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# Epileptogenic malformations of cortical development: when evolution goes awry

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**Background.** Since the time of its origin in a mammalian ancestor, perhaps 250 million years ago, the neocortex has undergone expansion in both relative and absolute size. The complexity of the brain in vertebrates is proportional to the elaboration of the mechanisms controlling cortical development. Malformations of cortical development (MCD) are classified into three major groups that recapitulate the main developmental steps: cell proliferation, neuronal migration, or postmigrational cortical organization and connectivity. The main clinical manifestations of MCDs are epilepsy and / or intellectual disability. Seizures are the most common clinical feature, at least 75% of patients with MCDs will have epilepsy. Recent advances in neuroimaging techniques and revolutionary achievements in molecular biology led to an explosive increase in our knowledge of cerebral cortex development and malformations of cortical development (MCD). So far, more than 100 genes were associated with one or more types of MCD. However, the genetic cause still remains unidentified in the majority of cases.

**Conclusions.** Investigations of human malformations of cortical development as of a model of impaired neurodevelopment gave a lot of important insights into normal and abnormal brain development processes and practical benefits to MCD patients and their families. Recent achievements in genetic technologies led to a real explosion in such knowledge and are expected to yield even more additional information in the near future.

**Key words:** malformation of cortical development, neuronal migration, evolution

## INTRODUCTION

The human brain is undoubtedly the most magnificent of all the organs comprised of an integrated network of more than 100 billion neuronal cells. Therefore its formation requires highly concerted actions of sophisticated developmental processes including neurogenesis, neuronal migration, and synaptogenesis. These processes were extensively studied for over 30 years in various species. The basic sequence of events that occurs during mammalian cortical development is shared between species (1), and murine models of cortical development traditionally served as the main tool for investigations. However, recent advances in neuroimaging techniques and revolutionary achievements in molecular biology led to an explosive increase in our knowledge of cerebral cortex development and

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malformations of cortical development (MCD) in humans. So far, more than 100 genes were associated with one or more types of MCD (2). The biological pathways include cell-cycle regulation in many steps (including mitosis and cell division), apoptosis, cell-fate specification, cytoskeletal structure and function, neuronal migration and many inborn errors of metabolism (2). However, the genetic cause still remains unidentified in the majority of cases (3).

## DISCUSSION

### Outline of cellular and molecular mechanisms of neuronal migration

Soon after closure of the neural tube the most anterior part of it becomes an enlarged vesicle called the prosencephalon. The wall of this structure consists of one continuous sheet of neuroepithelial progenitor cells. Prior to the onset of neurogenesis, most of these cells undergo symmetric cell division to produce two daughter progenitor cells. Later on, cells begin to undergo asymmetric cell divisions, and the fraction of cells differentiating into neurons increases, whilst the proportion of those remaining as progenitors decreases. It leads to the formation of transient pseudostratified epithelial layers called ventricular zone and subventricular zone. These proliferative zones are the places of origin of about 80% to 90% of all cortical neurons. GABAergic interneurons are produced in both subventricular zone (from which two-thirds of interneurons originate) and medial and caudal ganglionic eminences. Once a cell goes out of the cell cycle, it is committed to migrate away to its final resting place in the developing neocortex (4). However, before this journey starts, future pyramidal neurons stall in the subventricular zone for about 24 hours suggesting that the subventricular zone constitutes a unique 'permissive' environment for synchronizing migration between pyramidal neurons and interneurons generated at the same time (5). Indeed, despite of substantially different routes of migration the neurons born at the same time share the same laminar fate (4, 5). There are two main modes of migration: radial and tangential. Radial migration is the principal mode of migration within the developing brain involving approximately 80 to 90% of all cortical neurons. The first cohort of migrating neurons constitutes the preplate including Cajal-Retzius cells. With each subsequent wave of migration, the cells move past the neurons previously settled in the cortical plate. Thus, future adult brain layers II–VI are established according to an inside-out pattern and show a temporospatial gradient where the earliest born neurons will go on to form the deepest layers of the cortical plate and the later born neurons reside more superficially (4). Radial migration contributes to the formation of both cortical (i. e. laminar such as the cerebral cortex, hippocampus, or cerebellum) and nuclear structures (6). In a general sense, radial migration allows the transfer of topographic point-to-point information from the ventricular zone to the mantle (6). This kind of migration involves specialized glial cells called radial glia (6). These radial glial cells protrude a process that spans the full thickness of developing brain from the ventricular zone to the pial surface providing a scaffold for moving neurons (6). Recent evidence has shown that radial glial cells not only provide the primary pathways for directed migration, but also themselves are the neural progenitors (4). A subpopulation of neurons including the majority of interneurons and some cortical oligodendrocytes moves tangentially across the radial glia scaffold (4). Two major tangential routes have been identified: (1) from the medial ganglionic eminence to the neocortex and hippocampus, and (2) from the lateral ganglionic eminence to the olfactory bulb (4). Indeed, two modes of neuronal migration, radial and tangential, are just two mechanisms of cell dispersion and the same population of neurons may use both modes to reach their final destination.

