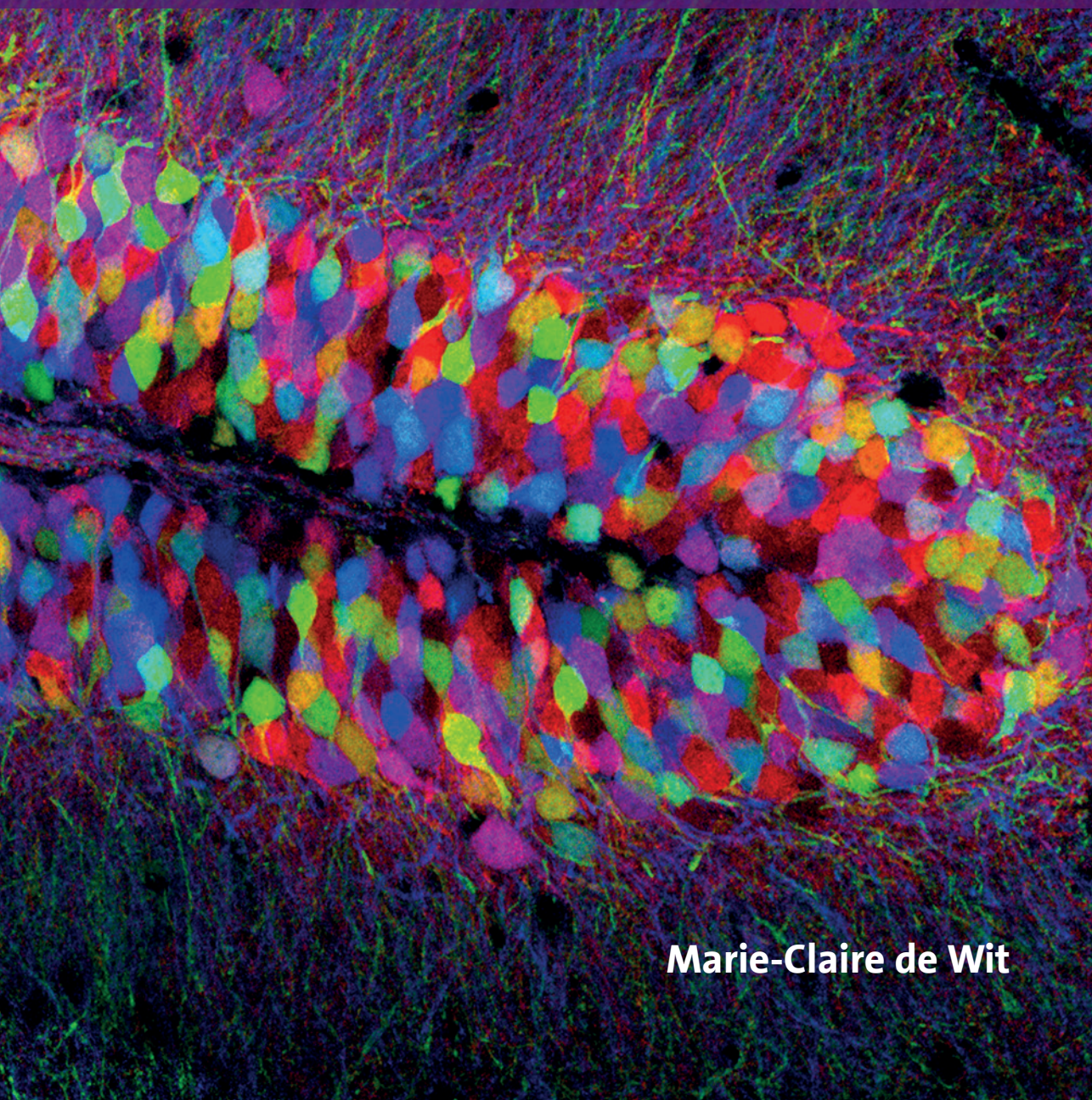
Malformations of the Human Cerebral Cortex

Patterns and Causes



Marie-Claire de Wit

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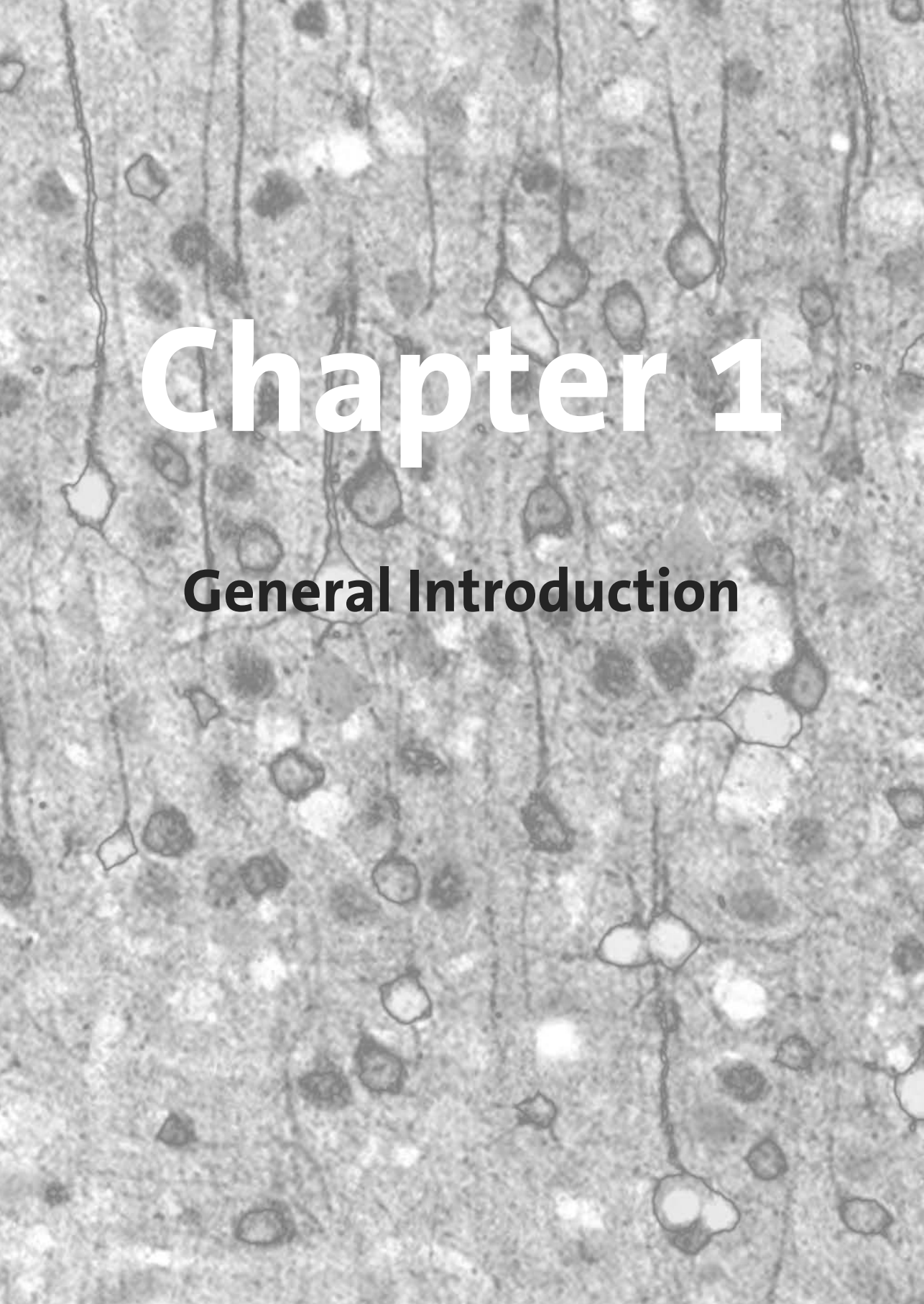


Chapter 1

General Introduction

GENERAL INTRODUCTION

1.1 MCD definition and impact

Malformations of cortical development (MCD) are a group of disorders characterized by a congenital abnormal structure of the cerebral cortex. In general, malformations are defined as structural abnormalities caused by a disturbance in cell organization or function within a tissue type. When a disturbance results in an abnormal structure of the cerebral cortex we call this: malformations of cortical development. MCD are heterogeneous as a group, as they include several different structural abnormalities, and they have a diverse array of causes, both genetic and environmental.

A patient with a MCD may present to a physician with different neurological symptoms. These signs and symptoms depend on the regions of the brain that are affected, on the severity of the malformation, and on how much the affected regions still have residual neurological functions or whether normal brain areas can compensate for loss of functions (plasticity). As MCD are cortical malformations, functions meant here are the cerebral cortical functions, such as the motor function of the limbs, language development and visual functions. For example, a child may present with a spastic hemiparesis if only one hemisphere is affected, with drooling and a speech disorder if both perisylvian areas are affected, or with severe psychomotor delay when the entire cortex is abnormal. Intellectual development can range from normal to severe mental retardation. As abnormalities of the cortex increase the risk of epilepsy, many MCD patients will present with epilepsy or develop epilepsy later in life.[1] Seizures can be the only clinical feature, and then the focal nature of the epilepsy based on the seizure semiology or the EEG may suggest the presence of a MCD. Although the treating physician may suspect a cortical malformation in some cases, it is impossible to determine the nature of the abnormality based on the clinical features alone. To diagnose a MCD imaging of the brain is essential, preferably by MRI scan. This means that any patient with congenital neurological deficits and/or epilepsy without an established cause should be evaluated by MRI to exclude/diagnose a MCD.

The prevalence and incidence of MCDs are not easy to estimate. Without a brain MRI, one cannot say which patients are affected and even then some MCD are missed if not specifically looked for. In a population based cohort of 96 American children with congenital hemiparesis, 18% had a MCD as the underlying abnormality.[2] In this population the prevalence of congenital hemiparesis was 5:10.000. In most patients with epilepsy a brain MRI is performed, so MCD have been reasonably well investigated in this group. Prevalence figures are reported from 5-10% in unselected groups to 5-20% in patients with intractable epilepsy, depending on age and selection of the cohort.[3-7] Epilepsy is generally reported between 0.5 and 1% of the population. Even with this limited information, it is clear that MCD are not rare and the morbidity associated with MCD is significant.

1.2 MCD subtypes

Malformations of cortical development can be classified into several subgroups, based on the MRI. The revised Barkovich classification is most widely accepted, and is based on the proposed underlying mechanisms with a mixture of genetic and imaging criteria.[8] Although this classification is not ideal due to these mixed criteria and the fact that not all patients fit in this model, it is useful to guide the diagnostic process and to group patients with a similar disease. Lissencephaly was the first type of MCD that was recognized as a cause of severe developmental disability over 50 years ago.[9] At the time a definite diagnosis could only be made by examination of the brain post-mortally. The advent of neuroimaging possibilities, in particular CT scanning in the seventies and MRI scanning in the eighties of the 20th century, enabled physicians to diagnose cortical abnormalities during life. Lissencephaly, being the 'first MCD', is also the subtype best described and understood. The brain MRI shows specific features of a thickened cortex with reduced or no gyration with a predilection for the occipital regions and EEGs show typical high voltage beta activity. Clinically, about all patients have epilepsy and psychomotor delay. The severity correlates with the severity of cortical involvement on MRI [this thesis, chapter 3.1].

At the same time much progress was made in the possibilities of genetic research. Over the last decades this has led to insights into the causes of malformations of cortical development. For some cortical malformations, e.g. lissencephaly, much is now known about pathogenesis and inheritance. In others, causes are yet to be discovered, and appropriate clinical and radiological syndrome classification will provide the basis for future research. An etiological diagnosis is the goal, as this offers parents the best information on the cause, prognosis, and recurrence risk and the option of prenatal diagnosis in a next child. Genetic counseling is important for parents of an affected child, but also for patients themselves especially in those types of cortical malformations that may show a variable phenotype in families, e.g. in filamin A related heterotopia.

In the forthcoming years, classification, etiological diagnoses, and clinical follow-up will provide possibilities for the development of improved treatment for these children and adults. At the moment no specific treatments are available and care is focused on treating the complications of cortical malformations, such as epilepsy and cerebral palsy. For some patients with intractable epilepsy, surgery can be successful, and for those the recognition of the cortical malformations responsible for their seizures is of vital importance. Finally, careful observation of patients with abnormalities of cortical development can aid understanding of, and research into, the processes of human brain development.

To reach for these goals the first priority lies in the recognition and description of patients with MCD. Cortical malformations are not always easy to recognize on brain CT or MRI, or their relationship with other problems is not noted. Correct interpretation can lead to a molecularly confirmed or syndrome diagnosis, and to improved care for the individual

patient. On a group level, correct interpretation will improve the knowledge of known phenotypes and allow recognition of new phenotypes.

In general, malformations of cortical development are caused by an interruption of normal cortical development due to a lack of normal gene expression, the production of abnormal protein or a disruptive factor such as ischemia or an infection. Many genes and proteins are involved in the development of the human brain and we are just beginning to understand their function and interaction. For the understanding of the clinical manifestations and underlying causes, basic knowledge about prenatal brain development is helpful. This allows timing of the underlying insult, but also more understanding of the pathophysiological process and of the classification of the MCD.

This introduction will focus on global understanding of cortical development, classification of malformations and known causes. It will not go into details on the development of the cerebellum, mesencephalon and midline structures. First the normal cortical development is briefly discussed to enable a more thorough description of the MCD subtypes in the later paragraphs.

2.1 Normal development of the cerebral cortex

In humans the formation of the cortex is completed around birth. At that time, the human brain contains around $1x10^{11}$ neurons. To reach this number, one can calculate that on average new neurons are being generated at the rate of about 250,000 per minute during the nine months of gestation.[10] The newborn brain weighs approximately 350-400 grams, while in adulthood this has grown to 1300-1400 grams. After birth, further brain development is focused on myelination and synaptic network formation and organization. It is said that there are more synapses in the cerebral cortex than there are stars in the galaxy (around $1x10^{15}$ synapses).[11] Also, the generation of glial cells continues during the first year. Some neural precursors remain and retain the ability to generate neurons on a modest scale. [12]

The development of the cerebral cortex during the nine months of gestation is a complicated and intricate process, and it can be best understood by dividing it in three steps. These are overlapping stages of proliferation and differentiation, migration and organization. The normal six-layered structure of the neocortex is strictly organized. There are two main types of neurons in the cortex: excitatory glutamatergic cortical neurons and locally projecting GABA-ergic inhibitory interneurons. Both groups have a large number of subtypes that can be differentiated morphologically, electrophysiologically or molecularly, however for the understanding of current insights in the organization of the cortex it is most informative to focus on these main groups.

2.2 Early embryonic phase of the prosencephalon

By the fourth week of embryonic development the neural tube has closed and on the anterior or rostral side of the embryo three vesicles form, the prosencephalon, the

mesencephalon and the rhombencephalon. The most caudal vesicles, the mesencephalon and the rhombencephalon, will give rise to the midbrain and the cerebellum. The most distal vesicle, the prosencephalon cleaves horizontally to separate the optic vesicle and olfactory bulbs, transversely to separate the diencephalon from the telencephalon and sagitally to divide the telencephalon into two ventricles (see figure 1). The telencephalon is the precursor of the cerebrum and the two ventricles are lined with the cells that will populate the brain and form the hemispheres. Many genes and proteins are crucial during this development, the most important being the cholesterol dependent Sonic hedgehog protein that is secreted by the prechordal mesoderm and induces a cascade of activation of other genes leading to the correct dorsoventral patterning.

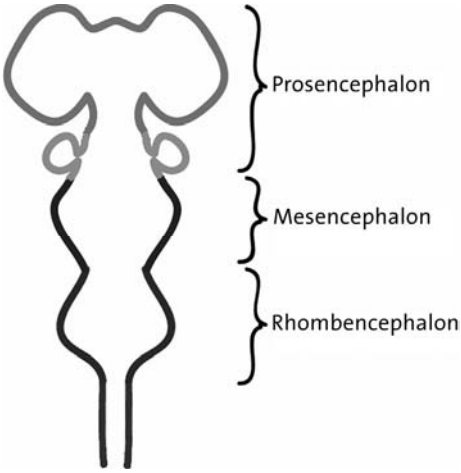


Figure 1: schematic representation of the early prosencephalon, including the eye-buds, mesencephalon and rhombencephalon.

2.3 Proliferation

The ventricular surface contains the germinal zones with the progenitor cells that will give rise to all neurons and glial cells. The most important time for the generation of neurons and glial cells is during the 2nd tot 4th month of gestation. Initially, proliferation takes place in the ventricular zone, close to the ventricular surface. In this first proliferative phase, progenitor cells divide symmetrically. This means that each mitosis creates two equal daughter progenitor cells. This symmetric mitosis follows a specific pattern in which a progenitor cell starts its mitosis in the periphery of the ventricular zone, then migrates to the ventricular surface, divides into two daughter cells and then both these progenitor cells migrate back to the periphery.[13] The significance of this back-and-forth pattern is not completely understood, but it is probable that the progenitor cells need to maintain contact with the ventricular surface. The number of cell-cycles that occurs during the first period of symmetric division determines the total number of cortical neurons, and therefore the size of the brain. The growth in this phase is what differentiates the size of human brains from that of primates.

During the third month of gestation the number of progenitor cells stabilizes and the second phase of proliferation starts. The progenitor cells are now recognizable as radial glial cells that are oriented perpendicular to the ventricular surface. Their radial extension spans the entire neocortical wall and maintains contact with both the ventricular and pial surfaces. The radial glial cells begin to divide asymmetrically, thereby producing one progenitor cell and one postmitotic neuron. These newborn neurons migrate to their destination in the cortex by using the radial extension of the radial glial cells as a guide in the migration to their destination (see figure 2).
In addition, the radial glial cells generate a secondary population of progenitor cells called intermediate progenitor cells. The intermediate progenitor cells migrate to the overlying area where they form the second germinal zone, called the subventricular zone. Here they generate more neurons for the upper cortical layers and appear to do so by symmetric division. The progenitor cells of the subventricular zone also produce part of the interneurons in humans. Another group of interneurons is produced in the ganglionic eminences, the future basal ganglia, and these will migrate tangentially.[14]
Finally the radial glial cells differentiate into astrocytes or ependymal cells. They probably also produce oligodendrocytes. Some remain postnatally as astrocytic stem cells that retain the ability to generate neurons and glia. By 20-24 weeks of gestation the total number of neurons is complete. Glial multiplication and differentiation mainly takes place from approximately 5 months of gestation to at least one year postnatally.

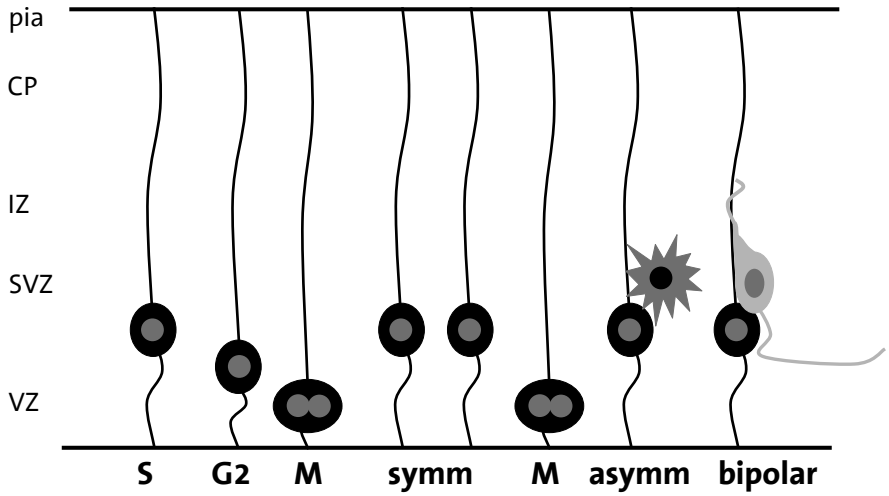


Figure 2: Subsequent stages of proliferation. The radial glial cells extend from the ventricular surface (VZ) to the outer, pial surface (pia). The neural progenitor cells show a cell-cycle dependent oscillation and move towards the VZ in preparation of mitosis. Symmetric division (symm) results in two equal progenitor cells and this process is repeated. Asymmetric divisions (asymm) create post-mitotic neurons that first appear multipolar, and then convert to a bipolar appearance with a migratory process wrapped around the glial extension and an axon trailing behind.

S: S-phase in cell cycle; duplication of chromosomes, G2: G2-phase in cell cycle; preparation for division, M: mitosis, symm: symmetric division, asymm: asymmetric division.

Important genetic pathways involved in the division of neural progenitors are the Notch receptor pathway, the ErB receptor pathway (ligand neuregulin) and the fibroblast growth factor receptor.[15.16,17] Beta-catenin signaling is important in the decision to differentiate or proliferate, and in the timing of migration.[18]

2.4 Migration

During the process of proliferation, postmitotic neurons already start their migration to their target area in the cortex. The neurons use the guidance of the radial glial cell by attaching to the radial fiber, moving outward and disengaging at the correct location.
This process is highly organized. Radial glial cells that border each other in the ventricular zone produce neurons that will border each other in the neocortex. A group of radial glial cells born to a single progenitor cell is called a proliferative unit. Radial glial cells each produce a proliferative unit that migrate along their radial extension to the cortex, each passing through those gone before to finally form the six-layered cortex in an inside-out fashion. The target layer and the order of these neurons therefore depend on the time of their origin. In other words, a proliferative unit gives rise to an ontogenetic column (see figure 3).[19,20] The first wave of symmetric division determines the number of proliferative units and the second wave of asymmetric division determined the size of the ontogenetic column. Also, the number of asymmetric divisions determines the number of neurons created in each radial column and therefore the thickness of the cortical layer.

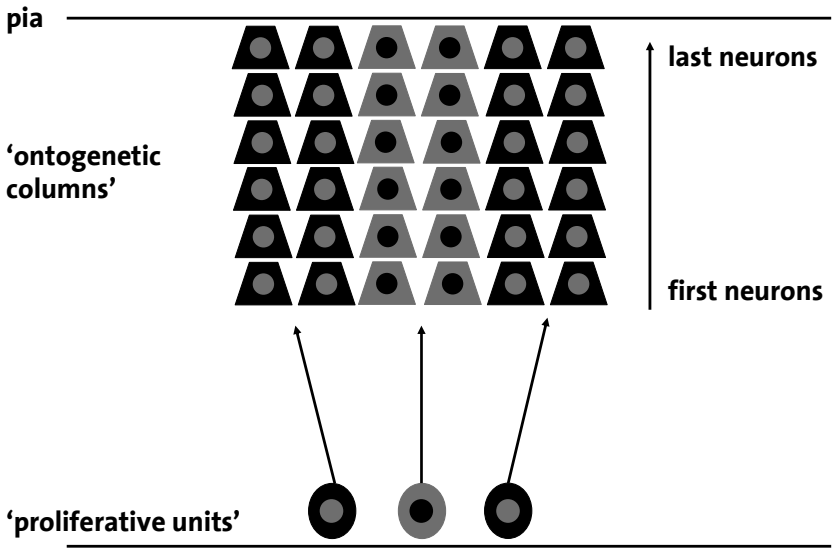


Figure 3: cortical organization based on the proliferation of neural progenitors at the ventricular surface ('proliferative units') producing postmitotic neurons in organized inside-out columns.

To migrate, neurons wrap a thin protrusion around the glial fiber and glide along in outward direction by forming and releasing neuron-glial adhesion. In this movement the neuronal centrosome enters the leading process first, followed by the cell soma. This is regulated by the PAR complex.[21] Different organelles move along the microtubule cytoskeleton and this is organized by cytoplasmic dynein with co-factors LIS1 and doublecortin. The motor behind neuronal movement is formed by actin-myosin contractility.[21]

Neurons produced in the subventricular zone migrate later than those generated in the ventricular zone, but follow the same pattern. Most radially migrating neurons are destined to become excitatory glutamatergic cortical projection neurons.[20]

Interneurons are the main inhibitory neurons in the brain and show a different migration pattern. In the rodent these GABA-ergic interneurons arise from the medial, lateral and caudal ganglionic eminences and migrate tangentially to their destination (see figure 4). In humans however, a large proportion interneurons are generated in the subventricular zone.[22] Most research has been done on interneurons of the olfactory system en hippocampus as these systems are being populated by new neurons even during adulthood.[12] Cortical interneurons do not use a radial glial fiber to guide their path, but are guided by chemical cues. They are polarized cells that display a leading process and a shorter trailing process. The leading process continuously branches out, and the branch best oriented towards the guidance cues in the environment becomes stabilized.[23]

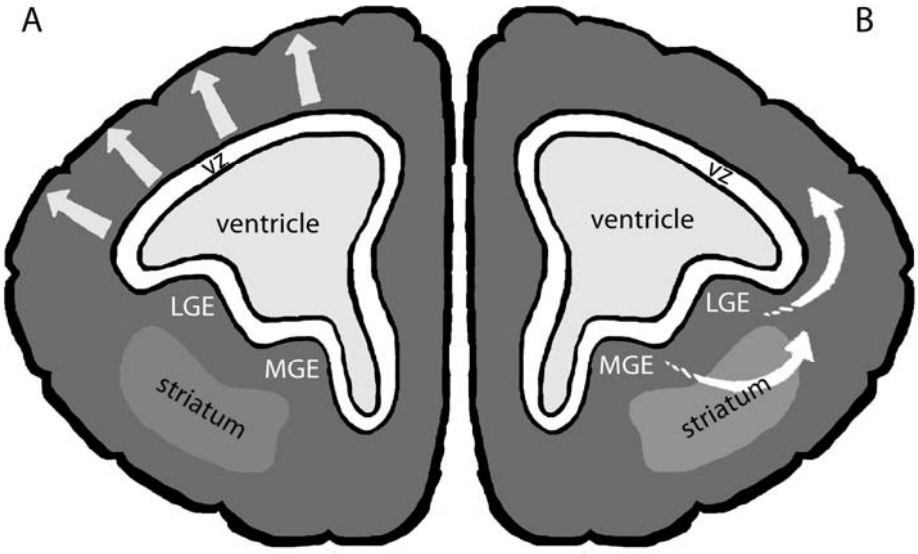


Figure 4: schematic coronal section of the embryonic neocortex, illustrating radial migration (A, straight arrows) to the cortex, as opposed to tangential migration (B, curved arrows) from the medial and lateral ganglionic eminence (MGE and LGE). VZ = ventricular zone.

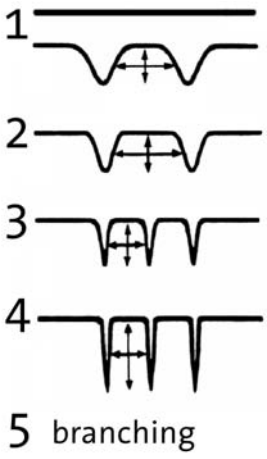
At the same time, between 9 and 20 weeks, the midline structures of the brain are formed: the corpus callosum, septum pellicidum, optic chiasm and hypothalamus. The corpus callosum is formed by cortical axons from the cingulate cortex that cross the midline attracted by specialized glial cells that express chemoattractants (of the Netrin family). They first form the rostrum, then the genu and lastly the posterior splenium. Disturbances of callosal development influence the final gyral pattern, especially that of the medial surface of the cortex (cingulate gyrus).

Gyration is largely a passive process driven by proliferation and migration. The human brain has a much larger surface area than a rodent brain, while the thickness of the cortical layer is only mildly larger. This follows from the increased number of symmetric cell divisions in the first phase of proliferation, resulting in a much greater number of ontogenic columns. Folding the cortex into gyri enables this larger surface area to fit in as small a volume as possible. This does not explain the fairly stereotypical form of the brain with recognizable sulci such as the calcarine sulcus or sylvian fissure. This pattern probably depends on tension by tracts of axonal cortico-cortical connections that are present from around the time of the intermediate zone onwards.[24]
At birth a human infant brain has the approximate same number of neurons it will have in adulthood. Further growth in volume results from glial cell proliferation and myelination. Finally the cortex will be 3.5-4 mm thick. The full gyration pattern emerges in the last part of gestation (after 35 weeks) and one should realize that it is not finished in preterm infants. [25,26,27]

Table 1 and figure 5 *(reprinted with permission, copyright Radiology 1996)*[25]:
Gyration stages I-V can be determined by scoring the development of sulci and gyri in different areas of the brain: gyration is first seen in the central sulcus, occipital medial (or calcarine) sulcus, followed by occipital, parietal and temporal-posterior regions and lastly in the temporal-anterior and frontal areas. The figure showed the stages of gyration: 1; no to shallow sulci, round gyri, 2: gyri broader than deepness of sulci, 3: equal broadness and deepness and gyri take on square aspect, 4: sulci are deeper than gyri are broad, square gyri, 5 branching into secondary and tertiary gyri.

	PCA (wk)	Central sulcus, Occipit med	Occipital, Parietal, Temp-posterior	Frontal, Temp-anterior
I	30-32	3	2-2.5	1
II	32-34	4	2.5-3	2-2,5
III	34-37	5	3-4	2.5-3
IV	37-40	5	4-4.5	3.5-4
V	>41	5	5	4.5-5

PCA: postconceptional age



2.5 Organization

From the fifth month of gestation to well into puberty further organization of the cortex consists of differentiation of neurons and glial cells, orientation and alignment of cortical neurons, sprouting of axons and dendrites, formation of synapses, selective elimination of cells and synapses and myelination. Some controversy exists as to whether postmitotic neurons are equipotent and assume their network function based on the environment they migrate into (tabula rasa hypothesis) or that they are genetically programmed to a specific function (the protomap hypothesis). The latter hypothesis has gathered some convincing experimental support.[24,28] Primordial cortical areas can assume their function by making the appropriate connections; for example the future somatosensory cortex needs to attract input from the ventroposterolateral nuclei of the thalamus.

Further differentiation of the cortex is orchestrated by the interplay between gradients of different molecules, the expression of different genes and the interaction between neurons. Different patterning centers secrete different families of signaling molecules. The list of molecules and genes involved is extensive and growing by the month, so it is not feasible to go into detail. Examples of important signaling molecules are fibroblast growth factor secreted by the anterior cortex, Wnts or bone morphogenetic protein by the posteromedial border, and epidermal growth factors by the lateral areas.[24] These gradients stimulate or suppress expression of important genes in the surrounding areas resulting in expansion and specialization of the different cortical areas.[27]

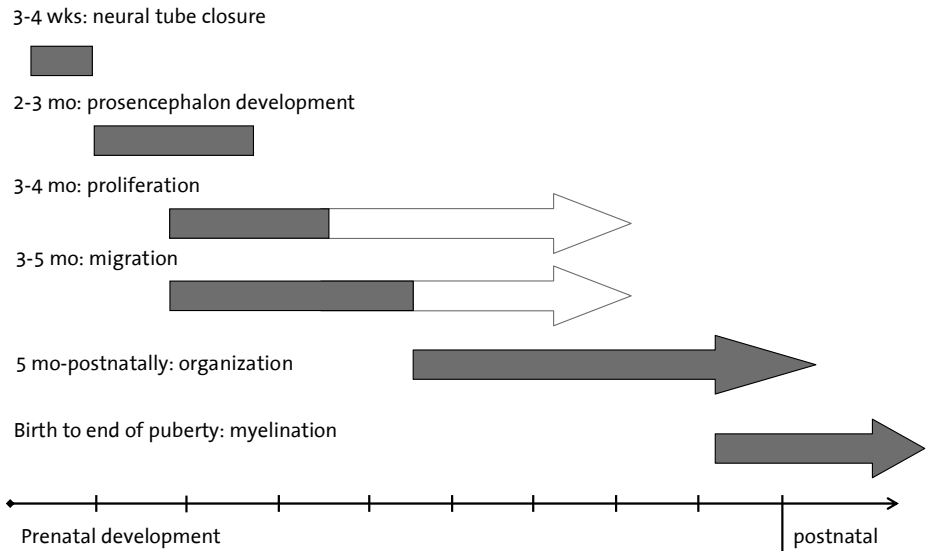


Figure 6: timeline of human cortical development, peak times. X-axis represents months of gestation.

3. Malformations of cortical development

At all stages of cortical development trouble may arise, and abnormal development can result in abnormal anatomy. The gold standard of the diagnosis is microscopic neuropathology, but improvements in neuroimaging techniques enable the diagnosis of many malformations of cortical development during life. The investigation of choice to visualize the central nervous system is MRI imaging, while ultrasound may give a good indication in neonates as well. Subtle abnormalities on MRI can be difficult to interpret in the neonatal period due to the high water content of the brain, and a repeat MRI in the second year of life may be necessary to reach a definite diagnosis. The appearance of the cortex also changes on MRI during the first years of life due to myelination, and MCD can become apparent on follow-up.[28,29]

The phenotype of the different malformations of cortical development depends on the stage of development that is disturbed. Disruption of neurulation resulting in neural tube defects can be located on the caudal or rostral side. Neural tube defects (dysraphism) are not subject of this thesis; however they can coincide with malformations of cortical development such as polymicrogyria or periventricular nodular heterotopia. This is not considered truly coincidental, but may be a consequence of a factor that disrupts both the process of neurulation as well as cortical development, or may be secondary to the concurrent hydrocephalus.[30,31] Disorders of prosencephalic cleavage, notably holoprosencephaly or commissural developmental anomalies, are not discussed.

The current classification of MCD is based on a mixture of MRI, clinical and genetic criteria.[8] It has changed over the recent years, and will have to be adapted to increasing knowledge on developmental processes and genetic factors. Also, not all patients fit neatly into the classification system; e.g. different types of MCD may occur in one patient, microlissencephaly fits both the categories of proliferation and migration disorders, and cortical dysplasia can be classified as a proliferation disorder when abnormal cells are thought to be present or a migration disorder if not.

The classification system in broadly based on the following categories:[8]

1. Malformations due to abnormal neuronal and glial proliferation or apoptosis
    - 1.1 Decreased proliferation/increased apoptosis: Microcephaly with/without normal cortex.
    - 1.2 Increased proliferation/decreased apoptosis: Megalencephaly with/without normal cortex.
    - 1.3 Abnormal proliferation (abnormal cell types)
2. Malformations due to abnormal neuronal migration
    - 2.1 Lissencephaly/subcortical band heterotopia spectrum
    - 2.2 Cobblestone complex
    - 2.3 Heterotopia
3. Malformations due to abnormal cortical organization (including late neuronal migration)
    - 3.1 Polymicrogyria and schizencephaly

These categories are described in more detail below.

Classification of the type of MCD based on MRI imaging is the first step towards finding the causative mechanism in a particular patient. This should be followed by family history, clinical history (including pregnancy and delivery), physical, dysmorphological, and neurological examination. Detailed classification is needed to guide genetic analysis as mutations in genes known to be involved in MCDs generally result in specific (neuroimaging) patterns.[1]

Causes of disruption of normal cortical development can be both environmental as genetic. The list of known causes is extensive and can be found in tables in the appendix.
In general causes can be:

- *Monogenetic*: These can be autosomal dominant (e.g. *LIS1*), autosomal recessive (e.g. *ARFGEF2*), and X-linked (e.g. *FLNA*).
- *Chromosomal abnormalities*: Chromosomal rearrangements can cause MCD as part of the phenotype. Most well known is Miller-Dieker syndrome, where *LIS1* and the surrounding genes on chromosome 17p are deleted causing complete lissencephaly with specific facial dysmorphism. In 22q11 microdeletion (velocardiofacial syndrome) polymicrogyria frequently occurs. For other microdeletion syndromes malformations of cortical development are less frequent, but are sometimes described. Associated phenotypical features may lead to specific suspicions (such as in cases of del 5q35, 5p- syndrome, 1p- syndrome etc).
- *Syndromal associations*: a large number of syndromes associated with MCD and with unknown underlying cause have been described.
- *Metabolic disorders*: Most well known are peroxisomal disorders in the Zellweger syndrome spectrum, but others have been described, such as congenital disorders of glycosylation and organic acidurias.
- *Infectious (prenatal)*: mainly associated with polymicrogyria.
- *Prenatal insults otherwise*: e.g. prenatal ischemia can cause polymicrogyria.

In the tables in the appendix causes are reported if the description of the MCD and the neuroimaging are clear, and if the MCD has been reported in more than one case. Reports of MCDs in aborted fetuses before gestational age of 30 weeks are disregarded as gyration is insufficient at that age to definitely classify an abnormality.

