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# Genetic Malformations of the Human Cerebral Cortex

Review

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If flies had a cerebral cortex, figuring out how it developed would be relatively simple. You could perform deliberate, random mutagenesis and screen flies for cortical abnormalities behaviorally, and you could examine them anatomically. You could group mutant flies according to their cortical phenotype, since different genes that cause similar phenotypes probably encode proteins that relate somehow in a biochemical pathway. Finally, you could order the mutated genes into epistatic relationships by performing specific breeding experiments.

Although the genetics of humans is not as malleable as that of the fly, millions of unfortunate individuals who suffer from cortical malformations attest to the fact that Nature has already performed widespread random mutagenesis on the human brain, producing a rich variety of mutations that disrupt cortical development in specific and surprising ways. Moreover, humans live under essentially constant behavioral screening of the cortex—we call it school, work, and play. Therefore, even subtle anomalies manifested by a single seizure or a mild learning disorder are usually detected. Rapid, noninvasive imaging of the human cortex (e.g., magnetic resonance imaging [MRI]) can link behavioral phenotypes to gross anatomical anomalies in a little more time than it takes to examine the fly's CNS. Although humans were formerly considered difficult subjects for positional cloning, technical limitations are rapidly vanishing. With genetic maps of ever-higher density, gene identification from small pedigrees or even single individuals with informative chromosomal rearrangements has become possible. But the richness of the genetics remains the cardinal feature of the human brain: human cortical malformations are frequently "genetically heterogeneous," meaning that more than one gene can cause a similar or identical phenotype when mutated. This property, formerly regarded as a major technical nuisance (because it complicates gene mapping studies), now appears as a golden opportunity to sketch out genetic pathways that can be fleshed out by in vitro studies and animal models.

Like any mutational screen, the one that affects humans is probably not complete. It is biased toward dominant and X-linked traits, and against recessives, given the common Western societal restrictions against consanguineous marriages. However, other major populations prefer consanguineous marriages for cultural reasons, allowing even autosomal recessive disorders to be seen with surprising frequency. The human mutational screen also is biased against detecting embryonic lethal phenotypes, but not as severely as might be expected because of the unique size of the human brain. Since cells in the human cortex undergo so many rounds of mitosis, spontaneous somatic mutations that produce mosaic brains of normal and mutant cells appear to be surprisingly common and allow analysis of otherwise lethal mutations in naturally occurring mosaics.

This review outlines human genetic disorders that produce a morphologically abnormal cerebral cortex. Other disorders are associated with a grossly normal cortex that nonetheless does not function properly—such as inherited epilepsy or mental retardation—or disturb the development of CNS glia, but these disorders are not covered for space reasons. I will also focus primarily on disorders in which the genetics and pathology are reasonably well characterized, rather than on disorders—such as schizophrenia—for which morphological disorders of the cortex have been reported but which are less cut and dried. Since this review provides a brief overview of a vast literature, I apologize at the outset for much important work that cannot be cited because of space constraints.

## Phenotypes and Nomenclature

The most common result of severe cortical malformations is profound mental impairment, epilepsy, and limb paralysis. It is therefore surprising that some individuals with dramatic brain malformations have remarkably normal intelligence and are detected only because of their epilepsy. Overall, morphological abnormalities of the cortex account for a substantial fraction (5%–15%) of epilepsy in adults (Hardiman et al., 1988; Brodtkorb et al., 1992; Hauser et al., 1993) and a higher proportion of epilepsy in children. However, the types of malformations seen are highly variable in any given series of patients studied, because the malformations are heterogeneous genetically, and because each malformation is individually rare (typically occurring in <1/10,000 people). Consequently, collection of sufficient numbers of patients with a specific malformation for genetic studies typically requires widespread, international collaboration.

The mechanisms of genes that cause human malformations have traditionally been roughly inferred from the locus of the malformation, but this information is increasingly being supplanted by more complete analysis in mouse models. Since neurons destined for the cortex are formed from progenitor cells located deep in the brain, along the lateral ventricles, malformations that produce collections of cells in the ventricular zone are interpreted as reflecting genes involved in cell proliferation and cell fate, particularly if the abnormally located cells also appear morphologically abnormal. Since neurons subsequently migrate from the ventricular zone to the cortex, there are a number of disorders that cause the accumulation of neurons at a variety of locations between the ventricular zone and the cortex and thus are thought to disturb neuronal migration. Although terms such as "neuronal migration disorder" are only a first approximation when discussing human disorders,

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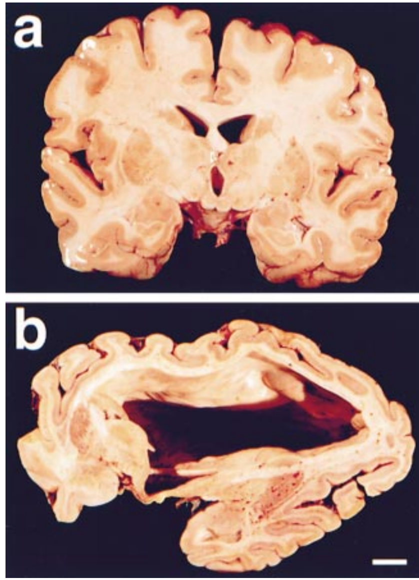


Figure 1. Human Holoprosencephaly

The photographs show slices of a normal brain (a) and a case of HPE (b). The slices are taken in the coronal or frontal plane. In HPE, the normal two hemispheres are replaced by a single hemisphere with a massively enlarged single midline ventricle and variable fusions of the ventral telencephalon. Scale bar, 1 cm.

they are nonetheless common parlance. Unfortunately, many human syndromes were first described a century ago by pathologists, before genetics or neurobiology was well established. Hence, terminology is generally imprecise and in Greek, with the classification of many cortical disorders relying on descriptions of the alterations in the patterns of gyri and sulci, since these are most readily visible radiographically or at postmortem examination. However, since the advent of disease gene cloning, it has been recognized that there is an imperfect correlation between abnormal patterns of gyration and specific histological structures or genetic conditions: mutations in the same gene can cause a range of gyral abnormalities, and a given gyral pattern can be caused by more than one gene. Recently, efforts have been made to systematize the classification of cortical malformations (Barkovich et al., 1996). The classifications used in this article should not be considered definitive. Classifications will improve as better morphological information becomes available, and as genes underlying specific conditions become identified and provide clearer insight for use in mechanistic classifications.