Although focal neurogenesis in the adult mammalian brain had been observed as early as the1960s, only in the past decade the concepts of adult neurogenesis and neuronal migration have been established and accepted (4). One of the most impressive phenomena in adult neurogenesis is the migration of neuroblasts from the lateral ganglionic eminence along the walls of the lateral ventricles and the rostral migratory stream to the olfactory bulb with an impressive 1% daily turnover rate (4).

Cellular neuronal migration mechanisms involve several basic repetitive steps conserved through the species and similar in a host range of moving cells. Three main repetitive steps occur in a cyclic manner: (1) the cell extends a growth cone converting to a leading neurite (the prospective

axon) setting a polarization; this leading neurite extends and contracts as it explores the microenvironment, and the cell can extend multiple processes with even transient polarity reversals (4); (2) translocation of the nucleus into the leading neurite is called nucleokinesis. Crucial in this process is the centrosome, which is normally positioned in front of the nucleus, moves into the neurite and pulls the nucleus enwrapped and interconnected to the centrosome through the microtubule cage behind (4); (3) finally, there is the retraction of the trailing process. Molecular mechanisms guiding directional movement of neurons in a given microenvironment are complex. Multiple extracellular guidance cues are interpreted through receptors that relay signals to a network of intracellular signaling pathways, ultimately converging onto the cytoskeleton (4) and giving the net result of how a neuron behaves in a given microenvironment. The major molecular pathways and systems involved are actins with associated proteins, microtubules with associated proteins, multiple kinases mediating neuronal positioning information through phosphorylation of the key components of multiple pathways and a huge range of extracellular molecules acting both in long-distance and short-distance (4). The mutations in the genes encoding these key players and regulators of neuronal migration give rise to the whole range of malformations of cortical development.

**Evolutionary perspective**

Since the time of its origin in a mammalian ancestor, perhaps 250 million years ago, the neocortex has undergone expansion in both relative and absolute size independently in several mammalian branches. This expansion is particularly apparent in anthropoid primates, including humans, in which the neocortex comprises up to 80% of the brain mass (6). This tremendous expansion occurs primarily in the surface area rather than in the thickness (6). While the surface area of the neocortex in a mouse, a macaque monkey, and a human has an approximate ratio of 1:100:1,000, respectively, the thickness barely varies by a factor of two (6). This expansion is mostly attributed to hugely increased proliferative capacities of neuronal progenitors. The duration of neurogenesis extends from 6 days in mice to 60 days in monkeys and approximately 15-fold more postmitotic cells in humans are generated compared to macaques (6). The most notable feature of the cerebral cortex in all species, particularly in primates, is its parcellation into distinct laminar, radial, and areal domains (6). In contrast to radial migration, which appears to play a general role in the formation of major subdivision in the brain, the relatively modern in evolutionary terms tangential migration is thought to increase the complexity of neuronal circuits because it allows neurons born from distinct ventricular zones to intermingle (6).

**Malformations of cortical development**

The term malformations of cortical development (MCD) meaning macroscopic or microscopic abnormalities of the cerebral cortex that arise as a consequence of disarrayed normal steps in the formation of cortex (7) and encompassing the whole range of disturbances in cortical development was introduced in 1996 (5). Several successive attempts to classify these developmental errors according to the first disturbed developmental step and – when more objective data were not available – on neuroimaging features were made. According to the last revision of MCD classification (5) three major groups of MCDs were distinguished that recapitulate the main developmental steps as malformations of cell proliferation, neuronal migration, or postmigrational cortical organization and connectivity (2).

(I) Malformations due to abnormal neuronal and glial proliferation and apoptosis include congenital microcephalies, megalencephalies, cortical dysgenesis with abnormal cell proliferation with or without neoplasia (as hemimegalencephaly and focal cortical dysplasias or dysembrioplastic neuroepithelial tumours, respectively).

(II) Malformations due to abnormal neuronal migration are divided into four subcategories: malformations resulting from abnormalities of the neuroependymal (initiation of migration), mainly including periventricular heterotopia; generalized abnormalities of transmantle migration, mainly including lissencephalies; localized abnormalities of transmantle migration, mainly subcortical heterotopia; and abnormalities due to abnormal terminal migration / defects in the pial limiting membrane, mainly including cobblestone malformations.

