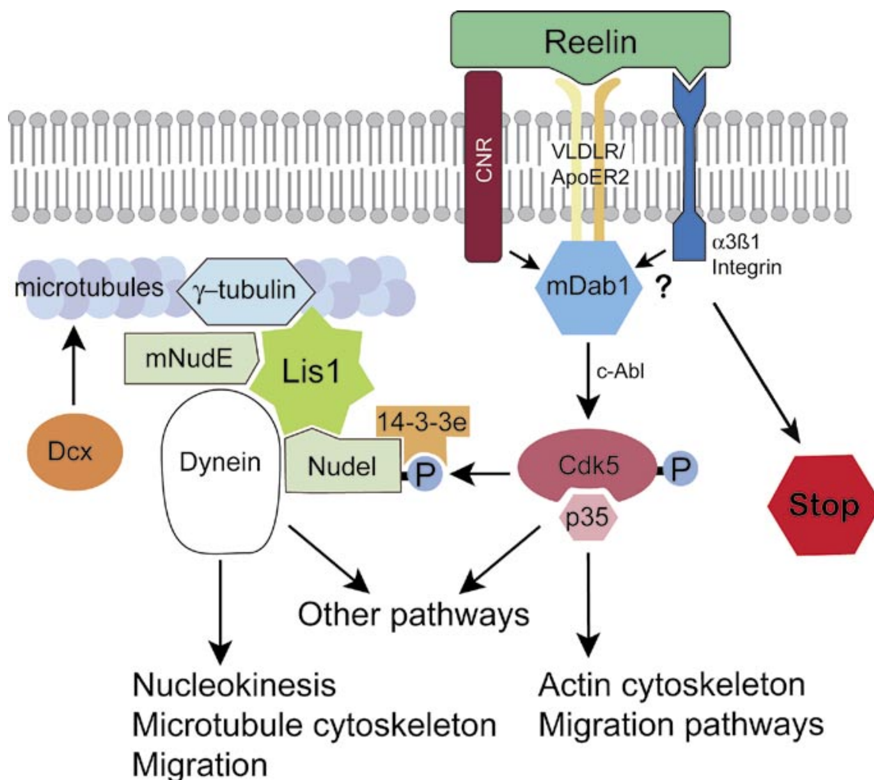

Figure 5. *Summary of the LIS1 and RELN signaling pathways for neural migration.* Extracellular Reln binds to one of three receptor complexes: cadherin-related neural receptor (Cnr), VldlR/ApoER2, or 3β1 integrin. Receptor stimulation causes activation of mDab1, which, via c-Abl or other pathways, can activate Cdk5/p35. Cdk5 can phosphorylate many intracellular targets, including regulators of the actin cytoskeleton and Nudel. Phosphorylated Nudel forms a complex with Lis1, mNudE, Dynein, and microtubules. This complex is required for nuclear positioning and cell migration. Dcx is also believed to modulate microtubule function. Adapted from Wynshaw-Boris and Gambello, 2001.

acts with NudE, the homolog of mNudE and NUDEL. Through a series of experiments they have shown that NUDEL, and possibly mNudE, directly interact with LIS1 and that together they regulate cytoplasmic dynein motor function and location within the cell (84). Disruption in *LIS1* results in abnormal localization of NUDEL and likely a defect in cytoplasmic dynein motor function leading to a defect in cell migration or at least nuclear migration. It is interesting to note that one feature that separates axon guidance, another form of migration found in the developing nervous system, from cell migration, is nuclear translocation. *LIS1* mutations may result in specifically a nuclear movement defect and therefore a preferential defect in cell migration rather than an axon guidance defect. Finally, the LIS1/NUDEL pathway has also been linked to the Reelin/Cdk5/p35 pathway described above. NUDEL is a direct target of Cdk5 (56, 72). The phosphorylation of NUDEL by Cdk5 appears to control its cellular localization and thus is likely to influence dynein motor function (Figure 5).