3.1 Disorders of proliferation

3.1.1 Microcephaly with simplified gyration (MSG)

MRI features of MSG

Fewer glia and/or neurons result in a small brain (microcephaly). This is generally associated with a more or less simplified gyral pattern. Gyration is less complex and the number of gyri is reduced. There is a continuum between normal gyration and a simplified pattern however, and a clear distinction is not always possible. It is more informative to

look at the complexity of the sulci formation, which can give an indication of the maturity of the gyral pattern. A useful measurement for this can be found in vd Knaap et al (see figure 5).[25] An even simpler, but still reliable measure of severity is to use a visual rating scale. This visual gyration score is defined in a 3-point rating scale, based on the classification of normal gyration during early brain development: (1) a normal pattern, where gyri and sulci are branched; (2) a simplified pattern, where the width of the gyri is equal to the depth of the sulci; and (3) a severely simplified pattern with shallow gyri and sulci.[32] In MSG there are no other malformations of the brain, except for aspecific abnormalities such as mild dilatation of the ventricles or a small pons or cerebellum.

Clinical features of MSG
In children with autosomal recessive microcephaly with simplified gyral pattern, head circumference is more than 3 SD below the mean. Children are generally mentally retarded, but show no specific neurological abnormalities such as epilepsy or spasticity. Syndromal variants of MSG, such as Amish lethal microcephaly or Nijmegen breakage syndrome usually do show more severe neurological deficits and a reduced life expectancy.

Causes of MSG
MSG can be a consequence of fewer proliferative cell cycles in early development, or of loss of progenitor cells or neurons by apoptosis. Classic microcephaly with simplified gyral pattern is also known as 'microcephalia vera', and several causative genes have been identified that are all involved in mitosis organization (see appendix). Mutations cause a reduction in the number of times that neuroblasts are able to divide, and therefore a reduction in the resulting total number of neurons and glial cells. MSG can also result from a loss of neuroblasts due to cell death, for example in DNA-repair disorders. In these cases the phenotype is more severe (see appendix).

Pathology of MSG
No specific microscopic abnormalities are found in patients with classic microcephaly with simplified gyral pattern.[33]

3.1.2 Megalencephaly

The opposite of MSG occurs in megalencephaly (large brain, more than 3 SD above normal). Increased proliferation of neurons or glial cells or an impairment of apoptosis can result in a large brain. Examples of megalencephaly are diseases that affect growth regulation pathways, such as tuberous sclerosis complex and neurofibromatosis type 1. This needs to be differentiated from macrocephaly (large head) caused by hydrocephalus, enlargement of the bony skull, subdural hematoma or effusion, storage disorders or leukencephalopathies. These disorders are not included in the scope of this thesis. Megalencephaly can also be seen combined with other congenital malformations, e.g. in megalencephaly-polymicrogyria-polydactyly-hydrocephalus (MPPH) syndrome (see also chapter 6.2).[34]

3.1.3 Disorders of differentiation

Local abnormalities of the cellular structure of the cortex are called focal cortical dysplasias. Cortical dysplasias are thought to either result from abnormal maturation or differentiation of neurons or from abnormal migration (based on 'migration lines' of neuronal tissue in the white matter proximal to the dysplasia).[35] Cortical dysplasias consisting of purely heterotopic 'normal' neurons (type 1 cortical dysplasia) should be considered 'true' migration disorders, but are also included in this paragraph for the sake of clarity. [36]

Clinical features of cortical dysplasia

Cortical dysplasias are highly epileptogenic, and are rigorously sought for in children with intractable focal epilepsy, as they are the most frequent cause of focal refractory seizures in this age group.[36,37] This may have resulted in detection bias, as non-epileptogenic cortical dysplasias are less likely to be found. Epilepsy surgery has a high chance of success in patients with cortical dysplasia, but higher in type II than in type I cortical dysplasia (see below).[38,39] Generally, small isolated cortical dysplasias do not result in other neurological deficits; however cortical dysplasias are also seen in the context of diseases such as tuberous sclerosis complex and hemimegalencephaly.

MRI features of cortical dysplasia

MRI features can be subtle. Some cortical dysplasias are only found by histopathological investigations after epilepsy surgery; 'MRI-negative cortical dysplasias'. Hopefully, these patients will benefit from improving MRI techniques. Clues to look for are focal cortical thickening, blurred gray-white matter junction, and related 'migration lines'. These are gray matter lines in the subcortical white matter. If these are visible and extend to the lateral ventricle, the abnormality is called a transmantle dysplasia. MRI features suggestive of a type II cortical dysplasia (with abnormal neurons) are increased cortical thickness, transmantle signs, and FLAIR or T2 weighted signal change.[37] Migration lines and the gray-white matter junction are best appreciated on T1 weighted imaging. T2 weighted images or FLAIR images show associated white matter hyperintensity or gliosis. Hippocampal atrophy and signal change are suggestive of type I cortical dysplasia.[37] Cortical dysplasias can also be part of a larger cortical malformation in hemimegalencephaly. Low-grade developmental tumors, the DNET (dysembryoplastic neuroepithelial tumor), ganglioglioma, and gangliocytoma are considered to be (related to) cortical dysplasias as they are probably the result of abnormal proliferation/differentiation. These tumors may have a solid mass, cysts, calcifications, and may enhance with gadolinium.

Causes of cortical dysplasia

One of the most important causes of focal cortical dysplasia due to abnormal proliferation is tuberous sclerosis complex. Growth regulation is impaired in this neurocutaneous disease, not only leading to increased number of neurons, but also the formation of abnormally large neurons that form the characteristic cortical 'tubers' or hamartomas of

the disease. It is a multi-organ disease and is not further explored in this thesis. Hemimegalencephaly is found sporadically or in association with hypomelanosis of Ito, Proteus syndrome, Klippel-Trenaunay, tuberous sclerosis complex, or linear naevus sebaceous. These syndromes are not included in the tables in the appendix.

Pathology of cortical dysplasia

Histopathologically, cortical dysplasias are graded by the Palmini grading system (see table 2).[36,39] Dysmorphic neurons are misshapen cells with abnormal orientation, size, cytoskeletal structure, and atypical dendritic processes. Balloon cells are abnormal cells with pale, eosinophilic cytoplasm and eccentric nucleus or nuclei, as some are multinucleated. They are usually larger than astrocytes and can have neural or glial immunohistochemical characteristics. Giant neurons are large neurons (compared with layer V pyramidal neurons) with central nuclei, but have a pyramidal morphology and are not dysmorphic. Ectopic neurons or heterotopias are microscopic or macroscopic clusters of normal appearing neurons that are not in their normal place, e.g. in the white matter.

Table 2: Palmini histopathologic grading system for cortical dysplasia.[36,39]

Grade	Severity	Histopathologic description
mMCD*	Very mild	Normal cortex with excess ectopic neurons
A		Only in or adjacent to the molecular layer (layer I) or
B		In the subcortical white matter outside of layer I.
Type I	Mild	Cortical disorganization and dyslamination without abnormal dysmorphic–cytomegalic neurons or balloon cells.
IA		Type IA is cortical disorganization with no other abnormalities.
IB		Type IB is cortical disorganization with immature or hypertrophic but not dysmorphic neurons.
Type II	Severe	Cortical disorganization and dyslamination with abnormal dysmorphic–cytomegalic neurons and balloon cells. Sometimes referred to as Taylor's type cortical dysplasia.
IIA		Type IIA contains dysmorphic–cytomegalic neurons without balloon cells.
IIB		Type IIB contains balloon cells

* mMCD, mild malformation of cortical development.

3.2 Disorders of migration

A complete inability to migrate results in a smooth cortex with a histopathologically inverted and thickened cortex; type 1 lissencephaly. Less severe migration disturbances that affect all or a subset of neurons give a milder MRI pattern in the same spectrum: pachygyria or subcortical band heterotopia. Another type of disorder of migration is seen in cobblestone lissencephalies, where neurons do start their radial migration, but fail to detach at the appropriate place and pass through the pial membranes. This can be due to abnormal membrane function due to deficient o-glycosylation. Neurons move on onto the cortex creating a cobblestone-like aspect of the surface. Cobblestone lissencephaly was previously called type II lissencephaly, and is often seen in association with congenital muscular dystrophy and eye abnormalities. A third type of migration disorder is periventricular nodular heterotopia. Clusters of gray matter line the ventricles consisting

of groups of neurons that failed to start migration. The overlying cortex may have a normal or abnormal appearance.

3.2.1 Lissencephaly

MRI features

In the lissencephaly-pachygyria-subcortical band heterotopia spectrum, MRI features can distinguish well between the different types of lissencephaly and give direction to additional genetic tests. In type 1 or classic lissencephaly the cortex is smooth and thickened (>10mm) with a cell sparse zone.[40] Lack of opercularization results in a typical 'figure of eight'-configuration (figure 7). Milder types of lissencephaly show thickened gyri (pachygyria) or fairly normal gyri with a band of gray matter below, the subcortical band heterotopia, consisting of neurons arrested during migration. Taking note of which areas of the brain are most affected aids investigations into the cause. This also goes for associated abnormalities of the corpus callosum or cerebellum. Type 1 lissencephaly affects mainly the cortex, more severely in the occipital than the frontal lobes. Its severity can be graded into six categories, grade 1 being most severe (see table 3 and figure 7).

Table 3: classic lissencephaly grades 1-6

Grade	MRI features
grade 1	complete agyria
grade 2	agyria with few shallow anterior sulci
grade 3	posterior agyria and anterior pachygyria
grade 4	diffuse pachygyria posterior>anterior
grade 5	mixed pachygyria and subcortical band heterotopia
grade 6	subcortical band heterotopia only

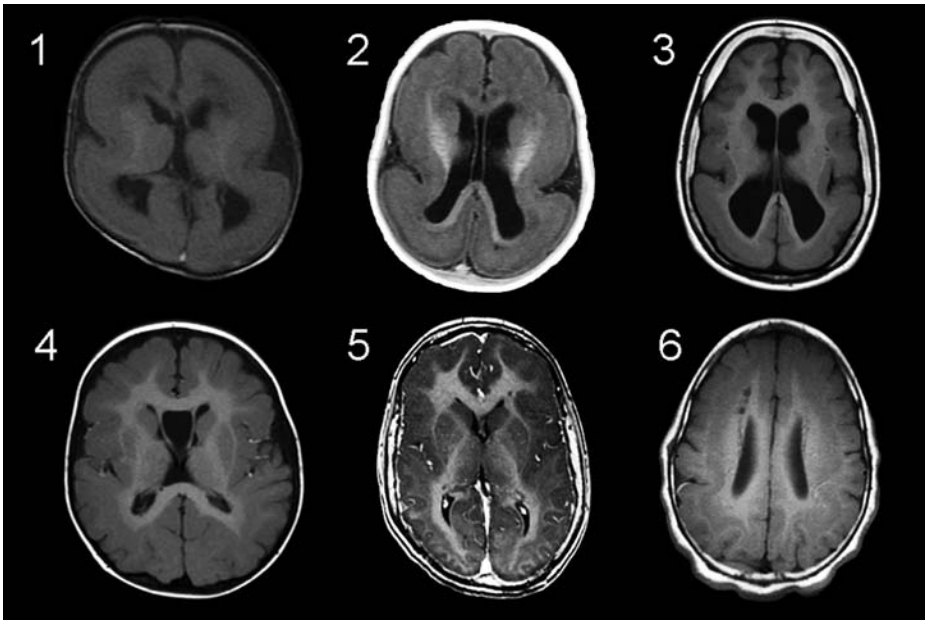


Figure 7: classic lissencephaly grades 1-6, note 'figure-of-eight' configuration in grade 1 lissencephaly.

Non-classic lissencephalies are type II lissencephaly or cobblestone lissencephaly (see also 3.2.2), and lissencephalies associated with abnormalities of the cerebellum or corpus callosum. Lissencephaly with cerebellar hypoplasia can be subdivided into types a-f (table 4). Naturally, not all patients always fit neatly into these categories, e.g. those patients with focal pachygyria.

Table 4: Lissencephaly with cerebellar hypoplasia (LCH) types.[41]

LCH types	MRI features
Type a	Vermis hypoplasia, lissencephaly/pachygyria with p>a or a>p gradient
Type b	Cerebellar hypoplasia with abnormal foliation, pachygyria a>p, cortex 5-10 mm, malformed hippocampus
Type c	Severe cerebellar and brainstem hypoplasia with diffuse agyria/pachygyria, severe microcephaly
Type d	Moderate cerebellar hypoplasia, microcephaly, agyria-pachygyria cortex 10-20 mm
Type e	Moderate vermis hypoplasia, frontal agyria, occipital simplified gyral pattern
Type f	Absent corpus callosum, microcephaly, agyria/pachygyria, mild brainstem hypoplasia

p = posterior, a = anterior

Clinical feature of lissencephaly

Lissencephaly type I was the earliest recognized MCD, and consequently much is known about the clinical features. Signs and symptoms are very diverse and range from normal intelligence to severe mental retardation. There is a relationship between the severity of the lissencephaly/pachygyria grading based on neuroimaging and outcome with respect to the severity of psychomotor retardation, motor symptoms and epilepsy.[40] Most to all patients have epilepsy. Lissencephaly type I is associated with facial dysmorphic features when part of the Miller-Dieker syndrome. This syndrome is caused by a microdeletion of 17p13.3 including the *LIS1* gene and the 14-3-3ε gene (also known as the *YWHAE* gene). These patients show bitemporal hollowing, small jaw, short nose with upturned nares, long upper lip with thin vermillion border and a flattened midface with vertical furrowing of the forehead, but these dysmorphic features may be subtle or absent. It is also associated with cardiac malformation, genital anomalies in boys, sacral dimple and clinodactyly (OMIM 247200). Interestingly, deletion of *YWHAE* without affecting the *LIS1*-gene results in craniofacial dysmorphic features, growth restriction, and on MRI of the brain some gliosis.[42] In a single patient periventricular nodular heterotopia have been reported.[43]

Causes of lissencephaly

In 1993 the first gene involved in malformations of cortical development was identified when mutations in *LIS1* on chromosome 17p were found to be responsible for autosomal dominant type I lissencephaly.[44] Since then several more genes have been found, summarized in the appendix. Lissencephaly type I results from a failure to complete radial migration (along radial glial fibers) of excitatory glutamatergic pyramidal neurons. If the clinical phenotype with facial dysmorphic features suggests Miller-Dieker syndrome, then FISH investigation of the 17p13.3 region should be the initial test. In classic lissencephaly with a posterior to anterior gradient *LIS1* mutations are very likely, and current advise is to order MLPA testing first, as intragenic and submicroscopic deletions are common, followed as needed by gene sequencing.[45] Lissencephaly with a reversely oriented gradient (anterior

to posterior) is found in mutations in *DCX*, and *DCX* mutations are also specific for subcortical band heterotopia in females. Deletions are commonly found.[46] The severity is not only determined by the type of mutation, but also by the result of X-inactivation in girls. In boys mutations in *DCX* are a rare cause of lissencephaly.[47] In animal models *DCX* mutations are not only associated with problems in radial migration of excitatory neurons, but also in GABA-ergic interneurons and neuronal maturation. This may be relevant to understand the epilepsy in these patients.[48,49] In patients with pachygyria combined with cerebellar hypoplasia, mutations in *RELN*, *VLDLR* or *TUBA1A* are more likely (see appendix).[50,51] In *TUBA1A* related lissencephaly, the pachygyria shows a predilection for the perisylvian regions which does not fit into the classic grading system.[51] *TUBA1A* mutations are specifically found in patients with lissencephaly (perisylvian) with cerebellar and/or brainstem hypoplasia and corpus callosum abnormalities (LCH type c or f). However, also in approximately 5% of classic lissencephaly patients *TUBA1A* mutations can be found. Phenotypes similar to *LIS1* phenotypes are related to the *TUBA1A* R402C mutation.[51] Very rarely lissencephaly with callosal agenesis and abnormal genitalia in boys is caused by mutations in X-linked *ARX*.[52] This homeobox gene causes a diverse array of phenotypes when disturbed, from mental retardation to various types of epilepsy or malformation of cortical development. It affects GABA-ergic interneuron migration primarily.

Pathology of lissencephaly

The histopathology of *LIS1*-related lissencephaly shows a typical 4 layered inverted cortex instead of the normal 6-layered pattern, most prominently in the occipital cortex. The 4-layered lissencephaly is characterized by a molecular layer (layer I) containing Cajal-Retzius cells, a pyramidal cell layer, a cell-sparse layer that is myelinated in patients older than 2 years and can be visible on MRI (layer III), and a thick layer of disorganized neurons (layer IV). Sometimes the boundary between the grey and white matter can be blurred by vertical clusters of axons. Microscopic white matter heterotopia are seen in some patients with documented *LIS1* mutations, but these are unlikely to be visible on MRI.[53]

In two published cases of lissencephaly based on *DCX* mutations, a vaguely 4-layered lissencephalic cortex in the frontal lobes was described, but it was not like the typical cortex associated with *LIS1* mutations. Layer I contained Cajal-Retzius neurons and was indistinguishable from *LIS1* related lissencephaly. Layer II contained pyramidal neurons but was relatively thin with only a few pyramidal neurons. In one of the 2 cases examined, there was a relatively well-formed layer III with sparse myelination. In both cases, this layer contained more neurons than in brains with *LIS1* mutations. Layer IV was thickened, but also contained more pyramidal neurons than were found in *LIS1* brains. At the junction between the grey and white matter there was a transition between the lissencephalic cortex to multiple small nodules of subcortical heterotopia that made up the subcortical band. The two lissencephalic brains with *DCX* mutations showed a transition to relatively normal 6-layered cortex ventrally and diffusely disorganized cortex occipitally.[53]

In two published cases of *ARX* mutation related lissencephaly histopathology showed a 3-layered cortex. There was a hypercellularity of the molecular layer that consisted of primarily small- to medium-sized neurons. Immediately below this layer was a relative

increase in pyramidal cells followed by a thick layer of small- and medium-sized neurons, including pyramidal neurons of various sizes. No hypocellular or myelinated layer was detected.[53]

In severe forms of *TUBA1A* related lissencephaly in four prenatally diagnosed patients various abnormalities were seen. Consistently observed were developmental anomalies in cerebral gyration (complete agyria usually without an identifiable Sylvian fissure and thinner cortical plate), the corpus callosum, hippocampus, cerebellum and brainstem, with various degrees of severity. Dystrophic axonal tracts with aberrant pathways were seen in the periventricular white matter suggesting abnormal axonal guidance. In one case of 35 weeks gestational age the cortex was abnormally thick and similar to the four-layered cortex of classical lissencephaly. Labelling studies suggested that migration abnormalities involved projection neurons as well as interneurons. The ventricles were at most only slightly enlarged. The white matter was poorly developed and the periventricular areas contained heterotopic immature neurons, which are rarely observed in classical lissencephaly. The cerebellum and brainstem were consistently affected with a variable degree of hypoplasia with heterotopic cells in the cerebellar hemispheres and olivary heterotopia in the medulla.[54]

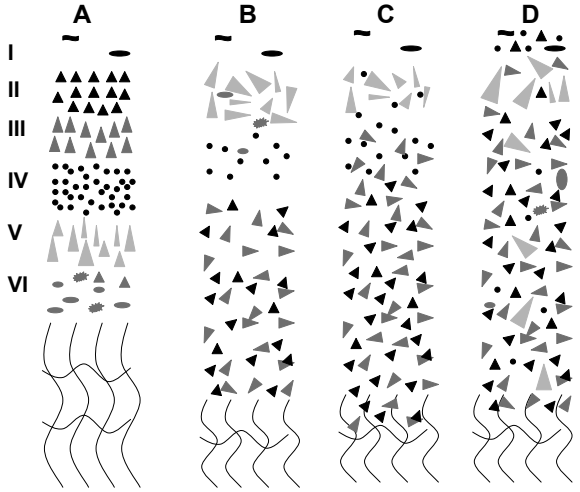


Figure 8: simplified representation of lissencephaly pathology.
A: normal cortex, with six layers (I: molecular layer, II: external granular layer, III: external pyramidal layer, IV: internal granular cell layer, V: internal pyramidal layer, VI, fusiform or multiform layer).
B: LIS1 related lissencephaly, C: DCX related lissencephaly, D: ARX related lissencephaly.

3.2.2 Cobblestone lissencephalies

MRI features of cobblestone lissencephaly

The MRI imaging of cobblestone or type II lissencephaly can be similar to type 1 lissencephaly in terms of agyria or pachygyria. In high quality imaging the uneven aspect of the cortex can be visible. Projections of white matter into the cortex result in an irregular gray-white matter junction. This can be better appreciated after the neonatal period. The

irregularity of the gray-white matter junction can appear similar to that seen in polymicrogyria, and mild cobblestone phenotypes and polymicrogyria may be indistinguishable on MRI.[55]

Clinical features of cobblestone lissencephaly
Cobblestone lissencephalies are typically seen in the context of three congenital muscular dystrophy syndromes: muscle-eye-brain disease, Fukuyama-muscular dystrophy and Walker-Warburg syndrome. Children with Walker Warburg syndrome are most severely affected of this group. They all have retinal anomalies and muscular dystrophy with elevated creatine kinase. Milder variants of muscle-eye-brain disease may show (near) normal mental development, limb-girdle muscular dystrophy and less severe eye abnormalities.

Causes of cobblestone lissencephaly
Cobblestone lissencephalies result from an overmigration of radially migrating neurons. Normally the glia limitans 'limits' further migration and defects result in migration of neurons into the subarachnoid space. Up until 2009, all genes found in cobblestone lissencephaly with muscular dystrophy and eye abnormalities caused defective O-glycosylation of alpha-dystroglycans (see appendix). Once this pattern was discovered, other genes in this pathway were quickly found and the extent of the phenotype described. Recently mutations in *TUBB2B* were found to cause an MRI pattern of asymmetric polymicrogyria, however neuropathology was compatible with cobblestone lissencephaly.[56] *TUBB2B* is involved in microtubule function and patients do not show muscular dystrophy or eye abnormalities. Other genes unrelated to O-glycosylation, have also been implicated in animal models.[57,58]

Pathology of cobblestone lissencephaly
In the cortex ectopic clusters of neurons are seen. There are projections of white matter with fibrovascular tissue between the clusters. In the white matter ectopic neurons can be found. Migrating neurons seem to have moved through the glia limitans onto the outer cortex creating an uneven surface.[56] This can be difficult to see on MRI.

3.2.3 Heterotopia

MRI features of heterotopia
Heterotopia are formed of neurons that have not migrated to their destination in the cortex. As they are otherwise normal neurons, they have all characteristics of gray matter on MRI. This distinguishes heterotopia from other periventricular abnormalities such as calcifications in congenital infections and subependymal noduli in tuberous sclerosis complex. Heterotopia can be unilateral or bilateral, and isolated versus continuous. Bilateral continuous periventricular heterotopia with an otherwise normal brain, except for an enlarged retrocerebellar space, is the classical phenotype of *FLNA* mutations. Distinctly different types of heterotopia are those found in the cerebral white matter. The

typical subcortical band heterotopia is considered to be part of the lissencephaly-pachygyria continuum and is discussed in section 3.2.1.

Clinical features of heterotopia

Clinical features are very diverse, may give no neurological complaints and can be a chance finding. Psychomotor development may be completely normal or delayed. Periventricular nodular heterotopias give a high risk of epilepsy, but seizures may be delayed until adulthood. Associated congenital anomalies may give a clue to the underlying cause, e.g. cardiac malformations are associated with *FLNA* mutations.

Causes of heterotopia

The most common monogenetic cause are loss-of-function mutations in X-linked *FLNA*.[59,60] This causes bilateral periventricular nodular heterotopia, but also cardiac malformations in the outflow tract and Ehlers-Danlos like skin and joint anomalies. Rarely bilateral PNH are caused by autosomal recessive mutations in *ARFGEF2*, also associated with microcephaly, severe mental retardation, movement disorder and epilepsy.[61] There are many syndromes associated with periventricular heterotopia (see appendix). For subcortical heterotopia or heterotopia in the white matter, no specific causes are known so far.

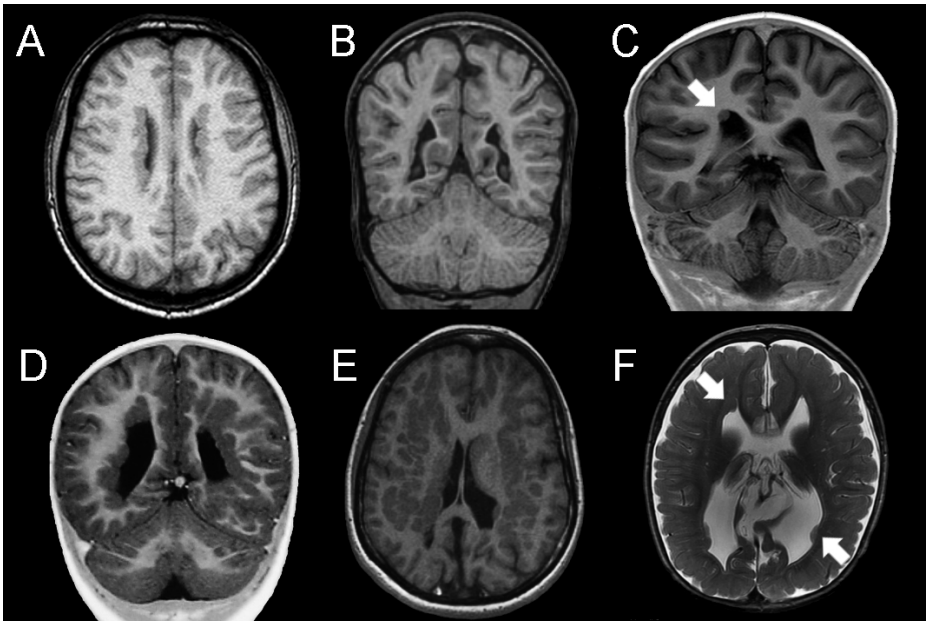


Figure 9: examples of MRI patterns of heterotopia. A: T1-weighted transversal image showing bilateral continuous periventricular nodular heterotopia in a patient with a *FLNA* mutation. B: T1-weighted coronal image showing bilateral, almost continuous periventricular nodular heterotopia in a patient with autosomal recessive *ARFGEF2* mutations. C: T1-weighted coronal image showing a unilateral, isolated periventricular nodular heterotopia (arrow) without known cause. D: T1-weighted coronal image showing extensive bilateral continuous periventricular and white matter heterotopia without known cause. E: T1-weighted transversal image showing bilateral subcortical heterotopia without known cause. F: T2-weighted transversal image showing bilateral isolated periventricular nodular heterotopia in a patient with a meningomyelocele, Arnold Chiari malformation and hydrocephalus.

Pathology of heterotopia

In bilateral periventricular nodular heterotopia due to *FLNA* mutations the heterotopia contains normal appearing neurons. Apart from the heterotopia, glomeroid vascular abnormalities have been reported in autopsy material.[62,63] Interestingly, nodular heterotopia show an intrinsic laminal specificity that is similar to that of the cortex .[64] This suggests that the neurons in the heterotopia fail to migrate, but do attempt to differentiate normally. PET studies also suggest that neurons in heterotopic gray matter participate in neural networks and brain function.[65,66]

3.3 Disorder of cortical organization:

Polymicrogyria is the most heterogeneous malformation of cortical development, both in appearance as in causes. It can be seen as an isolated malformation, but also in combination with periventricular nodular heterotopia or other brain malformations such as corpus callosum agenesis or cerebellar abnormalities.

3.3.1 Polymicrogyria

MRI features of polymicrogyria

Polymicrogyria is probably the most common MCD and also probably the most difficult malformation to recognize on MRI imaging. This is partly due to the marked change in its appearance during the first years of life, and partly to its heterogeneous nature. In newborns the cortex is thin and due to a lack of myelination the gray-white matter border is harder to appreciate. In these infants the polymicrogyric cortex shows numerous, very small ripples. After myelination the polymicrogyric cortex appears thickened with an unclear white-matter junction. If the resolution of the imaging allows, it can be appreciated that this 'fuzziness' results from the small undulations of the cortex. The surface of the cortex may appear lumpy-bumpy or may seem smooth due to fusion of the upper layer of the cortex across adjacent microgyri. Not surprisingly, the compaction of small gyri can be easily mistaken for a thickened cortex (pachygyria). This is a common mistake in interpreting MCD on MRI and a high level of suspicion is necessary.[1,67] When in doubt, repeat MRIs after the age of 18 months can be very useful as myelination changes the appearance of polymicrogyria (see figure 10).[68]

Polymicrogyria can also manifest as schizencephaly, which are clefts in the neocortex reaching the ventricle lined with PMG. Polymicrogyria can be difficult to distinguish radiologically from cobblestone malformation.

PMG subtypes can be recognized based on differences in topography, such as diffuse or generalized, frontal, posterior, mesial parieto-occipital, multilobar, and perisylvian forms. The perisylvian form accounts for 60–70% of patients, with all other forms less commonly seen.[69] The severity of perisylvian polymicrogyria may be graded using the system by Leventer, separately grading the left and right hemisphere if applicable (see table 5).[70] Other PMG subtypes cannot be graded with this system.

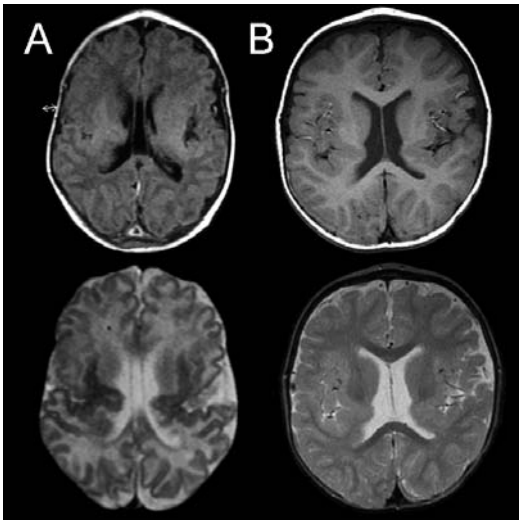


Figure 10: example of changing aspect of polymicrogyria over time in a boy with psychomotor retardation and spastic tetraplegia. A) upper panel T1 weighted and lower panel T2 weighted transversal MRI images at the age of three weeks, and B) the same images at the age of 29 months. Note the difficulty in recognizing the irregular gray-white matter junction in the early MRI. Also note the advanced myelination in the abnormal perisylvian regions, which can be an early indication of a cortical malformation.

Table 5: grading of perisylvian polymicrogyria severity

Grade	MRI features
Grade 1	Entire perisylvian region extending to other brain regions including one or both poles
Grade 2	Entire perisylvian region extending to other brain regions but not involving either pole
Grade 3	Entire perisylvian region
Grade 4	Posterior perisylvian region

Clinical features of polymicrogyria

Bilateral perisylvian polymicrogyria results in the so-called perisylvian syndrome. Motor function of the oral musculature is impaired, resulting in speech delay, dysarthria and dysphagia. This clinical syndrome has also been described as Worster-Drought syndrome, however also children without PMG can have this syndrome.[71] PMG can be unilateral or bilateral. In unilateral cases clinical features resemble cerebral palsy, with a congenital spastic hemiparesis. Epilepsy is common, but not obligatory.[1]

Causes of polymicrogyria

Polymicrogyria is the probably the MCD with the most heterogeneous causative mechanisms. Several non-genetic causes have been described, such as congenital cytomegalovirus infection or prenatal ischemia.[72-75] Several genes and microdeletion syndromes have been associated with PMG, but each seems to be responsible for only a small group of patients (see appendix). The most important established genetic cause is the 22q11 microdeletion syndrome.[70]

Pathology of polymicrogyria

PMG is characterized by an excessively folded cortical ribbon of miniature, individually thin gyri, which may be fused together or piled on top of each other. Predilection areas are around the insula and the central fissure. The normal 6-layered cortex can be reduced to four layers or no clear layers may be seen at all. The four layers consist of a molecular layer and two layers of neurons separated by an intermediate layer of few cells.[76] Pathology can be the only way to distinguish PMG from cobblestone complex, as brain MRI may be ambiguous. [77]

4. Scope of the thesis

During the last decade (pediatric) neurologists have benefited from increasing possibilities in imaging the human cerebral cortex. This is based on advancements in neuroimaging techniques, but also on the possibility of improving image quality by scanning young children under general anaesthesia. We have now recognized that malformations of cortical development (MCD) can explain epilepsy and neurological deficit in many patients. Genetic research in these patients has been able to identify several involved genes already; however the underlying cause of these malformations has remained elusive in many patients.

The aims of the studies described in this thesis are to establish the yield of etiological diagnosis in patients with MCD by detailed clinical and radiological description, classification, and by genetic testing, to describe the natural history of patients with MCD, and to describe new phenotypes and syndromes.

To achieve this aim a cohort of patients with malformations of cortical development has been recruited from 2001 onwards. This consisted of retrospectively ascertained patients that were re-evaluated and patients that were newly referred. All patients were periodically seen for evaluation of the history, and a neurological and dysmorphological exam. Brain MRIs were performed according to a standard protocol. This cohort of over 200 patients with different types of MCD forms the basis of the studies of this thesis. The cohort mainly consists of, but is not limited to, children.

In **chapter two** our cohort of patients with MCD is described and we show in how many patients an etiological diagnosis can be currently made by combining clinical, radiological, and genetic classification, with syndrome identification, family study, and diagnostic molecular testing.

In **chapter three** we describe new insights in the natural history of patients with different MCDs with known causes, in particular mutations in *LIS1* and *FLNA*.

In **chapter four** we discuss new phenotypes resulting from description of our cohort, in particular a new phenotype associated with *ARFGEF2* mutations and a group of patients with microcephaly with simplified gyral pattern with early onset diabetes.

In **chapter five** we describe the association of MCD and metabolic disease in a case of malonyl-CoA decarboxylase deficiency.

In **chapter six** we discuss syndromal associations observed in our patients. This involves a group of patients with periventricular nodular hetertopia and limb deficiency and patients with MPPH-MCD (megalencephaly, polydactyly, PMG, hydrocephalus syndrome, also known as macrocephaly capillary malformation).