### Disorder of Pattern Formation in the Forebrain: Holoprosencephaly

During normal development, the early forebrain (a.k.a. prosencephalon) divides and gives rise to the two cerebral hemispheres. In holoprosencephaly (HPE), there is failure of the normal midline separation of the two hemispheres resulting in a single forebrain ventricle and a single forebrain. There is continuity of the gray matter of the two cerebral hemispheres across the midline (Figures 1a and 1b). Holoprosencephaly is not rare by the standards of a genetic disease, affecting 0.58–1.2/10,000 births (Rasmussen et al., 1996). At least 12 chromosomal regions on 11 chromosomes have been implicated as HPE loci by either chromosomal anomalies or in some cases by linkage analysis (Golden, 1998; Ming and Muenke, 1998), suggesting the potential for a remarkable genetic dissection of a dramatic morphogenetic event.

So far, two HPE genes have been identified in humans, and there is preliminary evidence for three more. *HPE3* (chromosome 7q36) is caused by heterozygous mutations in the *sonic hedgehog* gene (*SHH*) (Roessler et al., 1996). *SHH* encodes a secreted protein required for ventral induction throughout the neuraxis, as well as for positional specification in the limb and elsewhere (Goodrich and Scott, 1998). Engineered mutations in *Shh* in mice show a remarkably similar holoprosencephalic phenotype in homozygotes (Chiang et al., 1996), though unlike the human phenotypes there is no heterozygote phenotype in mice. Other HPE genes are likely to be involved in *SHH* signaling, and preliminary evidence suggests that one such gene is *PATCHED*, which encodes a likely receptor and downstream target for SHH protein (Ming et al., 1998, Am. J. Hum. Genet., abstract). SHH protein requires cholesterol esterification to have normal activity. An animal toxin that interferes with this steroid modification causes HPE in animals (Golden, 1998). Moreover, at least one other human disorder of cholesterol metabolism, the Smith-Lemli-Optiz syndrome, is associated with HPE (Kelley et al., 1996) and has been postulated to interfere with the steroid modification required for normal SHH signaling.

Whereas the known properties of *SHH* made it an obvious candidate gene for HPE, genetics offers the advantage of allowing one to identify unexpected genes with the same phenotype. A second HPE locus on chromosome 13q32 has recently been shown to reflect mutations in *ZIC2*, a human homolog of the *Drosophila odd-paired* gene (Brown et al., 1998) that encodes a homeodomain-containing transcription factor. *ZIC2* is preferentially expressed in the dorsal region of the neural tube and in the developing extremity and defines the fates of neural cells in cooperation with the transcription factor Gli3 (Brown et al., 1998). Thus, although it can be imagined that ZIC2 operates somewhere downstream of SHH, it certainly provides a new entry point into the molecular specification of the forebrain. Other HPE genes will likely produce additional surprises: there are preliminary reports of two additional HPE genes—the transcription factors *SIX3* and *TGIF*—that are not known to be targets of SHH (Gripp et al., 1998, Am. J. Hum. Genet., abstract; Wallis et al., 1998, Am. J. Hum. Genet., abstract).

The division of the cerebral hemispheres along the dorsal midline is a complicated process about which little is known. Members of the bone morphogenetic protein (BMP) superfamily are critical for dorsal specification in other regions of the neuraxis (Hogan, 1996; Liem et al., 1997), and specific BMPs (e.g., BMP4 and BMP6) are expressed at high levels in the dorsal forebrain (Furuta et al., 1997), among the dorsal cells that invaginate along the dorsal midline to separate the hemispheres. The cells of the dorsal midline of the forebrain invaginate ventrally, separating the hemispheres, and then invaginate laterally into the medial walls of the two

hemispheres to form the choroid plexus, a specialized vascular structure within the adult lateral ventricles that secretes the cerebral spinal fluid. Thus, these midline cells undergo widespread migration, rearrangement, and repression of neural fates, and BMPs seem to be likely candidates to mediate many of these effects, given their known roles in dorsal patterning and the repression of neural differentiation. It is somewhat paradoxical about HPE that the defects in ventral specification that produce the midline defects and cyclopia also result in a failure of the dorsal invagination and separation of the two cerebral hemispheres. Perhaps *ZIC2* is especially important to this dorsal invagination.