In addition to its role in cell migration, cell proliferation may also be influenced by LIS1. A series of experiments again linked interactions of LIS1 with cytoplasmic dynein and dynactin (23). Both of these proteins are important in mitotic cell division and cytokinesis. Although a role for NUDEL or mNudE has not been directly investigated in cell division, given the data reviewed above it is interesting to speculate on such an association. Further evidence LIS1 may have a role in cell proliferation comes from observations in mutant mice and in humans with lissencephaly. Mice engineered to have incremental decreases in LIS1 show a progressive thinning of the cortex suggesting a cell proliferation defect (41). The brains of patients with lissencephaly are usually small, again implying a possible proliferation defect (26, 59). Together these data imply LIS1 may have a role in cell proliferation and in cell migration, both contributing to the human phenotype of lissencephaly.

Patients with MDS usually have lissencephaly at the severe end of the spectrum with complete agyria. In ILS the cortical malformation ranges between agyria and pachygyria (Figure 3). A patient with subcortical band heterotopia (SBH) and a *LIS1* mutation has also been reported (63). The cortical malformation with a *LIS1* abnormality is more severe posteriorly (21, 64, whereas patients with lissencephaly and *DCX* mutations show a more severe involvement of the frontal lobes. Recent data also suggest that the overall severity of the lissencephaly in ILS is related to the type and location of the mutation (11).

Mutations in the *DCX* gene located on the X-chromosome are associated with subcortical bond heterotopia, also known as "double cortex," in females (20, 31). Males with *DCX* mutations usually have classical lissencephaly (34, 63-65), but have also presented with subcortical bond heterotopia. Both the type of mutation and somatic mosaicism have been associated with this milder phenotype in males (34, 63). In pedigrees, where females have subcortical bond heterotopia and males lissencephaly, a DCX mutation has so far always been identified. In females with sporadic subcortical bond heterotopia the figures vary (19, 33, 54), but in those with bilateral diffuse bands, or thin frontal bands only, the mutation rate appears to be high. This again emphasizes the severity gradient of the cortical malformation in relation to the gene mutation, with a frontal, or more severe frontal, malformation of the brain associated with *DCX* mutations. Random versus skewed X-inactivation was thought to determine the thickness and extent of the band. However studies on lymphocytes did not confirm this hypothesis, although they may not reflect the inactivation ratio in the brain. However it has become clear that the type of mutation also determines the phenotypic spectrum in females (33, 54). Mutations in either *LIS1* or *DCX* both result in disruptions of cell migration. In the case of *LIS1*, cell migration appears to be slowed. Cells that must travel the furthest would be predicted to be the most retarded. This appears to be

the case. The superficial layer of the cortex, layer 2, is predominately populated by large pyramidal cells that usually reside in the deeper layers of the cortex. These are among the first cells to migrate from the VZ and because the intermediate zone is relatively thin earlier in development, they have the shortest distance to travel. In contrast, the later born cells normally destined for the more superficial layers must travel the farthest and are the cells found closer to the ventricle in classical lissencephaly, implying they are affected for a longer period of time or more severely affected.

Dcx was disturpted in mice using targeted mutagenesis, however these mice did not have a cortical defect (they did have a hippocampal cell positioning phenotype) (14). More recently, using a method to inhibit RNA function, Dcx function was reduced in a rat and mouse model that did result in subcortical band heterotopia resembling that seen in humans (4). Understanding the differences between these models and the intracellular function of Dcx is an areas of active investigation.

