

# Genetic Basis of Developmental Malformations of the Cerebral Cortex

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**W**idespread use of noninvasive brain imaging techniques, in particular magnetic resonance imaging, has led to increased recognition of genetic disorders of cortical development in recent years. The causative genes for many of these disorders have been identified through a combination of detailed clinical and radiological analyses and molecular genetic approaches. These disease genes have been found to affect different steps of cortical development, including proliferation of neuronal progenitor cells, neuronal migration, and maintaining integrity of the pial surface. In many cases, syndromes with similar clinical phenotypes are caused by genes with related biochemical functions. In this article, we review the recent advances in molecular genetic studies of the disorders of cortical development. The identification and functional studies of the genes associated with these developmental disorders will likely lead to improvement in diagnosis and facilitate our understanding of the mechanisms of cortical development.

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The large cerebral cortex that executes complex cognitive functions is a striking feature that distinguishes the human brain from that of other mammals. However, even now, relatively little is known about the genetic mechanisms that control the development of the human cerebral cortex. In recent years, molecular genetic studies of malformations of the human cerebral cortex have shed light on the genetic control of cerebral cortical development. The **Table** lists genes that are associated with malformations of the human cerebral cortex. These disorders are also clinically important because they are individually rare but collectively account for a number of cases of epilepsy, mental retardation, and other cognitive disorders.

## NORMAL DEVELOPMENT OF THE HUMAN CEREBRAL CORTEX

The neurons of the cerebral cortex are produced in the ventricular zone, which is a specialized proliferative region surrounding the ventricles. Then the postmitotic neurons leave the ventricular zone to migrate a considerable distance toward the surface to form the cerebral cortex. This migration takes place on a scaffold of specialized glial cells, called radial glia, though a mode of neuronal migration not dependent on radial glia is also known.<sup>17</sup> Once the neurons reach the cerebral cortex, newly arrived neurons have to pass the older neurons to form a normal 6-layered cortex. Therefore, the upper layers of the cerebral cortex are made up of later-born neurons, creating an “inside-out” pattern of cortical layering. Migration of neurons into the cerebral cortex appears to peak between the 11th and 15th week of gestation in humans.<sup>18</sup> It is not entirely clear when neuronal migration is finally

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### Genes Associated With Malformations of the Human Cerebral Cortex\*

Gene	Associated Disease	Gene Function
Microcephalin	Autosomal recessive microcephaly ( <i>MCPH1</i> )	Unknown; possibly DNA repair <sup>1</sup>
<i>ASPM</i>	Autosomal recessive microcephaly ( <i>MCPH5</i> )	Mitotic/meiotic spindle <sup>2</sup>
<i>NBS1</i> (nibrin)	Nijmegen breakage syndrome	DNA repair <sup>3,4</sup>
<i>EMX2</i>	Schizencephaly	Transcription factor <sup>5</sup>
<i>TSC1</i> (harmartin)	Tuberous sclerosis	Tumor suppressor <sup>6</sup>
<i>TSC2</i> (tuberin)	Tuberous sclerosis	Tumor suppressor <sup>7</sup>
<i>LIS1</i>	Lissencephaly (Miller-Dieker syndrome and isolated lissencephaly sequence)	Cytoplasmic microtubule regulator <sup>8</sup>
<i>DCX</i> (doublecortin)	Double cortex/X-linked lissencephaly syndrome	Microtubule-associated protein <sup>9,10</sup>
<i>RELN</i> (reelin)	Lissencephaly with cerebellar hypoplasia	Extracellular matrix protein <sup>11</sup>
<i>ARX</i>	X-linked lissencephaly with abnormal genitalia	Transcription factor <sup>12</sup>
<i>FLNA</i> (filamin A)	Periventricular nodular heterotopia	Actin-binding protein <sup>13</sup>
<i>FCMD</i> (fukutin)	Fukuyama type congenital muscular dystrophy	Unknown; possibly glycosyltransferase <sup>14</sup>
<i>POMGNT1</i>	Muscle-eye-brain disease	Glycosyltransferase <sup>15</sup>
<i>POMT1</i>	Walker-Warburg syndrome	Glycosyltransferase <sup>16</sup>

\*The genes are listed along with the associated diseases and their presumed functions. Several of these genes are not discussed in the text owing to space constraints. Several genes that affect brain development but are associated with metabolic disorders or dysmorphic syndromes are omitted.

completed, but the majority of the neurons appear to reach the cortex by the 24th week of gestation.<sup>19</sup> Genetic defects affecting these different steps of development lead to distinct disorders, which are discussed herein.

#### DISORDERS OF NEURONAL PROLIFERATION AND/OR SURVIVAL

##### Genetic Microcephaly Syndromes

Microcephaly, literally meaning “small head,” refers to a condition in which the brain fails to achieve normal growth. Clinically, microcephaly is present when the occipitofrontal circumference is less than –2 SDs below the mean for the person’s age and sex, though sometimes a stricter cutoff of –3 SD is used. The causes of microcephaly are diverse.<sup>20</sup> Here we limit our discussion to microcephaly syndromes with abnormalities limited to the central nervous system.