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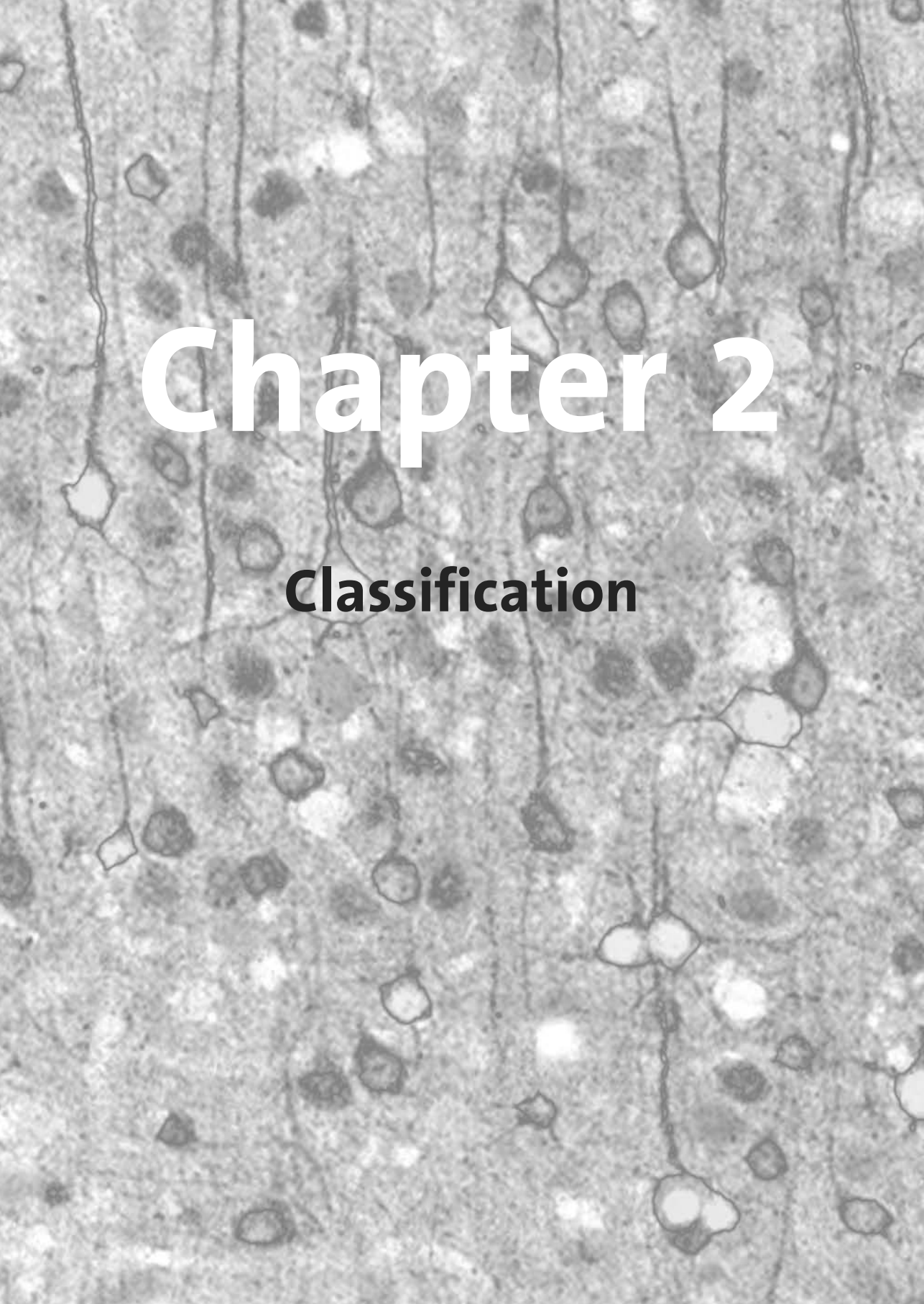

Chapter 2

Classification

2.1

Cortical brain malformations: results of clinical, neuroradiological and genetic classification

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ABSTRACT

Background:
Malformations of cortical development (MCDs) are a major source of handicap. Much progress in understanding the genetic causes has been made recently. The number of affected children in whom a molecularly confirmed diagnosis can be made is unclear.
Objective:
To evaluate the etiology of MCDs in children and the effect of a combined radiological, clinical, and syndrome classification.
Design:
A case series of 113 children with a radiological diagnosis of MCD from January 1, 1992, to January 1, 2006.
Setting:
The Erasmus MC–Sophia Children's Hospital, a secondary and tertiary referral center.
Patients:
Patients with MCD underwent a complete radiological, clinical, and neurological assessment and testing for known genes involved in the pathogenesis of MCD as appropriate for their phenotype.
Results:
We established an etiological diagnosis in 45 of 113 cases (40%). For 21 patients (19%), this included molecular and/or genetic confirmation (Miller-Dieker syndrome; *LIS1, DCX, FLNA, EIF2AK3*, or *KIAA1279* mutations; or an inborn error of metabolism). In 17 (15%), a syndrome with an unknown genetic defect was diagnosed. In 7 patients (6%), we found evidence of a gestational insult. Of the remaining 68 patients, 34 patients probably have a yet-unknown genetic disorder based on the presence of multiple congenital anomalies (15 patients), a family history with multiple affected persons (12 patients), or consanguineous parents (7 patients).
Conclusions:
In our cohort, combining diagnostic molecular testing with clinical, radiological, and genetic classification; syndrome identification; and family study provided a diagnosis in 40% of the cases of MCD. This contributes to the possibility of prenatal diagnosis and improved patient treatment and disease management.

CORTICAL BRAIN MALFORMATIONS

Effect of Clinical, Neuroradiological, and Modern Genetic Classification

Malformations of cortical development (MCDs) are a major source of mental retardation, motor dysfunction, and epilepsy in children. Up to 10% of children with epilepsy are estimated to have an MCD, and the percentage is greater in those with intractable epilepsy.[1] Not all patients with MCD develop epilepsy, however, and the level of motor and mental handicap varies. In recent years, much progress has been made in understanding the genetic and molecular basis of cortical development. Several essential genes have been identified (table 1). Also, MCDs have been described in patients with known syndromes such as the Adams-Oliver, Delleman, MICRO, and Kabuki make-up syndromes.[2-6] Illustratively, the London Medical Dysmorphology Database includes 46 syndromes with MCD as a requirement and 232 with MCD as a possible feature.

Accurate classification is important for patient care and for improving insight into different phenotypes and causes. The 2005 classification system for MCD distinguishes disorders of cell proliferation, migration, and cortical organization and is useful for guiding the diagnostic approach in individual patients.[7] This approach starts with a detailed brain magnetic resonance imaging (MRI) study, combined with medical and family history and a pediatric and neurological examination. Affected relatives, parental consanguinity, dysmorphic features, and typical MCD patterns can suggest genetic causes. Complications during pregnancy or congenital infections can be compatible with a fetal insult. Internal organ dysfunction and a progressive course may suggest a metabolic disease.[8]

Apart from monogenetic mutations, chromosomal anomalies should be looked for. Some microdeletions can cause distinctive MCD, particularly interstitial deletions of chromosome 17p in lissencephaly, duplications of 5p in nodular heterotopia, and microdeletions of 22q11 in polymicrogyria (PMG).[9-11]

The aims of the present study were to evaluate the effect of clinical, radiological, and genetic test results on etiological diagnosis in a series of consecutive cases of MCD in a university children's hospital; to describe the relationship between diagnosis and clinical features; and to evaluate the consequences of an etiological diagnosis for disease management and genetic counseling.

Table 1. Genes known to be involved in MCD

Gene	Chromosome	OMIM No.[a]	Protein	Inheritance	Description
MCPH1	8p	607117	Microcephalin	AR	MCPH phenotype of a simplified gyral pattern, microcephaly, and relatively good clinical function
ASPM	1q	608716	Abnormal spindle-like, microcephaly associated protein	AR	MCPH phenotype
CDK5RAP2	9q34	608201	CDK5 regulatory subunit–associated protein 2	AR	MCPH phenotype
CENPJ	13q12.2	609279	Centromeric protein J	AR	MCPH phenotype
SLC25A19	17q25.3	607196	Mitochondrial deoxynucleotide carrier	AR	Simplified gyration, early death, 2-ketoglutaric aciduria
EIF2AK3	2p12	226980	eIF2 kinase 3	AR	Simplified gyral pattern in Wolcott-Rallison syndrome
LIS1	17p13.3	607432	Platelet-activating factor acetylhydrolase, isoform 1b, α subunit	AD	Pachygyria with a posterior-to-anterior gradient
DCX	Xq22-23	300121	Doublecortin	XL	In boys, lissencephaly; in girls, subcortical band heterotopia
RELN	7q22	600514	Reelin	AR	Lissencephaly with cerebellar and brainstem hypoplasia
VLDLR	9p24	192977	Very low-density lipoprotein receptor	AR	Pachygyria with cerebellar hypoplasia
ARX	Xp22.13	300382	Aristaless-related homeobox protein	XL	Diverse phenotypes from lissencephaly, abnormal genitalia, and corpus callosum agenesis to XL mental retardation
TUBA3	12q12	602529	Tubulin	AD	Pachygyria, hypoplastic splenium, vermis, and medulla
FLNA	Xq28	300017	Filamin A	XL	Loss-of-function mutations, periventricular nodular heterotopia; sometimes with Ehlers-Danlos syndrome, other skeletal disorder, or cardiac malformation
ARFGEF2	20q13.13	605371	Brefeldin A–inhibited guanine nucleotide exchange protein 2	AR	Periventricular nodular heterotopia, congenital microcephaly, severe developmental delay
GPR56	16q13	604110	G protein–coupled receptor 56	AR	Bilateral frontoparietal PMG, white matter abnormalities
KIAA1279	10q22.1	609367	Unknown	AR	Diffuse PMG, Hirschsprung disease, dysmorphic features
FKRP	19q13.3	606596	Fukutin-related protein	AR	Cobblestone-type lissencephaly, muscular dystrophy, eye abnormalities
FCMD	9q31	607440	Fukutin	AR	Walker-Warburg syndrome
POMT1	9q34.1	607423	O-mannosyltransferase-1	AR	Walker-Warburg syndrome
POMGnT1	14q24.3	607439	O-mannosyltransferase-2	AR	Walker-Warburg syndrome
LARGE	22q12.3-13.1	603590	Acetylglucosaminyl transferase–like protein	AR	Muscular dystrophy, eye abnormalities, structural brain abnormalities
RAB3GAP1	2q21.3	602536	RAB3 glutamyl transpeptidase–activating protein	AR	Warburg MICRO syndrome with pachygyria, microcephaly, microphthalmia, congenital cataract, hypogonadism

Abbreviations: AD= autosomal dominant; AR= autosomal recessive; MCD= malformations of cortical development; PMG= polymicrogyria; XL= X-linked.
[a]Available at http://www.ncbi.nlm.nih.gov/omim.

METHODS

Patient population

The medical records of children with MCD referred to the Department of Pediatric Neurology or the Department of Clinical Genetics at the Erasmus Medical Center were retrospectively collected from January 1, 1992, through December 31, 2001, and prospectively from January 1, 2002, to January 1, 2006. We stopped inclusion when we started to recruit referrals actively. Information on the retrospective group was collected using the radiology reports as documented in the medical correspondence database of the Erasmus Medical Center–Sophia Children's Hospital using the terms simplified gyral pattern, lissencephaly, pachygyria, polymicrogyria, heterotopia, cortical dysplasia, and migration disorder. All patients with available neuroimaging studies were included. All neuroimaging findings (MRI and computed tomography) were reevaluated by a pediatric neurologist (I.F.M.d.C.), a clinical geneticist (G.M.S.M.), and a pediatric neuroradiologist (M.H.L.). From 1992 to 2005, we included the most recent MRI study if a diagnosis of MCD was made when the patient was younger than 16 years. Consensus was required and patients were classified into 4 groups on the basis of MRI criteria.[7,12] None of the patients had a molecularly confirmed diagnosis.

We excluded patients with neoplasms, tuberous sclerosis, and neurofibromatosis 1. We excluded those with hydranencephaly and holoprosencephaly, unless associated with obvious MCD such as periventricular nodular heterotopia. We excluded patients with infratentorial abnormalities only and patients with a normal cortex and microcephaly or macrocephaly.

Clinical evaluation

If possible, patients were examined at the multidisciplinary outpatient clinic. Epilepsy was diagnosed when seizures were present and electroencephalographic findings were abnormal. The level of mental retardation was defined as mild (IQ, 70-80), moderate (IQ, 50-69), or severe (IQ, <50), based on age-appropriate neurodevelopmental testing, performed by the school or by our clinic.

RESULTS

Patient classification

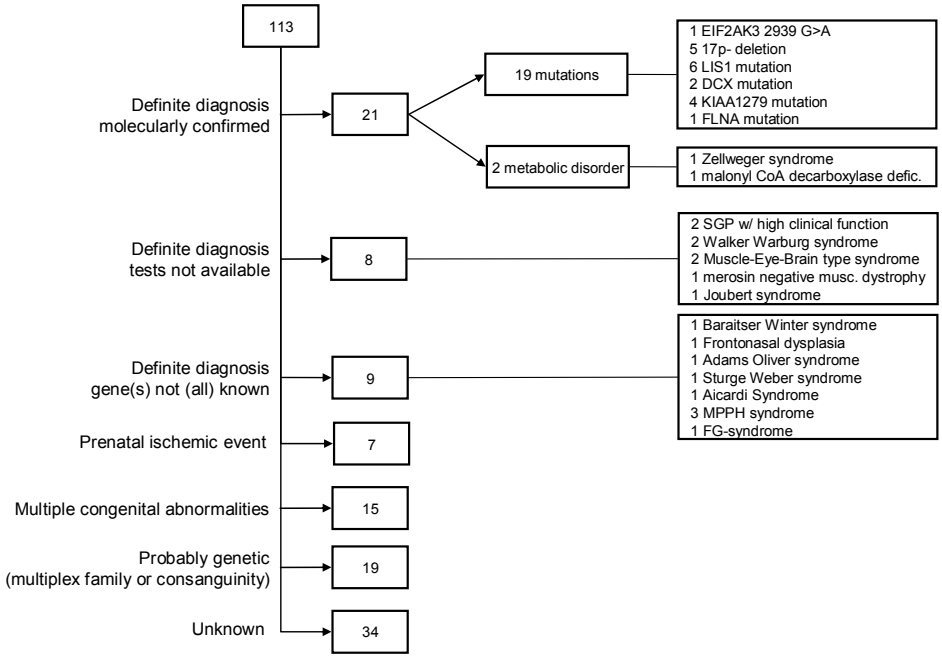
Diagnosis was confirmed retrospectively in 48 patients, with 65 patients added prospectively, resulting in a case series of 113 patients (55 boys and 58 girls). They were classified into 4 groups on the basis of MRI findings. Further classification was based on results of the physical examination and genetic and metabolic tests (Table 2 and Figure 1). In the retrospective search, 76 children were found to have a documented diagnosis of MCD. Thirteen were excluded because of missing brain imaging studies. In 15, the diagnosis was reversed. In the remaining 48 cases, an MCD was confirmed, although the classification was revised in 11 patients. In 6, a diagnosis of pachygyria was changed to PMG. The opposite change was made in 1 case. In 2 cases, a diagnosis of lissencephaly was

revised to simplified gyral pattern (SGP). A presumed cortical dysplasia was found to be PMG in 2 cases.

Table 2. Classification of patients

Group No.	Description (subgroup)	No. of Patients
1	Disorders of proliferation, congenital microcephalies	11
2	Disorders of migration, lissencephaly/heterotopia (all)	
	Agyria, pachygyria, subcortical band heterotopia (A)	24
	Cobblestone-type lissencephaly (B)	2
	Periventricular nodular heterotopia (C)	14
	Periventricular nodular heterotopia with PMG (D)	11
3	Disorders of cortical organization, PMG (all)	
	PMG and schizencephaly (A)	48
	Transmantle dysplasia (B)	1
4	MCDs secondary to inborn errors of metabolism	2
All		**113**

Figure 1. Flowchart of diagnostic results



Epidemiological findings

In the 10 years of retrospective analysis, we found 48 cases; in the 4 years of prospective analysis, 65 cases. This change may be owing to increased alertness and/or the quantity and quality of brain imaging. In addition, the number of first-patient visits increased greatly. At the time of the study, patients were not referred to us preferentially. Per year, 1700 to 2000 new patients were referred to the pediatric neurology outpatient clinic, making the incidence of MCD in our population approximately 8 cases for every 1000 new patients.

Clinical Overview

Before or during follow-up, 68 patients (60%) developed epilepsy. Significantly fewer patients with PMG had epilepsy than in the other groups (Table 3). They may develop epilepsy at an older age. Mental retardation was present in 106 of 113 patients, although the degree was mild in 27. The severity of mental retardation was correlated with the presence of epilepsy and its age at onset (P=.001). If a first seizure happened before 9 months of age (median age at onset), most of these patients (31 of 35 [91%]) showed moderate to severe mental retardation, whereas this was found in 17 (57%) of the 30 patients who developed seizures later in life. This probably reflects the severity of the underlying brain malformation. All 22 children who presented with neonatal seizures or infantile spasms developed severe mental retardation, and 8 died during infancy. Overall, 17 children died during follow up. Neurological motor problems were common, including a spastic tetraparesis in 26 children, a spastic hemiparesis in 19, and severe hypotonia in 18. A range of congenital major and minor anomalies was seen.

Table 3. Clinical features of epilepsy and mental retardation

Group No.	Subgroup	No. of patients	Moderate/severe mental retardation, no. (%)	Epilepsy, No. (%)	Median age at onset of epilepsy, y
All	Overall	113	79 (70)	68 (60)	0.8
1	Microcephaly	11	9 (82)	6 (55)	0.2
2A	Pachygyria	24	19 (79)	19 (79)	1.8
2C	PNH	14	8 (57)	10 (71)	2.9
2D	PNH with PMG	11	7 (64)	9 (82)	0.8
3	PMG	48	32 (67)	20 (42)*	1.6

Abbreviations: PMG, polymicrogyria; PNH, periventricular nodular heterotopia. *p= .001, χ^2 test.

Genetic Overview

The standard karyotype was normal in all patients who underwent testing unless otherwise specified. Further genetic analysis is described per group (Figure 1 and Table 4).

Groups

Congenital microcephalies with MCD

We found 11 cases of congenital microcephalies with MCD (group 1), including 7 with SGP and 4 with agyria or pachygyria. Two unrelated patients had SGP and diabetes mellitus. In patient 1, the cause was Wolcott-Rallison syndrome; in the other, this cause was excluded. Three of the remaining patients with SGP had consanguineous parents. Two patients with SGP had mild or no mental retardation and normal motor skills (e.g., Figure 2A). None had evidence of a gestational insult.

Patients with SGP are candidates for testing of the MCPH loci (Table 1), but tests are currently not available. The patient with Wolcott-Rallison syndrome had a homozygote missense mutation in *EIF2AK3* and was included in a previously published report.[13] In 3 of the 4 patients with agyria or pachygyria, interstitial deletions in 17p13.3 and *LIS1* mutations were excluded; for the remaining patient, no DNA was available.

Lissencephaly, pachygyria, and subcortical band heterotopia spectrum

Twenty-four patients were diagnosed as having syndromes or diseases that fell within the spectrum of lissencephaly, pachygyria, and subcortical band heterotopia (group 2A) (Table 4). In 16 of these patients, a genetic syndrome was diagnosed. Five had Miller-Dieker syndrome with typical facial features, severe mental retardation, and epilepsy (Figure 3A). Four patients with pachygyria and moderate or severe mental retardation had a *LIS1* mutation (Figure 3B). Two patients with milder retardation and MRI abnormalities had a somatic mosaic *LIS1* mutation (Figure 3C).[14] Two girls with subcortical band heterotopia and epilepsy had a *DCX* mutation (Figure 3D). One girl was diagnosed as having Baraitser-Winter syndrome (patient 25) (Figure 2B).[15] Patient 26 had a muscle-eye-brain type disease with muscular dystrophy, elevated levels of creatine kinase, and visual impairment. His MRI did not show a cobblestone-type lissencephaly, but rather occipital pachygyria with nonprogressive leukodystrophy (Figure 2C). Arguably, this patient could be classified as belonging to group 2B. One boy was diagnosed as having FG syndrome on the basis of dysmorphic features and urogenital abnormalities.[16] He is undergoing testing for the R916W mutation in the *HOPA* gene (OMIM 300188).[17]

Of the 8 patients with unknown etiology, 1 pedigree shows an affected mother and son, suggesting an autosomal dominant or X-linked inheritance, but the mutation analysis results of *LIS1*, *DCX*, and *ARX* were normal. One patient had a brother with microlissencephaly in group 1. There was no consanguinity in group 2A. Most of the patients had severe psychomotor retardation with or without epilepsy (16 of 24), whereas only 5 patients had normal development or a mild mental deficit. The 5 patients with Miller-Dieker syndrome had an interstitial deletion of 17p13.3 confirmed by fluorescence in situ hybridization analysis. Sequencing of *LIS1* was performed in 12 patients and showed pathological mutations in 4, including 2 nonsense mutations (W261X and G147X), 1 frameshift mutation (c.162delA), and 1 intron mutation (900+1G>C; IVS8+1G>C). In 2 patients, a somatic mosaicism was found (mosaic 162delA and R113X). Analysis of *DCX* was performed in 8 patients. In 2, a nonsense mutation was found (R303X and R272X). No mutations were found in 2 patients in whom *ARX* and *RELN* were tested. None of the

patients fit the typical phenotype of X-linked lissencephaly with abnormal genitalia for *ARX* or of pachygyria, cerebellar anomalies, and lymphedema for *RELN* (Appendix). In patient 25, results of microscopic analysis of a muscle biopsy were normal, and mutations in *FCMD* (OMIM 607440) and *FKRP* (OMIM 606596) were excluded.

Cobblestone-type lissencephalies

Two patients with cobblestone-type lissencephalies (group 2B) had Walker-Warburg syndrome (Table 4). Both died in infancy (Figure 2D). No muscle biopsy material was preserved for histochemical analysis of α-dystroglycans.[18] Mutations in *POMT1* and *POMGnT1* were excluded in both patients. Further genetic analysis will be performed when methods become available.

Nodular heterotopia

We found 14 patients with nodular heterotopia without cortical abnormalities (group 2C) (Table 4). One patient with a history of maternal cocaine abuse during pregnancy, mild retardation, epilepsy, and diffuse bilateral periventricular nodular heterotopia (BPNH) had an *FLNA* mutation (patient 38, Figure 3E).[19] We assumed there was an autosomal recessive syndrome in 3 siblings with microcephaly, BPNH, and cerebellar atrophy without *FLNA* or *ARFGEF2* mutations. One patient had a syndrome with an occipital meningocele and BPNH, a combination reported previously.[20] Patient 41 died in infancy of a severe cardiac anomaly and BPNH with enlarged ventricles. In another boy, maternal alcohol abuse during pregnancy was documented, but alcohol has not been clearly linked to BPNH in humans. Overall, symptoms ranged from drug-responsive epilepsy with normal or near-normal mental and motor development to epilepsy with severe handicap.

Testing of *FLNA* was performed in 10 patients in group 2C.[19] A pathogenic frameshift mutation (7104delG) was found in patient 40. In patient 41, a previously unreported missense change in exon 20 was found, but it was not possible to perform additional tests to prove pathogenicity. For 2 patients, no DNA was available. Testing of *ARFGEF2* was performed in 3 patients, with normal results.[21]

Heterotopia with overlying PMG

In 11 patients with heterotopia combined with overlying PMG (group 2D), 7 presented with severe psychomotor retardation, whereas 4 showed normal or near-normal mental development independent of epilepsy or the extent of MRI abnormalities (Table 4). We diagnosed frontonasal dysplasia in a patient with a frontal encephalocele and facial asymmetry. Considering the overlap with craniofrontonasal dysplasia, mutations in *EFNB1* were excluded (OMIM 300035). One girl had a large Xq chromosome deletion (patient 53). In 2 patients, there was parental consanguinity. A standard karyotype of patient 53 showed a deletion of Xq21qter, including the *FLNA* and *DCX* loci. This finding was excluded in her parents. It was not possible to conduct X chromosome inactivation studies to analyze the relevance.

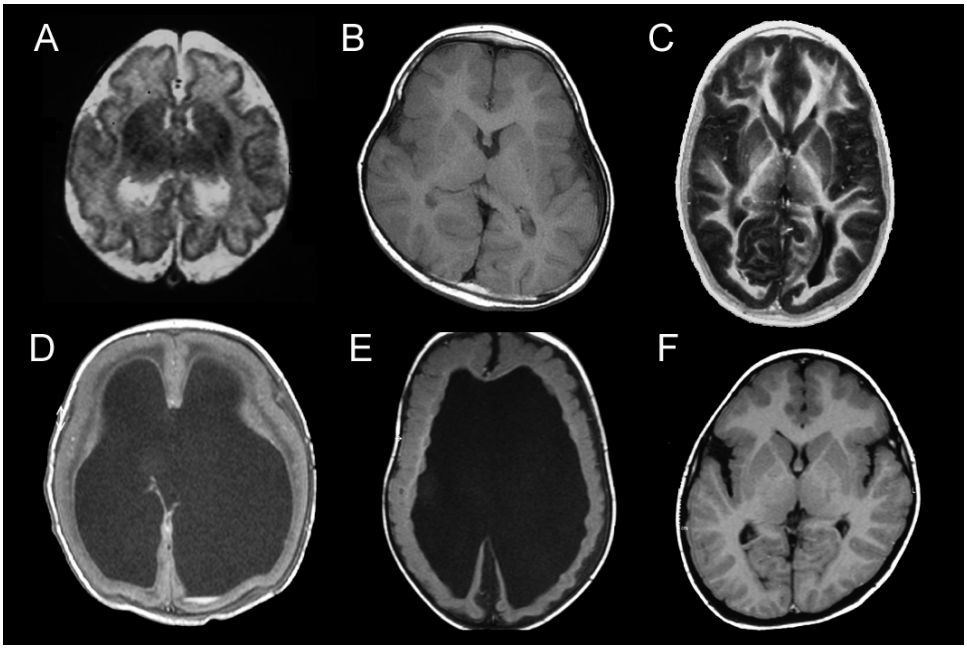


Figure 2. Magnetic resonance images of key patients with a definite diagnosis without known molecular defects. All images are T1-weighted, except where indicated. A, T2-weighted image of patient 2, a boy aged 29 months with a simplified gyral pattern and high clinical function. A reduced number of gyri, shallow sulci, and normal cortical thickness are seen. B, Patient 25, a girl aged 6 months with Baraitser-Winter syndrome. The cortex in the frontal lobes is thickened. C, Patient 26, a boy aged 6 years with a muscle-eye-brain–type syndrome. Smooth gyration in the occipital lobes is evident. D, Patient 36, a newborn boy with Walker-Warburg syndrome. Gyration is absent and the ventricles are enlarged. E, Patient 68, a boy aged 3 years with Adams-Oliver syndrome. Periventricular heterotopic nodules of gray matter and irregularity of the gyral pattern suggest polymicrogyria (PMG). F, Patient 71, a boy aged 5 years with merosin-negative muscular dystrophy. Irregular gyration is seen in the frontoparietal regions, most pronounced around the sylvian fissure, suggestive of PMG.

PMG and schizencephaly

We established a causative diagnosis in 20 of 48 cases with PMG and schizencephaly (group 3A) (Table 4). Four patients belonged to a pedigree with Goldberg-Shprintzen syndrome.[22] Ten cases were diagnosed as having a syndrome or a monogenetic disorder associated with PMG. One patient fulfilled criteria for Adams-Oliver syndrome (Figure 2E), another for Sturge-Weber syndrome, another for Aicardi syndrome, and another for Joubert syndrome. [2,7,23,24] Patient 67 had a muscle-eye-brain type disease with schizencephaly, congenital cataracts, and muscular dystrophy. Patient 71 was diagnosed as having a merosin-negative muscular dystrophy (Figure 2F). Three patients, 2 of whom are from the same pedigree, were found to have a syndrome consisting of megalencephaly, perisylvian PMG, postaxial polydactyly, and hydrocephalus.[25] In an additional 7 patients, there was documented evidence of a gestational insult that could have caused PMG. Congenital cytomegalovirus infection was excluded in 9 patients with unexplained PMG; in 2 additional patients, this infection could not be excluded. We suspected a genetic cause in 6 patients with multiple congenital abnormalities and in 4 with consanguineous parents or

multiple affected family members. In 11 of 48 patients, we found no clues to an underlying cause.

In all patients examined after 2001, microdeletions at the 22q11 locus were excluded. Analysis of *KIAA1279* in patients with Goldberg-Shprintzen syndrome was performed as described by Brooks et al.[22] Testing for mutations in *GPR56* was performed in 13 cases of bilateral perisylvian PMG, with negative results. Mutations in *AHI1* (OMIM 608894), associated with Joubert syndrome, were excluded in patient 72. In patient 67, analysis of α-dystroglycans in muscle biopsy specimens was ongoing at last follow-up. In patient 71, *LAMA2* (OMIM 156225) analysis was not available at the time of diagnosis.

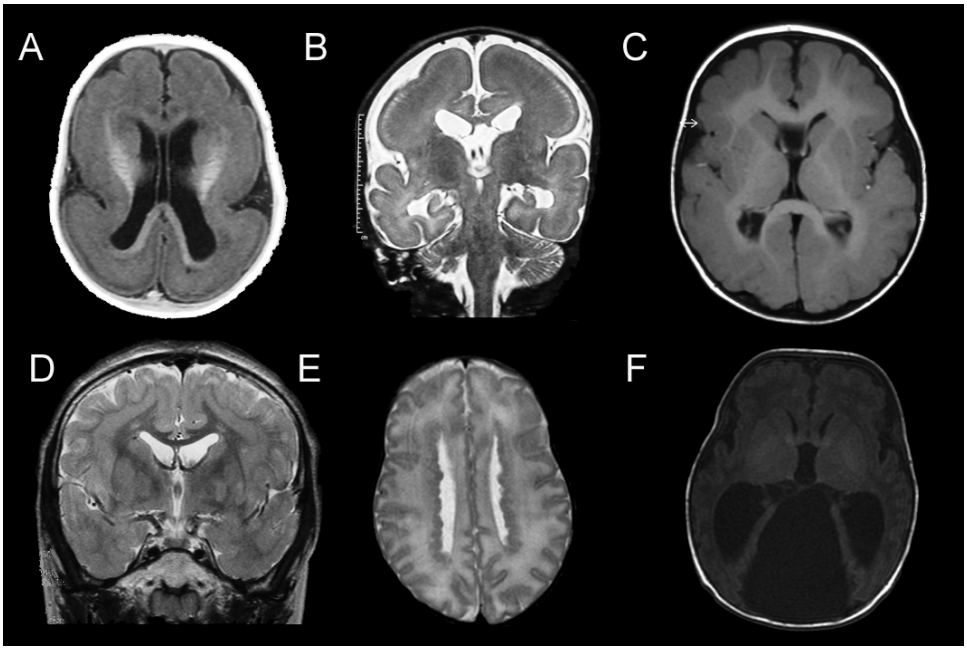


Figure 3: Magnetic resonance images of key patients with a definite diagnosis with a known molecular defect. A, Axial T1-weighted image of patient 13, a girl aged 5 months, with Miller-Dieker syndrome. The cortex is smooth and thickened with a slight posterior-to-anterior gradient. B, Coronal T2-weighted image of patient 18, a girl aged 6 years, with a *LIS1* mutation. The cortex is smooth and thickened with relative sparing of the temporal lobe. C, Axial T1-weighted image of patient 21, a boy aged 14 months, with a mosaic *LIS1* mutation. A less severe phenotype is seen, but still with a reduced number of gyri and cortical thickening. D, Coronal T2-weighted image of patient 23, a girl aged 6 years, with a *DCX* mutation. A thick band of subcortical gray matter ("double cortex") is seen. E, Axial T2-weighted image of patient 38, a girl aged 5 weeks, with an *FLNA* mutation. A bilateral periventricular ribbon of heterotopic nodules is evident. F, Axial T1-weighted image of patient 113, a girl aged 1 week, with Zellweger syndrome. Irregular and small gyri, suggestive of diffuse polymicrogyria, and partial agenesis of the corpus callosum are seen.

Metabolic causes

Metabolic causes were found in 2 patients (group 4) diagnosed as having Zellweger syndrome and malonylcoenzyme A decarboxylase deficiency, an inborn metabolic disease (Table 4). Polymicrogyria is associated with Zellweger syndrome, but diffuse severe PMG with cerebellar abnormalities is rare (Figure 3F).[26] To our knowledge, pachygyria and nodular heterotopia have not been described in previous reports of malonyl-coenzyme A decarboxylase deficiency.[27]

Peroxisomal fatty acid oxidation in fibroblasts and a mutation in *PEX10* (OMIM 602859) confirmed the diagnosis in patient 113. Malonyl-coenzyme A decarboxylase in fibroblasts of patient 112 was deficient, and *MLYCD* (OMIM 606761) analysis showed absence of a transcript.[27]

COMMENT

This study of a large consecutive group of patients with MCD shows the effect of combining radiological, clinical, and molecular analysis. After radiological classification, analysis for associated congenital anomalies and dysmorphic features, and genetic tests, a definite diagnosis of the underlying cause was found in 38 cases (34%). For 21 patients (19%), this included molecular and/or genetic confirmation, and in 17 (15%), this was a syndrome diagnosis with an unknown genetic defect. In another 7 patients, we found evidence of a gestational insult, for a total of 45 etiological diagnoses (40%). These results improved patient care and gave parents opportunities for reproductive choices and prenatal diagnosis. Some smaller studies have been published, most of which focus on a specific MCD subgroup.[12,19,28-30] One cohort study of similar size reviewed radiological and clinical findings, without syndrome diagnoses or molecular diagnostic testing.[31]

Neuroradiological Data

Our retrospective revision of all neuroimaging studies showed that 26 of 63 patients (41%) had received previous misdiagnoses. A quality MRI of the brain and a skilled neuroradiologist are essential for a correct classification and the choice of diagnostic tests. The 2005 MCD classification is useful, although some patients remain hard to classify, especially those with combinations of heterotopia and abnormal cortex.[7,12]

Laboratory and Molecular Data

For some MCD phenotypes such as Walker-Warburg syndrome, genetic heterogeneity is wide, making molecular confirmation difficult.[18] In others such as microcephaly with SGP, tests for known genes are not routinely available. Telomere multiplex ligation-dependent probe amplification is also becoming available to assist in diagnosis. More extensive testing can further increase the number of molecularly confirmed diagnoses. As expected from previous observations, genetic causes were most often confirmed in our patients with lissencephaly or pachygyria.[29,32] Mutations in *LIS1* or *DCX* explained the lissencephaly and pachygyria in more than half of our patients. The genetic causes of PMG are still largely elusive. Mutations in *GPR56* and *KIAA1279* only explain a small percentage of PMG cases.[22,33] We tested *GPR56* in 13 patients with bilateral frontoparietal PMG, but found no

mutations. None of these patients had the typical pons hypoplasia and white matter abnormalities, further confirming the specificity of the phenotype associated with *GPR56* mutations.[30]

Implications for Clinical Care

In our cohort, most patients were diagnosed as having MCDs when they developed seizures. Patients with PMG have a lower incidence of epilepsy, and motor dysfunction often prompted medical attention. The overall risk of psychomotor retardation is high, but 34 of our 113 patients had an IQ greater than 70. Epilepsy is a negative prognostic factor, particularly infantile spasms or neonatal seizures. In 1 patient with BPNH, we found a pathogenic *FLNA* mutation, whereas her developmental delay previously was accredited to prenatal exposure to cocaine. This supports the predictive power of a specific radiological pattern for genetic testing. We did not find mutations in patients whose MRI pattern was not typical for the gene tested. We recommend careful classification before ordering genetic tests.

Future Perspective

Nine patients in our cohort fit a known syndrome with an unknown genetic cause. Another 15 patients presented with an apparently unique syndrome, meaning a combination of multiple anomalies likely to result from an underlying genetic cause. Furthermore, a genetic cause is likely in patients with affected family members with or without consanguinity (12 patients) or in patients with consanguineous parents only (7 patients). These 3 groups are candidates for whole-genome analysis by means of new techniques designed to discover other genes involved in brain development. This approach has been successful in cohorts of patients with congenital malformations and mental retardation.[34] In addition, consanguineous families with multiple affected members can be explored by improved linkage techniques.

CONCLUSIONS

Our cohort represents a heterogeneous group but closely follows clinical practice, where most patients are referred with an unclassified MCD. We show that classification based on radiological, clinical genetic, and neurological examinations combined with genetic testing can yield important information about monogenetic, syndromal, and metabolic causes and can lead to improvement of patient care and genetic counseling. This requires a multidisciplinary team specialized in neuroradiology, pediatric neurology, and genetics. Even then, the underlying cause remains elusive in more than 50% of patients, and the suspicion of an underlying genetic cause remains in many of our unclassified cases. This encourages exploitation of new genome-wide techniques.

Table 4. Clinical patient characteristics by diagnosis

Group No.	No. of patients	Diagnosis	Sex/No.	Mental retardation, no. of Patients				Epilepsy (Age [a])	Molecular Defect	No. of patients		Patient No.
				Severe	Mod	Mild	None			Consanguinity	Family history	
Definite Diagnosis With Known Genetic Defect												
1	1	Wolcott-Rallison syndrome	F	1				1 (2.9)	*EIF2AK3* mutation	1	1	1
2A	5	Miller-Dieker syndrome	M/1, F/4	5				5 (0.4)	17p13.3 Deletion			12-16
	6	*LIS1* mutation	M/3, F/3	4	1	1		5 (1.0)	*LIS1* mutation			17-22
	2	*DCX* mutation	F/2	1		1		2 (1.0)	*DCX* mutation			23, 24
2C	1	*FLNA* mutation	F			1		1 (1.3)	*FLNA* mutation			38
3A	4	Goldberg-Shprintzen syndrome	M/1, F/3	2	2			0	*KIAA1279* mutation	4	4	63-66
4	1	Peroxisomal Zellweger syndrome	F	1				1 (0.0[b])	*PEX10* mutation			113
	1	Metabolic other	F		1			0	*MLYCD* mutation	1		112
Total	**21**											
Definite Diagnosis Without Known Genetic Defect												
1	2	SGP with high clinical function	M/1, F/1			1	1	0		1		2,3
2A	1	Baraitser-Winter syndrome	F		1			0				25
	1	Muscle-eye-brain–type syndrome	M				1	1 (8)				26
	1	FG syndrome	M	1				0				35
2B	2	Walker-Warburg syndrome	M/1, F/1	2				2 (0.0[b])				36,37
2D	1	Frontonasal dysplasia syndrome	M				1	1 (0.8)				52
3A	1	Muscle-eye-brain–type syndrome	M	1				1 (0.8)				67
	1	Adams-Oliver syndrome	M	1				1 (0.7)				68
	1	Sturge-Weber syndrome	M	1				1 (1.6)				69
	1	Aicardi syndrome	F	1				1 (1.3)				70
	1	Merosin-negative muscular dystrophy	M	1				0			1	71
	1	Joubert syndrome	M	1				0				72
	3	MPPH syndrome	F/3	3				3 (0.2)			2	73-75
Total	**17**											

Probably causative prenatal event											
3A	7	PMG	M/3, F/4	1	3	3		0			84-90
No definite diagnosis											
1	8	Congenital microcephaly with MCD	M/6, F/2	7	1			5 (0.1)	4	2	4-11
2A	8	Pachygyria	M/5, F/3	5	1	2		6 (0.2)		3	27-34
2C	13	Heterotopia	M/6, F/7	6	2	4	1	9 (3.0)	4	3	39-51
2D	10	Heterotopia and PMG	M/4, F/6	6	1	3		9 (0.9)	2		53-62
3A	28	PMG	M/16, F/12	12	3	10	3	13 (2.0)	6	4	76-83, 91-110
3B	1	Cortical dysplasia	F			1		1 (0.3)			111
Total	**68**										

Abbreviations: MCD, malformations of cortical development; Mod, moderate; MPPH, a syndrome consisting of megalencephaly, perisylvian PMG, postaxial polydactyly, and hydrocephalus; PMG, polymicrogyria; SGP, simplified gyral pattern.
[a]Indicates median age at onset in years. [b]Indicates within 48 hours of birth.