The Reeler mouse and its function have been described in part above. Since Reelin is an extracellular matrix molecule, a receptor for Reelin would be required for signaling to the migrating cell. Reelin has been found to bind to cadherin-related receptors (CNRs) (74), at least 2 members of the LDL receptor family (17, 40, 80), and α3β1-integrin (22). Binding of Reelin to α3β1-integrin functions as a stop signal, however the downstream components within the cell that regulate the migration stop are not known. Upon contact with Reelin the CNRs initiates phosphorylation of the cytoplasmic second messenger mDab1, possibly through a CNR associated tyrosine kinase Fyn (74) or through the LDL receptor (17, 40). The Scrambler and Yotari mutant mice have been identified as mutations in the *mDab1* gene (75). Scrambler, Yotari and *mDab1-/-* all exhibit a Reeler phenotype further supporting the notion that they lie the same pathway. Phosphorylated mDab1 can interact with a variety of proteins including the SH2 domain of Src (44). Src has been shown to interact with actin and affect cytoskeletal remodeling (7, 52, 78). Src deficient cells show strong adhesion to surfaces and low migration capacity (50). Thus, these data tie Reelin signaling directly into cell migration.

mDab1 also activates the protooncogene c-Abl. Once activated, c-Abl can phosphorylate Cdk5, a process that is enhanced by Cables, thus activating Cdk5 (82). Cdk5 and p35 (another activator of Cdk5) have also been implicated in directing neurons to the appropriate location within the cerebral cortex (12, 30, 61). Both are highly expressed in the developing CNS and mice engineered to be homozygous mutant for Cdk5 or p35 also show a cortical defect similar, although not identical, to the Reeler phenotype (30). Cdk5 co-localization with p35, Rac and Pak-1 in neurons (57). They suggest that a Rac dependent hyperphosphorylation of Pak-1 results in a dynamic down-regulation of actin polymerization and enhancement of new focal complex formation during cell migration and process outgrowth (57). Activation of Pak has also been shown to result in a loss of stress fibers and focal adhesions (53). These data indicate that the Rac family of GTPases along with Src family members can regulate cytoskeletal remodeling and therefore transduce guidance signals from the cell membrane to the cytoskeleton.

The most recently identified gene for human lissencephaly is *ARX*. A mouse mutant for this gene has also been generated and shows a defect in at least radial cell migration (49). Further studies are in progress to better understand the role of this transcription factor in cell migration and the pathogenesis of lissencephaly.

FUTURE DIRECTIONS AND THERAPY

Several other syndromes with lissencephaly as a component have been identified (*http://www.geneclinics.org*). The best characterized of these is lissencephaly with cerebellar hypoplasia (LCH). Several families have been published suggesting autosomal recessive inheritance (43, 47, 71). Additional variants of lissencephaly have been identified radiographically and pathologically, and we predict that mutations affecting proteins related to cytoskeletal dynamics such as DCX and LIS1 along with proteins involved in guiding cells to the appropriate location such as Reelin, will eventually be identified as the basis of these related disorders.

Therapy may be well established for some disorders while in other disorders there may be no treatment. It may be possible for the discussion of future directions to interface with the previous section on pathogenesis.

The fields of developmental biology, genetics, pathology, neurology and neuroimaging have come together to begin providing detailed explanations for the pathogenesis of several human conditions resulting from cell migration anomalies. The rate of progress in this field of study will no doubt provide many advances over the next few years. Linking the known genes into pathways from extracellular signaling to cytoskeletal dynamics will be important for a complete understanding of the processes involved. Finding additional molecules in these pathways along with defining the genetic defects in other families and other syndromes will no doubt provide further insights into cell migration during normal CNS development and in the pathogenesis of human malformations.

REFERENCES

1. Alter M, Liebo J, Desnick SO, Strommer B (1968) The behavior of the reeler neurological mutant mouse. *Neurology* 18:289.

2. Anton ES, Marchionni MA, Lee KF, Rakic P (1997) Role of GGF/neuregulin signaling in interactions between migrating neurons and radial glia in the developing cerebral cortex. *Development* 124:3501-3510.

3. Asou H, Miura M, Kobayashi M, Uyemura K, Itoh K (1992) Cell adhesion molecule L1 guides cell migration in primary reaggregation cultures of mouse cerebellar cells. *Neurosci Lett* 144:221-224.