## Disorders of Cell Fate, Proliferation, and Specification

### *Schizencephaly*

Schizencephaly represents a cleft, unilateral or bilateral, extending from the pial surface of the cortex all the way to the ventricular surface (Barkovich and Kjos, 1992b). The term is used more commonly in the radiographic literature, while neuropathologists prefer the term porencephaly, to refer to essentially an absence of cortex where cortex belongs (Friede, 1975). Typically, the sides of the cleft are lined with cortex that contains numerous small gyri, or "polymicrogyria" (Figures 2a and 2b). Schizencephaly/porencephaly is quite variable and is undoubtedly causally heterogeneous. Some cases show an otherwise fairly normal cortex and mild clinical symptoms (usually seizures or cognitive difficulties). In other cases, there is a generalized malformation of the cortex accompanied by severe mental retardation and seizures (Barkovich and Kjos, 1992b). Schizencephaly was classically regarded as a vascular condition, perhaps reflecting in utero vascular insufficiency (Barkovich and Kjos, 1992b), and there is very convincing evidence for a vascular cause of some cases of schizencephaly (Friede, 1975). However, the first identification of mutations in the *EMX2* gene in humans with clinically severe schizencephaly brought a surprise (Brunelli et al., 1996). *EMX2* is a human homolog of the fly gene *empty spiracles*, which encodes a homeodomain-containing protein that is preferentially expressed in the developing cerebral cortex and is required for normal development of the cortex in mice (Pellegrini et al., 1996). Since *EMX2* mutations are not seen in most cases of schizencephaly, it is not known whether other genes expressed in cortical or vascular progenitor cells also cause schizencephaly, or whether nongenetic vascular mechanisms account for other cases of schizencephaly.

### *Tuberous Sclerosis*

The term tuberous sclerosis (TSC) was coined to describe lesions of the brain that somehow resembled potato tubers in their gross consistency (Figure 3a). Tuberous sclerosis is a dominantly inherited multiorgan disorder with a high rate of spontaneous mutations. Tumors, cysts, and other malformations (called hamartomas) occur in many tissues, including kidney, bone, skin, and heart (Kwiatkowski and Short, 1994). In the brain, there are two striking malformations. In the cortical gray matter, there are "cortical tubers," otherwise called focal dysplasias (Figure 3a), in which the normal six-layered cortex is thickened and disordered ("dysplastic"), characterized by very large, filament-rich neurons and bizarre, enlarged glia-like cells. The dysplasias also contain giant cells referred to as "balloon cells"

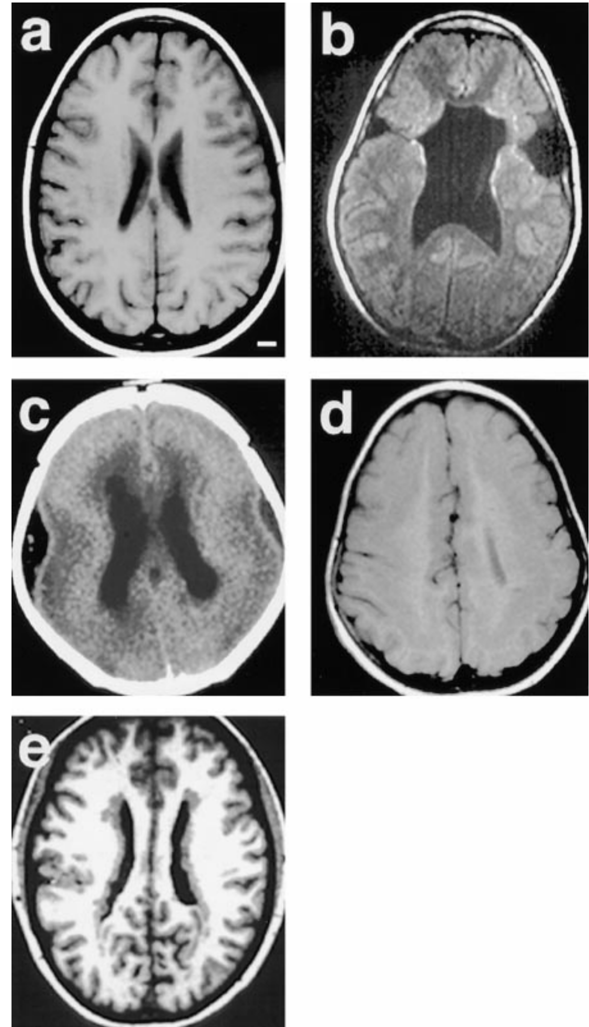


Figure 2. MRI of Malformations of the Human Cortex

The photographs show MRI images of humans taken with standard settings typically used in clinical practice, in order to illustrate the sensitivity of MRI to reveal anatomical abnormalities of the human brain. The images are each taken in the "axial" plane, with the plane and level illustrated being roughly equivalent to a slice taken through the head above the ears, parallel to the brim of a hat on one's head. A slice through a normal cortex is shown in (a), showing the dark ventricles deepest beneath the cortex, with the white matter above that and the thin cortical gray matter, which is arranged in folds (gyri). A case of schizencephaly is shown in (b) and is defined by the deep clefts that run from the outside of the cortex into the ventricles. This MRI was taken with slightly different settings than in (a), so the colors are a little different. A patient with classical lissencephaly is shown in (c) (courtesy of M. Berg) to illustrate the complete lack of the usual cortical folds. A case of double cortex is shown in (d) (courtesy of G. Holmes) and contains a second layer of gray matter embedded within the white matter beneath the normal cortex. The case of periventricular heterotopia in (e) shows a fairly normal gyral pattern but accumulations of gray matter along the ventricles bilaterally. The text contains explanations and definitions of the conditions. Scale bar in (a), 1 cm.