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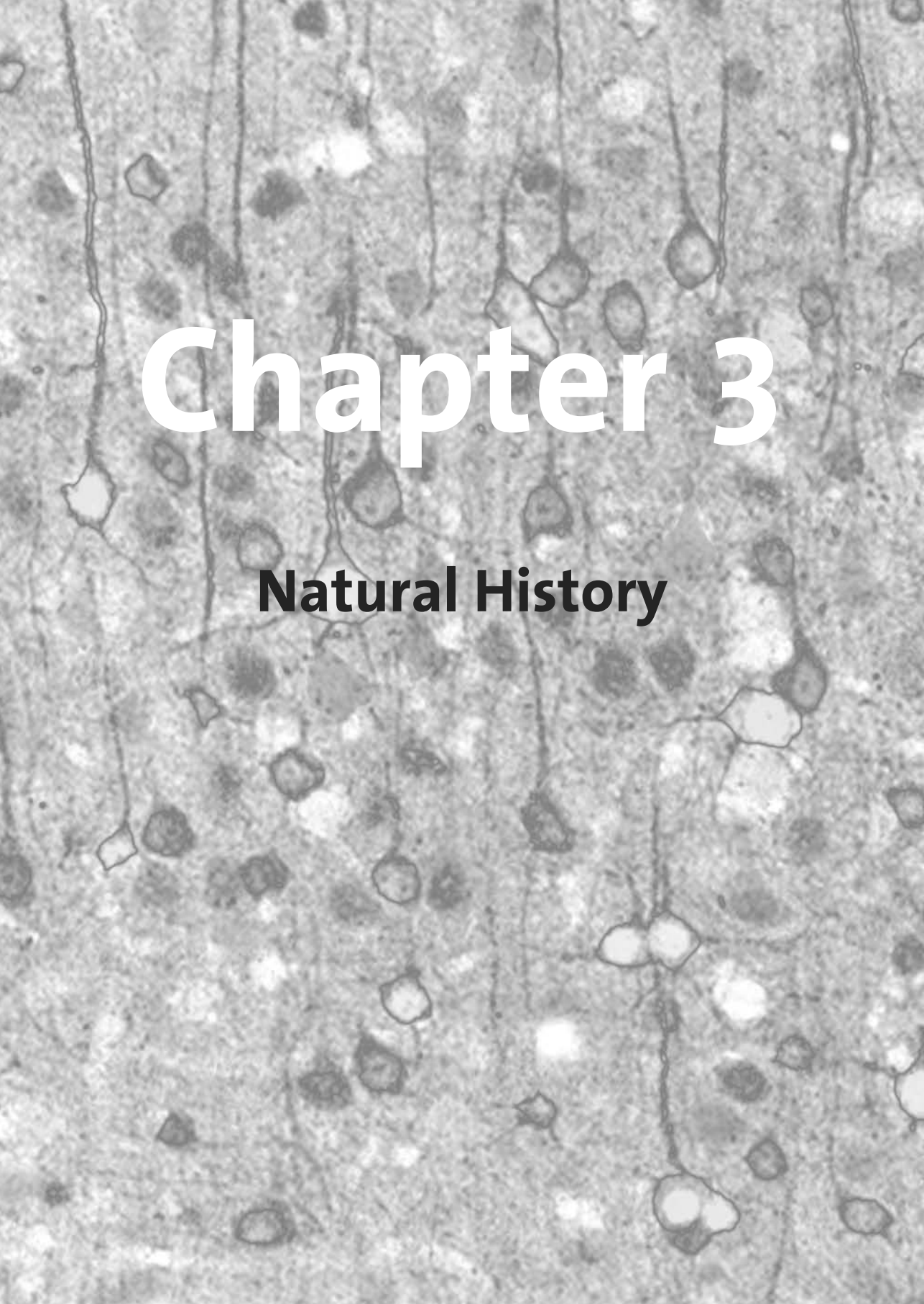

Chapter 3

Natural History

3.1

Long term follow-up of lissencephaly

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ABSTRACT

All patients with classic or type 1 lissencephaly born between 1972 and 1990 in the Netherlands were at the time enrolled in an observational study. We attempted to contact these 24 patients again to obtain long-term follow-up and survival information. Eleven patients were alive. All patients showed severe mental retardation, intractable epilepsy and complete dependence on care. Life expectancy showed a significant correlation to the severity of the lissencephaly on neuroimaging.

Molecular analysis of the *LIS1*-gene was not possible at the time of the original study and was now requested by eight parents. This revealed a pathogenic mutation or large deletion of *LIS1* in seven patients.

Our study provides information about the long-term course of lissencephaly, the relationship between lissencephaly severity and prognosis, and shows that renewed attention to genetic counseling remains valued by families of a patient with a severe congenital neurological disease.

LONG TERM FOLLOW-UP OF LISSENCEPHALY

INTRODUCTION

Lissencephaly type 1 ('smooth brain') has long been recognized as a cause of severe mental retardation. In lissencephaly type 1 the cerebral cortex lacks the characteristic gyri and sulci and is thicker than normal, either throughout the cortex or more severely in the posterior than the anterior regions. The advance of neuroimaging techniques enabled the diagnosis of lissencephaly during life, and sparked more research into its causes. Improving techniques for genetic studies yielded a first answer when (partial) monosomy of chromosome 17 was found in patients with lissencephaly and typical facial dysmorphic features (Miller-Dieker syndrome).[1] The identification of 17p13.3 as the region involved in Miller-Dieker syndrome in 1989 changed genetic counseling and made prenatal testing possible in some cases.[2] Ten years later the *LIS1* gene (also called the *PAFAH1B1* gene) was identified in this region, which causes autosomal dominant lissencephaly or pachygyria with a posterior to anterior gradient.[3] Several other genetic causes were later found in the lissencephaly, pachygyria and subcortical bandheterotopia (SBH) spectrum. *DCX* causes X-linked lissencephaly or SBH with an anterior to posterior gradient.[4] Autosomal recessive lissencephaly associated with cerebellar and brainstem abnormalities is caused by *RELN* mutations.[5] In the *RELN* pathway, another gene called VLDLR can cause a similar but milder phenotype.[6] Mutations in *ARX* can cause X-linked lissencephaly, but there is a wide variation in phenotypes associated with mutations in this gene.[7] Mutations in *TUBA1A* cause autosomal dominant lissencephaly, often with cerebellar and callosal malformation.[8]

Most cases of type 1 lissencephaly are explained by mutations in *LIS1, DCX* or by 17p13.3 haploinsufficiency. Clinical signs reported are epilepsy, mental retardation and spasticity. Not much has been published on the follow-up of lissencephaly patients or their life expectancy. Groups of patients are described in general terms and follow-up is reported to early childhood and only in a few cases until adulthood.[9-13] To improve knowledge about the natural course of lissencephaly type 1 and provide better prognostic information for parents, we attempted to contact all lissencephaly patients born between 1972-1990 identified in a nation-wide study done in the Netherlands who, if alive, would now be adults.[11,14-16] This is the largest reported cohort of consecutive patients in the literature. At the time of the original study, parents had given informed consent for DNA to be stored and to be used if and when further genetic testing would become feasible.

METHODS

During the original study of de Rijk-van Andel 24 type 1 lissencephaly patients born in the Netherlands in the period 1972-1990 were included.[11,14-16] At the end of the original follow-up in 1990, 17 patients were still alive. Between 2006 and 2008, we attempted to contact these 17 patients. If possible, the patient was examined at our clinic, and if not an interview was done by telephone with the parents or caretaker. All original brain imaging (CT scans and MRI scans if available) was reevaluated and lissencephaly was graded according to the following lissencephaly patterning scale: grade 1: complete agyria, grade

2: agyria with few shallow anterior sulci, grade 3: posterior agyria and anterior pachygyria, grade 4: diffuse pachygyria posterior>anterior, grade 5: mixed pachygyria and subcortical band heterotopia (SBH), grade 6 SBH only.[17] If the patient had died, age and cause of death were recorded. At the time of the original study, DNA from leukocytes of patients alive at diagnosis was stored with the parents' informed consent. If genetic analysis had not previously been done, we offered the parents the option testing for 17p13.3 deletion, sequence analysis of *LIS1* and *DCX* where appropriate for the phenotype and MLPA analysis for deletions in/of these genes. Repeating neuroimaging studies or EEG was considered not justified as it would not benefit the patients.

RESULTS

Diagnosis

In two patients lissencephaly was diagnosed at autopsy and grading was done based on the autopsy report, for all other patients neuroimaging was available. Lissencephaly grading was done based on brain CT (examples in figure 1). Both CT and MRI had been made in five patients, and grading based on MRI was the same as on CT. The majority had a grade 1 or 2 lissencephaly (33% and 46%). Five patients had a grade 3 or 4 lissencephaly (see table).

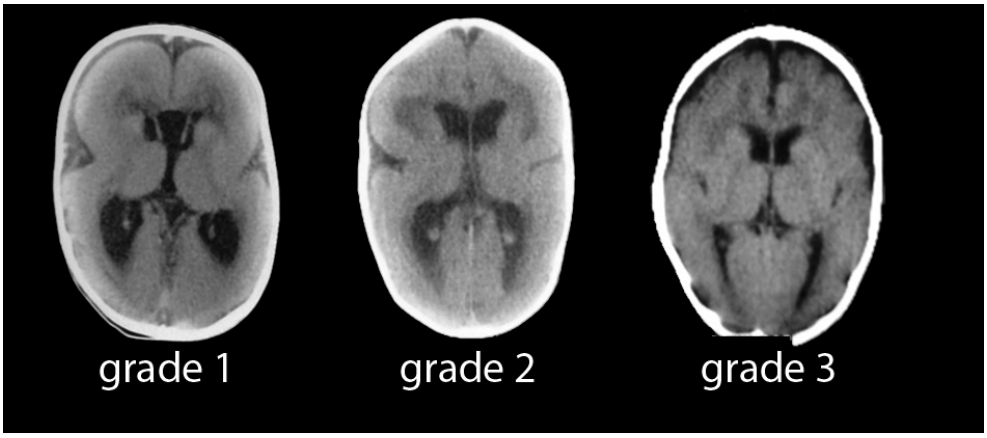


Figure 1: examples of CT scans and grading. Grade 1 (patient 24, female, CT scan made at the age of 9 months) shows complete agyria, 'figure of eight'-configuration due to lack of opercularization and thickened cortex. Also note the cavum septum pellicidum. Grade 2 (patient 17, male, CT scan made at the age of 4.5 months). Note the few shallow sulci in the frontal lobes and some opercularization. Grade 3 (patient 4, male, CT scan made at the age of 1 yr). Note diffuse course gyri and shallow sulci predominantly in the anterior regions and the further opercularization of the insular region.

Survival

During the original study period 7 patients (29%) died at ages between 0 and 9 years (table, figure 2 and 3). One patient was lost to follow-up and we have been informed that she died but we could not recover the age or cause of death (pat 5 in table). All other parents agreed to visit the clinic or gave information over the telephone. Five patients had died after 1990, between the ages of 3 and 19. The cause of death was either status epilepticus or pneumonia. Eleven patients (46%) are alive, now aged 18 to 27 years (see survival curves in figure 2). There is a significant association between survival and the severity of the lissencephaly (Log-Rank test p=0.002).

Clinical signs (table)

All patients show severe psychomotor retardation. In 15 cases parents report having (had) some contact with their child, in nine there is or has never been any contact. In one patient contact was lost at the onset of seizures. All have intractable epilepsy with age of onset between a few days to 2 years old (average 6 months). Five patients presented with seizures in the neonatal period, 17 with infantile spasms and two with multifocal epilepsy (at age 6 months and age 2 years). There is no significant correlation between age of onset of seizures and survival. In two patients seizures are reasonably controlled by anti-epileptic drugs, in the others seizures remain frequent (daily to weekly). The EEGs have been described before and show typical generalized fast activity with high amplitude and/or high amplitude sharp- and slow-wave complexes in almost all cases.[16] None of the patients ever had a normal EEG. Two patients had shown some motor development to head balance, clapping hands and belly crawling, but had lost these milestones later in life during periods of infectious disease and seizures. All patients show axial hypotonia, and four patients have a severe scoliosis. Spasticity is mild, but more prominent in three patients. All but one of the parents said that having a child with lissencephaly has a severe and negative impact on their family and their quality of life, but that their child seems content most of the time and does not suffer. One mother states that she feels that her child does not have any quality of life.

Genetic analysis

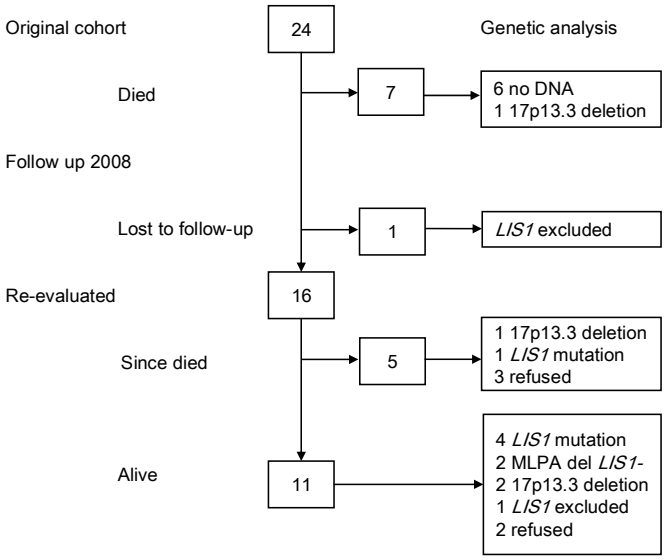
During the original study, of all patients alive at the time of inclusion (18/24) DNA had been stored with informed consent. At the time microscopic high resolution chromosome banding was done in all patients and in one patient had shown a probable chromosome 17p13.3 deletion consistent with Miller-Dieker lissencephaly syndrome (pat 7). This was confirmed with FISH analysis and this patient died during the original study period. At the current follow-up, we found that since 1990 in three more patients a diagnosis had been made of Miller-Dieker syndrome due to a deletion at chromosome 17p13.3 (pat 13, 20, and 24). One of these had since died. For 13 patients without a molecularly confirmed diagnosis DNA was still stored.

Five patients had died after 1990. Parents of one of these patients parents wanted further testing to aid reproductive choices in the sisters of the patient and a heterozygous LIS1

mutation (c.334insA) was detected (pat 23). Parents of two patients wanted to leave the decision to their other children; one couple did not have other children.
Eleven patients are alive now (46%). In two a chromosome 17p13.3 had been diagnosed after 1990. Of the remaining nine patients, seven families requested further genetic testing to confirm the diagnosis and to aid reproductive choices in the siblings of the patients. Two families preferred not to have any more medical tests.

Direct sequence analysis of the coding exons and exon intron boundaries showed heterozygosity for a frameshift, nonsense or splice site mutation in the *LIS1* gene in four patients (see table). MLPA analysis showed an intragenic *LIS1* deletion in one patient spanning at least the 6 most 3' exons, whereas another patient had a total *LIS1* deletion possibly further extending into the Miller Dieker region. In one patient *LIS1* sequencing and MLPA testing of the *LIS1* gene were normal (pat 8). Additional sequence analysis of *TUBA1A* was also normal. When a gene defect had been found, parents and siblings were invited for genetic counseling. In parents that were tested, mutations were absent (two couples). When mutations were found in the patients, most parents expressed that they were pleased that any doubts about influences during pregnancy and recurrence risks had now been dispelled.

Figure 2: flowchart of follow-up results



Discussion

Our cohort study confirms that children with type 1 lissencephaly have a severe mental and motor retardation and that the epilepsy is intractable in all cases. Epilepsy treatment is still important, because seizures can lead to loss of skills or death as also demonstrated in this cohort. Life expectancy is limited but with supportive care focused on the prevention of infectious complications and scoliosis many of these children can reach adulthood. All patients in this cohort have severe lissencephaly phenotypes, as milder phenotypes such as subcortical band heterotopia were not recognized during life in 1980-1990 due to the limitations of neuroimaging. In general, the phenotype may be as mild as having epilepsy with normal mental development.[18] We show that life expectancy is related to the severity of the lissencephaly on neuroimaging, with a significantly worse outcome for complete agyria as compared to the milder diffuse pachygyria. Location of the mutation in the *LIS1*-gene have been postulated not to directly predict disease severity.[19,20] However, *LIS1* missense mutations are though to result in a milder phenotype.[18] Also, the severity of the effect of the mutation on the *LIS1*-protein does have a relationship with the severity of the lissencephaly grading on neuroimaging.[13,21,22] In our cohort all mutations found in LIS1 are nonsense mutations or intragenic deletion, and we did not find any missense mutations. We had no indication of somatic mosaicism in our cohort, although this can never be ruled out entirely. Possibly, our findings are caused by selection bias toward the more severe phenotype due to the neuroimaging possibilities of that time, indirectly supporting the notion that missense mutations generally result in milder phenotypes.

With the techniques available at the time of the original study only microscopic chromosome deletions could be detected by high resolution chromosome banding and a deletion of 17p was found in one patient then. After 1990 a deletion of chromosome 17p13.3 had been found in three more patients. When we offered parents further genetic analysis using current techniques, the majority welcomed this, and in 8 out of 14 patients genetic testing confirmed the clinical diagnosis. These results confirm the high predictive value of classic lissencephaly on neuroimaging for mutation in *LIS1*. In one patient we were unable to confirm a genetic cause with all available options, including *TUBA1A* analysis (pat 8). Possibly she has a *LIS1* dysfunction that could not be proven with our methods (MLPA and sequencing) or the available material (DNA isolated from leukocytes).

Our study shows the importance of the interpretation and classification of neuroimaging for prognosis and counseling of parents of a child with a severe congenital neurological disease. It also reminds us that genetic studies are still valued by parents and siblings of adult disabled patients to understand the cause and to answer any lingering doubts about recurrence risk.

Acknowledgments:

Dr. D. Pilz, Department of Medical Genetics, Cardiff University School of Medicine, UK, tested patient 8 for *TUBA1A* mutation.

Table: Patient characteristics

No	sex	Lissencephaly grade	Genetics	Age (yrs)	Died (age)	Motor skills	MR	Epilepsy onset	Epilepsy type
1	M	2 (CT,MRI)	*LIS1* c. 569-insTAA	25		Briefly able to belly crawl	++	2 yrs	Multifocal seizures
2	M	2 (CT)	NA		6 mo	None	+++	9 wk	Neonatal seizures
3	F	3 (CT)	*LIS1* c.900+1G>C	24		Hypotonic tetraparesis, can clap hands	++	6 mo	Infantile spasms
4	M	3 (CT, MRI)	*LIS1* c.162delA	27		Hypotonic tetraparesis	++	3 mo	Infantile spasms
5	F	1 (CT)	*LIS1* seq., MLPA negative	?	Lost to FU age 5	Hypotonic tetraparesis	+++	5 mo	Infantile spasms
6	F	2 (CT)	Refused	25		Hypotonic and spastic tetraparesis	++	6 mo	Infantile spasms
7	F	1 (CT)	17p13.3 deletion		3 yrs	None	+++	6 mo	Infantile spasms
8	F	3 (CT)	*LIS1* seq., MLPA, *TUBA1A* negative	26		Hypotonic tetraparesis, achieved sitting	++	6 mo	Infantile spasms
9	M	1 (autopsy)	NA		9 yrs	Hypotonic tetraparesis	++	7 mo	Infantile spasms
10	F	1 (autopsy)	NA		3 yrs	Hypotonic tetraparesis	+++	3 mo	Infantile spasms
11	F	3 (CT)	*LIS1* c.782G>A	22		Stands with support	+	2 mo	Neonatal seizures
12	F	1 (CT)	Refused		19 yrs	Hypotonic tetraparesis, no skills	++	3 mo	Infantile spasms
13	M	2 (CT,MRI)	17p13.3 del (FISH)	24		None	++	4 mo	Infantile spasms
14	F	1 (CT and autopsy)	NA		1 mo	Floppy infant	+++	1 wk	Neonatal seizures
15	F	1 (CT and autopsy)	NA		7 mo	Floppy infant	+++	5 mo	Infantile spasms
16	F	2 (CT and autopsy)	NA		6 yrs	Hypotonic tetraparesis	+++	2 mo	Infantile spasms
17	M	2 (CT)	Refused		12 yrs	Hypotonic tetraparesis	++	3 mo	Infantile spasms
18	M	4 (MRI)	Refused	20		Spastic tetraparesis	+++	2 mo	Infantile spasms
19	M	2 (CT)	Refused		16 yrs	Hypotonic tetraparesis	++	6 mo	Multifocal epilepsy
20	F	2 (CT)	17p13.3 deletion	21		None	+++	3 mo	Neonatal seizures
21	M	2 (CT, MRI)	*LIS1* deletion (MLPA)	22		Tetraparesis, headbalance, can stand with full support	++	6 mo	Infantile spasms
22	M	2 (CT)	*LIS1* del exon 6-11 (MLPA)	19		Hypotonic tetraparesis	++	3 mo	Infantile spasms
23	M	2 (CT, MRI)	*LIS1* c.334insA		20 yrs	Hypotonic tetraparesis	++	-	Intractable epilepsy
24	F	1 (CT)	17p13.3 deletion		2 yrs	Hypotonic tetraparesis	++	3 wk	Neonatal seizures

No: original patient numbers. NA: not available. MR: Mental retardation +++ no contact, ++ little contact and laughing, + some play, no language
MLPA: lissencephaly kit MRC Holland, Amsterdam, the Netherlands, containing probes for each of the *LIS1* exons and including probes of the *DCX, POMT1, POMGnT1, FLNA* genes.

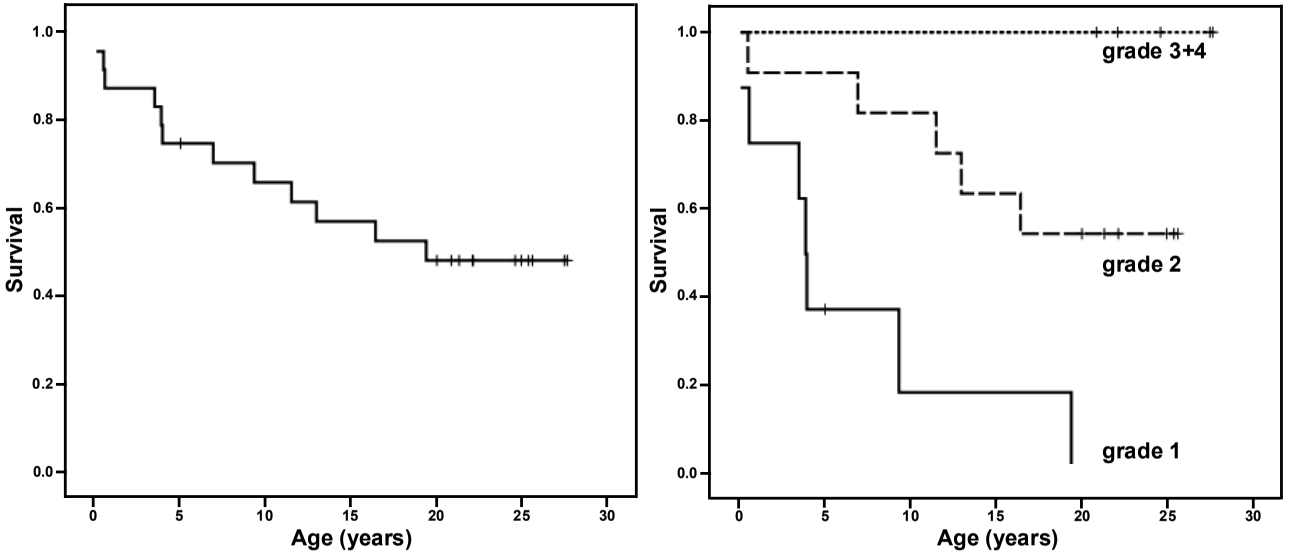


Figure 3: Kaplan-Meier survival curves: on the left the overall survival and on the right survival stratified by lissencephaly grade with a significant difference in survival between grades (Logrank p=0.002).

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3.2

Filamin A mutation, a common cause for periventricular heterotopia, aneurysms and cardiac defects

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ABSTRACT

Filamin A is an important gene involved in the development of the brain, heart, connective tissue and blood vessels. A case is presented illustrating the challenge in recognising patients with filamin A mutations. The patient, a 71-year-old woman, was known to have heart valve disease and bilateral periventricular nodular heterotopia when she died of a subarachnoid haemorrhage. Autopsy showed typical cerebral bilateral periventricular heterotopia and vascular abnormalities.
Postmortally, the diagnosis of a filamin A mutation was confirmed. Recognition during life may prevent cardiovascular problems and provide possibilities for genetic counselling.

FILAMIN A MUTATION, A COMMON CAUSE FOR PERIVENTRICULAR HETEROTOPIA, ANEURYSMS AND CARDIAC DEFECTS

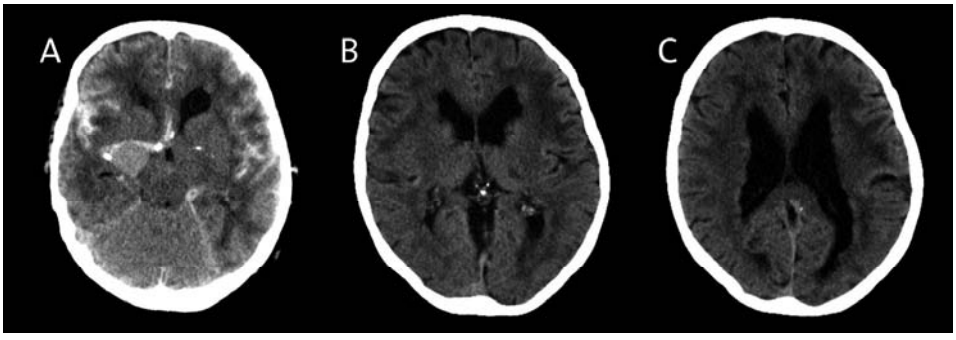
Filamin A (*FLNA*) is an important gene involved in the development of the brain, heart, connective tissues and blood vessels. Patients with *FLNA* mutations may develop symptoms at any age. We present a case that shows distinctive features of a *FLNA* mutation, which was only recognised at the time of her death.

CASE REPORT

A 71-year-old woman presented with an acute loss of consciousness. Brain CT showed a subarachnoid haemorrhage, leukoaraiosis and bilateral periventricular nodular heterotopia (BPNH) (fig 1). CT angiography confirmed several fusiform aneurysms inaccessible for coiling or surgery. The patient had cardiac problems from the age of 24 years with palpitations, chest pains and fatigue. At 32 years a severe aortic valve insufficiency was treated by valve replacement. Progressive heart failure led to heart transplantation at the age of 56 years. A VVIR-pacemaker was implanted because of symptomatic ventricular arrests. One year before admission she had developed temporary cognitive problems during a urinary tract infection. The consulting neurologist had ordered a brain CT (figure 1) as MRI was impossible with the pacemaker.

This showed fusiform aneurysms of the right internal carotid artery and both medial cerebral arteries, BPNH and leukoaraiosis. The patient had graduated normal primary school and was a housewife and mother of a healthy son. Her mother had died of heart failure at the age of 69 years. Her father died at the age of 72 years of a heart attack. The patient had a healthy twin brother and a sister, both childless. Despite optimal ICU support, the patient died of a rebleed 13 days later. Her husband consented to autopsy.

Figure 1: Brain imaging studies. (A) CT scan showing blood in the subarachnoid spaces and an aneurysm of the right middle cerebral artery. (B, C) Earlier CT scan showing mildly enlarged lateral ventricles with bilateral periventricular nodules of grey matter. The white matter shows bilateral hypodense areas suggestive of leukoaraiosis.



Brain pathology

There was a subarachnoidal haemorrhage due to a ruptured aneurysm of the left internal carotic artery. The vessels of the circle of Willis showed severe atherosclerosis. There were extensive hypertensive microvascular changes. There were subependymal proliferations consistent with ependymitis granularis (i.e. proliferation of ependyma, usually due to longstanding hydrocephalus). The cerebellum, pons and mesencephalon appeared normal. There were numerous periventricular nodular heterotopia along the walls of both the lateral ventricles extending into the parahippocampal area (fig 2). These consisted of neuronal cells and glia with a mature morphology. A discrete cortical malformation consisting of an unclear white to grey matter junction was seen in the right temporal region, but otherwise the cortex appeared normal. In the grey and white matter of both hemispheres, including the heterotopia, capillary proliferations were present. Many of these vessels displayed a glomeruloid-like architecture, and were present individually and in groups. The capillary lumina were lined by CD31 and CD34 positive cells, parts of which also stained for smooth muscle actin. The distribution of the proliferated microvasculature seemed random without concentration areas. These 'glomeruloid microvascular anomalies' have been described in a patient with a *FLNA* mutation, but not in other diseases.[1]

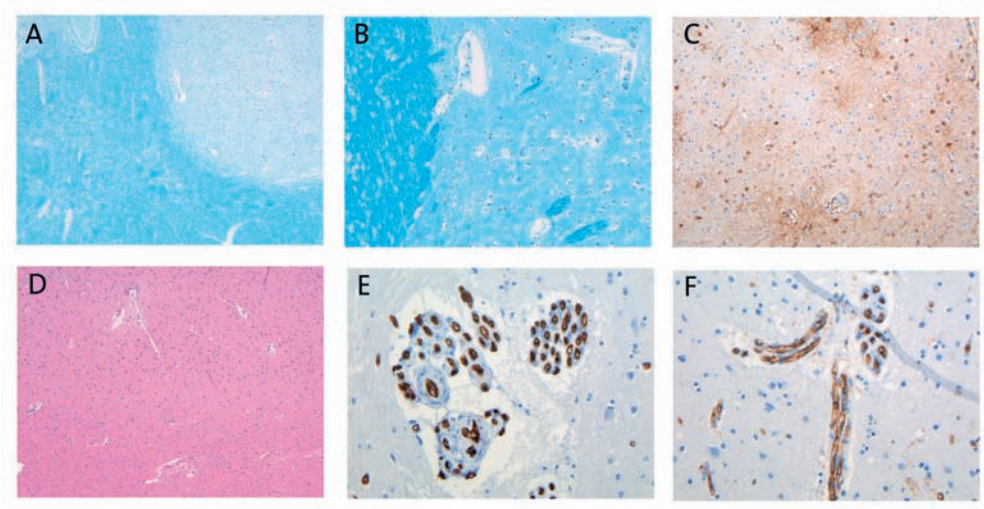


Figure 2: Microscopic findings in the brain at autopsy. (A, B) Kluver staining of heterotopia. (C) CFAP staining of heterotopia. (D) Area of temporal cortex dysplasia. (E, F) CD34 and CD31 staining of glomeruloid capillary proliferations.

Genetic analysis

Direct sequence analysis of the 48 coding exons and exon–intron boundaries of the *FLNA* gene showed a new pathogenic frameshift mutation 3045del5 in exon 21, not present in 100 controls. This mutation affects the C terminus binding site and/or the ability of the protein to dimerise and will probably produce instable mRNA that will be degraded by the nonsense mediated decay system.

DISCUSSION

In this patient, a common cause for the cardiac valvular disease, cerebral cortical malformation and arterial aneurysms, was suspected and confirmed at post mortem. BPNH usually presents with epilepsy, but surprisingly our patient never had seizures. In BPNH, neuronal progenitors fail to migrate to the cortex during prenatal brain development. In 1998 mutations in the X-linked *FLNA*, encoding the filamin A protein, were found in female BPNH patients.[2] In males, loss of function mutations are often fatal, but milder mutations cause a phenotype similar to females.[3] Many patients have additional cardiac abnormalities, particularly aortic or mitral valve disease.4 Other associated abnormalities include persistent ductus arteriosus, aortic root aneurysm and idiopathic thrombocytopenia.[2,4-6] A subgroup of BPNH patients caused by *FLNA* mutations show additional features of joint hypermobility and aortic aneurysms, similar to Ehlers Danlos syndrome.[7] One of these patients survived a subarachnoid haemorrhage.[8] Recently, in three families, X-linked mitral valvular dysplasia with varying degrees of aortic valve insufficiency was found to be caused by *FLNA* mutations.[9] In these families, there was no evidence of neurological disease, although no brain imaging was done. All of the clinical signs described above are associated with loss of function mutations. Skewing of X-chromosome inactivation level in females, and the severity of the mutation in both sexes, determine the phenotypic variation. Gain of function *FLNA* mutations cause another phenotype with skeletal dysplasia (frontometaphyseal dysplasia, Melnick–Needles syndrome or oto-palato-digital syndrome type I and II).[10] These syndromes are also associated with cardiac defects. Filamin A is the non-muscle isoform of filamin and is widely expressed. Two filamin A proteins associate to form a flexible V-shaped homodimer. This dimer crosslinks two F-actin filaments, creating a three dimensional network inside the cell. The C-terminal side can link to different membrane proteins, thereby anchoring the cytoskeleton to the cell membrane. The mechanism leading to BPNH has not been fully elucidated. Filamin A is needed for cell migration.[11,12] Still, the motility of many cell types in subjects with defective filamin A does not seem to be affected, both in humans and in mice.13 Filamin A also has an important function in the formation of intercellular junctions and the anchoring of the cell to the extracellular matrix. Flna knockout mice show cardiac and vascular developmental disorders and midline abnormalities.[14] Vascular endothelial cells in Flna null mice show defective cell–cell contacts and adherens junctions resulting in abnormal angiogenesis with disorganised blood vessels with aberrant branching.[12] Our patient showed that vascular aneurysms can be associated with *FLNA* mutations in the absence of other Ehlers Danlos type symptoms.

CONCLUSION

In patients with *FLNA* mutations, neurological, cardiovascular or orthopaedic symptoms may dominate the phenotype. Recognition may lead to prevention of complications and also allows genetic counselling for patients and their relatives. Although periventricular nodular heterotopia are the clue to the diagnosis, cardiovascular symptoms may dominate the patient's history. Neurologists that diagnose periventricular nodular heterotopia should consider referring their patients for a cardiac evaluation.

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3.3

Combined cardiological and neurological abnormalities due to filamin A gene mutations

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ABSTRACT

Background
Cardiac defects can be the presenting symptom in patients with mutations in the X-linked gene *FLNA*. Dysfunction of this gene is associated with a congenital malformation of the cerebral cortex, but can also lead to cardiac abnormalities, especially in the left ventricular outflow tract. We noticed that some patients diagnosed at the Neurogenetics Clinic had first presented to a cardiologist, suggesting that earlier recognition may be possible if the diagnosis is suspected.
Methods and results
From the Erasmus MC cerebral malformations database 24 patients were identified with cerebral bilateral periventricular nodular heterotopia (PNH) without other cerebral cortical malformations. In six of these patients, a pathogenic mutation in *FLNA* was present. Five of these a cardiac defect was also found in the outflow tract. Four had presented to a cardiologist before the cerebral abnormalities were diagnosed.
Conclusions
The cardiological phenotype typically consists of aortic or mitral regurgitation, coarctation of the aorta or other left-sided cardiac malformations. Most patients in this category will not have a *FLNA* mutation, but the presence of neurological complaints, hyperlaxity of the skin or joints and/or a family history with similar cardiac or neurological problems in a possibly X-linked pattern may alert the clinician to the possibility of a *FLNA* mutation.