4. Bai J, Ramos RL, Ackman JB, Thomas AM, Lee RV, LoTurco JJ (2003) RNAi reveals doublecortin is required for radial migration in rat neocortex. *Nat Neurosci* 6:1277-1283.

5. Bix GJ, Clark GD (1998) Platelet-activating factor receptor stimulation disrupts neuronal migration in vitro. *J Neurosci* 18:307-318.

6. Boulder C (1970) Embryonic vertebrate central nervous system: revised terminology. *Anat Rec* 166:257-261.

7. Brown MT, Cooper JA (1996) Regulation, substrates and functions of src. *Biochim Biophys Acta* 1287:121-149.

8. Caviness V (1982) Neocortical histogenesis in normal and reeler mice: a developmental study based upon [3H]thymidine autoradiography. *Dev Brain Res* 4:293-302.

9. Caviness VS Jr (1975) Architectonic map of neocortex of the normal mouse. *J Comp Neurol* 164:247-263.

10. 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 (2000) The location and type of mutation predict malformation severity in isolated lissencephaly caused by abnormalities within the LIS1 gene. *Hum Mol Genet* 9:3019-3028.

11. Cardoso C, Leventer RJ, Ward HL, Toyo-Oka K, Chung J, Gross A, Martin CL, Allanson J, Pilz DT, Olney AH, Mutchinick OM, Hirotsune S, Wynshaw-Boris A, Dobyns WB, Ledbetter DH (2003) Refinement of a 400-kb critical region allows genotypic differentiation between isolated lissencephaly, Miller-Dieker syndrome, and other phenotypes secondary to deletions of 17p13.3. *Am J Hum Genet* 72:918-930.

12. Chae T, Kwon YT, Bronson R, Dikkes P, Li E, Tsai LH (1997) Mice lacking p35, a neuronal specific activator of Cdk5, display cortical lamination defects, seizures, and adult lethality. *Neuron* 18:29-42.

13. Chong SS, Pack SD, Roschke AV, Tanigami A, Carrozzo R, Smith AC, Dobyns WB, Ledbetter DH (1997) A revision of the lissencephaly and Miller-Dieker syndrome critical regions in chromosome 17p13.3. *Hum Mol Genet* 6:147-155.

14. Corbo JC, Deuel TA, Long JM, LaPorte P, Tsai E, Wynshaw-Boris A, Walsh CA (2002) Doublecortin is required in mice for lamination of the hippocampus but not the neocortex. *J Neurosci* 22:7548-7557.

15. D'Arcangelo G, Homayouni R, Keshvara L, Rice DS, Sheldon M, Curran T (1999) Reelin is a ligand for lipoprotein receptors. *Neuron* 24: 471-479.

16. D'Arcangelo G, Miao GG, Chen SC, Soares HD, Morgan JI, Curran T (1995) A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. *Nature* 374:719-723.

17. D'Arcangelo G, Nakajima K, Miyata T, Ogawa M, Mikoshiba K, Curran T (1997) Reelin is a secreted glycoprotein recognized by the CR-50 monoclonal antibody. *J Neurosci* 17:23-31.

18. de Rijk-van Andel JF, Arts WF, Hofman A, Staal A, Niermeijer MF (1991) Epidemiology of lissencephaly type I. *Neuroepidemiology* 10: 200-204.

19. 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 (1998) doublecortin is the major gene causing X-linked subcortical laminar heterotopia (SCLH). *Hum Mol Genet* 7:1063-1070.

20. des Portes V, Pinard JM, Billuart P, Vinet MC, Koulakoff A, Carrie A, Gelot A, Dupuis E, Motte J, Berwald-Netter Y, Catala M, Kahn A, Beldjord C, Chelly J (1998) A novel CNS gene required for neuronal migration and involved in X- linked subcortical laminar heterotopia and lissencephaly syndrome. *Cell* 92:51-61.