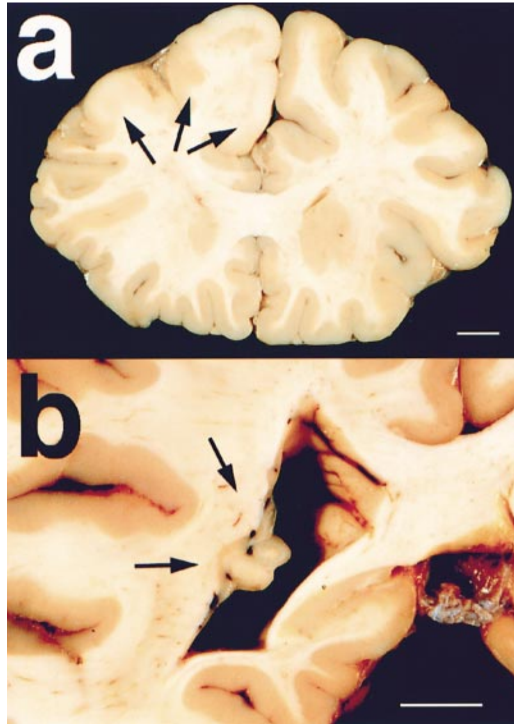


Figure 3. The Cerebral Cortical Lesions of Tuberous Sclerosis

The photograph in (a) shows a sectioned human brain (similar to Figure 1a in the plane of section, though at a slightly different level of section) showing large cortical tubers (arrows) in which the normal distinction between gray matter (which appears brown in this fixed brain) and white matter is lost, due to the aberrant cell types in the lesion. The gyri containing the tubers, or focal dysplasias, are enlarged. The photograph in (b) shows a subependymal nodule, consisting of poorly differentiated neural cells often protruding into the ventricular region. These nodules can transform into tumors. Photos courtesy of J. Joseph. Scale bar in (a), 1 cm; in (b), 400 μm.

that stain for both neuronal and glial markers and that seem to be of uncertain lineage (Vinters et al., 1992). Beneath the cortex, the brain in tuberous sclerosis also shows nodular collections of small cells along the surface of the lateral ventricle that resemble ventricular cells and are called subependymal nodules (Figure 3b) or, more descriptively, "candle drippings." These nodules often contain numerous balloon cells and can in some cases become transformed into glial tumors referred to as subependymal giant cell astrocytomas (Kwiatkowski and Short, 1994).

The tuberous sclerosis hamartomas and tumors outside of the nervous system appear to arise through a tumor suppresser gene model, with the lesions reflecting inheritance of a germ-line TSC mutation from one parent, combined with the spontaneous loss of the second TSC allele in the clonal cells of each lesion (Green et al., 1994; Henske et al., 1995). However, while the same "two-hit" mechanism, which was first developed by Knudson for retinoblastoma, appears to hold for the subependymal astrocytomas of the brain, there is no evidence for loss of both TSC alleles in tubers or subependymal nodules either in human tuberous sclerosis (Henske et al., 1996; Wolf et al., 1997) or in the Eker rat, which carries a spontaneous mutation in the *TSC2* gene (Yeung et al., 1997). Presumably, the brain malformations represent some sort of second event after inheritance of a germline TSC mutation, but the type of second event that occurs is still not certain.

A very similar clinical and anatomical picture of tuberous sclerosis is caused by mutations in two different, nonlinked genes, and both of them have now been cloned. The *TSC2* gene was cloned some years ago, though its function is still not completely understood (European Chromosome 16 Tuberous Sclerosis Consortium, 1993). The *TSC2* gene encodes a widely expressed protein called tuberin, which contains a domain that has GAP activity for Rap1, a small G protein that relays membrane signals to the MAP kinase pathway (Wienecke et al., 1995). Tuberin has also been implicated as a GAP for Rab5 (Xiao et al., 1997) and as a potential transcriptional coregulator (Henry et al., 1998). The defects in cell type specification that characterize tuberous sclerosis lesions, as well as the tendency of some tuberous sclerosis lesions to transform into tumors, potentially implicates tuberin in regulating cell proliferation or cell fate specification, though very little is known about how tuberin might do this. Very recently, a fairly close TSC2 homolog in flies, encoded by the *gigas* gene, has been identified (Ito and Rubin, 1999). In *gigas* mutant flies, cells undergo DNA replication in the absence of mitosis, forming giant, multiploid cells and disturbing the normal morphogenesis of the retina. The giant neural cells in tuberous sclerosis dysplasias have been suggested to represent similar multiploid cells due to defective cell cycle replication (Ito and Rubin, 1999). The *TSC1* gene on chromosome 9 causes a clinically very similar condition, but encodes a large novel protein, called hamartin, whose function is even less well understood (van Slegtenhorst et al., 1997) but for which a good fly homolog also exists (Ito and Rubin, 1999).

Focal cortical dysplasias (FCDs) are actually encountered more frequently as single lesions in individuals with no other signs or symptoms of tuberous sclerosis than in the setting of tuberous sclerosis. The sporadic FCDs show a range of pathologies, but many closely resemble tuberous sclerosis lesions, leading to the suggestion by Andermann that they represent a "forme fruste" of tuberous sclerosis (Andermann et al., 1987). Sporadic FCDs may reflect one or more spontaneous mutations at the TSC and/or other loci, especially since the TSC genes are large and subject to high rates of spontaneous mutation; however, this suggestion is unproven.

### Disorders of Neuronal Migration

For mice, neuronal migration defects can only be demonstrated by studying neurogenesis and migration directly, and hence the term is used loosely when applied to human diseases where the same level of analysis is not possible. The best-characterized mouse neuronal migration disorders are the *reeler* (Caviness and Sidman, 1973) and *mdab1* (Gonzalez et al., 1997; Howell et al., 1997; Sheldon et al., 1997; Ware et al., 1997) mutants, and the engineered mutations in *cdk-5* (Ohshima et al., 1996; Gilmore et al., 1998) and its regulator, *p35* (Chae et al., 1997). There are, however, a large group of human disorders that are most consistent with disorders of

migration, and they frequently present radiographically with associated disorders of the gyral pattern of the cortex.