(III) Malformations due to abnormal postmigrational development are composed of polymycrogyrias of different etiology, focal cortical dysplasias

due to late developmental disturbances and post-migrational developmental microcephalies (5).

Reduced proliferation or increased apoptosis in neurogenesis leads to congenital microcephalies, while vice versa results in megalencephalies; abnormal foci of proliferation produce focal or diffuse dysgenesis and dysplasia (5). If neuronal migration is disturbed, the neurons can remain at the ventricular surface (periventricular heterotopia), arrest in the white matter (subcortical band heterotopia) or form a disordered, often thickened, cortical plate with abnormal and simplified gyration (pachygyria) or a smooth appearance of the cortical surface (lissencephaly). Sometimes migration disturbances manifest by overmigration of neurons to the pial surface resulting in cobblestone lissencephaly. The third category, disorders of cortical organization or late migration, comprises mostly the polymicrogyrias, a heterogeneous group of malformations with multiple small gyri and an abnormally thin or thick cortex, sometimes so severely affecting the brain structure as to cause clefting between the ventricular and meningeal surface (schizencephaly) (8).

The ideal classification should rely on extensive knowledge of biological pathways which is incomplete though constantly growing at present (2). Recent investigations show that boundaries between disorders of neuronal proliferation, migration and subsequent cortical organization are fading, as exemplified by identification of a broad range of malformations in humans with mutations in *WDR62*, *DYNC1H1*, and *TUBG1* genes (2). These findings support the notion that MCD-related genes are implicated in many developmental stages that are genetically and functionally interdependent (2).

As a group, MCDs result in a variable burden of disability ranging from mild adult-onset to the severest congenital presentations. The main clinical manifestations of MCDs are epilepsy and / or intellectual disability. Seizures are the most common clinical feature and it was estimated that at least 75% of patients with MCDs will have epilepsy (7), however, motor and cognitive dysfunctions are also very common (9). Most of the MCDs contain areas of the cortex that are intrinsically epileptogenic (9). Epileptic seizures are seen in about 90% of lissencephaly (1), 80% to 90% of periventricular nodular heterotopias (2), majority of hemimegalencephaly patients and various proportions of other categories of MCD patients (7). The seizures are frequently intractable and pharmacoresistant, as in hemimegalencephaly and lissencephaly, but sometimes amenable to surgical treatment. However, the epileptogenic zone may be more extensive than the lesion visualised on MRI alone and in some cases, FCD may be shown histologically in a surgical specimen but would not have been detected at all preoperatively on the best MRI (10). Microdysgenesis as an MCD visible only on microscopy was first described in autopsied brains of patients with idiopathic generalized epilepsies (10). Microdysgenesis may underlie epilepsy currently considered cryptogenic so that MCD may be even more important as a group than acknowledged at the moment (10). Indeed, it was recently shown that juvenile myoclonic epilepsy due to *EFHC1* gene mutation can in fact be a disorder of impaired neuronal development. *EFHC1* is a microtubule-associated protein and *EFHC1* impairment in the developing rat neocortex causes a marked disruption of radial migration, with defects in the radial glia scaffold organization and in the locomotion of post-mitotic neurons (11). Mutations in the *ARX* gene produce the whole range of clinical manifestations from isolated epileptic encephalopathy or isolated mental retardation to the multiple malformation syndrome including lissencephaly. Recently, it was suggested that all these clinical entities involving *ARX* mutations are due to disturbed interneuron development (12). Therefore, the true impact of MCDs on human pathology is difficult to assess and as the current diagnostics of MCDs relies mainly on neuroimaging studies and sometimes on neuropathological investigations of a surgically resected specimen, even the true prevalence of MCDs is currently unknown. It is supposed that 25% to 40% of intractable childhood epilepsy could be attributable to MCDs (7, 13). Better detection rates require specialized MRI protocols (13, 14) and with proper neuroimaging the diagnoses of MCDs can sometimes be made before the 24th week of gestation (2) and can help to provide appropriate genetic counselling and testing.

While some forms of MCDs as lissencephalies, hemimegalencephalies and others are considered to be "purely" genetic, the etiological structure of others are more diverse as in polymicrogyrias and schizencephaly which can be caused by different