21. Dobyns WB, Truwit CL, Ross ME, Matsumoto N, Pilz DT, Ledbetter DH, Gleeson JG, Walsh CA, Barkovich AJ (1999) Differences in the gyral pattern distinguish chromosome 17-linked and X-linked lissencephaly. *Neurology* 53:270-277.

22. Dulabon L, Olson EC, Taglienti MG, Eisenhuth S, McGrath B, Walsh CA, Kreidberg JA, Anton ES (2000) Reelin binds 3β1-integrin and inhibits neuronal migration. *Neuron* 27:33-44.

23. Faulkner NE, Dujardin DL, Tai CY, Vaughan KT, O'Connell CB, Wang Y, Vallee RB (2000) A role for the lissencephaly gene LIS1 in mitosis and cytoplasmic dynein function. *Nat Cell Biol* 2:784-791.

24. Feng Y, Olson EC, Stukenberg PT, Flanagan LA, Kirschner MW, Walsh CA (2000) LIS1 regulates CNS lamination by interacting with mNudE, a central component of the centrosome. *Neuron* 28:665-679.

25. Fishell G, Hatten M (1991) Astrotactin provides a receptor system for CNS neuronal migration. *Development* 113:755-765.

26. Friede RL (1989) *Developmental Neuropathology.* 2nd ed. 1989, Berlin: Springer-Verlag. 577.

28. Galileo DS, Majors J, Horwitz AF, Sanes JR (1992) Retrovirally introduced antisense integrin RNA inhibits neuroblast migration in vivo. *Neuron* 9:1117-1131.

29. Garces JA, Clark IB, Meyer DI, Vallee RB (1999) Interaction of the p62 subunit of dynactin with Arp1 and the cortical actin cytoskeleton. *Curr Biol* 9:1497-1500.

30. Gilmore EC, Ohshima T, Goffinet AM, Kulkarni AB, Herrup K (1998) Cyclin-dependent kinase 5-deficient mice demonstrate novel developmental arrest in cerebral cortex. *J Neurosci* 18: 6370-6377.

31. Gleeson JG, Allen KM, Fox JW, Lamperti ED, Berkovic S, Scheffer I, Cooper EC, Dobyns WB, Minnerath SR, Ross ME, Walsh CA(1998) Doublecortin, a brain-specific gene mutated in human X-linked lissencephaly and double cortex syndrome, encodes a putative signaling protein. *Cell* 92:63-72.

32. Gleeson JG, Lin PT, Flanagan LA, Walsh CA (1999) Doublecortin is a microtubule-associated protein and is expressed widely by migrating neurons. *Neuron* 23:257-271.

33. Gleeson JG, Minnerath SR, Fox JW, Allen KM, Luo RF, Hong SE, Berg MJ, Kuzniecky R, Reitnauer PJ, Borgatti R, Mira AP, Guerrini R, Holmes GL, Rooney CM, Berkovic S, Scheffer I, Cooper EC, Ricci S, Cusmai R, Crawford TO, Leroy R, Andermann E, Wheless JW, Dobyns WB, Walsh CA, et al (1999) Characterization of mutations in the gene doublecortin in patients with double cortex syndrome. *Ann Neurol* 45:146-153.

34. Gleeson JG, Minnerath S, Kuzniecky RI, Dobyns WB, Young ID, Ross ME, Walsh CA (2000) Somatic and germline mosaic mutations in the doublecortin gene are associated with variable phenotypes. *Am J Hum Genet* 67:574-581.

35. Goldowitz D, Cushing RC, Laywell E, D'Arcangelo G, Sheldon M, Sweet HO, Davisson M, Steindler D, Curran T (1997) Cerebellar disorganization characteristic of reeler in scrambler mutant mice despite presence of reelin. *J Neurosci* 17:8767-8777.

36. Grumet, M (1992) Structure, expression, and function of Ng-CAM, a member of the immunoglobulin superfamily involved in neuron-neuron and neuron glia adhesion. *J Neurosci Res* 31:1-13.