### *What Is Lissencephaly?*

Lissencephaly, meaning literally "smooth brain," refers to a genetically, clinically, radiographically, and histologically heterogeneous group of conditions that all are manifested radiographically by a simplification or complete loss of the gyri and sulci that characterize the normal human brain (Figure 2c). Agyria ("no gyri"), a related term, is used roughly synonymously, whereas pachygyria refers to gyri that are both reduced in number and unusually thick, often ten times thicker than normal, producing a cortex that is quite small in surface area but not obviously reduced in neuronal numbers. The abnormalities of gyration appear to be a secondary and nonspecific consequence of widespread cortical malformation and are easily apparent radiographically (Figure 2c). Although the gyral pattern is frequently used as a marker for the migrational disturbance, the relationship is quite imprecise. There are at least two relatively common and distinct histological patterns of lissencephaly: "classical" lissencephaly, also known as Type I or Bielschowsky type; and "cobblestone" lissencephaly, also known as Type II. However, there are probably additional histological patterns associated with lissencephaly that are not as well characterized.

### *Isolated Lissencephaly Sequence*

The most common genetic cause of classical lissencephaly is gross disruption of a gene on chromosome 17p13 called *LIS1*, or more properly *PAFAH1B1* (Reiner et al., 1993). *PAFAH1B1* mutations cause a range of severity of lissencephaly referred to as the "isolated lissencephaly sequence" (Dobyns et al., 1993). Most affected patients undergo spontaneous, heterozygous deletions of *PAFAH1B1*, or larger deletions encompassing adjacent genes, causing a syndrome of multiple congenital anomalies called the Miller-Dieker syndrome (Dobyns et al., 1993). Point mutations have also been described in *PAFAH1B1* with a similar phenotype (Lo Nigro et al., 1997). *PAFAH1B1* mutations account for about 50%–60% of cases of lissencephaly in the United States (Pilz et al., 1998). The disorder typically affects the cortex and hippocampus most severely, with the cerebellum being relatively spared; however, most parts of the brain are in some way affected (Dobyns et al., 1993). The cortex in classical lissencephaly shows a well-formed pial surface and marginal zone, with a subjacent layer of pyramidal cells that likely corresponds to some of the cells of the deeper layers of the cortex and/or subplate; beneath the pyramidal layer is a narrow, cell-poor zone, and then a dense, very deep jumble of neurons below (Dobyns, 1987; Dobyns et al., 1993). Unlike most of the mouse neuronal migration disorders in which cortical function can be remarkably spared, affected children with lissencephaly are usually profoundly crippled neurologically, with little meaningful cognitive abilities or neurological development in the most severe cases.

The mouse homolog of *PAFAH1B1* has recently been mutated, and the mice show defects that are considerably more complex than a mere migrational disturbance (Hirotsune et al., 1998). Mice heterozygous for a complete loss-of-function mutation show a very subtle defect in cortical development, much milder than heterozygous humans. Mice with homozygous strong loss-of-function alleles are lethal at early embryonic ages, whereas compound heterozygotes with incomplete loss-of-function mutations show neonatal lethality with severe migrational, architectural, and perhaps proliferative abnormalities of the cortex (Hirotsune et al., 1998). Thus, *PAFAH1B1*, which is widely expressed in all tissues and which is expressed in dividing as well as migrating neural cells, may be required at many stages of neuronal development.

What is the cellular role of *PAFAH1B1*? *PAFAH1B1* encodes a protein containing multiple WD40 repeats (stereotyped tryptophan and aspartate residues at intervals of 40 amino acids) that form domains allowing potentially extensive protein–protein interactions (Reiner et al., 1993). After its identification in *LIS1*, *PAFAH1B1* was reidentified as a noncatalytic subunit of platelet activating factor (PAF) acetylhydrolase—hence, the use of the gene name *PAFAH1B1*. PAF acetylhydrolase is the major degradative enzyme for PAF, a bioactive lipid that is involved in regulating the shape and function of platelets and that appears to mobilize neuronal calcium (Hattori et al., 1994). However, PAFAH1B1 was later isolated independently as a regulatory protein that binds the activated form of a tyrosine kinase related to Syk (Brunati et al., 1996). Which of these multiple, seemingly distinct, functional interactions of *PAFAH1B1* are the critical ones directing neuronal migration are not yet certain but are likely to be most easily sorted out by the functional connection of *PAFAH1B1* to other genes required for migration.

The evolutionary conservation of *PAFAH1B1* is remarkable, and studies in nonmammalian organisms suggest a role in the translocation of the nucleus via a microtubule-based mechanism. *PAFAH1B1* has a convincing ortholog in *Aspergillus nidulans*, called *nudF* (Xiang et al., 1995), that is required for translocation of the nucleus along an elongated cellular process called the mycelium (Morris et al., 1998a). *nudF* interacts with *nudA*, which encodes the heavy chain of cytoplasmic dynein, a microtubule motor protein implicated in retrograde organelle transport in neurons (Hirokawa et al., 1990). *nudF* also interacts with *nudC* and *nudE*, which encode novel proteins. A vertebrate homolog of *nudC* (Morris et al., 1998b) has been independently identified as encoding a LIS1-interacting protein in a two-hybrid screen and is highly expressed in the developing brain (Morris et al., 1998b). These data suggest that PAFAH1B1 may represent a link to the microtubule network in neurons. Moreover, there is direct evidence that PAFAH1B1 can be precipitated with microtubules and can increase microtubule stability (Sapir et al., 1997). Once again, analysis of *Aspergillus*, or study of fly homologs of *PAFAH1B1*, may provide genetic systems for the cellular analysis of *PAFAH1B1* function.