genetic, vascular, toxic, infectious factors and multiple inborn errors of metabolism (2, 13). So far, more than 100 genes were associated with one or more types of MCD (2)] and genetic testing of MCD patients entered into a common clinical practice. For example, 12 lissencephaly-associated genes have been identified to date (*ARX, RELN, VLDLR, ACTB, ACTG1, DCX, DYNC1H1, KIF2A, LIS1, TUBA1A, TUBB2B, TUBG1*) accounting for roughly 90% of lissencephaly patients (2, 15), more than 20 genes and at least 8 copy number variations (CNV) were associated with polymicrogyria (2), *ARFGEF2* and *FLNA* gene mutations and several CNVs are known to result in periventricular nodular heterotopias (2). After the completion of the Human Genome Project more than 10 years ago approximately 23,000 human genes were identified; however, the function of the majority of these genes remains unknown. Although the discovery of genetic alterations involved in different human pathological phenotypes occur at an ever increasing pace, to date ~3,700 monogenic human phenotypes have been associated with a specific disease gene. The discovery of genes which, when mutated, cause brain malformations has historically been an arduous, time consuming, and costly endeavor (16). The real explosion in knowledge of molecular mechanisms of normal and abnormal cortical development and in MCD diagnostics came with the advent of next-generation sequencing (NGS) technologies and a wider application of array comparative genomic hybridization. At present, it is possible to sequence the complete genome of an individual in a matter of weeks and at a cost equivalent to sequencing ≈10 average sized genes by Sanger sequencing (16). A variation of this NGS strategy is to sequence 1% to 2% of the genome that encodes proteins, termed Whole Exome Sequencing (WES) (16). WES, in particular, has led to the identification of more than 150 novel disease genes with numerous ones implicated in brain development (16). At least one rare copy-number variation was detected in 22.5% of patients from a cohort of 169 patients with various structural brain malformations investigated by array comparative genomic hybridization (3). Due to all these achievements, more and more MCD patients and their families are able to profit from the advantages given by exact genetic diagnosis, including natural history and recurrence risks prognostication, patient management decisions, options for prenatal testing and relief of psychological burden, all of them having a significant positive impact on a patient and a family. These methods, at least when applied to the usual investigations of patients leukocyte DNA, are of limited value in postzygotic mosaicism cases. Several patients with mosaic *FLNA*, *LIS1* and *DCX* mutations were described (2) and somatic mutations as a cause for multiple neurodevelopmental disorders including lissencephaly and hemimegalencephaly were suggested (17). Therefore, the authors of the recent extensive review of MCDs propose that many additional types of megalencephaly, dysplastic megalencephaly (including classic hemimegalencephaly), focal cortical dysplasias, lissencephaly, polymicrogyria, and heterotopia will prove to be caused by mosaic mutations (2).

## CONCLUSIONS

Investigations of human malformations of cortical development as of a model of impaired neurodevelopment gave a lot of important insights into normal and abnormal brain development processes and practical benefits to MCD patients and their families. Recent achievements in genetic technologies led to a real explosion in such knowledge and are expected to yield much more additional information in the near future.

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## EPILEPTOGENINĖS ŽIEVĖS VYSTYMOSI MALFORMACIJOS: KAI SUTRINKA EVOLIUCIJOS PROCESAI

*Santrauka*

**Įvadas.** Nuo to laiko, kai maždaug prieš 250 milijonų metų žinduolių pirmtakuose išsivystė smegenų žievė, ji stipriai išaugo ir savo santykiniu, ir absoliučiu dydžiu. Stuburinių smegenų sudėtingumas proporcingas smegenų žievės vystymąsi reguliuojančių mechanizmų sudėtingumui. Žievės vystymosi malformacijos (ŽVM) pagal pagrindinius vystymosi etapus skirstomos į tris pagrindines grupes: ląstelių proliferacijos sutrikimai, neuronų migracijos sutrikimai ir pomigracinių žievės organizacijos bei jungčių sudarymo procesų sutrikimai. ŽVM dažniausiai pasireiškia epilepsija ir / arba intelektine negalia. Traukuliai – dažniausias klinikinis simptomas, mažiausiai 75 % pasireiškia ir su ŽVM diagnozuojama epilepsija. Pastarųjų metų neurovaizdinių tyrimų pasiekimai ir revoliuciniai pokyčiai molekulinės biologijos srityje reikšmingai praplėtė žinias apie smegenų žievės vystymąsi ir vystymosi sutrikimus. Šiuo metu su viena ar keliomis ŽVM susieti daugiau nei 100 žmogaus genų. Vis dėlto genetinės sutrikimų priežastys vis dar lieka nežinomos daugumai pacientų su ŽVM.

**Išvados.** Žmogaus žievės vystymosi malformacijų tyrimai, kaip nervų sistemos vystymosi sutrikimų modelis, suteikė reikšmingų žinių apie smegenų vystymąsi bei jo sutrikimus, o taip pat davė praktinės naudos pacientams su ŽVM ir jų šeimoms. Ypač daug informacijos sukaupta pasitelkiant pastarųjų metų pasiekimus genetinių technologijų srityje, dar daugiau žinių tikimasi gauti netolimoje ateityje.

**Raktažodžiai:** žievės vystymosi malformacijos, neuronų migracija, evoliucija