COMBINED CARDIOLOGICAL AND NEUROLOGICAL ANOMALIES DUE TO FILAMIN A MUTATIONS

INTRODUCTION

Cardiac defects in adults are usually sporadic and rarely considered to be part of a hereditary syndrome. The presence of other congenital malformations or a positive family history should alert the clinician to the possibility of an underlying, possibly genetic cause.[1,2] Combined neurological and cardiac disease is well recognized in neuromuscular disorders[3]; however a cardiac defect can also be the presenting symptom in patients with a congenital malformation of the cerebral cortex due to mutations in the X-linked gene *FLNA* (OMIM 300017). Dysfunction of this gene leads to abnormalities in outflow tract development, often manifesting as a mitral or aortic valve insufficiency and a cerebral migration disorder, characterized by clusters of grey matter along the ventricles consisting of neurons that failed to migrate to the cortex during prenatal development.[4] Some mutations in *FLNA* can lead to craniofacial and skeletal abnormalities, including otopalatodigital (OPD) syndrome types 1 (OMIM 311300) and 2 (OMIM 304120), Melnick-Needles syndrome (MNS, OMIM 309350) and frontometaphyseal dysplasia (FMD, OMIM 305620). The filamin proteins (FLNA, FLNB and FLNC) are products of different genes and splice variants. Filamins stabilize F-actin networks in the cell and link them to cellular membranes by binding to transmembrane receptors or ion channels, thereby regulating cell morphology, membrane integrity and cell locomotion.[5] FLNA and FLNB are widely expressed, while FLNC is restricted to the striated muscle. Mutations in *FLNC* have been associated with myofibrillar myopathy.[6] FLNA is highly expressed in early myotubes, developing myofibrils and migratory neurons. During myofibril development FLNA is replaced by FLNB.[5] There seems to be some functional redundancy of both proteins.[7] Mutations in *FLNB* have only been described in skeletal chondrodysplasia, like Larsen syndrome (OMIM 150250), spondilocarpotarsal dysostosis (OMIM 272460), atelosteogenesis I and III (OMIM 108720 and 108721) and boomerang dysplasia (OMIM 112310). Among these, only Larsen syndrome occasionally presents with cardiac outflow tract defects and none with cerebral PNH. In this study, we evaluated patients known with a *FLNA* mutation for cardiac abnormalities.

METHODS

We used our database of patients with malformations of cortical development to determine how often we see a combination of cerebral periventricular nodular heterotopia (PNH) and cardiac abnormalities due to *FLNA* mutations, to describe the patient characteristics and to provide information for the cardiologist as to when they should be alert to the possibility of an associated cerebral malformation. *FLNA* mutations were identified by direct sequencing of exons and intron exon boundaries (reference sequence NM_01110556.1).

RESULTS

From our ongoing database of patients with malformations of cortical development, we identified 24 patients with bilateral PNH without other cerebral cortical malformations.[8] In six patients this was due to a pathogenic mutation in *FLNA*. Five of these *FLNA* patients had a cardiac defect in the outflow tract. Details of these five patients are found in the table and clinical descriptions below. Four presented to a cardiologist before the diagnosis of the cerebral abnormalities was known. In the 18 other patients with bilateral PNH without other cerebral malformations pathogenic mutations in *FLNA* were excluded, and none of these had a cardiac defect as evaluated by a cardiologist. Patients with other malformations of cortical development were not all screened by a cardiologist, so the incidence of cardiac defects in this group cannot be inferred from our data.

Five case reports

1. The first patient is a boy (patient 1 in Table and Figure 2), the third child of healthy parents of Somalian descent. A prenatal ultrasound at 20 wks showed an unclassified cardiac abnormality. Pregnancy and term birth (38 wks) were uncomplicated. Birth weight was 2635 grams, head circumference 34 cm (normal). Cardiac ultrasound and catheterization showed a cardiac malformation consisting of a situs solitus, AVVA concordant, mono-atrium, mitral atresia, hypoplastic left ventricle, double outlet right ventricle, patent arterial duct, severe hypoplasia of the transverse aortic arch and coarctation of the aorta. Physical exam showed a normal abdomen, cryptorchidism, a closed palate and normal facies, apart from minor anomalies of a preauricular pit on the left side and mildly posteriorly rotated ears. Neurological evaluation in the neonatal period showed an alert infant with normal movements, reflexes and muscle tone. Ophthalmologic screening was normal. EEG showed no epileptiform discharges. Brain MRI showed wide cerebral ventricles with bilateral PNH, a normal cortex, and a bifid septum pellucidum, and an enlarged retrocerebellar space with normal cerebellum and brainstem. Thorax CT angiogram performed to classify the cardiac abnormality additionally showed incomplete fusion of the distal third part of the sternum. The patient died at the age of 2 months of heart failure. Autopsy was not allowed and the family was lost to follow-up.

Genetic testing: *FLNA* sequencing of DNA extracted from leukocytes showed two sequence changes. The missense change c.5290G>A (p.A1764T) has been reported before in a woman with PNH and has been described as pathogenic, although there is no definitive evidence.[1] The second missense change was found in exon 20 (c.3035C>T) resulting in a serine to leucine substitution at position 1012 of the protein and has not been reported before in PNH or in the OPD-spectrum. This is a change in rod 1 (repeat 1-15) before the hinge 1 domain and is predicted to be a non-tolerated amino acid change in two different computer models. It is uncertain whether the second missense change contributes to the phenotype.

2. During the first pregnancy of an unrelated healthy Dutch couple prenatal ultrasound and prenatal MRI had shown a girl with a possibly enlarged heart and mildly enlarged cerebral ventricles (patient 2 in Table and Figure 2). Delivery at term was uncomplicated. Birth weight was 2830 gr and head circumference 37 cm (+2SD). Postnatally, cardiac

ultrasound showed a secundum atrial septal defect, coarctation of the aorta and mild aortic regurgitation. The coarctation was surgically corrected at the age of 16 days by resection and end-to-end anastomosis. She showed normal to mildly delayed cognitive development and delayed motor development with hypotonia and severe hyperlaxity. Facial features show a broad forehead, prominent orbital ridges, deep set eyes with downslant, and a flat midface. She has never had seizures, and EEG showed no epileptiform discharges. Brain MRI showed bilateral PNH and an enlarged retrocerebellar cyst (Figure 1). At the age of 3 months she developed dyspnoea due to congenital lobar emphysema of the right middle pulmonary lobe with bronchomalacia. She was successfully treated with a pulmonary lobectomy.

Genetic analysis: *FLNA* sequencing of DNA extracted from leukocytes showed a missense change c.220G>A in exon 2 (p.G74R). Family studies showed the mother, not the father, to be carrier of the same mutation. Brain MRI of the mother showed bilateral PNH, typical of *FLNA* mutations. She subsequently developed epilepsy at age 27 years. Cardiological evaluation, including ultrasound, of the mother was normal. The mother has hyperlaxity. This missense change was absent in leukocyte DNA of the maternal grandparents. These have no *FLNA* related complaints or symptoms, although brain MRI was not done. Its de novo occurrence in the family renders it very likely that c.220G>A, p.G74R is a pathogenic mutation.

3. A woman with a normal IQ and an otherwise unremarkable history underwent aortic valve replacement for severe aortic valve regurgitation at age 40. Details on the pathology were lost. At age 55 she was diagnosed with heart failure and one year later she underwent heart transplantation. Macroscopic pathology showed a dilated heart and a dilated aortic root. The left ventricle shows subendocardial fibrosis. At age 60 she was diagnosed with severe venous varicosis of the legs and at age 70 an infrarenal aortic aneurysm with a 4.7 cm diameter was found and treated conservatively. At age 71 symptoms of mild cognitive decline prompted a brain CT showing bilateral PNH as a chance finding. Eight months later she died of a subarachnoid hemorrhage from a ruptured fusiform carotid aneurysm. She never had an epileptic seizure and showed no hyperlaxity of joints or skin. Family history revealed that her mother had heart problems from a young age and died from heart failure at age 69, details could not be recovered. The patient had a healthy twin brother and a sister and a healthy son. The autopsy showed severe generalized atherosclerosis with mild dilatation of the thoracic aorta and an aneurysm of the abdominal aorta of 7 cm. Brain autopsy showed symmetrical bilateral PNH and bilateral fusiform carotid aneurysms with widespread glomeruloid microvascular changes in the cerebral cortex. [9]

Genetic analysis: direct sequence analysis of *FLNA* in DNA extracted from leukocytes showed a pathogenic frame shift mutation c.3045del5 in exon 21.

4. A six-year old girl presented with a cardiac murmur and was diagnosed with a mild aortic stenosis (9mm gradient) and regurgitation with a normal left ventricle diameter that remained stable over the years (patient 4 in Table and Figure 2). She is now 46 years old and has a normal IQ. She had a first generalized epileptic seizure at age 28. Brain CT

showed bilateral PNH. Dysmorphic evaluation showed no abnormalities and no hyperlaxity. She has no children and one healthy brother.
Genetic analysis: direct sequence analysis of *FLNA* in DNA extracted from leukocytes showed a pathogenic frame shift mutation c.3582delC in exon 22.
5. A girl presented shortly after birth with cyanosis during feeding and was diagnosed with a ventricular septal defect and aortic regurgitation (patient 5 in Table and Figure 2). At age 24 the ventricular septal defect was surgically corrected and she received an aortic bioprosthesis. At age 36 yrs she underwent a Bentall procedure. At age 40, she presented to a neurologist because of a complicated migraine attack with aphasia. A brain MRI showed bilateral PNH and an enlarged retrocerebellar space (Figure 1). Family history revealed that her mother died suddenly at age 57 from a rupture of the aorta. The patient has one sister with mild aortic regurgitation, she has been invited for counselling. The patient has a healthy son.
Genetic analysis: direct sequence analysis of *FLNA* in DNA extracted from leukocytes showed a pathogenic mutation frame shift mutation c.6635delTCAG in exon 41.

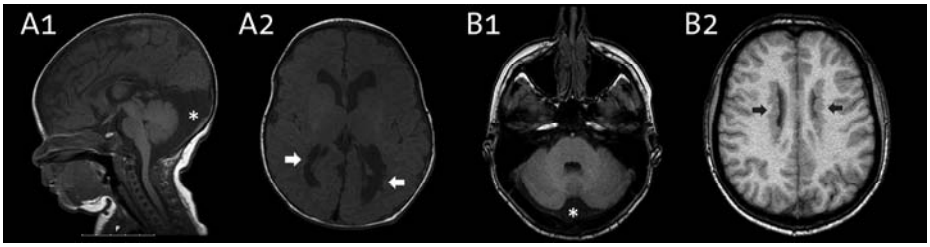

Figure 1: neuroimaging characteristics of a child (patient 2) in A1 and A2, and an adult (patient 5) in B1 and B2. All are T1 weighted MRI images. Note the periventricular nodular heterotopia (denoted by arrows) and the enlarged retrocerebellar space (denoted by a star).

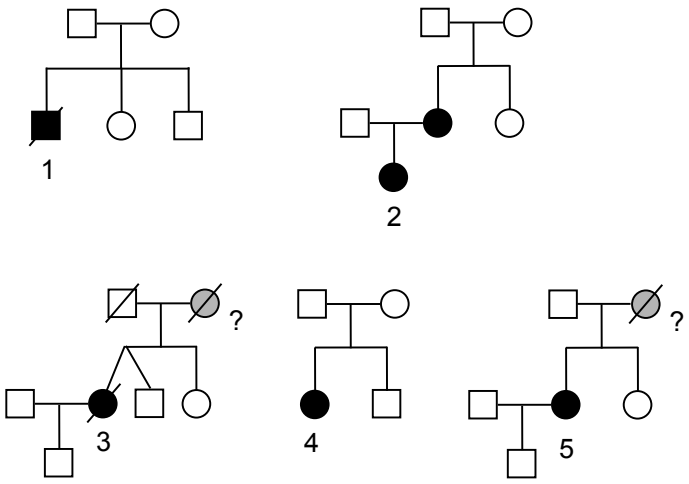

Figure 2: pedigrees of the described cases. FLNA mutation carriers in black, probably affected but deceased persons in grey. Numbers refer to the described cases.

Table: patient characteristics.

Case	Sex	Cardiological phenotype	Neurological phenotype	Outcome	Mutation
1	M	Mono-atrium, mitral atresia, hypoplastic LV, double outlet RV, aortic coarctation	Normal	Died age 2 months of heart failure	c.5290G>A
2	F	Aortic coarctation	Hypotonia	Now 18 m old	c.220G>A
3	F	Severe aortic valve insufficiency	Normal	Died aged 71 yrs of subarachnoid haemorrhage	c.3045del5
4	F	Mild aortic valve stenosis and regurgitation	Epilepsy	Now 45 yrs old	c.3582delC
5	F	Severe aortic valve regurgitation	Normal	Now 41 yrs old	c.6635delTCAG

DISCUSSION

Five out of six patients with a pathogenic mutation in *FLNA* from our database show a combination of cardiac disease and bilateral cerebral PNH. Four patients presented to a cardiologist before or at the time of their neurological workup. Neurological signs were absent or mild at that time and the diagnosis of bilateral PNH was made later due to epileptic seizures (case 4), hypotonia (case 2) or during the workup of non-related complaints. This suggests that the cardiologist had the first opportunity to recognise these patients. Recently also X-linked mitral valvular dystrophy without neurological signs or symptoms of epilepsy was found to be caused by mutations in *FLNA*, however brain imaging was not reported.[10] The cardiological phenotype is not always this specific. FLNA knock out mice show midline skeletal defects and early male lethality due to cardiac malformations in atrioventricular septal and outflow tract development.[11] Human patients also show abnormalities in the outflow tract ranging from patent ductus arteriosus, mitral or aortic valvular abnormalities to coarctation of the aorta, and ascending aorta aneurysm.[12-14] Cerebral PNH in *FLNA* patients is caused by impaired migration of later born neurons due to disrupted cell adhesion and abnormal ventricular ependymal function.[15] This shows that pathways involved in cell adhesion can both affect the neuro-epithelium and vascular development. Apart from cerebral and cardiovascular developmental defects *FLNA* mutations can also cause connective tissue abnormalities, and autopsy studies show abnormal glomeruloid microvascular proliferations in the brain.[9,15] A combination of PNH and Ehlers-Danlos syndrome with joint hyperlaxity and aorta aneurysms caused by a mutation in *FLNA* has been described in females.[16] Neurological phenotypes associated with PNH are diverse and range from epilepsy and normal development to patients with multiple congenital anomalies and mental retardation. In males mutations are often prenatal lethal. Less severe mutations with residual filamin A function are found in males with PNH. One male patient has been described with PNH and a lethal complex cardiac malformation, including atrial and ventricular septal defect and persistent left superior caval vein.[12] Interestingly, gain of function mutations of *FLNA* are associated with syndromes with craniofacial and skeletal abnormalities, including otopalatodigital syndrome types 1 and 2, Melnick-Needles syndrome (MNS) and frontometaphyseal dysplasia.[17] In these syndromes several male patients have been described with heart defects, cryptorchidism and umbilical hernia, but no PNH.[17] The combination of mitral and/or aortic regurgitation and skeletal abnormalities with hyperlaxity is also found in

autosomal dominant Marfan syndrome (OMIM 154700), in the allelic Shprintzen-Goldberg syndrome (OMIM 182212) and in Loeys-Dietz syndrome type 1A and B (OMIM 609192 and 610168). Some neuromuscular abnormalities are described in these patients, but no cerebral cortical developmental abnormalities. Although we did not find any cases, cardiac defects are reported to be associated with some other cerebral PNH phenotypes. Patients with Williams syndrome, caused by a microdeletion of chromosome 7q11.22-23, have distinctive facial dysmorphias, 'elfin face', and often cardiac defects, such as supravalvular aortic stenosis, mitral or pulmonary valve abnormalities, and atrial or ventricular septum defect. One case has been described with associated PNH.[18] In chromosome 6q terminal deletion syndrome, brain MRI commonly shows hypoplasia of the corpus callosum, and a few patients have been described with associated PNH.[19] About half of 6q terminal deletion patients are reported to have cardiac abnormalities, primarily ventricular or atrial septum defect. Distal duplications of chromosome 5 p have been associated with PNH in two patients, one of which also had atrial septum defect and mitral and tricuspid valve prolapse.[20] Genetic factors related to similar cardiac malformations without PNH are being widely investigated, but still only few genes are known to cause a developmental defect of the atrioventricular septum and the outflow tract in humans.[21-23]

CONCLUSION

Patients with mutations in *FLNA* show a cardiological phenotype with aortic or mitral regurgitation, coarctation of the aorta or other left-sided malformations. Although patients with cardiac defects in this category are numerous and most will not have a *FLNA* mutation, the presence of neurological complaints, hyperlaxity of the skin or joints and/or a family history with similar cardiac or neurological problems in a possibly X-linked pattern should alert the clinician to the possibility of a *FLNA* mutation. Recognition will enable genetic testing and genetic counselling for patients and their family.

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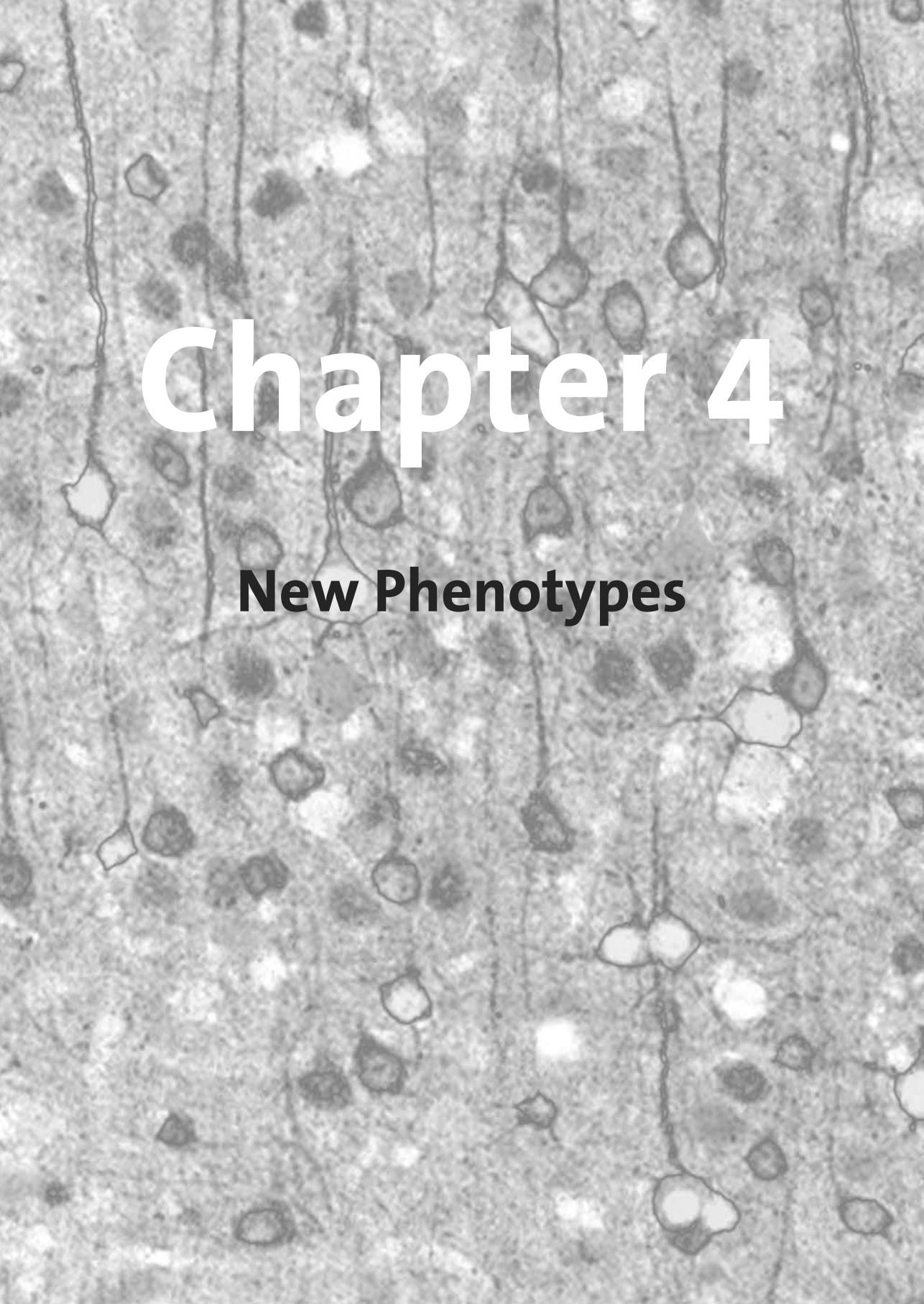

Chapter 4

New Phenotypes

4.1

Movement disorder and neuronal migration disorder due to *ARFGEF2* mutation

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ABSTRACT

We report a child with a severe choreadystonic movement disorder, bilateral periventricular nodular heterotopia (BPNH), and secondary microcephaly based on compound heterozygosity for two new *ARFGEF2* mutations (c.2031_2038dup and c.3798_3802del), changing the limited knowledge about the phenotype. The brain MRI shows bilateral hyperintensity of the putamen, BPNH, and generalized atrophy. Loss of *ARFGEF2* function affects vesicle trafficking, proliferation/apoptosis, and neurotransmitter receptor function. This can explain BPNH and microcephaly. We hypothesize that the movement disorder and the preferential damage to the basal ganglia, specifically to the putamen, may be caused by an increased sensitivity to degeneration, a dynamic dysfunction due to neurotransmitter receptor mislocalization or a combination of both.

MOVEMENT DISORDER AND NEURONAL MIGRATION DISORDER DUE TO ARFGEF2 MUTATION

INTRODUCTION

In 2004 mutations in the gene *ARFGEF2* encoding ADP-ribosylation factor guanine nucleotide exchange factor 2, were found to cause autosomal recessive bilateral periventricular nodular heterotopia (BPNH) in four patients from two Turkish pedigrees.[1] Clinical features reported were microcephaly, feeding difficulties, severe mental retardation, quadriparesis, and epilepsy. More details on the phenotype were not reported and since then, to our knowledge, no additional patients have been published. We discuss a patient with a different phenotype caused by two new *ARFGEF2* mutations.

CASE

Our patient is the only child of non-consanguineous parents from Dutch descent. Prenatal ultrasounds showed growth retardation. She was born at 38 weeks of gestation with an Apgar score of 10 after 5 min, birth weight 2,790 g, and length 46 cm. Occipito-frontal circumference was 34.2 cm at the age of 3 weeks (−1.5 SD). During the first year developmental delay became apparent. She showed social smiling at 2 months and grasping at 5 months, and rolled over at the age of 8 months. There were excessive extension movements. She had persistent feeding problems, severe drooling, and frequent vomiting. At the age of 9 months a pediatrician noted dystonic-spastic paraplegia with axial hypotonia. With physical therapy she showed some progression, but did not reach normal milestones. At the age of 4 years feeding difficulties prompted a gastrostomy. Despite adequate caloric intake, growth is unsatisfactory with height progressing at 2.5 SD below normal. Occipito-frontal circumference growth went from the −1.5 SD to 0.5 cm below the −2.5 SD curve. She never had seizures. Now, at the age of 7 years, she makes eye contact, smiles, and understands some words. She vocalizes, but does not produce any words. Pupillary responses are normal. There is no nystagmus. Axial muscle tone is low and variable in the extremities. Muscle strength is normal. She shows a severe extrapyramidal movement disorder with irregular repetitive jerking movements of all extremities and the face (chorea) and sustained abnormal movements and posturing (dystonia). This is exacerbated by emotions and during infections when large proximal jerking movements may resemble ballism. There is no rigidity or ataxia. Tendon reflexes are lively and symmetric, with extensor plantar responses (Babinski sign). She can sit with support, but cannot stand. The movement unrest can be so severe that she becomes exhausted and is partially responsive to treatment with high doses of benzodiazepines.

Brain MRI at the age of 13 months showed BPNH, myelination delay, and generalized atrophy, a second MRI at age 4 years and 10 months showed improved myelination, stable or minimally progressive generalized atrophy but also symmetric atrophy of the basal ganglia, specifically of the globus pallidus and the putamen with focal T2- hyperintensity of the putamen (Figure). In retrospect, inhomogeneous abnormalities of the putamen can also be seen on the first MRI and suggest early degeneration resulting in gliosis.

EEGs show delta-activity without differentiation or epileptiform activity. Metabolic testing of urine and plasma, including amino acids, lactate, pyruvate, and creatine kinase was normal. The family history is unremarkable.
Sequence analysis of all 39 coding exons and intron–exon boundaries of the *ARFGEF2* gene on chromosome 20 shows compound heterozygosity for two variations that have not been previously reported. At the DNA level c.2031_2038dup and c.3798_3802del using reference sequence NM_006420.2 and at protein level p.Gln680ProfsX2 and p.Phe1267-GlyfsX17, respectively. Both these variations lead to frame shift followed by a premature stop-codon and are therefore considered pathogenic mutations. Parents are heterozygote for found mutations.

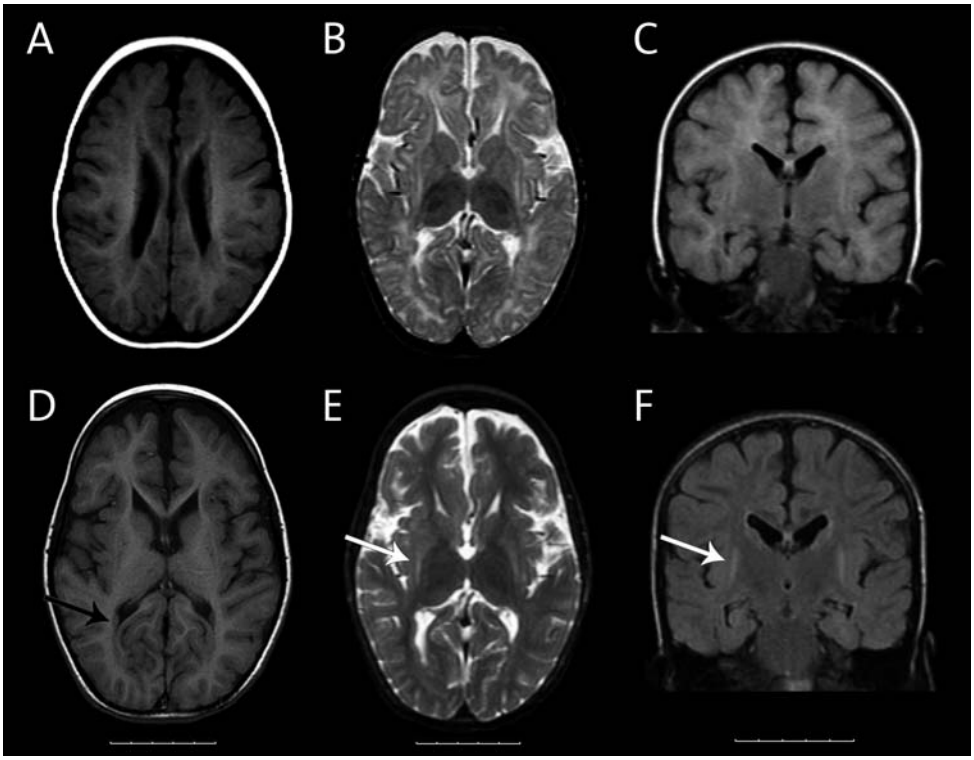

Figure: Brain MRI made at the age of 13 months (images A, B, and C) and at the age of 4 years and 10 months (images D, E, and F). A and D T1-weighted images showing bilateral periventricular nodular heterotopia with the intensity of gray matter (black arrow in image D). B and E T2-weighted images showing hyperintensity of the putamen and atrophy of the putamen and globus pallidus (white arrow in image E). C and F Flair images, coronal view, showing bilateral hyperintensity of the putamen, more clearly visible on image F made at a later age (white arrow), and mild atrophy of the hippocampi.

DISCUSSION

Mutations in ARFGEF2 can cause autosomal recessive bilateral periventricular nodular heterotopia (BPNH) with microcephaly.[1] Our patient shows that a choreadystonic extrapyramidal movement disorder can be part of the phenotype. The movement disorder is reflected in MRI abnormalities of the basal ganglia, specifically of the putamen, in addition to generalized atrophy and BPNH. Anatomically, putamen and caudate nucleus form the dorsal striatum and are the major afferent center of the basal ganglia, receiving excitatory input from the cerebral cortex, thalamus, and brain stem. The putamen receives projections from the cortical motor and somatosensory areas, the midbrain, and the raphe nuclei. The striatum projects to the globus pallidus and substantia nigra, mainly with GABAergic neurons. Output is mediated by local inhibitory interneurons.[2] Similar choreadystonic movement disorders and putamen degeneration on MRI are seen in Huntington's disease, Wilson's disease and Leigh syndrome.

ARFGEF proteins are guanine nucleotide exchange factors (GEFs) that activate ADP-ribosylation factors (ARFs). ARFGEF2 protein, also known as BIG2, is one of three GEFs expressed in the brain during the period of neural proliferation and migration with high expression in the cortical ventricular zone and ganglionic eminences, the future basal ganglia.[1] ARFs regulate the formation of coated vesicles from the Golgi, trans-Golgi network (TGN), and endosomes. In recycling endosomes BIG2 loss of function result in failure to deliver proteins to the cell membrane.[3-5] This was shown for the GABA(A) receptor, but also for the transferrin receptor and the tumor necrosis factor receptor.[1,4,6,7] Other cell surface receptors are likely to be also affected. To understand our patient's phenotype, different consequences of *ARFGEF2* loss of function need to be considered. The BPNH are similar to those seen in patients with loss-of-function mutations in the X-linked filamin A gene (*FLNA*). *FLNA* is the most commonly identified genetic cause for BPNH, found in approximately one third of patients.[8] In heterozygous females BPNH is associated with epilepsy and/or developmental delay or may be an accidental finding. In males FLNA mutations are often fatal, but may result in a similar phenotype to affected females .[8,9] In both *ARFGEF2* and *FLNA* patients the neuroepithelium lining the ventricles is disrupted leading to failed migration of later-born neurons.[9] The similarity may be solely due to failed transport of the filamin A protein to the cell membrane by BIG2 dysfunction or to a final common pathway disrupting vesicle trafficking.[9,10] However, despite the similar brain phenotype, *FLNA* patients do not have extrapyramidal movement disorders or microcephaly. Speculatively, in *ARFGEF2* neuronal migration may be more severely affected due to disruption in GABA(A) receptor function in the embryonic period, as GABA(A) antagonists have been shown to impair neuronal motility in rodents in vitro and in vivo.[11,12] Microcephaly can be due to diminished neuronal proliferation and BIG2 inhibition in vitro experiments seem to suggest this occurs.[1] Increased apoptosis can also cause microcephaly and atrophy. Other BIG2 inhibition experiments show some signs of endoplasmic reticulum stress in cultured cells.[13] Possibly, ER stress increases susceptibility to apoptosis in specific neurons. Our patient's normal occipito-frontal circumference at birth shows that congenital microcephaly is not obligatory. Why the putamen should show a specific sensitivity for neuronal loss is unknown. Finally, *ARFGEF2* is expressed

postnatally and neurotransmitter receptor function can be impaired by defective exosome function.[3,6] Disruptions in the recycling of receptors also hinder dendritic arborization.[9] This may have contributed to the movement disorder. Our patient is compound heterozygote for two new mutations and new patients are needed to determine if a genotype–phenotype relation exists for *ARFGEF2* mutations. BPNH caused by autosomal recessive *ARFGEF2* mutations is more than an anatomical malformation. We suggest that the clinical features of the choreadystonic movement disorder, BPNH, and microcephaly of our patient can be understood in the light of abnormal vesicle transport resulting in neuroependyma disruption, decreased proliferation of neurons, and/or increased sensitivity to apoptosis and lifelong exosome dysfunction affecting neurotransmitter receptor function and neuronal development.

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4.2

Microcephaly with simplified gyral pattern associated with early onset insulin dependent diabetes mellitus

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ABSTRACT

Two families are presented with a child suffering from microcephaly with a simplified gyral pattern of the brain (SGP) and early onset insulin dependent diabetes mellitus (IDDM). The first patient was diagnosed postmortally with Wolcott–Rallison syndrome, after her younger brother developed IDDM, and a homozygous mutation in the eukaryotic translation initiation factor 2-alpha kinase 3 was found. The younger brother did not undergo magnetic resonance imaging (MRI). The patient from the second family has no *EIF2AK3* mutation. SGP is considered to arise from decreased neuronal proliferation or increased apoptosis at an early stage of embryonal development, but insight into the pathways involved is minimal. *EIF2AK3* is involved in translation initiation. It has been proposed that loss of function mutations reduce the ability of the cell to respond to endoplasmic reticulum stress, resulting in apoptosis of pancreatic Langerhans cells. Our findings suggest that in some cases, early onset IDDM and SGP can arise from common mechanisms leading to increased apoptosis.

MICROCEPHALY AND SIMPLIFIED GYRAL PATTERN OF THE BRAIN ASSOCIATED WITH EARLY ONSET INSULIN-DEPENDENT DIABETES MELLITUS

INTRODUCTION

In congenital microcephaly, magnetic resonance imaging (MRI) of the brain may reveal a relatively normal looking cortex (true microcephaly) or a diverse spectrum of brain malformations. One of those malformations is a simplified gyral pattern (SGP). in which the number of gyri and sulci is reduced, while cortex thickness is normal. This differentiates SGP from pachygyria or microlissencephaly with abnormally thick cortex. The same phenotype is also described in the literature as microcephaly with simplified gyration (MSG). SGP is thought to arise from decreased proliferation and/or increased apoptosis during embryogenesis.[1] Most cases are unexplained, although four genes have been identified that cause primary microcephaly with mental retardation without significant neurological deficit and a relatively well preserved gyral pattern (*MCPH1, ASPM, CDK5RAP2* and *CENPJ*). These genes are all involved in mitosis, as shown by premature chromosome condensation in affected cells. Mutations are thought to cause decreased proliferation of neuroblasts.[2] As far as we know, there are no known gene defects that cause SGP by the mechanism of increased apoptosis.

Persistent early onset insulin-dependent diabetes mellitus (IDDM) is rare. Neonatal onset persistent IDDM (with symptoms within the first 3 months of life) has an estimated incidence of one in 800,000 live births.[3] Three main mechanisms contribute to the development of IDDM: defects in insulin signaling leading to insulin resistance, defects of insulin secretion leading to hypoinsulinaemia and impaired development or apoptosis of pancreatic beta-cells leading to decreased pancreatic beta-cell mass.[4] One of the monogenetic early onset IDDM syndromes in which inappropriate apoptosis is proposed to be the pathological mechanism is Wolcott–Rallison syndrome (WRS, OMIM 226980). This syndrome is characterized by neonatal or early onset IDDM associated with multiple epiphyseal dysplasia and other variable clinical features and is caused by mutations in the eukaryotic translation initiation factor 2-alpha kinase 3 gene (*EIF2AK3*). In the literature, one patient with WRS has been described that also had a brain developmental disorder resembling pachygyria or SGP.[5] Other monogenetic IDDM syndromes where inappropriate apoptosis has been proposed to be the underlying mechanism are Wolfram syndrome (OMIM 222300), Friedreich ataxia (OMIM 229300), thiamine-responsive megaloblastic anaemia (OMIM 249270) and Werner syndrome (OMIM 277700).[4]

Here, we present three cases from two families with early onset IDDM, two of which also show a simplified gyral pattern of the cerebral cortex. In one family, Wolcott–Rallison syndrome is the cause of IDDM; the other shows a similar phenotype but without an *EIF2AK3* mutation.