### *X-Linked Lissencephaly and the Double Cortex Syndrome*

Another, X-linked locus causes classical lissencephaly in males that is virtually indistinguishable from *PAFAH1B1* mutations, and the responsible gene was mapped and recently cloned (des Portes et al., 1998; Gleeson et al., 1998). Females with mutations in this X-linked lissencephaly gene, abbreviated *DCX*, show a remarkable

malformation called "double cortex" or (more commonly in the clinical literature) subcortical band heterotopia (Figure 2d) or subcortical laminar heterotopia (Barkovich et al., 1989, 1994). The females by definition represent a mosaic state, since brain cells in the female normally inactivate one or the other X chromsome during development. It is natural to assume that the normal cortex contains cells that express genes from the normal X chromosome and that the abnormal band contains cells expressing the mutant X chromosome. However, that has not been directly shown.

*DCX* encodes a protein, called doublecortin, without known enzymatic activity. DCX protein is expressed intensely in all migrating neurons in the developing brain and is persistently expressed only in those few regions of the adult brain that undergo continuing migration, such as the olfactory bulb (J. G. Gleeson, P. Lin, L. Flanagan, and C. A. W., unpublished data). DCX protein appears to localize in the soma of migrating neurons, forming a meshwork in the cytoplasm of the soma that resembles the "cages" of microtubules previously described by Hatten and coworkers (Gregory et al., 1988; Rivas and Hatten, 1995). Like PAFAH1B1, DCX can be precipitated with microtubules from brain. Moreover, DCX dramatically stimulates microtubule polymerization in a dose-dependent fashion (J. G. Gleeson, P. Lin, L. Flanagan, and C. A. W., unpublished data). The interaction of both *DCX* and *PAFAH1B1* with microtubules suggests that the very similar phenotype caused by mutations in these two genes reflects a common or linked mode of action (Gleeson et al., 1998) on microtubule dynamics.

The amino acid sequence of DCX is also intriguing because of a potential biochemical connection between human lissencephalies and mouse neuronal migration disorders. DCX shows a consensus substrate site for c-Abl, a nonreceptor tyrosine kinase that is known to regulate cytoskeletal dynamics (Gleeson et al., 1998). Mutations in *disabled 1* cause abnormal neuronal migration in mice (Howell et al., 1997; Sheldon et al., 1997; Ware et al., 1997). The *disabled 1* gene encodes a c-Abl binding protein related to *Drosophila disabled*, which is so named because it interacts genetically with the fly *Abl* gene (Gertler et al., 1989). Although *disabled* mutations in mice do not have the same phenotype as *PAFAH1B1* mutations in mice, both mutants appear to be characterized by abnormal neuronal migration, suggesting possible interactions between the mouse and human mutants. While c-Abl or some Abl-like tyrosine kinase represents a possible biochemical link between Dab1 and DCX, it remains to be demonstrated that these proteins physically interact or function in concert.

***Other Lissencephalies***

Additional loci cause radiographic lissencephaly, although many of them have not been well characterized and may show histological patterns distinct from classical lissencephaly. Autosomal recessive loci associated with lissencephaly or pachygyria have been reported (Norman et al., 1976; Straussberg et al., 1996). Autosomal recessive lissencephaly or pachygyria in combination with profound congenital cerebellar hypoplasia has also been reported (Hourihane et al., 1993; Farah et al., 1997). Severe microlissencephaly with neonatal death represents a particularly severe, autosomal recessive disorder with a very small, misshapen brain and very poor survival, but it also has not been studied in detail (Barkovich et al., 1998). Zellweger syndrome is a disorder of the peroxisome that results in defective lipid metabolism and is characterized by widespread gyral disturbances, arrest of neuronal migration, and disturbances of myelination (Evrard et al., 1978; Barkovich and Peck, 1997). Therefore, potentially three to five additional lissencephaly loci exist that resemble classical lissencephaly or that arrest neurons in different ways, allowing for extensive genetic analysis of the guidance of migrating cortical neurons.

***Periventricular Heterotopia***

Whereas lissencephalies, as well as mouse mutations in *Reelin*, *mdab1*, *cdk5*, and *p35*, show some preserved migration (and instead are characterized by misdirected or incomplete migration), periventricular heterotopia reflects the complete failure of migration of some cell types and results in persistent accumulations of neurons ("heterotopias") in the periventricular region into adulthood (Figure 2e). Although these nodules are in the same approximate location as the nodules seen in tuberous sclerosis (and are frequently mistaken for them), the nodules in these two conditions are quite distinct radiographically and histologically. While the nodules in tuberous sclerosis contain undifferentiated "balloon" cells that most resemble glia, the nodules in periventricular heterotopia represent remarkably mature-appearing neurons of multiple size classes and showing well-developed dendrites (Eksioglu et al., 1996). Periventricular heterotopias are seen in several conditions, and there are likely to be multiple causative genes (Barkovich and Kjos, 1992a). However, the most common cause of periventricular heterotopia appears to be an X-linked gene that is dominant in females and lethal to most males.

The first pedigrees of periventricular heterotopia were only described in print in 1993 and 1994, following the widespread use of MRI in clinical practice (DiMario et al., 1993; Kamuro and Tenokuchi, 1993; Huttenlocher et al., 1994). Affected patients in all of these pedigrees were exclusively female and characterized by a shortage of male offspring and an excess of miscarriages, leading to the suggestion of an X-linked, dominant, male lethal gene. This suggestion was later confirmed by linkage analysis, which placed the gene in Xq28 (Eksioglu et al., 1996), and subsequent identification of the periventricular heterotopia gene as *filamin 1* (*FLN1*) (Fox et al., 1998). *FLN1* encodes a protein that has been critically implicated in control of cell shape, migration, filopodia formation, and chemotaxis (Matsudaira, 1994). Since Filamin 1 directly interacts with both receptors and actin itself, it forms a potential focus upon which the signaling molecules required for the guidance of migration may assemble.