Microcephaly vera (primary autosomal recessive microcephaly) is characterized by microcephaly at birth, relatively normal early motor milestones, and mental retardation of variable severity. Epilepsy is uncommon. So far, 6 genetic loci that lead to clinically indistinguishable phenotypes have been identified, and these loci were named *MCPH1* through *MCPH6*.<sup>21,22</sup> Other pedigrees that do not map to these loci suggest that 1 or more additional loci are yet to be identified.<sup>21</sup>

The causative gene has recently been identified in 2 of the recessive microcephaly syndromes. The gene for *MCPH1*, microcephalin, encodes a novel protein of unknown function.<sup>1</sup> It contains 3 BRCT (BRCA1 C-terminal) domains. Many proteins containing this domain function in DNA repair, and perhaps microcephalin has a related function. The *MCPH5* gene, *ASPM*, is the homologue of a *Drosophila* gene, *asp* (abnormal spindle).<sup>2</sup> *Drosophila* *Asp* localizes to the centrosome during cell division and seems to be important in maintaining the integrity of the centrosomal microtubule organizing center.<sup>23</sup> *ASPM* functions in mammals have not been studied, but the mouse *Aspm* gene is highly expressed in the areas of the brain with active neurogenesis, suggesting its role in the prolifera-

tion of neuronal progenitor cells.<sup>2</sup> *ASPM* and its homologues contain small (about 20 amino acids) motifs called IQ domains (because they contain conserved isoleucine and glutamine residues), and the number of IQ domains is consistently larger in organisms with larger brain size. This suggests an interesting possibility that this increase in the IQ domains in the *ASPM* homologues has played a role in the evolutionary expansion of the brain, though other mechanisms might explain this correlation.

#### DISORDERS OF NEURONAL MIGRATION

##### Classical Lissencephaly

The word *lissencephaly* derives from the Greek words *lissos*, meaning “smooth,” and *enkephalos*, meaning “brain.” In classical lissencephaly, normal gyration of the cerebral cortex is absent or severely reduced and the surface of the brain appears smooth. Pathologically, the cortex is greatly thickened and shows 4 layers instead of the normal 6 layers. Patients with classical lissencephaly usually have severe developmental delay, epilepsy, and are often microcephalic.

Classical lissencephaly is seen in association with abnormalities of 2 genes: *LIS1* on chromosome 17p<sup>8</sup> and *DCX* (doublecortin) on chromosome Xq.<sup>9,10</sup> Deletions of the genomic region including *LIS1* cause Miller-Dieker syndrome, a syndrome with lissencephaly and unique facial features, whereas small deletions or point mutations in *LIS1* cause the “isolated lissencephaly sequence,” in which facial features of Miller-Dieker syndrome are absent.<sup>24</sup> *DCX* causes lissencephaly in males, which is almost indistinguishable from *LIS1* mutations, whereas females with heterozygous *DCX* mutations have “double cortex” syndrome. In double cortex syndrome, the gyral formation of the brain is essentially normal, but there is a band of heterotopic neurons (also called “subcortical band heterotopia”) halfway between the cortical surface and the lateral ventricles. These female patients usually have epilepsy and mild to moderate mental retardation.

Both *LIS1* and *DCX* proteins appear to be regulators of microtubules. Microtubules are dynamic components

of the intracellular cytoskeleton and are important in regulating cell shape and motility. DCX is expressed in the post-mitotic neurons and has been shown to interact directly with and increase the stability of microtubules.<sup>25-27</sup> On the other hand, regulation of microtubules by LIS1 appears more complicated. Interestingly, insights into the function of LIS1 came from its homologue in the filamentous fungus, *Aspergillus nidulans*. In *Aspergillus*, the LIS1 homologue, nudF, is involved in translocation of the nucleus through pathways involving other nuclear migration proteins, such as nudE. Translocation of the nucleus is necessary for a neuron to migrate, and a remarkably similar biochemical pathway appears to be present in migrating neurons. In mammals, mNudE, a homologue of nudE, interacts with LIS1, and defects in central nervous system lamination were observed when this interaction was disrupted in *Xenopus* embryos.<sup>28</sup> The function of mNudE appears to involve regulation of the microtubule organizing center, and it may act as a link between LIS1 and  $\alpha$ -tubulin, which plays a key role in initiating microtubule polymerization at the microtubule organizing center.