37. Hatten M, Mason C (1990) Mechanisms of glial-guided neuronal migration in vitro and in vivo. *Experientia* 46:907-916.

38. Hatten ME (1999) Central nervous system neuronal migration. *Annu Rev Neurosci* 22: 511-539.

39. Hattori M, Adachi H, Tsujimoto M, Arai H, Inoue K (1994) Miller-Dieker lissencephaly gene encodes a subunit of brain platelet-activating factor acetylhydrolase [corrected, erratum Nature 1994 370:391]. *Nature* 370:216-218.

40. Hiesberger T, Trommsdorff M, Howell BW, Goffinet A, Mumby MC, Cooper JA, Herz (1999) Direct binding of Reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation. *Neuron* 24:481-489.

41. Hirotsune S, Fleck MW, Gambello MJ, Bix GJ, Chen A, Clark GD, Ledbetter DH, McBain CJ, Wynshaw-Boris A (1998) Graded reduction of Pafah1b1 (Lis1) activity results in neuronal migration defects and early embryonic lethality. *Nat Genet* 19:333-339.

42. Hong SE, Shugart YY, Huang DT, Shahwan SA, Grant PE, Hourihane JO, Martin ND, Walsh CA (2000) Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. *Nat Genet* 26:93-96.

43. Hourihane JO, Bennett CP, Chaudhuri R, Robb SA, Martin ND (1993) A sibship with a neuronal migration defect, cerebellar hypoplasia and congenital lymphedema. *Neuropediatrics* 24:43-46.

44. Howell BW, Gertler FB, Cooper JA (1997) Mouse disabled (mDab1): a Src binding protein implicated in neuronal development. *Embo J* 16:121-132.

45. Husmann K, Faissner A, Schachner M (1992) Tenascin promotes cerebellar granule cell migration and neurite outgrowth by different domains in the fibronectin type III repeats. *J Cell Biol* 116:1475-1486.

46. Kato M, Dobyns WB (2003) Lissencephaly and the molecular basis of neuronal migration. *Hum Mol Genet* 12 (suppl 1):R89-96.

47. Kerner B, Graham JM Jr, Golden JA, Pepkowitz SH, Dobyns WB (1999) Familial lissencephaly with cleft palate and severe cerebellar hypoplasia. *Am J Med Genet* 87:440-445.

48. Kitagawa M, Umezu M, Aoki J, Koizumi H, Arai H, Inoue K (2000) Direct association of LIS1, the lissencephaly gene product, with a mam-

malian homologue of a fungal nuclear distribution protein, rNUDE. *FEBS Letters* 479:57-62.

49. 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 (2002) Mutation of ARX causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. *Nat Genet* 32:359-369.

50. Klinghoffer RA, Sachsenmaier C, Cooper JA, Soriano P (1999) Src family kinases are required for integrin but not PDGFR signal transduction. *Embo J* 18:2459-2471.

51. Lambert de Rouvroit C, Goffinet AM (1998) A new view of early cortical development. *Biochem Pharmacol* 56:1403-1409.

52. Lowell CA, Soriano P (1996) Knockouts of Src-family kinases: stiff bones, wimpy T cells, and bad memories. *Genes Dev* 10:1845-1857.

53. Manser E, Huang HY, Loo TH, Chen XQ, Dong JM, Leung T, Lim L (1997) Expression of constitutively active alpha-PAK reveals effects of the kinase on actin and focal complexes. *Mol Cell Biol* 17:1129-1143.

54. Matsumoto N, Leventer RJ, Kuc JA, Mewborn SK, Dudlicek LL, Ramocki MB, Pilz DT, Mills PL, Das S, Ross ME, Ledbetter DH, Dobyns WB (2001) Mutation analysis of the DCX gene and genotype/phenotype correlation in subcortical band heterotopia. *Europ J Hum Genet* 9:5-12.