***Kallmann Syndrome***

One of the most remarkable migrations in the developing brain takes place among neurons that ultimately secrete leuteinizing hormone-releasing hormone (LHRH) from the hypothalamus. These neurons are actually formed in the olfactory placode. The postmitotic neurons migrate from the placode up the nervus terminalis, enter the forebrain behind the olfactory bulb, and then migrate up the olfactory tract and ultimately into the hypothalamus

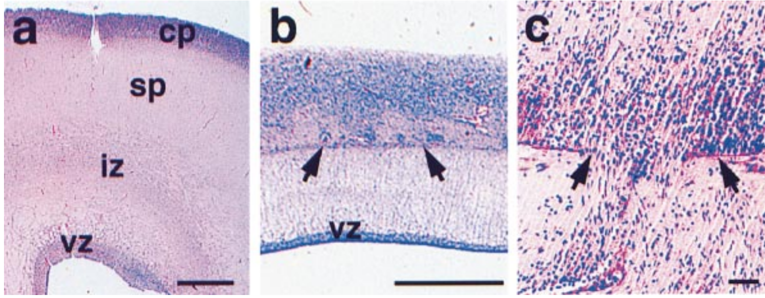


Figure 4. Cellular Appearance of Cobblestone (Type II) Lissencephaly

The photomicrographs contrast (a) a normal human fetal brain (22 weeks gestation) with the brain of a fetus of the same age with cobblestone lissencephaly due to Walker-Warburg syndrome (b and c). The brain in cobblestone lissencephaly (b) is less than half of normal thickness and lacks the clear stratification of the ventricular zone (vz), intermediate zone (iz), subplate (sp), and cortical plate (cp) seen in the normal brain (a). In both pictures, the ventricular surface is down and the pial surface is up. In (b), the location of the normal pial surface is indicated by arrows. The dark blue nuclei represent migrating cells that have penetrated the pia and flowed out randomly over the outer surface of the pia. The photomicrograph in (c) shows a higher power view of one of the defects in the pia, with immature neuroblasts migrating through it. Scale bar in (a) and (b), 1 mm; in (c), 100 $\mu$m.

(Wray et al., 1989). As might be expected, this complicated trajectory can be interrupted in several ways, causing congenital hypogonadism (since LHRH is necessary for normal gonadal function) in a syndrome called Kallmann syndrome. Kallmann syndrome is frequently associated with hypoplasia of the olfactory bulbs and olfactory cortex, called arhinencephaly, and lack of the sense of smell (Birnbacher et al., 1994; Quinton et al., 1996). An X-linked Kallmann syndrome gene, *KAL1*, has been cloned (Franco et al., 1991; Legouis et al., 1991). *KAL1* encodes a protein that contains several of the structural domains that characterize neural cell adhesion molecules, but very little is known about its action. Patients with *KAL1* mutations also show bizarre mirror-symmetric movements of their hands, and there is evidence that this reflects an aberrant projection from the ipsilateral motor cortex (Mayston et al., 1997), suggesting additional potential roles for the *KAL1* gene product in axon guidance. Additional autosomal recessive and autosomal dominant pedigrees have been reported, but linkage information is not available on these loci (Hermanussen and Sippell, 1985).

## Disorders of the Integrity of the Pial Surface

At least three other disorders, corresponding to at least two other genes, profoundly disturb neuronal migration and cause a smooth brain when viewed radiographically and are thus also referred to as lissencephaly. However, the histological architecture of this second form of lissencephaly, referred to as Type II lissencephaly or cobblestone lissencephaly (Dobyns and Truwit, 1995), is completely different from classical lissencephaly seen with *PAFAH1B1* or *DCX* mutations. In cobblestone lissencephaly, the entire structure of the cortical mantle is effaced (Figures 4a–4c). The precise location of the pial surface is difficult to ascertain because the pia is disrupted and discontinuous, but fragments of the pia can be found roughly halfway between the ventricular surface and the outermost cortical cells. This implies that most of the cortical neurons have actually migrated through the pial surface and essentially left the brain, migrating over the outer surface of the pia and forming piles of neurons that become confluent in a disordered mess that can be analogized to lava floes. Microscopically, the neurons in the cortical region show no evidence of lamination or organization. Other brain regions, especially the cerebellum, are also affected. This histological pattern can appear grossly as anything from a lissencephalic brain to other gyral disorders such as polymicrogyria, depending on the severity of the disruption. Similar migrational patterns among cortical neurons have been observed after simple disruption of the pial surface with needles (Rosen et al., 1992) or toxins, suggesting that the ultimate disorder in cobblestone lissencephaly is the stability of the pia.

### *Fukuyama Muscular Dystrophy*

Fukuyama congenital muscular dystrophy (FCMD) is a well-characterized, autosomal recessive condition in which relatively mild cobblestone lissencephaly is accompanied by a severe congenital muscle disorder as well (Fukuyama et al., 1981). It is seen most commonly in Japan and is rare elsewhere, and this difference in incidence appears to reflect a very strong founder effect in the Japanese population. The Japanese founder effect derives from a single allele present in most cases ascertained in Japan, representing an ancient retrotransposon insertion (Kobayashi et al., 1998). The gene maps to chromosome 9q31–33 (Toda et al., 1993) and was recently cloned (Kobayashi et al., 1998). The gene encodes a novel protein (called Fukutin) that appears to be a secreted protein. Fukutin's presence in the extracellular matrix provides a plausible model for how it may be necessary for stability of both muscle fibers and the pial surface of the cortex.