Family 1

Case 1, a girl, was born to consanguineous Turkish parents, third degree relatives, after an uneventful pregnancy. At the age of 6 weeks, she was admitted with hyperglycaemia and diagnosed with IDDM. It was noted that her head circumference was 3 SD below the mean. At the age of 2.5 years, she was admitted for analysis of insufficient growth and psychomotor retardation. A brain MRI showed a simplified gyral pattern with normal cerebellum, brainstem and corpus callosum (Figure 1, upper row). Routine metabolic screening was normal, including thyroid function, and antibodies against Langerhans cells were absent. During this admission, she deteriorated into a comatose state with myoclonic jerks of the upper extremities. Electroencephalogram (EEG) registration revealed a status epilepticus which was treated with midazolam and phenytoin, later switched to valproate. She slowly gained consciousness over the following days but had lost some developmental milestones. Hand X-rays showed coneshaped proximal phalanges, osteoporosis and cortical sclerosis (Figure 2). Over the following years, her epilepsy proved therapy resistant and the IDDM difficult to control. Around the age of 3, she was not able to walk independently due to a spastic–ataxic tetraparesis and only spoke some words. She was able to make eye contact, communicate and play. Further analysis showed cochlear hearing loss of 60 dB on the right side and 50 dB on the left. At the age of 4.7 years, she developed hepatic dysfunction during a staphylococci sepsis and died. A few years later, Wolcott–Rallison syndrome was diagnosed in a younger brother caused by a pathogenic *EIF2AK3* mutation. Retrospectively, we concluded that case 1 suffered from the same disorder.

Case 2 is a boy, the younger brother of case 1. A few days postpartum, he was found to have IDDM. His head circumference was normal at 1.5 to 2 SD below the mean. His neurological examination was normal, but psychomotor development was delayed. At the age of 22 months, he has just learned to walk but does not speak any words. Liver enzymes were repeatedly found to be mildly elevated, and he was admitted several times for rising liver enzymes during mild infections. He suffers from frequent and intractable vomiting. Antibodies against Langerhans cells are absent and thyroid function is normal. His EEG shows a normal reactive background pattern with aspecific abnormal sharp waves bilaterally in the temporal and parieto-occipital regions. He has never had an epileptic seizure. The suspicion was raised of him having an autosomal recessive form of infantile diabetes, and in particular, considering the sister, Wolcott–Rallison syndrome. X-rays of hands and knees revealed hypoplastic epiphyses and mild osteopenia without any structural changes. Further investigations showed a homozygous pathogenic mutation in the *EIF2AK3* gene. Parents denied permission for brain MRI.

Family 2

Case 3 is a boy, born at 40 weeks gestation to third degree related parents, originally from Morocco. At birth, his head circumference was 2.5 SD below the mean. Two older sisters were healthy with normal head circumference. At the age of 3 months, he was admitted with generalized seizures. An EEG showed multifocal epileptic discharges with an abnormal background pattern. A brain MRI showed a simplified gyral pattern with thin

corpus callosum and normal cerebellum and brainstem (Figure 1b). Epilepsy proved therapy-resistant. At the age of 8 months, he was diagnosed with IDDM, which proved difficult to control. Physical exam showed severe psychomotor retardation, obesity and small genitalia. Additional investigations showed a mild hypothyroidism with TSH, 5.26 mU/l (reference 0.4–4.3) and FT4, 26.2 pmol/l (reference 12–26). Plasma lipids were elevated with cholesterol, 8.2 mmol/l (reference 2.0–5.0), triglycerides, 2.07 mmol/l (reference 0.30–1.30), low density lipoprotein (LDL) cholesterol, 6.11 mmol/l (reference <4.20) and normal high density lipoprotein (HDL)-cholesterol 1.16 mmol/l (reference 0.90–2.75). Further analysis including routine metabolic screening in urine and plasma, cerebrospinal fluid (CSF) screening for IgG, neurotransmitters and lactate/pyruvate and ophthalmologic screening was normal. Antibodies against Langerhans cells were absent and liver function was normal. Skeletal X-rays were never done. He died at the age of 1.5 years due to pneumonia. Parents refused autopsy.

MATERIALS AND METHODS

EIF2AK3 mutation screening: DNA was extracted from peripheral blood collected on ethylenediamine tetraacetic acid (EDTA), and mutation screening was performed by sequencing all the coding regions of the gene on genomic DNA for case 2 and in case 3 and his parents, as previously described.[6]

RESULTS

We identified a new homozygous mutation in case 2, corresponding to a G>A substitution at position 3,037 on *EIF2AK3* cDNA sequence (Genbank number: NF 004836.3). This mutation has not been identified in 95 healthy controls nor in any of the previously screened WRS patients. This mutation causes a G to E missense mutation at position 957 of the protein (Genbank number: NP 004827.3), in the second serine/threonine kinase domain of the protein. Based on previous genetic and functional investigations of *EIF2AK3* mutations in WRS patients by our group and others, the mutated protein is likely to be nonfunctional, due to the loss of kinase activity.[7,8] No mutation was identified in EIF2AK3 in case 3 from the second family, and the implication of this gene could be excluded on the basis of heterozygosity of common single nucleotide polymorphisms in this gene.

DISCUSSION

Wolcott–Rallison syndrome causes IDDM and epiphyseal dysplasia with variable manifestations including hepatic failure and psychomotor retardation [for review, see reference 8]. Mental retardation is frequently reported in WRS without mention of head circumference or brain imaging, but the degree of brain developmental disorder may vary within one family as seen in cases 1 and 2. Microcephaly has been reported in a single case of WRS, and a loss of function mutation in *EIF2AK3* was identified in this patient.[7]

Later, a patient with WRS was reported to have brain atrophy with pachygyria and simplified gyri on MRI.[6] Her head circumference was on the tenth percentile. The published figure is insufficient to classify the brain malformation, but cortical thickness does not seem to be increased, so the description could also be compatible with SGP. Our patient

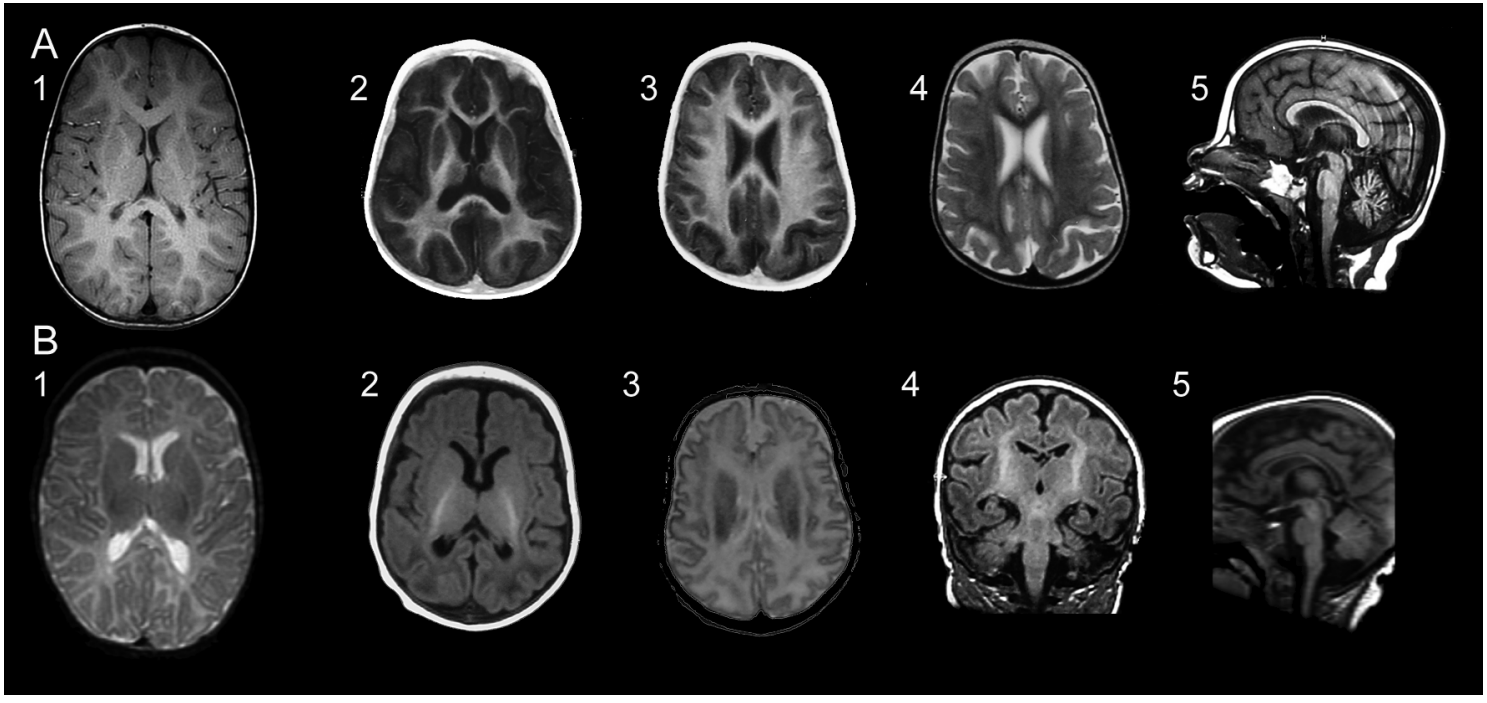

Figure 1: Brain MRIs.
A Upper row, case 1 (family 1): image 1 shows an age-matched control (T1 weighted image); images 2 and 3 show a T1 weighted, and image 4, a T2 weighted axial view at the age of 2.5 years. Note the decreased amount of gyri and sulci, but normal cortical thickness and white matter. Image 5 is a T1 weighted midsagittal view showing normal corpus callosum, brainstem and vermis.
B Lower row, case 3 (family 2): image 1 shows an age-matched control (T2 weighted image), images 2 and 3 show axial T1 weighted and T2 weighted images at the age of 3 months. Note the insufficient gyration and shallow sulci with mildly widened subarachnoid spaces and ventricles. Myelination and cortical thickness are normal for age. Image 4 is a T1 weighted coronal view through the hippocampi showing equal involvement of parietal and temporal lobes. Image 5 is a T1 weighted midline sagittal reconstruction showing a thin, but fully formed, corpus callosum and normal brainstem and vermis.

supports the idea that mental retardation in WRS may result from malformations of cortical development occurring during early gestation and that simplification of the gyral pattern and reduction of brain volume are variable features of the phenotype. In early onset IDDM, hypo- or hyperglycaemia occur only after birth so it cannot influence brain development during the first trimester of pregnancy. The underlying pathogenic mechanism is unclear. The *EIF2AK3* gene codes for one of four eukaryotic EIF2alpha kinases (eukaryotic translation initiation factor2-alpha kinase 3, also known as murine PERK: pancreatic endoplasmic reticulum kinase). PERK/EIF2alpha kinase 3 regulates the cellular response to endoplasmic reticulum (ER) stress. When an imbalance between protein synthesis and processing occurs, leading to accumulation of unfolded protein in the ER, EIF2alpha kinase is activated and phosphorylates eukaryotic initiation factor 2 alpha (EIF2alpha). EIF2alpha is an essential factor for protein synthesis by recruiting tRNA to ribosomes; its phosphorylation results in suppression of protein synthesis. *EIF2AK3* is also involved in the next steps in the cellular response to ER stress: the second being removal of excess unfolded protein, and the third, ultimately, apoptosis. It has been proposed that loss of *EIF2AK3* function leads to failure of protection against ER stress and uncontrolled apoptosis.[9] PERK knockout mice are born with normal exocrine and endocrine pancreas function but develop progressive Langerhans cell apoptosis and a phenotype with neonatal diabetes, skeletal defects and growth retardation.[10] Animal experiments have shown PERK to be present in the brain and to be important in the reaction to stress by inhibition of protein synthesis in situations of ischemia, status epilepticus and essential amino acids deficiency.[11-13]

The EIF2alpha activity level is further regulated by the EIF2beta protein. Both proteins are required to allow translation initiation. Mutations in the different subunits of EIF2-beta are known to cause a leukodystrophy syndrome called vanishing white matter disease.[14-15] It is unclear why the white matter of the brain is relatively more affected than the grey matter. Deterioration of patients with vanishing white matter disease is often triggered by head trauma or intercurrent infections, and uncontrolled apoptosis in response to stress is a likely explanation. Early neuronal apoptosis can be one of the mechanisms leading to SGP.[1] The mechanisms leading to *EIF2AK3* activation are not fully understood, and moreover, it is unknown whether *EIF2AK3* is expressed during embryonic brain development.[16] Putatively, *EIF2AK3* mutations are an example of how an insufficient cellular response to stress during brain development in utero could lead to abnormal apoptosis. Consequently, a second (epigenetic or environmental) event leading to ER stress added to the genetic mutation might be needed to cause abnormal brain development in WRS. This coincidence of factors could partly account for the wide clinical heterogeneity of the neurological deficits in WRS.[5,8] In case 3 (Family 2), the association of early onset IDDM and SGP on brain imaging was suggestive of WRS, by analogy of case 1, although skeletal abnormalities were not looked for. The medical history offers no support for any of the other previously mentioned monogenetic IDDM syndromes. Parental consanguinity suggests a genetic recessive cause, but analysis showed no mutation at the *EIF2AK3* locus. This observation suggests that pancreatic Langerhans cell apoptosis and brain development can be linked through other mechanisms and that *EIF2AK3* may not be the only gene associated with

IDDM, microcephaly and SGP. Possibly, further research into mechanisms of inappropriate apoptosis can shed more light on the pathological mechanism of simplified gyral pattern.

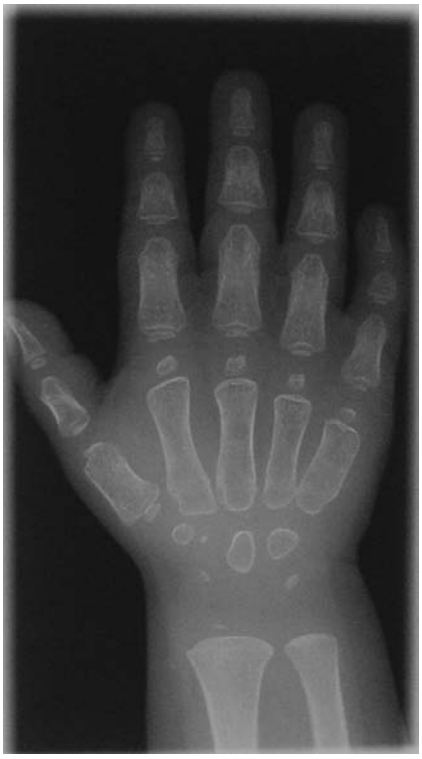

Figure 2: Hand X-ray of case 1: diffuse osteoporosis with cortical sclerosis, most pronounced in the metacarpals, and 'cone-shaped' proximal phalanges.

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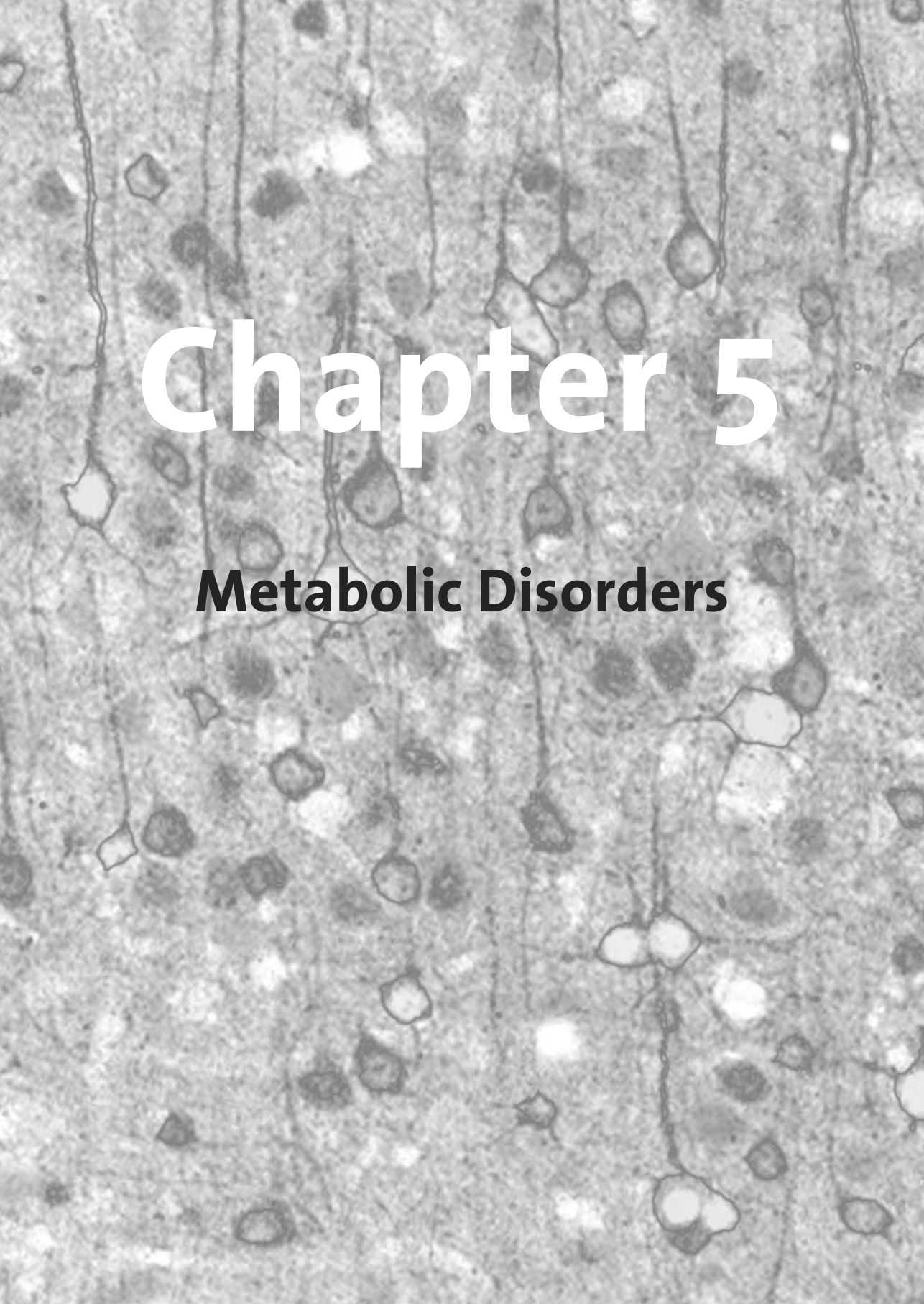

Chapter 5

Metabolic Disorders

5.1

Brain abnormalities in a case of malonyl-CoA decarboxylase deficiency

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Mol Genet Metab. 2006 Feb;87(2):102-6

ABSTRACT

Malonyl-CoA decarboxylase (MCD) deficiency is an extremely rare inborn error of metabolism that presents with metabolic acidosis, hypoglycemia, and/or cardiomyopathy. Patients also show neurological signs and symptoms that have been infrequently reported. We describe a girl with MCD deficiency, whose brain MRI shows white matter abnormalities and additionally diffuse pachygyria and periventricular heterotopia, consistent with a malformation of cortical development. *MLYCD*-gene sequence analysis shows normal genomic sequence but no messenger product, suggesting an abnormality of transcription regulation. Our patient has strikingly low appetite, which is interesting in the light of the proposed role of malonyl-CoA in the regulation of feeding control, but this remains to be confirmed in other patients. Considering the incomplete understanding of the role of metabolic pathways in brain development, patients with MCD deficiency should be evaluated with brain MRI and unexplained malformations of cortical development should be reason for metabolic screening.

BRAIN ABNORMALITIES IN A CASE OF MALONYL-CoA DECARBOXYLASE DEFICIENCY

INTRODUCTION

Malonyl-CoA decarboxylase (MCD) catalyzes the conversion of malonyl-CoA to acetyl-CoA and carbon dioxide. MCD deficiency (OMIM 248360) causes autosomal recessive malonic aciduria. Symptoms are psychomotor retardation, spasticity/hypotonia, seizures, cardiomyopathy, and episodes of metabolic acidosis. Seventeen cases have been published but brain pathology is poorly described (Table 1). The role and localization of MCD in the cell is controversial. Cytoplasmic malonyl-CoA is a potent inhibitor of mitochondrial fatty acid oxidation by inhibiting the mitochondrial outer membrane enzyme carnitine palmitoyltransferase I (CPT1). Heart and skeletal muscle contain a CPT1-isoform that is substantially more sensitive to inhibition by malonyl-CoA than liver CPT1, where cytoplasmic malonyl-CoA serves as a precursor for fatty acid synthesis.[1] Both isoforms are encoded by different genes, *CPT1B* and *CPT1A*. A third brain-specific CPT1 protein-isoform with high affinity for malonyl-CoA has been described.[2]

Symptoms of cardiomyopathy and metabolic acidosis triggered by stress can be explained by inhibition of mitochondrial -oxidation by continuous high levels of malonyl-CoA in cytoplasm [3] The carboxy-terminal of the MCD-protein contains a peroxisomal targeting sequence suggesting a peroxisomal function, but there is also a potential mitochondrial targeting sequence at the N-terminus and mitochondrial localization has been shown in rats.[1,4,5] In 1999, a mutation in a 2.1-kb cDNA fragment was identified on chromosome 16q24.3 encoding the human MCD gene *MLYCD* (OMIM 606761).[1] Wightman et al. further elucidated a probable mitochondrial and peroxisomal targeting sequence in the *MLYCD* gene and several different frame-shift, stop codon, and mislocalizing mutations in MCD-deficient patients.[6] We present a patient with a malformation of cortical development and a *MLYCD* abnormality, further delineating the neurological consequences of MCD deficiency.

CASE REPORT

Our patient is the daughter of Moroccan third degree consanguineous parents, born at 32 weeks by emergency c-section due to maternal pre-eclampsia. Family history shows five healthy children, two neonatal deaths, several early and late miscarriages. Birth weight was 1255 g (-1/-2 SD) with Apgars 5/9/10. A brain ultrasound at one month showed generalized atrophy. At nine months old she presented with feeding problems, failure to thrive and developmental delay. At two years growth was insufficient (height/ weight/head circumference -2SD), she was irritable and showed little to no language development. Metabolic screening showed a compensated metabolic acidosis and hyperammonaemia of 113 μmol/L (normal <50 μmol/L), elevated levels of urine malonic acid (1447mmol/mol creatin, reference <3mmol/mol creatin), methylmalonic acid (12mmol/mol creatin, reference <5mmol/mol creatin), malonylcarnitine (0.36 μmol/L,

reference 0.02–0.08 μmol/L), and propionylcarnitine (1.02 μmol/L, ref 0.14–0.94 μmol/L) in plasma.
Brain MRI showed generalized atrophy, major white matter loss, thickened cortex, and nodular heterotopia. (Fig. 1) Cardiological evaluation was normal. With a low fat/high protein diet and carnitine supplements she gained some weight. Follow up acylcarnitine analysis showed invariably elevated malonylcarnitine (0.49 μmol/L). At the age of 4.5 years she walks only with support and shows no language development. There is mild spasticity. Developmental level is estimated at one year. Length is at -2SD, length/weight at -1SD, OFC at -2.5SD and she consistently refuses food.

Laboratory investigation
A complete MCD deficiency was measured in cultured skin fibroblasts (0.96 nmol/mg/h, reference 10–31 nmol/mg/h). Genomic sequencing of the five MLYCD exons and flanking areas revealed no mutation in the patient and both parents. RT-PCR amplification of cDNA, followed by examination on 1% agarose gel even after cycling 40x, showed no product in the patient, whereas the control displayed the expected bands. Control genes showed a normal amplification product (Fig. 2). This abnormality has not been reported and suggests a mutation in a transcription regulatory site.

Biochemical and molecular methods
MCD enzyme activity was assayed in cultured skin fibroblasts according to a modification of the method of Scholte.[7]

MLYCD analysis
Genomic DNA was isolated from peripheral blood using standard protocols. The primers were designed to amplify the five exons including at least 50 bases of flanking genomic sequences based on the reference sequence of *MLYCD* as deposited in GenBank (Accession No. for the mRNA NM_012213 and for the *MLYCD* gene Entrez GeneID 23417). Genomic DNA of the patient and both the parents was isolated from leucocytes, using the Puregene DNA isolation kit from Gentra.
All five exons were multiplied by PCR and sequenced including at least 50 bp up- and downstream intronic sequence. Exon 1 and 5, because of their size were amplified in two overlapping pieces.

Genomic primers

Exon 1.1 forward	GCGGGGCAGTAACCTTTAG
Exon 1.1 reverse	CCACCGTAGAAGCTCACGA
Exon 1.2 forward	CTACGAGCTGCGCGAGAA
Exon 1.2 reverse	AGGGGGCAAGTGAGGACTAC
Exon 2 forward	TGTGCTGACCACAACACAGA
Exon 2 reverse	GAATGGAGATAGATTAGCTTTTAGCC
Exon 3 forward	CGAATAGTATGAATAGGAGTCAGCA
Exon 3 reverse	AAACACAAGGGGCTCTATGG

Exon 4 forward	CTTCTCGTCCCAGCAACAG
Exon 4 reverse	CCCACGAGGTTCGCTGAC
Exon 5.1 forward	CCTCTGTTGGTAACGTACCTG
Exon 5.1 reverse	TCTGCAGGTGGAAGTTGG
Exon 5.2 forward	GCTGCAGACTCCGCTGAT
Exon 5.2 reverse	GCTTGGAAAGCTGCTTCAGA

RT-PCR

Total RNA was isolated from the patients and a control fibroblast cell line using the RNAeasy Mini Kit (Qiagen). First strand cDNA synthesis was performed with the SuperScript system for RT-PCR (Invitrogen) with random hexamer primers. The quality of the cDNA was tested by RT-PCR of two unrelated genes, *ARX* (Aristaless like OMIM 300382), and glycerol kinase (*GK1* OMIM 300474), which both multiplied as expected, when analysed on a 1% agarose gel (see Figure 2). RT-PCR of the malonyl-CoA decarboxylase cDNA was performed using the following overlapping primers:

RT I forward	AGTAACCTTTAGCCACACTTGG
RT I reverse	CCACCGTAGAAGCTCACGA
RT II forward	CTACGAGCTGCGCGAGAA
RT II reverse	AGGGGGCAAGTGAGGACTAC
RT III forward	AATGAATGGGGTGCTGAAAG
RT III reverse	AAGCCATTTGGTGAAACCAG
RT IV forward	TCGTCAAGGAGTTGCAGAGA
RT IV reverse	TTTGAAACTGGGCCACTAGG
RT V forward	AGACCCTCAAGCTCCTCCTC
RT V reverse	AACTTTGGCTTTGCTTGGAA

Table 1

Summary of neurological symptoms and neuroimaging findings of all malonyl CoA decarboxylase patients in the literature

Reference	S	Age at presentation	Metabol. acidosis	PMR	Epileptic seizures	Head circumference	Brain imaging
Brown et al. [16]	M	5 years	+	Mild	-	Normal	ND
Haan et al. [17]	M	10 months	+	+	+ (during crisis)	Normal	CT: normal
Matalon et al. [18]	M	7 months	+	+	+	ND	ND
Macphee et al. [19]	M	9 years	ND	+	+	ND	ND
Macphee et al. [19]	F	1 years	+	+	+	ND	ND
Krawinkel et al. [20]	M	4 days	+	+	-	ND	Brain MRI: enlarged extracerebral spaces suggesting atrophy
Yano et al. [21]	M	4 years	+	+	+	Borderline microcephaly	ND
Buyukgebiz et al. [22]	F	died 8 days old	+	N/a	N/a	ND	Not performed
Ozand et al. [23]	M	9 months	+	+	+	P50, decreased to <p5	MRI: frontotemp atrophy, abnormal intensity putamen, caudate, periventricular white matter.
Ozand et al. [23]	M	12 days	-	+	-	P50	CT: hypodense myelin
Ozand et al. [23]	M	died 5 months	+	+	ND	ND	ND
Gao et al. [5]	M	died 10 months	ND	ND	-	ND	Not performed
Wightman et al. [6] San1	F	6 months	+	-	+	ND	ND
Wightman et al. [6] San2	F	7 months	-	-	-	ND	ND
Wightman et al. [6] San3	M	12 years	ND	+	+	ND	CT: leftsided cerebral infarction.
Wightman et al. [6] Rib1	F	21 months	-	+	-	+2.5SD	MRI: altered white matter signals frontal lobes
Wightman et al. [6] Dou1	M	6 day	+	-	-	Normal	ND
Our patient	F	2 years	+	+	-	-2SD	MRI: white matter loss, pachygyria, nodular heterotopia

References in brackets. ND, not described.

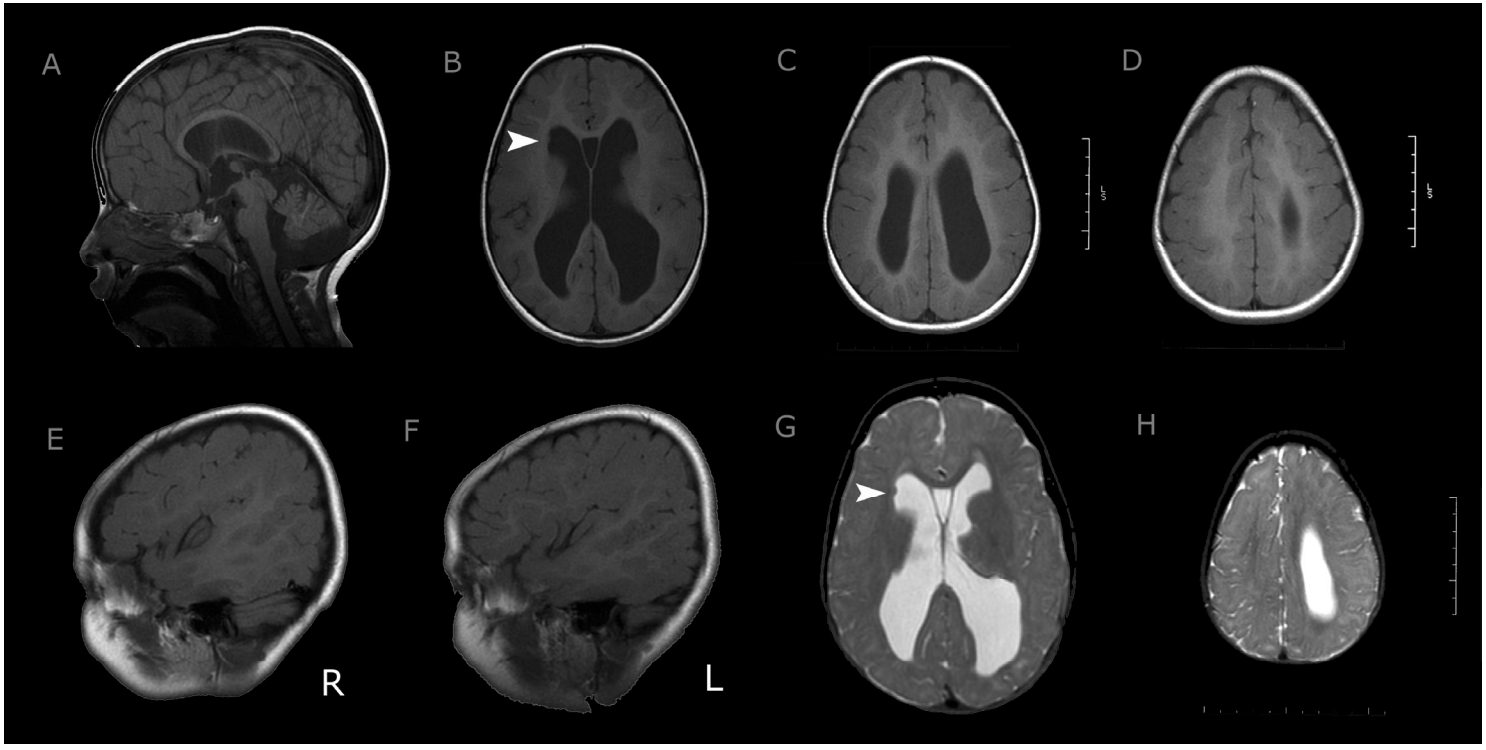

Figure 1: Brain MRI of the patient at the age of three years: (A) T1 midsaggital image showing thin corpus callosum, reduced volume of the cerebellar vermis and brain stem. (B) T1 image showing dilated ventricles, diffuse white matter loss, and diffuse pachygyria. The arrow denotes a heterotopic nodule in the frontal horn of the right lateral ventricle. (C) and (D) More rostrally T1 images showing diffuse pachygyria (E) and (F) right and left T1 sagital images showing the sylvian fissure. (G) T2 image at the level of (B) with arrow denoting heterotopia, (H) rostral T2 image.

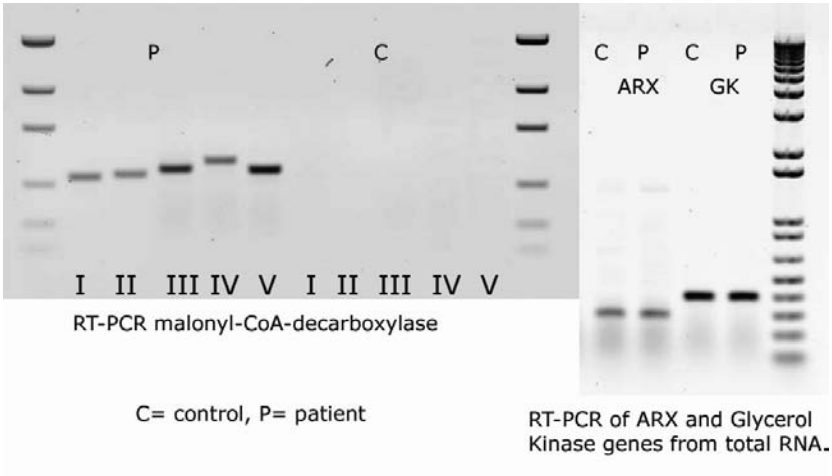

Figure 2: Left panel: RT-PCR products of malonyl-CoA decarboxylase cDNA in the patient and a control. The sequence has been divided in five different products (I–V). None of the products is amplified in the patient, suggesting a mutation in a transcription regulatory site. Right panel: RT-PCR of *ARX* (lanes 1 and 2) and glycerol kinase (*GK*, lanes 3 and 4) genes from total RNA, showing normal amplification products in the patient. C, control; P, patient.

Discussion

Except for the cortical malformation and lack of cardiac complaints, our patient phenotype is similar to the 17 previously described patients. While 12 of 17 patients are developmentally delayed and 8 of 17 had epileptic seizures, brain imaging is only reported in six cases (Table 1). In three cases this is a brain CT, insufficient to rule out neuronal migration disorders. In two patients the imaging showed atrophy and in three altered white matter signals (once combined), suggesting an influence of MCD deficiency on brain development. In our patient, brain atrophy and periventricular white matter loss are striking. These cannot be explained by metabolic crises, as our patient did not have periods of hypoglycaemia or severe acidosis. We found pachygyria and periventricular nodular heterotopia consistent with a neuronal migration disorder caused by an early gestational insult. In both pachygyria and periventricular heterotopia there is a premature arrest of neuronal migration. During intrauterine development neurons are required to migrate along radial glial cells and detach when they reach the target layer of the cortex. [8] Mutations in many genes cause periventricular heterotopia, lissencephaly, and subcortical band heterotopia. Not all cases of pachygyria/lissencephaly and nodular heterotopia are explained by mutations in these genes.[8] Inborn errors of metabolism rarely cause migration disorders. Zellweger syndrome and other peroxisomal biogenesis disorders can present with gyral abnormalities, heterotopia, polymicrogyria-like abnormalities, pachygyria, and cerebellar hypoplasia. Dysregulation of the glutamatergic pathway in Zellweger mice has been suggested to play a role.[9] If the MCD-protein also has a role in peroxisomal function, disturbance of this function could contribute to the observed brain development abnormalities.

Deficiency of the inner mitochondrial membrane enzyme CPT2 has been associated with cortical defects such as heterotopia and polymicrogyria.[10] CPT2 has a close interaction with CPT1 and could therefore be influenced by malonyl-CoA levels. The brain-specific CPT1 subunit (CPT1C) has high affinity for malonyl-CoA.[2] Disturbed interaction between malonyl-CoA and CPT1C in our patient may have contributed to abnormal brain development. In mouse-brain high levels of MCD are co-expressed in regions with high levels of lipogenic enzymes such as the hypothalamus and cortex. It suggests an important but so far unrecognized role in lipid synthesis in brain. Membrane function is known the be important in neuronal migration and also other lipid synthesis disturbances could contribute to migration disorders.[11,12]

One more striking symptom in our patient is her refusal to eat. Many children with inborn errors of metabolism suffer from failure to thrive, but her food refusal was consistent and unusual. It is well established that malonyl-CoA influences appetite control, apart from being an important factor in the balance of glucose–fatty acid metabolism. Recent studies in animals have demonstrated that increasing hypothalamic malonyl-CoA reduces appetite and feeding behavior, while starvation reduces hypothalamic malonyl-CoA level.[13,14] Hypothalamic malonyl-CoA levels thus function as a final common signal to regulate food intake.[15] It is possible that the feeding problems in our patient are influenced by high hypothalamic malonyl-CoA.