55. Morris SM, Albrecht U, Reiner O, Eichele G, Yu-Lee LY (1998) The lissencephaly gene product Lis1, a protein involved in neuronal migration, interacts with a nuclear movement protein, NudC. *Curr Biol* 8:603-606.

56. Niethammer M, Smith DS, Ayala R, Peng J, Ko J, Lee MS, Morabito M, Tsai LH (2000) NUDEL is a novel Cdk5 substrate that associates with LIS1 and cytoplasmic dynein. *Neuron* 28: 697-711.

57. Nikolic M, Chou MM, Lu W, Mayer BJ, Tsai LH (1998) The p35/Cdk5 kinase is a neuron-specific Rac effector that inhibits Pak1 activity. *Nature* 395:194-198.

58. Noctor SC, Flint AC, Weissman TA, Wong WS, Clinton BK, Kriegstein AR (2002) Dividing precursor cells of the embryonic cortical ventricular zone have morphological and molecular characteristics of radial glia. *J Neurosci* 22:3161-3173.

59. Norman MG et al (1995) *Congenital Malformations of the Brain: Pathological, Embryological, Clinical, Radiolological and Genetic Aspects.* New York:Oxford University Press.

60. Ogawa M, Miyata T, Nakajima K, Yagyu K, Seike M, Ikenaka K, Yamamoto H, Mikoshiba K (1995) The reeler gene-associated antigen on Cajal-Retzius neurons is a crucial molecule for laminar organization of cortical neurons. *Neuron* 14:899-912.

61. Ohshima T, Ward JM, Huh CG, Longenecker G, Veeranna, Pant HC, Brady RO, Martin LJ, Kulkarni AB (1996) Targeted disruption of the cyclin-dependent kinase 5 gene results in abnormal corticogenesis, neuronal pathology and perinatal death. *Proc Natl Acad Sci U S A* 93: 11173-11178.

62. Ono K, Tomasiewicz H, Magnuson T, Rutishauser U (1994) N-CAM mutation inhibits tangential neuronal migration and is phenocopied by enzymatic removal of polysialic acid. *Neuron* 13:595-609.

63. Pilz DT, Kuc J, Matsumoto N, Bodurtha J, Bernadi B, Tassinari CA, Dobyns WB, Ledbetter DH (1999) Subcortical band heterotopia in rare affected males can be caused by missense mutations in DCX (XLIS) or LIS1. *Hum Mol Genet* 8:1757-1760.

64. Pilz DT, Matsumoto N, Minnerath S, Mills P, Gleeson JG, Allen KM, Walsh CA, Barkovich AJ, Dobyns WB, Ledbetter DH, Ross ME (1998) LIS1 and XLIS (DCX) mutations cause most classical lissencephaly, but different patterns of malformation. *Hum Mol Genet* 7:2029-2037.

65. Pinard JM, Motte J, Chiron C, Brian R, Andermann E, Dulac O (1994) Subcortical laminar heterotopia and lissencephaly in two families: a single X linked dominant gene. *J Neurol Neurosurg Psychiatry* 57:914-920.

66. Rakic, P 1972 Mode of cell migration to the superficial layers of fetal monkey neocortex. *J Comp Neurol* 145:61-83.

67. Rakic, P (1990) Principles of neural cell migration. *Experientia* 46:882-891.

68. Ramon y Cajal S (1929) *Ètudes sur la neurogenesis de quelques vertebres.* Translated by Guth L, 1960, Springfield Ill: CC Thomas.

69. Reiner O, Carrozzo R, Shen Y, Wehnert M, Faustinella F, Dobyns WB, Caskey CT, Ledbetter DH (1993) Isolation of a Miller-Dieker lissencephaly gene containing G protein beta-subunit-like repeats. *Nature* 364:717-721.

70. Rio C, Rieff HI, Qi P, Khurana TS, Corfas G (1997) Neuregulin and erbB receptors play a critical role in neuronal migration. *Neuron* 19: 39-50.