### *Muscle-Eye-Brain Disease*

Another cause of cobblestone lissencephaly that is typically accompanied by dysplasia of the retina and congenital myopathy, muscle-eye-brain disease (MEB) is especially commonly diagnosed in Finland, evidently representing another founder effect. MEB shows patterns of cobblestone lissencephaly and cerebellar malformation that are generally more severe and widespread than FCMD (Barkovich, 1998). Thought at one time to be allelic to FCMD because it is also similarly autosomally recessively inherited, linkage studies have excluded MEB from the region of the FCMD gene on 9q31–33 (Ranta et al., 1995), and MEB has recently been mapped to chromosome 1 (Cormand et al., 1999). The causative gene for MEB is unknown, but the similar histological patterns and similar involvement of both muscle and brain in MEB and FCMD suggest a possible functional link between their gene products.

### *Walker-Warburg*

Walker-Warburg syndrome (WWS) once again shows retinal abnormalities, congenital muscular dystrophy, and cobblestone lissencephaly that is generally the most severe and widespread of the three forms of cobblestone lissencephaly. Some patients have especially severe cerebellar malformation and defects of the skull as well (referred to as encephalocele) (Barkovich, 1998). Although no linkage analysis is available, WWS also appears to be autosomal recessive and may be allelic to either MEB or FCMD.

## Disorder of Less Certain Etiology: Symmetrical Polymicrogyria

Similar to schizencephaly, there is convincing evidence that another cortical malformation, polymicrogyria, can be caused by hypoxic-ischemic injury or by infections. Nonetheless, there is increasing evidence for inherited forms of polymicrogyria that are focal—i.e., do not involve the entire cortex—yet symmetrical. For example, patients have been described with polymicrogyria involving both frontal lobes or involving both perisylvian regions (Graff-Radford et al., 1986; Kuzniecky et al., 1994; Gropman et al., 1997). In addition, biparietal polymicrogyria (Guerrini et al., 1997) and bioccipital polymicrogyria (Ferrie et al., 1995) have all been observed. The disorders are frequently associated with mild to severe mental retardation and seizures, with additional symptoms determined by the location involved. Perisylvian polymicrogyria is sometimes associated with relatively selective dyslexia (Galaburda et al., 1985). Polymicrogyria has been reported in multiple members within a kindred (e.g., Ferrie et al., 1995), strongly suggesting a genetic cause and allowing genetic mapping studies. Whether the inherited forms of bilateral polymicrogyria represent one or more genes and whether they represent defects of proliferation, migration, or postmigratory architecture will only be clarified following cloning of the respective genes.

## Conclusions

Genetic analysis of human cortical development has already yielded up several genes that are clearly critical to the normal development of the cortex. Disorders affecting virtually every stage of neuronal development have been identified: from regional specification, to cellular proliferation and fate determination, to migration, and through postmigratory development (though the last phase was not a subject of this review). Gene cloning has provided a wealth of genes to study—both confirmation of genes that we already knew were important to flies or mice and novel genes that potentially guide our attention in new directions. The increasing clinical study of the human cortex will only make this resource richer.

Disorders of neuronal migration into the cortex appear to be particularly commonly encountered in humans, and there may be several reasons for this. For example, mutations in neurogenic genes in flies and mice are frequently early embryonic lethal, and so the same is likely true in humans. In contrast, neuronal migration disorders, affecting a slightly later stage of development, may be severe enough to cause a recognizable phenotype, without being frequently lethal at embryonic stages. The numerous human disorders promise to provide a reasonably extensive analysis of the regulation of this critical process.

Preliminary analysis, albeit incomplete, seems to group the genes that regulate cortical neuronal migration into two broad categories. On the one hand, a large number of genes required for normal migration—including *FLN1* in humans and *disabled 1*, *Reelin*, *cdk5*, and *p35* in mice—have been implicated directly or indirectly in the control of actin dynamics. Some of the corresponding proteins are also intensely expressed in the leading processes of migrating cells as well as growing axons (Letourneau and Shattuck, 1989; Fox et al., 1998; Nikolic et al., 1998). All of these genes except *FLN1* have already been strongly implicated in controlling axon outgrowth and choice point selection either in flies or in vertebrates (Gertler et al., 1989; Nikolic et al., 1996; Del Rio et al., 1997), and *FLN1* has also been directly implicated in the control of filopodia formation (Ohta et al., 1999). In contrast, a second family of genes, consisting of *PAFAH1B1* and *DCX* and potentially the vertebrate homologs of the *Aspergillus* "*nud*" genes, do not have known roles in actin dynamics and have been implicated in microtubule dynamics. Do all of these genes form a single biochemical pathway, or are there multiple pathways or intersecting pathways?

One potential model that reconciles the various neuronal migration genes is to recognize that neuronal migration has both similarities to and differences from axon outgrowth. Like axon outgrowth, the initial phase of neuronal migration consists of the extension of a long leading process that looks and acts like an axonal growth cone. The genes that have roles both in axon outgrowth and in neuronal migration are plausibly involved in this phase of neuronal migration. However, neuronal migration entails a second process that is not duplicated in the growing axons. The second phase of neuronal migration involves the adherence of the leading process to the substrate and the translocation of the nucleus and cell body toward the leading process, producing a net forward movement of the entire cell (Gregory et al., 1988; Rivas and Hatten, 1995). This second phase of neuronal migration has the most similarity to the translocation of nuclei that characterizes *Aspergillus*, and it may be that the neuronal migration proteins (PAFAH1B1 and DCX) that interact with microtubules orchestrate this second process of neuronal migration. However, improved mechanistic studies, as well as the identification of additional interacting genes, await the further development of animal models in flies and mice.

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