In light of the incomplete knowledge of the influence of metabolic pathways on brain development patients with MCD deficiency should be evaluated with brain MRI. Unexplained malformations of cortical development should be reason for metabolic screening by urine organic acid and plasma acylcarnitine profiling in addition to measurement of plasma very long chain fatty acids which is routine.

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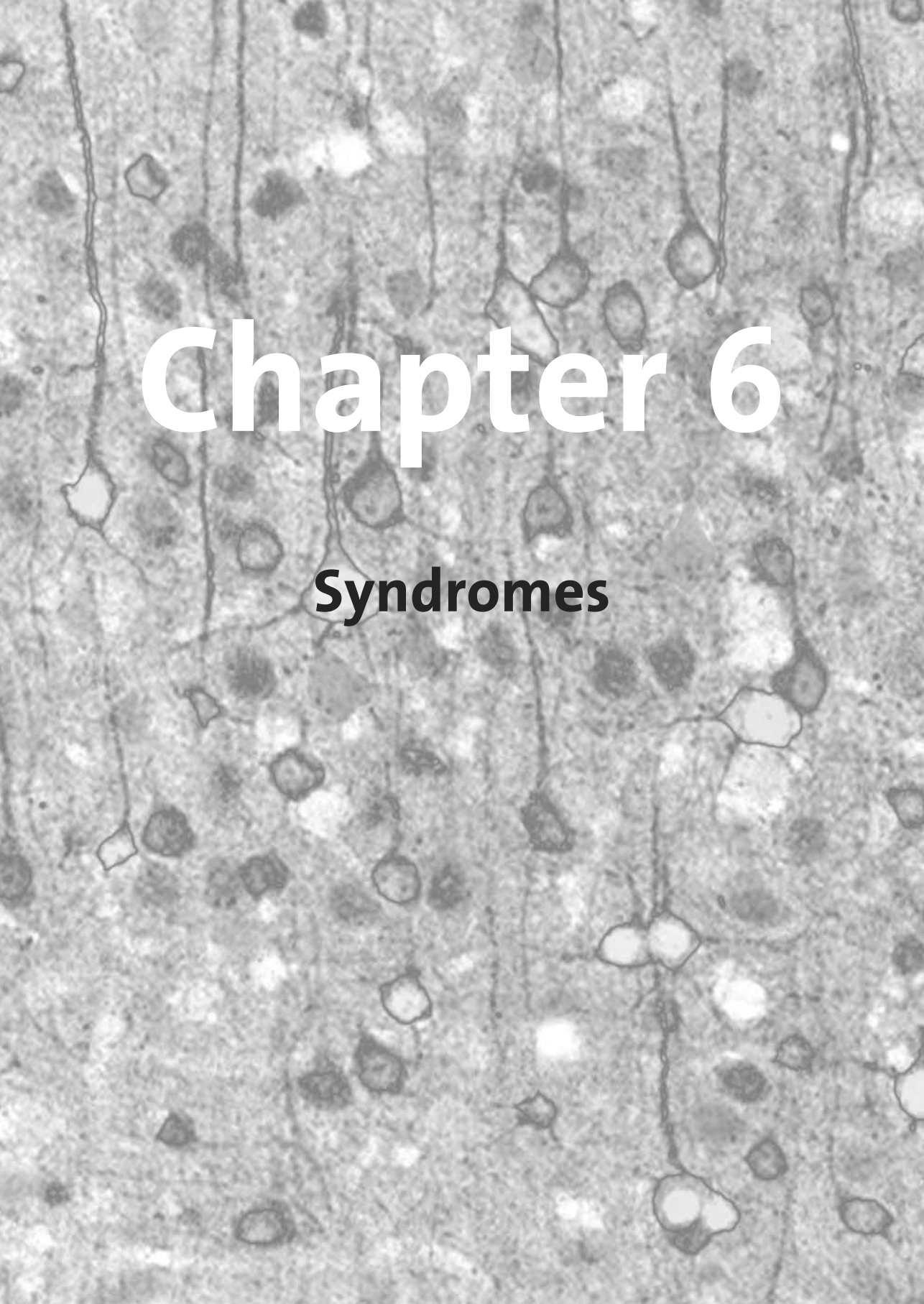

Chapter 6

Syndromes

6.1

Periventricular nodular heterotopia and distal limb deficiency: a recurrent association

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ABSTRACT

Malformations of cerebral cortical development, in particular periventricular nodular heterotopia (PNH), and distal transverse limb deficiency have been reported as associated congenital anomalies. Patients with PNH and transverse limb deficiency can be classified as having amniotic band sequence or Adams-Oliver syndrome (AOS). Controversy exists whether these should be considered separate entities. In some AOS patients, autosomal recessive inheritance has been shown, but in most patients causes are unknown, and both environmental and genetic factors have been implicated. We present three patients with PNH and distal transverse limb deficiency to support the hypothesis that these should be considered part of one group of disorders, and highlight the variable severity of the clinical and neuroradiological phenotype. Chromosome abnormalities were excluded by copy number analysis on 250K SNP microarray data. Research done on limb deficiency as on PNH caused by mutations in known genes, suggests the involvement of vascular developmental pathways. The combination of limb deficiency and PNH may have a common causative mechanism. Recognition and grouping of patients with this combination of abnormalities will help elucidating the cause.

PERIVENTRICULAR NODULAR HETEROTOPIA AND DISTAL LIMB DEFICIENCY

A recurrent association

INTRODUCTION

Malformations of cerebral cortical development (MCD) such as periventricular nodular heterotopia (PNH), or polymicrogyria (PMG) can occur as an isolated malformation or in combination with a diverse range of congenital abnormalities.[1] One reported association is with limb anomalies. A combination of limb deficiency and PNH has also been described in the amniotic band sequence (OMIM 217100) and Adams–Oliver syndrome (AOS, OMIM 100300). Both diagnoses have even been suggested in the same patient, highlighting the overlapping findings.[2,3] Amniotic band sequence refers to congenital disruption with distal limb deficiencies and constriction rings, associated with other congenital anomalies.[4] It remains controversial whether these are caused by external constriction by amniotic bands or by genetic factors that cause both the congenital anomalies and the formation of amniotic bands. AOS is a variable syndrome of scalp defects and distal limb deficiencies. The severe variant with central nervous system involvement is thought to be autosomal recessive.[3,5,6] Other patients with PNH and limb deficiencies are not classified as AOS or amniotic bandsequence.[7] In this report we focus on malformations of cortical development, particularly PNH, associated with distal transverse limb deficiency and suggest these may have a shared pathogenesis. To support this, we present three patients with PNH and distal limb deficiency.

PATIENTS

Patient 1

This boy was born to non-consanguineous parents at 32 weeks of gestation after an uneventful pregnancy. At birth defects of the limbs were noted and he was diagnosed with amniotic band sequence. The left hand is normal, but with a ring constriction at the wrist. Radiographs are normal. The right hand has a normal thumb. There is cutaneous syndactyly, partial between 2 and 3, 4 and 5, and complete between 3 and 4, and a lack of finger creases. There are nails present on digit 2, the fused digits 3–4 and digit 5. Radiographs show a normal thumb and normal metacarpals. Digits 2–5 lack phalanges. Digits 2 and 4 have fused phalanges, digits 3–5 hypoplastic proximal phalanges. The left foot shows short toes 1–3 without nails, with partial cutaneous syndactyly of toes 1 and 2, and complete syndactyly of toes 2 and 3. Toes 4 and 5 appear normal. Radiographs show absence of the distal phalanx of digits 1, 2, and 3, hypoplasia of the middle phalanges of digits 2 and 3, and fusion of the middle and distal phalanges of digits 4 and 5. The right foot also shows short toes 1–3 without nails, and partial cutaneous syndactyly between toes 2 and 3. Radiographs show hypoplasia of the distal phalanx of digits 1 and 3, and absence in digit 2. There is fusion of the middle and distal phalanges of digits 4 and 5 (Figs. 1A and 4A). The patient developed intractable epilepsy at age 19. Brain MRI showed bilateral symmetric occipital PNH (Fig. 2A). Psychomotor development was normal, and he finished high school. There are no facial anomalies. Occipitofrontal circumference is

normal. He has amblyopia of the left eye with divergent strabismus. Neurological findings are normal.

Patient 2

This girl was born at term by caesarean section, which was prompted by fetal distress. She was dysmature weighing 1,900 g, which was attributed to maternal cannabis, oxazepam, and tramadol use and cigarette smoking. These substances are not reported to cause MCD or limb defects. She has distal deficiencies of three limbs (Fig. 4B). On the left hand digits 2, 3, and 4 are absent. The left thumb is normal, and the fifth finger is short. Radiographs show a normal thumb, lack of the middle or distal phalanx of the fifth finger, short metacarpals 2, 3, and 4 and absence of all phalanges of digits 2, 3, and 4 (Fig. 1B). On the right hand there is a normal thumb and short fifth finger. Digit 2 is hypoplastic with a normal nail. Digits 3 and 4 are rudimentary with ring constrictions, and have no nail. Radiographs show normal metacarpals and thumb. The fifth finger shows no (ossification of the) middle phalanx, and is otherwise normal. Digit 2 shows a delta-shaped proximal phalanx, rudimentary ossification of the middle phalanx, and a hypoplastic distal phalanx. Digit 3 shows rudimentary ossification of the proximal phalanx and absence of other bones. Digit 4 shows a hypoplastic proximal phalanx. The left foot is short with a bud for digits 1 and 5 and dimples at the location of the other digits. Radiographs show absence of metatarsal bones and toes. On the right, there is a clubfoot with cutaneous syndactyly between 2 and 3, otherwise normal toes and nails (Fig. 4B). Radiographs are normal (Fig. 1B). Minor facial anomalies include bilateral epicanthic folds and telecanthus, upward slant of the palpebral fissures, small bitemporal diameter, and prominent upper lip (Fig. 3A). Brain MRI shows a large PNH at the left anterior ventricle (Fig. 2B). She had not developed epilepsy up to the present age of 2 years. Repeated EEGs were normal. Psychomotor development is mildly delayed, and she recently acquired walking. OFC grows at -0.5 SD. On neurological exam she was found to have bilateral abducens nerve palsy and symmetric facial palsy, compatible with Möbius syndrome (OMIM 157900). Muscle tone and strength are normal. Tendon reflexes are symmetrically normal and plantar responses are flexor.

Patient 3

This boy was born at term by caesarean, prompted by fetal distress, to non-consanguineous parents. He had hydrocephalus with OFC at +3.5 SD and received a ventriculo-peritoneal shunt, 1 day after birth. His OFC growth curve dropped to -1 SD over the course of 5 years. At 7 months he developed infantile spasms. MRI showed bilateral PNH with abnormal overlying cortex, consistent with PMG and partial agenesis of the corpus callosum (Fig. 2C). He has limb deficiencies of the left foot with a short first and fourth toes, short toes 2 and 3 with constrictions, all without nails, and normal fifth (Fig. 1C). Radiographs show hypoplasia of the distal phalanx of digit 1, hypoplasia of the proximal phalanx of digits 2–4 and absent middle and distal phalanges of digits 2–5. The right foot shows cutaneous syndactyly between digits 2 and 3, and normal radiographs (Fig. 4C). The hands are normal. Other findings included a supernumerary nipple, bilateral

ptosis, short palpebral fissures, and bilateral epicanthal folds (Fig. 3B). He is included in our cohort study of children with MCD.[1] He is now 8 years old and has severe psychomotor retardation with intractable epilepsy, spastic tetraplegia, and scoliosis.

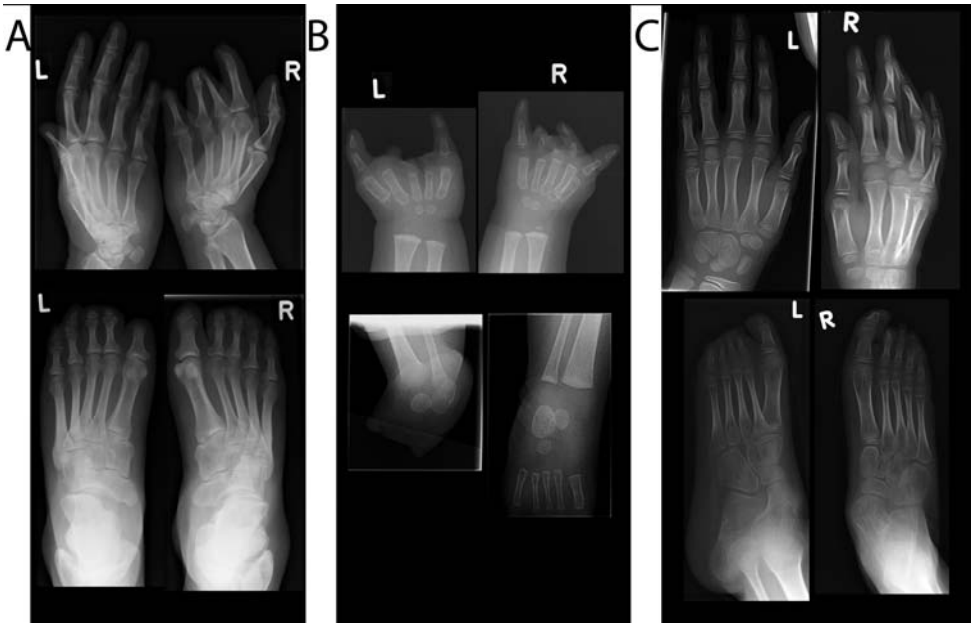

Figure 1: Radiographs of hands and feet. A: Patient 1 at 39 years. Upper panel: normal left hand and transverse limb deficiency of the right hand. Lower panel: feet with deficiencies on both sides. B: Patient 2 at 3.5 months. Upper panel: deficiencies of both hands. Lower panel: absence of metatarsal bones and toes of the left foot, and a normal right foot. C: Patient 3 at 9 years. Upper panel: normal hands. Lower panel: deficiencies of the left foot and a normal right foot.

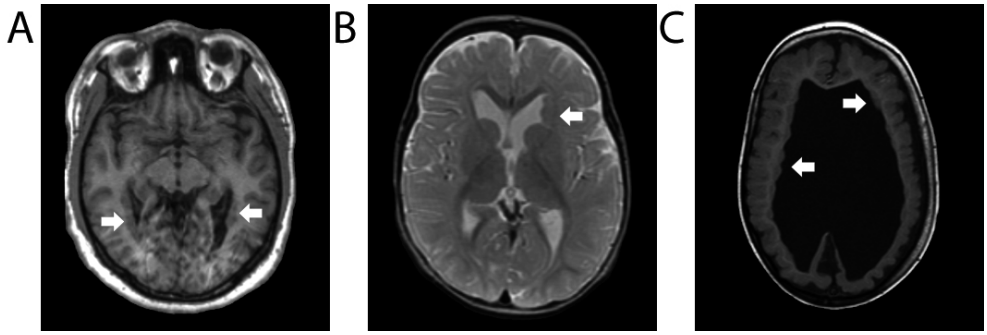

Figure 2: Brain MRI of all patients. A: Patient 1 at age 39 years, T1 weighted image. Note bilateral PNH in the occipital horns of the lateral ventricles (arrows). B: Patient 2 at age 11 months, T2 weighted image. Note a large PNH in the frontal horn of the left lateral ventricle (arrow). Ventricles are mildly enlarged for age. C: Patient 3 at age 3 years. T1 weighted image. Note bilateral PNH (arrows), hydrocephalus and diffuse polymicrogyria.

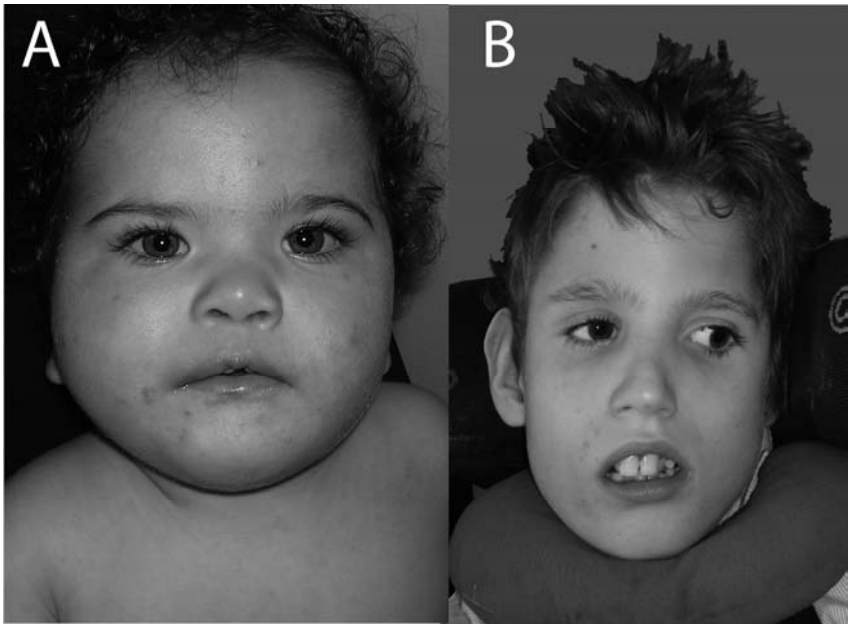

Figure 3: Facial appearance. A: Patient 2 at age 10 months. Note bilateral epicanthic folds and telecanthus, mild upward slant of palpebral fissures, narrow bitemporal diameter, and prominent upper lip. B: Patient 3 at age 9 years. Note bilateral ptosis, short palpebral fissures, bilateral epicanthal folds, and prominent upper central incisors.

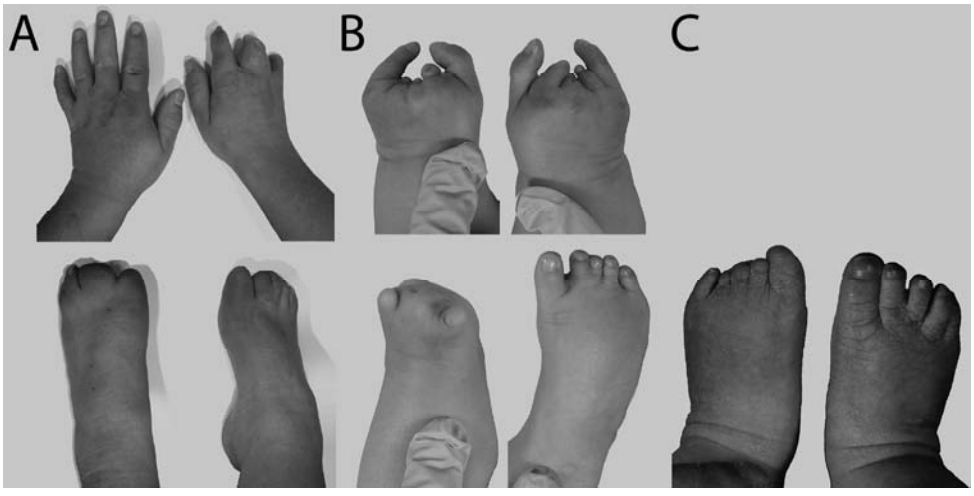

Figure 4: Limbs. A: Patient 1 at age 39 years. Upper panel: normal left hand and cutaneous syndactyly on the right hand. Lower panel: feet show absence of nails on digits 1–3, and cutaneous syndactyly. B: Patient 2 at age 3 months. Upper panel: absence of digits 2, 3, and 4, and short fifth finger on the left hand. On the right hand digit 2 is hypoplastic, digits 3 and 4 are rudimentary with ring constrictions, and the fifth finger is short. Lower panel: short left foot with a bud for digits 1 and 5 and absence of the other digits. On the right, a clubfoot with cutaneous syndactyly between digits 2 and 3. C: Patient 3 at age 6 months. Upper panel: hands are normal and were not photographed. Lower panel: left foot with short toes 1–4, all without nails. The right foot shows cutaneous syndactyly between digits 2 and 3.

Genetic analysis

Standard G banding karyotyping showed apparently normal chromosomes in all patients. *FLNA* sequence analysis was done in Patients 1 and 2 with normal results. This was not done in Patient 3 as the MRI phenotype did not fit the *FLNA*-pattern. DNA-analysis of all patients on Affymetrix 250K NspI SNP arrays, by the CNAG copy number method, did not show any pathogenic chromosome rearrangement. Copy neutral LOH analysis showed several homozygous areas on different chromosomes only in Patient 3, suggesting parental consanguinity.

DISCUSSION

The combination of periventricular nodular heterotopia (PNH) or other cortical malformations and distal transverse limb deficiency is a rare but consistent finding (Table I). PNH and distal limb deficiencies have been reported in patients classified as severe AOS, amniotic band sequence, or with other congenital anomalies. Several case reports describe patients with PNH, limb deficiency, and microphthalmia, cleft palate or midface hypoplasia.[2,8] The phenotype of our patients overlaps with the severe variant of AOS, although our patients do not have scalp or skin defects.[3,5,6] The extent of the digital defects in our patients is highly variable, as also seen in AOS patients. A combination of PNH with scalp defect without limb deficiency has also been described.[9] In a recent overview of PNH-phenotypes, four patients (one male) with limb deficiency were included.[7] Limb deficiency has been described with other cortical malformations, but the combination with PNH seems more commonly found (Table I).

Vascular disruption has been implicated in limb deficiencies, supported by the association with facial clefts, maternal bleeding, twin pregnancies, and other birth defects.[10-12] The pathogenesis of AOS is also thought to be related to vascular problems, such as vascular disruption, abnormal development of small vessels, or abnormal pericyte recruitment to blood vessels.[5,13,14] AOS is associated with other presumably vascular determined congenital defects, such as Poland sequence (OMIM 173800).[15] Möbius syndrome, as in Patient 2, can occur with limb defects such as oligodactyly, brachydactyly, syndactyly, polydactyly, or Poland sequence and again abnormal vascular development is implicated.[16,17] AOS is also associated with cardiac developmental anomalies and cutis marmorata.[18]

PNH is a neuronal migration disorder resulting from an inability of early neurons to migrate from the subventricular zone to the cortex. Mutations in the genes coding for filamin A (*FLNA*, OMIM 30017), and for BIG2 (*ARFGEF2*, OMIM 605371) have been found in humans with PNH. The most frequent genetic cause of PNH without limb deficiencies, found in approximately 30% of patients, are mutations in the X-linked *FLNA* gene.[7] In the absence of normal filamin A function, impaired migration of later neurons is due to disrupted cell adhesion and abnormal ventricular ependymal function.[19] *FLNA* mutations also cause cardiovascular developmental defects and connective tissue abnormalities, and autopsy studies additionally show abnormal glomeruloid microvascular proliferations in the brain in patients with *FLNA* related PNH.[19,20] PNH is not likely to be directly caused by vascular disruption, but the association with cardiac and vascular anomalies in *FLNA*

patients shows that pathways involved in cell adhesion can affect both the neuro-epithelium and vascular development. Other proteins that regulate axon guidance during brain development, such as Semaphorins, Netrins, Slits, and Ephrins, are also involved in vascular patterning and endothelial cell migration, processes which are essential for limb bud formation.[21,22] Pathways that affect neuronal migration can also affect vascular development, which may increase the chance of prenatal vascular disruption.

Vascular factors have also been implicated in other MCD, mostly polymicrogyria (PMG). Gestational insults, such as cytomegalovirus infection or ischemia, can cause PMG.[1,23] Embryonic vascular factors have been implicated in perisylvian PMG with or without limb anomalies in 22q11 microdeletion syndrome.[24] Congenital constriction bands with limb deficiencies or AOS are associated with bilateral perisylvian PMG, septo-optic dysplasia and periventricular leukomalacia.[5,25-27]

In some patients limb deficiencies seem to have resulted from external constriction by amniotic bands; however the recurrent association with cerebral malformation suggests a common, possibly genetic cause in other patients. Our observation suggests that patients with PNH and limb deficiencies manifest abnormalities with a common pathogenesis.

Table I: Comparison of published cases of patients with a combination of distal limb deficiency and cerebral anomalies

Authors	Sex	PNH	Brain imaging	Scalp/ skull defect	Other reported findings
Örstsavik et al., 1995 (sibs) [28]	F	-	CT: Periventricular calcification and atrophy	+	Vitreoretinal anomaly
	M	-	Autopsy: partial agenesis corpus callosum, PVL	+	Vitreoretinal anomaly
Fryns et al., 1996 [29]	M	-	CT: porencephalic cyst L hemisphere	+	-
Savarirayan et al., 1999 (sibs) [13]	M	-	CT: unilateral PMG	+	-
	F	-	ND	+	Pulmonary valve stenosis
Chitayat et al., 1992 [30]	M	-	CT angiography : abnormal brain vasculature.	+	Bicuspid aortic valve
Romani et al., 1998 [31]	M	-	CT : periventricular calcification, wide ventricles	+	-
Mempel et al. 1999 [32]	M	-	MRI : bilateral PMG	+	Cutis marmorata, cardiac defect
Amor et al., 2000 (sibs) [5]	M	-	CT: unilateral PMG	+	Cutaneous telangiectasia
	F		MRI: bilateral PMG	-	Prominent veins, lymphoedema
Unay et al., 2001 [33]	F	-	CT: periventricular calcification	+	-
Piazza et al., 2004 [34]	M	-	MRI: periventricular calcification	+	Cutis marmorata, pulmonary hypertension
Patel et al., 2004 (unrelated) [14]	M	-	MRI: periventricular calcification with infarction, hypoplastic veins	+	Cutis marmorata, cardiac defect
	M	-	CT: periventricular calcification	+	Cutis marmorata, cardiac defect. Twin pregnancy.
Parrini et al., 2006 (unrelated) [7]	M	+	MRI: diffuse bilateral PNH*	-	-
	F	+	MRI: diffuse bilateral PNH	-	-
	F	+	MRI: diffuse bilateral PNH*	-	-
	F	+	MRI: diffuse bilateral PNH*	-	-
Musumeci et al., 2006 (unrelated) [35]	F	+	MRI: bilateral PNH and agenesis corpus callosum	-	Arthrogryposis, café au lait spots
	F	+	MRI: diffuse bilateral PNH	-	-
McGoey and Lacassie, 2008 (sibs) [6]	F	-	MRI: partial agenesis corpus callosum, periventricular calcification *	-	Cutis marmorata prominent veins
	F	-	CT: periventricular calcification *	-	Hemangioma face and occiput
Prothero et al., 2007 [36]	M	-	MRI: periventricular calcification, wide ventricles	+	Falciform retinal folds
Brancati et al., 2008 [3]	F	+	MRI: diffuse bilateral PNH	-	Prominent veins on trunk
Papadopoulou et al., 2008 [27]	M	-	MRI: PVL and calcifications	+	-
Balasubramanian and Collins, 2009 (sibs) [37]	M	-	MRI: periventricular calcification*	-	Cardiac defect
	F	-	MRI: PVL *	+	-
Current report (unrelated)	M	+	MRI: occipital bilateral PNH	-	-
	F	+	MRI: unilateral PNH	-	Möbius syndrome
	M	+	MRI: bilateral PNH and PMG	-	-

PNH: periventricular nodular heterotopia; PMG: polymicrogyria; PVL: periventricular leukomalacia.
* MRI imaging not published, only description available

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6.2

Unbalanced der(5)t(5;20) translocation associated with megalencephaly, perisylvian polymicrogyria, polydactyly and hydrocephalus

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ABSTRACT

The combination of megalencephaly, perisylvian polymicrogyria, polydactyly and hydrocephalus (MPPH) is a rare syndrome of unknown cause. We observed two first cousins affected by an MPPH-like phenotype with a submicroscopic chromosome 5q35 deletion as a result of an unbalanced der(5)t(5;20)(q35.2;q13.3) translocation, including the *NSD1* Sotos syndrome locus. We describe the phenotype and the deletion breakpoints of the two MPPH-like patients and compare these with five unrelated MPPH and Sotos patients harboring a 5q35 microdeletion. Mapping of the breakpoints in the two cousins was performed by MLPA, FISH, high density SNP-arrays and Q-PCR for the 5q35 deletion and 20q13 duplication. The 5q35 deletion area of the two cousins almost completely overlaps with earlier described patients with an atypical Sotos microdeletion, except for the *DRD1* gene. The five unrelated MPPH patients neither showed submicroscopic chromosomal aberrations nor *DRD1* mutations. We reviewed the brain MRI of ten Sotos patients and did not detect polymicrogyria in any of them. In our two cousins, the MPPH-like phenotype is probably caused by the contribution of genes on both chromosome 5q35 and 20q13. Some patients with MPPH may harbor a submicroscopic chromosomal aberration and therefore high-resolution array analysis should be part of the diagnostic workup.

INTRODUCTION

Polymicrogyria (PMG) is generally considered to be a post-migratory disorder of cortical organization mostly associated with motor and sensory defects and often with epilepsy and psychomotor retardation. Prenatal insults like intoxications, infections and hypoperfusion can cause PMG.[1] The recent discovery of *KIAA1279* mutations in Goldberg-Shprintzen syndrome with diffuse PMG and other familial PMG syndromes indicate that genetic factors play an important role.[2-6] New chromosomal loci have been associated with PMG.[7,8] One of the PMG syndromes of unknown etiology is the recently described megalencephaly with perisylvian polymicrogyria, polydactyly and hydrocephalus (MPPH).[9-12] Megalencephaly is thought to be a proliferation defect of the cerebral cortex. Although it has been associated with overgrowth syndromes like Sotos and Cutis marmorata telangiectasia congenita (M-CM) syndrome, hamartomatoses like Pallister-Hall, Bannayan-Zonana syndrome and metabolic disorders, no gene mutations are known causing exclusively megalencephaly in humans.[13,14] Overlap between MPPH and M-CM has recently been suggested.[15]

We describe a family with two first cousins presenting with megalencephaly and polymicrogyria resembling MPPH syndrome. They both carry a derivative chromosome 5q as a result of the unbalanced segregations of a translocation t(5;20)(q35.2;q13.3) resulting in a chromosome 5q35.2-qter deletion encompassing the NSD1 locus for Sotos syndrome, and a partial trisomy of chromosome 20q13.3-qter. We have fine-mapped the deletion breakpoints and compare these with the phenotype and breakpoints of Sotos microdeletion patients. We also genotyped five other MPPH patients using high resolution SNP array analysis and sequenced the DRD1 gene as a possible candidate gene for MPPH.

PATIENT DESCRIPTION

Patient 1 and 2 are first cousins, both from healthy Dutch parents (Fig. 1A). Their mothers are sisters. **Patient 1** (III-1 in Fig 1A) is a girl examined at the age of 2 years for a severe psychomotor retardation, axial hypotonia, spasticity and epilepsy. The pregnancy was complicated by polyhydramnion, intrauterine growth retardation and pre-eclampsia. The delivery followed in the 37th week with a birth weight of 2180 grams (-2 SD) and OFC of 33.0 cm (0 SD). The neonatal period was complicated by *E.Coli* sepsis, cholestasis and liver dysfunction. She also presented with a small ASD type II and hypothalamic hypothyroidism with low TSH values. In time she developed kyphoscoliosis, pectus carinatum and signs of rickets, which did not improve with vitamin D supplementation. She experienced several urinary infections caused by grade IV vesicoureteral reflux. Psychomotor development was delayed, but at the age of 1.5 years a status epilepticus caused regression. Examination at the age of 2 years showed macrocephaly (OFC at +3 SD, height at 0 SD), high broad forehead, large fontanel, hypertelorism with epicanthic folds, short upturned nose with hypoplastic nostrils, down turned corners of the mouth with thick vermilion of the lips, high arched palate, small pointed chin with a vertical groove, large low-set ears, barrel shaped chest with kyphoscoliosis, postaxial polydactyly of the 5th right toe. She had severe head lag, was unable to sit unsupported, showed a spastic tetraparesis, made eye contact and had no speech development. Brain MRI revealed

megalencephaly, asymmetric mild dilatation of the lateral ventricles (right > left) and right perisylvian polymicrogyria (Figure 2A). Family history showed two miscarriages, one concerning an anencephalic embryo.

Patient 2 (III-2 in Fig. 1A and B) was born to the sister of Patient 1's mother after a pregnancy complicated by polyhydramnion and fetal hydrops with hydrothorax. The delivery in the 35th week was complicated by perinatal asphyxia, shearing of umbilical cord, haemorrhage and fetal shock. She was macrosomic and macrocephalic at birth (weight 3160 grams, +2.5 SD; OFC 38 cm, +3.5 SD). The neonatal period was complicated by multiorgan failure, persistent hydrothorax, cholestasis, rickets, hypothalamic hypothyroidism with low TSH and FT4, vesicoureteral reflux causing urosepsis and a non-symptomatic cardiac ASD type II. She developed severe intractable epilepsy and tetraparesis and progressive hydrocephalus requiring a ventriculo-peritoneal shunt at the age of 1 year. At that age, physical exam showed length at 0 SD, no psychomotor development, prominent forehead, deep-set eyes, short nose with hypoplastic nares, down turned mouth corners, pointed chin. Abdominal ultrasound showed hepatomegaly with foci suspected for hepatoma. She died of progressive respiratory and heart failure during pneumonia at the age of 1.5 years. Brain CT at the age of 1 year showed macrocephaly with generalized hydrocephalus, cavum septum pellucidum and abnormal gyral pattern in the perisylvian regions most consistent with PMG. (Figure 2B). Family history revealed one miscarriage.

Patient 3 is a Dutch full term born girl with a birth weight of 2170 gram (- 2.5 SD), who was diagnosed with a large ASD, a perimembranous VSD and patent ductus arteriosus. OFC at the age of 1 week was 32.5 cm (-2.5 SD). Generalized hypotonia and developmental delay persisted after heart surgery, which justified neurological investigation. Brain MRI at the age of 5 months, 1 and 3 year showed prominent frontal lobes, deep sulci, delayed myelination of periventricular white matter, apparently normal cortical development (Fig. 2C). Her development is severely delayed. Hypercalcemia with nephrocalcinosis and severe hypotonia persist at the age of 2 years, with height and OFC at 0 SD. Facial features and congenital anomalies suggested the diagnosis of Sotos syndrome.

Patients 4, 5 and 6 have been clinically described by Mirzaa et al. (corresponding to LP95-025, LR02-064 and LR04-181) as affected by MPPH. [9]

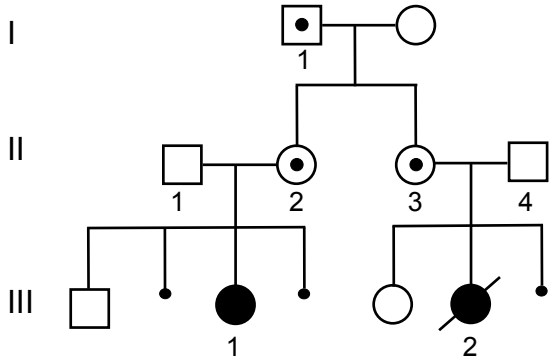
Patient 7 has been diagnosed by L.G. and W.B.D. as having features of MPPH. Clinical details are reported separately.[11]

Patient 8 is a full term girl born with macrocephaly (OFC 41 cm) who showed squint and no visual awareness at the age of 3 months. Head circumference grows along the +4 SD. A brain MRI at the age of 3 months showed megalencephaly with mild ventricle dilatation, delayed myelination, periventricular white matter loss, cavum septum pellucidum, frontoparietal and perisylvian polymicrogyria (Fig 2D). She did not present with polydactyly. The clinical and MRI findings are compatible with MPPH.

Clinical and radiology data of Sotos and MPPH patients are summarized in table 1.

Figure 1:

A



A: Pedigree of family with patient 1 (III-1) and 2 (III-2). (A) Pedigree, with two affected first cousins, patient 1 and 2 (filled symbols), carrying an unbalanced 46,XXder(5)t(5;20)(q35.3;q13.3) translocation. Black dots indicate carriers of the balanced t(5;20)(q35.2;q13.3) translocation.

B

B: Patient 2 at the age of 3 months [Color figure can be viewed in the online issue of the Am J Med Genet, available at www.interscience.wiley.com].