71. Sabry MA, al Saleh Q, Farah S, Obenbergerova D, Simeonov S, al Awadi SA, Farag TI (1997) Another Arab patient with overlap of Varadi-Papp/Opitz trigonocephaly syndromes? *Am J Med Genet* 68:54-57.

72. Sasaki S, Shionoya A, Ishida M, Gambello MJ, Yingling J, Wynshaw-Boris A, Hirotsune S (2000) A LIS/NUDEL/cytoplasmic dynein heavy chain complex in the developing and adult nervous system. *Neuron* 28:681-696.

73. Scheffer IE, Wallace RH, Phillips FL, Hewson P, Reardon K, Parasivam G, Stromme P, Berkovic SF, Gecz J, Mulley JC (2002) X-linked myoclonic epilepsy with spasticity and intellectual disability: mutation in the homeobox gene ARX. *Neurology* 59:348-356.

74. Senzaki K, Ogawa M, Yagi T (1999) Proteins of the CNR family are multiple receptors for Reelin. *Cell* 99:635-647.

75. Sheldon M, Rice DS, D'Arcangelo G, Yoneshima H, Nakajima K, Mikoshiba K, Howell BW, Cooper JA, Goldowitz D, Curran T (1997) Scrambler and yotari disrupt the disabled gene and produce a reeler-like phenotype in mice. *Nature* 389:730-733.

76. Stitt T, Hatten M (1990) Antibodies that recognize astrotactin block granule neuron binding to astroglia. *Neuron* 5:639-649.

77. Stromme P, Mangelsdorf ME, Shaw MA, Lower KM, Lewis SM, Bruyere H, Lutcherath V, Gedeon AK, Wallace RH, Scheffer IE, Turner G, Partington M, Frints SG, Fryns JP, Sutherland GR, Mulley JC, Gecz J (2002) Mutations in the human ortholog of Aristaless cause X-linked mental retardation and epilepsy. *Nat Genet* 30: 441-445.

78. Thomas SM, Brugge JS (1997) Cellular functions regulated by Src family kinases. *Ann Rev Cell Dev Biol* 13:513-609.

79. Toyo-oka K, Shionoya A, Gambello MJ, Cardoso C, Leventer R, Ward HL, Ayala R, Tsai LH, Dobyns W, Ledbetter D, Hirotsune S, Wynshaw-Boris A (2003) 14-3-3epsilon is important for neuronal migration by binding to NUDEL: a molecular explanation for Miller-Dieker syndrome. *Nat Genet* 34:274-285.

80. Trommsdorff M, Gotthardt M, Hiesberger T, Shelton J, Stockinger W, Nimpf J, Hammer RE, Richardson JA, Herz J (1999) Reeler/Disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2. *Cell* 97:689-701.

81. Zheng C, Heintz N, Hatten M (1996) CNS gene encoding astrotactin, which supports neuronal migration along glial fibers. *Science* 272:417-419.

82. Zukerberg LR, Patrick GN, Nikolic M, Humbert S, Wu CL, Lanier LM, Gertler FB, Vidal M, Van Etten RA, Tsai LH (2000) Cables links Cdk5 and c-Abl and facilitates Cdk5 tyrosine phosphorylation, kinase upregulation, and neurite outgrowth. *Neuron* 26:633-646.

83. Vallee RB, Faulkner NE, Tai C (2000) The role of cytoplasmic dynein in the human brain developmental disease lissencephaly. *Biochim Biophys Acta* 1496:89-98.

84. Wynshaw-Boris A, Gambello MJ (2001) LIS1 and dynein motor function in neuronal migration and development. *Genes Dev* 15:639-651.

85. Xiang X, Osmani AH, Osmani SA, Xin M, Morris NR (1995) NudF, a nuclear migration gene in Aspergillus nidulans, is similar to the human LIS-1 gene required for neuronal migration. *Mol Biol Cell* 6:297-310.