### Lissencephaly With Cerebellar Hypoplasia

This condition is characterized by an abnormally thick and simplified gyral pattern of the cerebral cortex as well as hypoplasia of the cerebellum. It shows autosomal recessive inheritance, and clinical features include hypotonia, severe developmental delay, seizures, and nystagmus. Mutations in the *RELN* (reelin) gene have been found in some of these patients.<sup>11</sup> *RELN* is the human homologue of a mouse gene, *Reln* (reelin), which was identified as the causative gene for the “reeler” mutant mouse.<sup>29</sup> Reeler mutants show severe hypoplasia of the cerebellum (leading to a “reeling” gait) and disorganized layering of the cerebral cortex. Reelin protein is secreted by Cajal-Retzius cells, which are early-born neurons of the cerebral cortex. There is evidence that reelin functions in arresting migrating neurons at the proper position,<sup>30</sup> but its exact function is still not completely understood.

### X-linked Lissencephaly With Abnormal Genitalia

Most recently, yet another lissencephaly syndrome has come into focus. X-linked lissencephaly with abnormal genitalia is associated with agenesis of the corpus callosum and ambiguous or underdeveloped genitalia. Recently, mutations in the Aristaless-related homeobox transcription factor gene, *ARX*, have been found in these patients.<sup>12</sup> Studies in mice suggest that this gene is important for neuronal proliferation, as well as migration and differentiation of interneurons, in the embryonic forebrain.<sup>12</sup> The identification of *ARX* mutations greatly broadens the potential range of mechanisms that ultimately lead to loss of cerebral gyrification.

### Periventricular Nodular Heterotopia

In this condition, clusters of neurons fail to migrate out of the ventricular region and form neuronal nodules along the walls of the lateral ventricles. Therefore, it most likely

represents a deficit in the initiation of migration. It is also a genetically heterogeneous condition, but many cases are associated with mutations in the *FLNA* (filamin A) gene on the X chromosome.<sup>13</sup> Females affected with heterozygous *FLNA* mutations typically have epilepsy, but usually there are no cognitive abnormalities. It is speculated that some neurons are not affected by the mutations because of the process of X chromosome inactivation in females. On the other hand, males with hemizygous *FLNA* mutations are thought to usually die in utero, though there are rare cases of surviving males.<sup>31</sup> *FLNA* is a large cytoplasmic actin-binding protein, which probably acts as a link between extracellular signals and actin cytoskeleton.<sup>32</sup> The actin cytoskeleton, like microtubules, is important in regulating cell shape and motility. Recently, cases of periventricular nodular heterotopia that are not due to *FLNA* mutations have also been reported.<sup>33,34</sup>

## DISORDERS OF THE INTEGRITY OF THE PIAL SURFACE

### Cobblestone Dysplasia

Cobblestone dysplasia (also known as type II lissencephaly) is a type of cortical malformation characterized by disorganization of the cortical layers, overmigration of neurons through the pial surface onto the outside of the brain, and proliferation of gliovascular tissue on the surface of the brain. The term *cobblestone* is applied because of the nodular appearance caused by its surface abnormalities.

Cobblestone dysplasia is seen in association with at least 3 human genetic disorders, which show some clinical overlap: Fukuyama type congenital muscular dystrophy, muscle-eye-brain disease, and Walker-Warburg syndrome. All 3 disorders are transmitted as autosomal recessive traits and associated with cobblestone dysplasia, muscular dystrophy, and various ophthalmologic abnormalities. Cerebellar polymicrogyria, with or without cyst, is also seen in all 3 conditions, suggesting widespread abnormalities in the central nervous system.

In recent years, causative genes for these conditions have been reported: the *FCMD* (fukutin) gene in Fukuyama type congenital muscular dystrophy<sup>14</sup>; the protein O-mannose  $\beta$ -1, 2-*N*-acetylglucosaminyltransferase (*POMGNT1*) gene in muscle-eye-brain disease<sup>15</sup>; and the protein O-mannosyltransferase (*POMT1*) gene in some cases of Walker-Warburg syndrome.<sup>16</sup> *POMGNT1* and *POMT1* proteins are glycosyltransferases, and although the biochemical properties of the *FCMD* protein are not well understood, sequence analysis suggests that it may also be an enzyme that modifies cell-surface glycoproteins or glycolipids.<sup>35</sup> Recent evidence suggests that functional disruption of dystroglycan is central to pathogenesis of these disorders.<sup>36</sup> In patients with Fukuyama type congenital muscular dystrophy and muscle-eye-brain disease, hypoglycosylation of dystroglycan has been demonstrated, and this disrupts the ability for dystroglycan to bind its ligands such as laminin.<sup>37</sup> It is speculated that this loss of binding leads to the disruption of the integrity of the pial surface, and neurons migrate through these breaches.

## CONCLUSIONS

Recent advances in molecular genetic studies of malformations of the human cerebral cortex have led to identification of many genes that are important regulators of cortical development. It has become clear that syndromes with similar clinical phenotypes are often caused by genes with related biochemical functions (eg, DCX and LIS1, glycosyltransferases). However, as we have seen, many of the biological pathways involving these genes are still not completely understood. Cloning of new disease genes and studies of the functions of the known disease genes will likely lead to further elucidation of important biological pathways in the development of the human cerebral cortex.

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