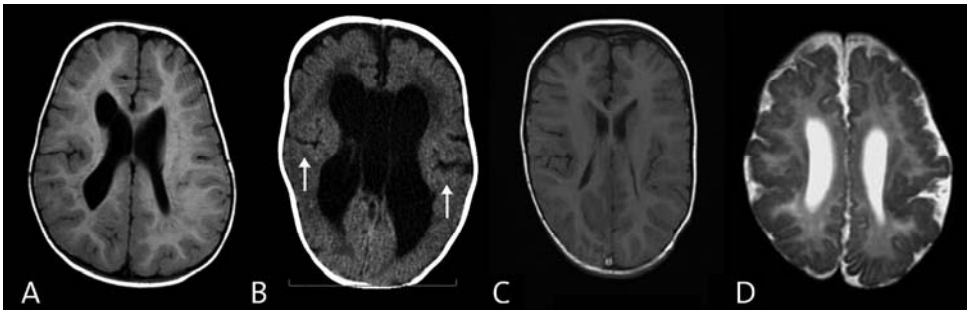


Figure 2: Brain imaging. (A) T1 weighted axial brain MRI of patient 1 at the age of 2 years showing asymmetry of lateral ventricles with right-sided porencephalic cystic dilatation and perisylvian polymicrogyria (arrows). (B) Brain CT scan of patient 2 at the age of 1.5 years showing hydrocephalus and bilateral wide sylvian fissures and cortical anomaly compatible with perisylvian polymicrogyria. (C) T1 weighted axial brain MRI of patient 3 at the age of 3 years, showing prominent frontal lobes and no polymicrogyria. (D) T2 weighted axial brain MRI of patient 8 at the age of 3 months, showing enlarged lateral ventricles and bilateral cortical polymicrogyria most prominent in perisylvian areas.

METHODS

Karyotyping and MLPA

Standard G banding karyotyping was performed in all patients. A subtelomeric MLPA to detect chromosomal imbalances using DNA from patient 1 and 2 was performed using the P036 and P070 kits (MRC-Holland, Amsterdam, The Netherlands).

FISH analysis

Fish analysis was performed in patients III-1, III-2, and their relatives and pt 3. The BAC clones used were all selected from the University of California Santa Cruz UCSC browser. The clones were purchased from BACPAC Resources. DNA was semi-automatically isolated with a AutoGenPrep 3000 robot (Autogen, Holliston, MA), after a whole genome amplification (WGA) (Repli-g kit Qiagen Benelux BV, The Netherlands), the DNA was digested and labelled with Bio-16-dUTP or dig -11-dUTP by a Random Prime labelling system (Invitrogen, Carlsbad, CA). Before use the probes were validated on metaphase cells from a healthy person to confirm their cytogenetic location. The FISH experiments were performed according to standard protocols with minor modifications. FISH slides were analysed with an Axioplan 2 Imaging microscope (Zeiss, Sliedrecht, The Netherlands), images were captured using the fluorescent software Isis (MetaSystems, Altlussheim, Germany). Names and location of the BAC clones from 5pter: RP1-24H17; 5p13.2: RP11-253B9; 5qter: RP11- 1240G13; NSD specific 5q35.3 probes: RP1-118M12 and RP11-47L17; 20pter: RP5-1061L1; 20qter: RP1-81F12.

SNP-array analysis

Whole genome analysis was performed by microarray analysis using Affymetrix GeneChip 50 and 250K arrays. The assays were carried out according to the standard protocol. Briefly, 250 ng of total genomic DNA was digested with either *Hind*III (50K) or *Nsp*I (250K). Appropriate adaptors were ligated to the four base-pair overhang of the DNA fragments. A single primer was used for amplification and PCR products were purified. After fragmentation with DNAse I, products were labeled with biotin and hybridized to the array. The arrays were washed and stained with streptavidin-phycoerythrin in the Affymetrix fluidics station 450 and scanned in the GeneChip Scanner 3000 G7. The images of the scans were collected and intensities measured in the Affymetrix GeneChip operating Software (GCOS). Genotype data analysis was performed in Affymetrix GeneChip Genotyping analysis software (GTYPE) using the Dynamic Model algorithm (DM) for 50K analysis and the BRLMM algorithm for 250K analysis.

Copy Number analysis

Copy number analysis was performed on the allele intensities of each SNP by using the software package: Copy Number Analyzer for Affymetrix GeneChip (CNAG version 3.0, Nannya et al, Cancer Res. 2005 Jul 15;65(14):6071-9). For real-time PCR, primers and sequence analysis see supporting information Supplementary Methods which may be found in the online issue of the Am J Med Genet, available at www.interscience.wiley.com.

Table 1: Clinical and MRI findings of MPPH and Sotos syndrome patients.

Patient with Sotos or MPPH	Laboratory diagnosis	Brain MRI (age)	Clinical presentation	Reference
Patient 1 MPPH	del	Megalencephaly, wide ventricles, unilateral perisylvian polymicrogyria	Macrocephaly, PMR, epilepsy, spastic tetraparesis, postaxial polydactyly right foot, VUR, hypothyroidism, rickets, ASD II	This report
Patient 2 MPPH	del	Megalencephaly, hydrocephalus, abnormal perisylvian gyral pattern on CT, cavum septum pellucidum	Macrocephaly, macrosomia, profound PMR, epilepsy, spastic tetraparesis, no polydactyly, hypothyroidism, hepatoma, cholestasis, VUR, ASD II	This report
Patient 4 MPPH	-	Asymmetric megalencephaly, bilateral perisylvian PMG, short CC, hydrocephalus	Macrocephaly, profound MR, seizures, spastic quadriplegia; no polydactyly	LP95-025 in ref. 9
Patient 5 MPPH	-	Megalencephaly, bilateral perisylvian PMG, hydrocephalus, enlarged LV	Macrocephaly, profound MR; postaxial polydactyly 4 limbs	LR02-064 in ref. 9
Patient 6 MPPH	-	Symmetric megalencephaly, perisylvian PMG, hydrocephalus	Macrocephaly, profound MR, seizures, hypotonia, polydactyly 4 limbs	LR01-181 in ref. 9
Patient 7 MPPH	-	Symmetric megalencephaly, perisylvian PMG, hydrocephalus	Macrocephaly, large fontanel, hypertelorism, depressed nasal bridge; postaxial polydactyly 3 limbs	Ref. 11
Patient 8 MPPH	-	megalencephaly, bilateral frontoparietal PMG, delayed myelination	Macrocephaly at birth, squint, no visual awareness at 3 months	This report
Sotos 1 (patient 3 in text)	del	Dolichocephalic skull, prominent frontal lobes (8 m)	Severe PMR, hypotonia	This report
Sotos 2	mut	Prominent perivascular spaces, cerebellar arachnoidal cyst (11y)	Motor delay	This report
Sotos 3	mut	Cavum septum pellucidum, ventriculomegaly (5 y)	PMR, epilepsy from 2 yr	This report
Sotos 4	mut	Cavum septum pellucidum, prominent perivascular spaces (3 y)	Mild PMR	This report
Sotos 5	mut	Ventriculomegaly, cerebellar arachnoidal cyst (3 y)	Behavioral problem, mild LD	This report
Sotos 6	mut	Ventriculomegaly (5 m)	PMR and hypotonia at age 5 m	This report
Sotos 7	mut	Ventriculomegaly, thin corpus callosum, diffuse atrophy, prominent frontal lobes (1.5 y)	PMR, intrauterine asphyxia, neonatal hypoglycaemia	This report
Sotos 8	mut	Ventriculomegaly, thick corpus callosum (7 months)	VSD, West syndrome, moderate PMR	This report
Sotos 9	del	Ventriculomegaly, diffuse atrophy (1 y)	Moderate PMR	Patient 8 in ref.23
Sotos 10	mut	Ventriculomegaly, diffuse atrophy, gliosis of WM (8 y)	Mild PMR, mild hemiplegia	This report

PMR= psychomotor retardation, LD= learning disability, WM= white matter, del = *NSD1* microdeletion by FISH, mut= intragenic mutation *NSD1*, y=year

RESULTS

Cytogenetic, MLPA and FISH analysis

Standard G banding chromosome investigation (550 bands) was normal in all patients. MLPA analysis with subtelomere probe kits in patients III-1 and III-2 and their mothers II-2 and II-3, respectively, revealed a loss of signal at the subtelomeric region of chromosome 5q35 and a gain of signal for chromosome 20q13 only for the patients III-1 and III-2. FISH analysis was performed with chromosome 5qter and 5pter BAC probes, probes specific for the NSD1 locus on chromosome 5q35.3 and chromosome 20 probes. Results confirmed an unbalanced translocation in both patients III-1 and III-2 resulting in the karyotype 46,XX,der(5)t(5;20)(q35.2;q13.3). Further FISH analysis with probes on chromosomes 5q and 20 q revealed a balanced t(5;20)(q35.2;q13.3) translocation in both mothers II-1 and II-3 of patient III-1 and III-2, respectively, and the grandfather I-1. Targeted FISH analysis of the sporadic patient 3, performed in the suspicion of Sotos syndrome, showed a microdeletion with the *NSD1*-specific probe RP11-47L17.

SNP array analysis

To define the deletion breakpoints in patient 1 and to investigate the genome of patients 3-8 for chromosomal abnormalities, DNA of these patients was hybridized on either 100K or 250K SNP arrays (Affymetrix). Patient 1 showed a 5.7 Mb terminal deletion on chromosome 5q35.2-qter. The proximal deletion breakpoint on chromosome 5q35.2 is rs11953281, as indicated by copy number analysis (CNAG).[16] The breakpoint, confirmed by Q-PCR analysis is between the genes *LOC645398* (present) and *DRD1* (absent). In addition, an amplification of 8 Mb was seen for chromosome 20q13.3-qter. Copy number analysis indicated rs4811719 as the proximal SNP bordering the amplification, present in 2 copies, and rs6014787 as the first SNP present in 3 copies. The breakpoint was confirmed by Q-PCR between *LOC100131175* (2 copies) and the *TFAP2C* gene.

Comparison with other 5q35.3 microdeletion patients

Many of the symptoms of patients 1 and 2 resembled Sotos syndrome, however overgrowth was not evident in patient 1 and the phenotype was much more severe. Therefore we also studied in detail the genotype of patient 3 with a similarly severe phenotype and lack of overgrowth. Japanese Sotos microdeletion patients have been described with broader and more severe phenotypic abnormalities than Caucasian microdeletions.[17,18,19] SNP array analysis for patient 3 showed an interstitial 5q35.3 deletion spanning about 2 Mb, between rs9313730 and rs11745917, involving about 60 genes. This deletion is within the common microdeletion range of Caucasian Sotos patients.[20,21] The most proximally deleted gene in patient 3 is *THOC3* and the most distally (telomeric) located is *LOC653314*. We have aligned the deletions in our patients 1-3 with the chromosome 5q deletions spanning the *NSD1* locus fine mapped by FISH, as reported by Tatton-Brown et al. and deletions mapped by QMPSF by Saugier-Veber et al.[20,21] All the described patients were diagnosed with Sotos syndrome, including patient COG025 Tatton-Brown et al., whose deletion extends until 5qter.[20] Two other patients, COG231 and COG342, have the largest reported deletions extending on the proximal site and overlap

with our patients 1 and 2 (Fig. 4B).[20] However, the most proximal probe (SOT21) used for the COG231 and COG342 patients gives uninformative results, possibly excluding *FLJ16171* and *DRD1* from the deleted area. Patients 14 and 15 from Saugier-Veber et al. also have unusually large deletions.[21] Here the breakpoint is somewhere between the *FLJ16171* and *SFXN1* genes, also giving inconclusive results for *DRD1*. Clinical details in both studies from Tatton-Brown et al. and Saugier-Veber et al. did not include MRI findings of the patients but the general conclusion was that, except for a more common deep learning disability and lack of overgrowth, the microdeletion patients presented with the same features of the patients with *NSD1* mutation.[20,21] In the five unrelated MPPH patients 4-8, SNP array analysis did not reveal pathogenic rearrangements. DNA of patients 4,5,6 and 8 was analysed by 100K SNP array, of patient 7 by 250K SNP array. Assuming that the MPPH phenotype of our patients 1 and 2 could be (in part) related to a larger 5q35 deletion than previously described in Sotos patients we tested *DRD1* as a possible candidate and sequenced it in MPPH patients 4-8. No mutations were found.

Review of brain imaging in Sotos syndrome

Sotos syndrome has not been previously associated with polymicrogyria. After the observation of polymicrogyria in patients 1 and 2 we reviewed brain MRIs of 10 Sotos patients with intragenic *NSD1* mutations and/or microdeletions detected by FISH, diagnosed in our and other academic centers, including patients with a more severe phenotype like our patient 3 and the patient 8 described by Sogaard et al.[23] Almost constant MRI findings were megalencephaly with prominent frontal lobes, wide lateral ventricles, cavum septum pellucidum and wide Virchow-Robin perivascular spaces. In none typical polymicrogyria was observed (Table 1). We therefore conclude that PMG does not belong to the phenotype of Sotos syndrome.

DISCUSSION

We describe two first cousins, with a submicroscopic deletion of a 5.7 Mb region on chromosome 5q35.2-qter, including the *NSD1* locus and an 8 Mb 20q13.3-qter amplification, as the result of a derivative chromosome 5 of a familial balanced translocation t(5;20)(q35.2;q13.3). Development and head circumference of the relatives bearing the balanced translocation are normal. The features of our patients overlap with the recently described MPPH syndrome, including the polydactyly in patient 1 and perisylvian polymicrogyria on brain MRI of both patients.[9] No patients with chromosomal rearrangements at 5q and 20q have been described with these features.

Macrocephaly with megalencephaly and mild enlargement of the cerebral ventricles is typical of Sotos syndrome. Patients with large telomeric deletions of chromosome 5q35 including *NSD1* seem, with some exception, to be severely affected, but no brain MRI has been reported.[24-27] We reviewed the brain MRI of our patient 3, the patient 8 of Sogaard et al. and eight other molecularly confirmed Sotos patients and consistently observed megalencephaly, mostly of frontal lobes, with apparently normal cortex, enlarged ventricles but no signs of high pressure hydrocephalus (Table 1).[23] Therefore we conclude that PMG and high-pressure hydrocephalus are not part of the Sotos phenotype and the

MPPH-like features of patients 1 and 2 are probably not caused by *NSD1* haploinsufficiency. Rare deletions distal to and excluding the *NSD1* locus have been associated with a relatively mild clinical phenotype, but systematic analysis of the neuroimaging of the patients has not been reported.[24,28]

The deletion breakpoint of the patients 1 and 2 on chromosome 5 was mapped between the gene of unknown function *FLJ16171* (present) and *DRD1* (deleted) and it is to our knowledge the largest microdeletion in the 5q35.2qter area. In- or exclusion of *DRD1* in areas of other Sotos microdeletion patients was not clear from literature data; therefore we tested *DRD1* as a candidate for MPPH, but found no mutation in 5 unrelated MPPH patients. Nagai et al. reported severe phenotypic abnormalities in Japanese patients with microdeletions at the *NSD1* locus, including cardiac, urinary and endocrine problems compared to patients with intragenic *NSD1* mutations.[17] This genotype-phenotype correlation, although less evident, was also confirmed in Caucasian Sotos patients.[22] Low copy repeats (LCRs) have been considered responsible for non-allelic homologous recombination (NHAR) as a mechanism causing the microdeletion, supporting the definition of Sotos syndrome as a genomic disorder.[29] In the 5q35 area however, different mechanisms seem to be responsible for NAHR. LCRs (SoSPREP and SoSDREP) at 5q35.3 define the recurrent 1.9 Mb deletion in Sotos patients.[30] However, atypical shorter non-recurrent deletions have been described, and in some of these Alu-mediated NAHR is suggested.[18,19,20] In addition in the 5q35.1 area the contribution of highly homologous LINE elements seems to be responsible for induction of NAHR.[31]

In unbalanced translocations, the general assumption is that developmental defects are mostly the result of genetic haploinsufficiency. In a large series of over 5000 patients with developmental defects much fewer pathogenic microduplications than microdeletions have been detected.[32] In our patients 1 and 2, however, the 20q13.3-qter amplification could also contribute to the phenotypic abnormalities.

The amplification breakpoint on chromosome 20q13.3 of patient 1 and 2 was mapped between *LOC388799* (2 copies) and the known gene *TFAP2C* (3 copies). Previously reported chromosome 20q amplifications are much larger than in our patients, being 20q11.2-qter and 20q13.1-qter.[32,33,34] Most of these are not pure trisomies, but are combined with complex rearrangements.[35] Most common features in these patients are brachycephaly, bulging forehead, deep-set eyes, short nose, large ears, dimpled chin, heart defects, hydrocephalus, cortical atrophy, cerebellar atrophy and phenotypes vary from mildly to severely affected.[33,34,36,37] Some of these features are easily recognizable in our patients 1 and 2. A submicroscopic 20q13.1-qter duplication, extending 1Mb proximally of our patients' duplication, was recently reported in a patient with mental retardation, without clinical detail.[38]

The chromosome 20q13 area contains some genes subjected to genomic imprinting.[39] The best known is *GNAS1*, involved in the pathogenesis of Albright osteodystrophy.[40] It is not clear whether the extra maternal chromosome 20q13 material in our patients contributes to some of their phenotypic manifestations through imbalances in the imprinted *GNAS* cluster.[41] Paternally inherited deletions at the 20q13.3 locus including *GNAS* have been associated with major phenotypic abnormalities, suggesting that, besides the *GNAS* cluster,

also other genes like i.e. *TF2PC* might influence imbalances between maternal and paternal alleles.[42]

MPPH syndrome has been described so far in sporadic patients, therefore it is considered to result from de novo dominant mutations. We show MPPH-like features in two patients with a 5q35.3 deletion and 20q13.3 trisomy, but we excluded submicroscopic rearrangements in 5 typical MPPH patients. Our findings support the hypothesis that MPPH is a heterogeneous disorder, with more mechanisms involved in its pathogenesis, some of which are apparently linked to the rearrangements of genes at terminal regions of chromosome 5q and 20q.[15] We therefore advise high-resolution microarray analysis in the workup of patients with MPPH.

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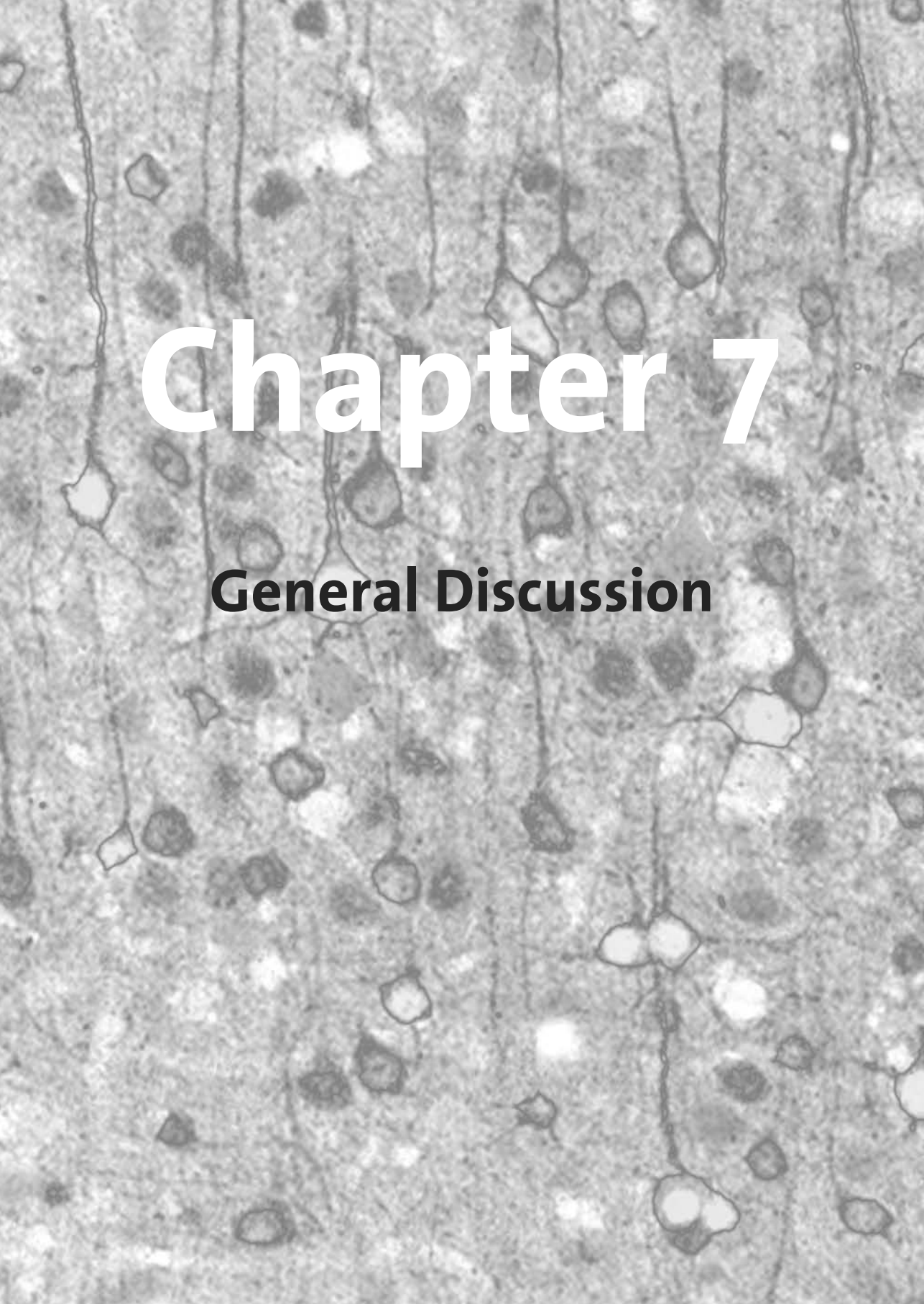

Chapter 7

General Discussion

GENERAL DISCUSSION

Malformations of cortical development (MCD) are a group of disorders characterized by a congenital abnormal structure of the cerebral cortex. These MCD include different structural abnormalities, and they have a diverse array of causes, both genetic and environmental. Although different subgroups of MCD are rare or very rare, MCDs as a group are responsible for significant morbidity in psychomotor developmental delay and epilepsy. For example, up to 20% of children with intractable epilepsy are found to have an MCD. Improved neuroimaging and genetic laboratory techniques have revealed new information on patterns and causes of MCD. Unfortunately, the underlying cause often still remains elusive and specific treatments are not available.

The primary aim of this thesis was to study patients with malformations of cortical development to determine if a multidisciplinary approach including clinical and imaging classification of the malformation combined with available diagnostic laboratory techniques, would lead to an increase of etiological diagnoses, improvements in description of the phenotypes, and recognition of 'new' syndromes. We expect that a multidisciplinary approach will improve the diagnostic process in these patients, and can also improve care and treatment. Here I will also make recommendations regarding the best care for these patients based on our findings regarding neuroimaging, clinical observation and genetic testing.

Neuroimaging

The diagnosis of MCD begins with imaging of the brain with MRI. A clinically useful classification should therefore be based on neuroimaging characteristics and lead to recommendations for additional diagnostic testing. Information on sex, dysmorphological information (e.g. microcephaly) or other clinical features that can be directly observed from the patient should also be incorporated in this system. The currently most used classification system for MCD is the Barkovich classification updated in 2005.[1] This classification is based on the proposed underlying disturbance of normal developmental processes, and uses a mix of morphological (e.g. lissencephaly) and etiological categories (e.g. specific gene mutations). This makes it difficult to classify and compare patients before all genetic tests are done, and we found that not all patients fit into defined categories (chapter 2).[2] Also, it results in changes in the classification system as new underlying mechanisms are found, but also when new phenotypes are found to be associated with known causes. For example the 22q11 microdeletion is classified as a subgroup of bilateral perisylvian PMG, while we have since found that it can also be found in unilateral PMG.[3,4] Although the Barkovich classification is the best available, in a next revision morphological classification and genetic classification could be more clearly separated.

During our studies we learned that a detailed MRI classification should lead the choice for additional metabolic or genetic tests (chapter 2).[2,3] Many genetic and metabolic tests are labour-intensive and time-consuming, but also very expensive. As basing tests on faulty information obviously does not lead to the desired result, MRI reports should be as up-to-date as possible. In reevaluation of older MRIs by our multidisciplinary team (neuroradiologist, pediatric neurologist and clinical geneticist) we found the original radiological diagnosis regularly to be incorrect (chapter 2).[2] Even MRIs reported in the literature should be evaluated critically. [3]

Using MRI scanners with a higher resolution (Tesla values) and more advanced software settings (e.g. scans based on the diffusivity of water molecules) differences in MCD can be visualized more accurately. For example, it can be difficult to distinguish polymicrogyria and cobblestone malformation on MRI and more detailed imaging may improve understanding of these malformations.[5] Possibly, in the future molecular neuroimaging with targeted contrast agents can further improve the accuracy of neuro-imaging, be it with MRI, PET scan or otherwise.[6,7,8]
Besides creating more detailed MRI images, recognition of MCD may also be improved by computerized pattern recognition. Such computerized recognition is for example already widely used in ECG reading, but its application in brain imaging will be more challenging due to the facts that MRIs cannot easily be made in exactly the same position at different instances, that the appearance of the brain changes with age, and that different types of artifacts are often present. Obviously, to obtain good quality images the patient needs to lie still in the scanner. This is difficult in the case of children or patients with cognitive impairment or psychiatric disorders, making it necessary to use general anesthesia.
Different protocols can be chosen to make MRI images, each aimed at visualizing specific pathologies or specific parts of the brain. If a MCD is not expected beforehand, the image may not be ideal to classify the malformation or small abnormalities may be missed. In our cohort, classification was sometimes difficult due to suboptimal imaging and repeat MRIs are not always feasible considering the burden of the general anesthesia, costs and waiting lists (chapter 3.1). Also, the MRI aspect of the brain changes considerably during the first two years of life due to myelination making it difficult to compare scans of different patients or of the same patient made at different ages. We recommend repeating an MRI after the age of 18-24 months, if the diagnosis of a possible MCD is uncertain in an infant.

Clinical observations

To make a diagnosis of MCD, neuroimaging information needs to be combined with observations on neurological deficits, psychomotor development, epilepsy, dysmorphic clinical features, other congenital anomalies and growth pattern. Pattern recognition can lead to a syndrome diagnosis relatively quickly.[9] Description of clinical features is also important when the underlying cause of a specific subgroup of MCD has been discovered, to determine the extent and variation of the phenotype. Firstly, in the case of a genetic cause, this enables to determine genotype-phenotype relationships and what the in- and exclusion criteria for a genetic test should be. Secondly, this knowledge improves the

information we give patients and parents on prognosis and it can also improve genetic counseling as to what recurrence risk parents may expect for their next child. Many examples of the use of clinical observations can be found in this thesis. We reported that epilepsy or congenital microcephaly are not always present in *ARFGEF2* mutations, but that a combination of a movement disorder based on basal ganglia abnormalities combined with periventricular nodular heterotopia should also prompt DNA testing of this gene (chapter 4.1).[10] We showed that there is a wide variability in the phenotype of patients with *FLNA*-gene related periventricular nodular heterotopia and that epilepsy is not obligatory (chapter 3.2 and 3.3).[11] We reported that the brain phenotype of the rare malonyl-CoA decarboxylase deficiency can include pachygyria and periventricular nodular heterotopia (chapter 5).[12] We showed that the combination of microcephaly with simplified gyral pattern and early onset diabetes can be seen in mutations of *EIF2AK3*, but also that this phenotypic syndrome is genetically heterogeneous and that more genes must be involved to explain a similar phenotype seen in other patients (chapter 4.2).[13] Further research in these patients is ongoing.

If the underlying cause cannot be determined, groups of patients with similar features that probably share a common cause can be identified, and new syndromes can be delineated. Identifying new syndromes is the first step to enable further genetic research into its cause. We described, for example, a group of unrelated patients that shared a similar MCD combined with similar defect of their hands and feet (chapter 6.1).[14]

Genetic tests

In our studies we showed that we are able to make an etiological diagnosis in almost half of the patients, using current standard techniques, however in a minority this includes a molecularly established diagnosis, e.g. by a metabolic or genetic test.[2] A molecularly confirmed diagnosis enables physicians to give parents the best possible answer on questions regarding the cause of the problems of their child, his or her prognosis, and what the recurrence risk is in the family. We also noted that genetic causes seem to be more common than non-genetic causes and care should be taken not to attribute the MCD too easily to problems during the pregnancy. This may result in an incorrect estimate of the recurrence risk. For example in our cohort, the periventricular nodular heterotopia of a particular child were thought to be caused by maternal cocaine abuse during pregnancy, but further testing based on the specific MRI pattern revealed a pathogenic *FLNA* mutation (chapter 2).[2] On a higher level, molecularly confirmed diagnoses will help physicians and scientists to understand the underlying problems and search for the best possible treatments.

With the advance of genetic laboratory techniques, more genetic abnormalities will be detected. An important recent new option is whole genome analysis using the microarray technique that allows detection of small chromosomal deletions or duplications (copy number variations). This will improve the yield of diagnoses, also in our cohort (unpublished data). It also comes with new challenges, as it is not always apparent what the meaning of the found abnormalities is or what the relationship is to the signs and symptoms of the patient. [15]

In the near future new technologies will become available that enable testing of several genes in one patient in a relatively short time by targeted arrays and resequencing chips. This also includes techniques allowing sequencing of the whole genome in each patient. It will make genetic tests less time consuming and more cost-effective. MCD classification will not become obsolete however, not only with respect to choosing the appropriate gene chip, but mostly to interpreting the enormous bulk of genomic data from an individual patient. The interpretation of this data will require the integration of knowledge in different fields, ideally expanded into a systems biology approach.

For the time being, if a MCD patient has a phenotype not directly suggesting the involvement of a known gene, but a genetic cause is likely due to the family history or due to the presence of multiple congenital anomalies and/or dysmorphic features, the genetic cause can be difficult to find. Only very rarely a MCD is found in several patients over different generations in a family tree, making the traditional approach of linkage not feasible. Using homozygosity mapping from genomic microarray data (usually SNP-based arrays), can allow locus identification in smaller consanguineous families.[16] Information on submicroscopic copy number variations found by array techniques in different patients with similar phenotypes may be combined to find new loci. In a family with two first cousins with perisylvian polymicrogyria we found an unbalanced der(5)t(5;20)(q35.2;q13.3) translocation by array technique (chapter 6.2).[17] As both these girls show a phenotype similar to the MPPH-syndrome (megalencephaly, perisylvian polymicrogyria, polydactyly and hydrocephalus) our findings suggest that the underlying genetic cause for this syndrome may be found in the affected chromosome regions of our patients(chapter 6.2).[17] We were unable to confirm this, and such findings may remain unique for one family. Alternatively, this might indicate that the MPPH-syndrome is not an homogeneous disorder but rather an expression of different disease mechanisms.[18]
When a possible chromosomal locus is identified by any of the above described techniques, candidate genes can be more easily identified by using knowledge of the underlying defective mechanism or pathway in different MCD, combined with knowledge of the pathways involved and information on possibly involved genes available from animal studies.

Some important pathways in MCD are:

- *Cell proliferation*; in patients with microcephaly with simplified gyral pattern mutations have been detected in genes involved in mitosis and mitotic spindle integrity (see appendix). These patients have a relatively mild neurological phenotype with mental retardation. Patients with similar neuroimaging but severe mental retardation, epilepsy, and early death have been found to have mutation in genes involved in apoptosis or DNA repair (see appendix and chapter 4.2).
- *Microtubules, the cytoskeleton and the microtubule organizing center*; the most common genes found in lissencephaly, subcortical band heterotopia and nodular heterotopia: *LIS1, DCX, FLNA* and *TUBA1A,* encode proteins involved in the function of microtubules and the cytoskeleton.

- *Signaling molecules that direct migrating neurons*; *Reelin* acts as a signal to stop migrating neurons through binding to receptors on their surface. Mutations can result in lissencephaly with cerebellar and brainstem hypoplasia. A receptor for *Reelin* is encoded by *VLDRL*, and mutations in this gene result in a similar phenotype.
- *Glycosylation of alpha-dystroglycan*; the genes, *Fukutin, FKRP, POMT1, POMT2,* and *POMGnT1,* harbor mutations in most patients with cobblestone lissencephaly and encode proteins involved in glycosylation of transmembrane and extracellular matrix molecules (see appendix). Problems in the integrity of the basement membranes allow migrating neurons to pass beyond the pial membrane and settle within the meningeal space.
- *Transcription factors*; *TBR2* and *ARX* encode transcription factors and mutations can be found in some patients with polymicrogyria and lissencephaly respectively. *ARX* specifically regulate migration of inhibitory GABAergic neurons, but the target genes and mechanisms are still largely elusive.
- *Endosomal vesicle transport*; *ARFGEF2* and *RAB3GAP1* are associated with nodular heterotopia and polymicrogyria. They both encode proteins involved with the trans-Golgi network and vesicle trafficking to the plasma membrane.

Coordination of care and treatment of MCD patients

To be able to find the underlying cause of the MCD in a greater number of patients and to improve information on prognosis and care, it is necessary to carefully examine the patient from different angles. The patient group is heterogeneous and variable. For recognition and classification of the malformations, a multidisciplinary team offers the best setting with collaboration between an experienced neuroradiologist, pediatric neurologist, and clinical geneticist. As MCD subtypes are rare, follow-up of patients would ideally be coordinated in a few specialized centers or shared by a group of physicians specifically interested in this patient category. Personal and shared experience improves the quality of the observations. Recognition of rare phenotypes or syndromes depends on seeing a large number of patients, and both national and international collaboration is important to collect sufficient information to delineate new syndromes, find new genes and reliably describe genotype-phenotype relationships.

Although MCDs are a developmental malformation of the cerebral cortex, and therefore already present at birth, the signs and symptoms of the condition evolve during life. Information on the course of the disorder will help to make rational choices in treatment options regarding rehabilitation, epilepsy, cognitive development and behavioral problems. Prognosis with regard to psychomotor development is variable, and likely dependent on the MCD-subtype and extent of cortical development.[2] For example, by long term follow-up of children with type 1 lissencephaly we have improved the accuracy of the information we are able to give parents about the condition of their child and have shown that survival is correlated to the severity of the lissencephaly (chapter 3.1). Epilepsy is a common complication of MCD, and in some subgroups, e.g. cortical dysplasia, epilepsy

surgery can be a good treatment option. This makes an early and accurate diagnosis more valuable.

New treatments might be targeted against complications of the MCD (e.g. epilepsy) or against the underlying defect (pathway dysfunction in the cell). Of course, anatomical abnormalities of the cerebral cortex in MCD cannot be corrected, so therapy aimed at the underlying defect would only be potentially beneficial if the target pathway also has a function in postnatal brain function. More in general on treatment, new options can arise from observation of patients or through understanding of the underlying mechanism of disease. Observation of a large number of patients, experience with different treatment options and trial-and-error, enable a clinician to recognize what might be promising treatments. These should then be tested in a clinical trial to confirm the usefulness of such a treatment. We know from observation, for example, that in the treatment of seizures caused by Tuberous Sclerosis Complex some anti-epileptic drugs are more likely to be successful than others (vigabatrin in this case) and this was confirmed in clinical studies.[19] Something similar may well be the case for other cerebral malformations. Another example is treatment of patients with cerebral palsy with a specific form of rehabilitation therapy that includes constraint of the 'good arm', which shows promising effect in clinical trials and can even induce a change in cortical function on fMRI.[20,21]
Understanding of the underlying mechanism of disease is emerging from the laboratory, but in medicine, and pediatric neurology in particular, treatment advances are lagging behind the discovery of genes and pathways responsible for the hereditary diseases and congenital anomalies. Over the last years, insight in the underlying mechanism of particular diseases has resulted in improved treatment in some patients with inborn errors of metabolism (notably enzyme replacement therapy in, e.g., Pompe's disease). Other treatments based on influencing the underlying mechanism of disease are expected to come in the near future (e.g. influencing gene expression by exon skipping therapy in muscular dystrophies). An example in MCD is again found in Tuberous Sclerosis Complex, where treatment with drugs targeted against the underlying pathway dysfunction shows promising results.[22,23] Hopefully, this type of insight may also lead to therapies preventing the occurrence of intractable epilepsy (anti-epileptogenic therapy), and to treatments that may improve cognitive defects in specific syndromes. The basis of these advances lies in translational research, and bridging the gap between the basic sciences and the bedside will further benefit patients and their parents in the future.

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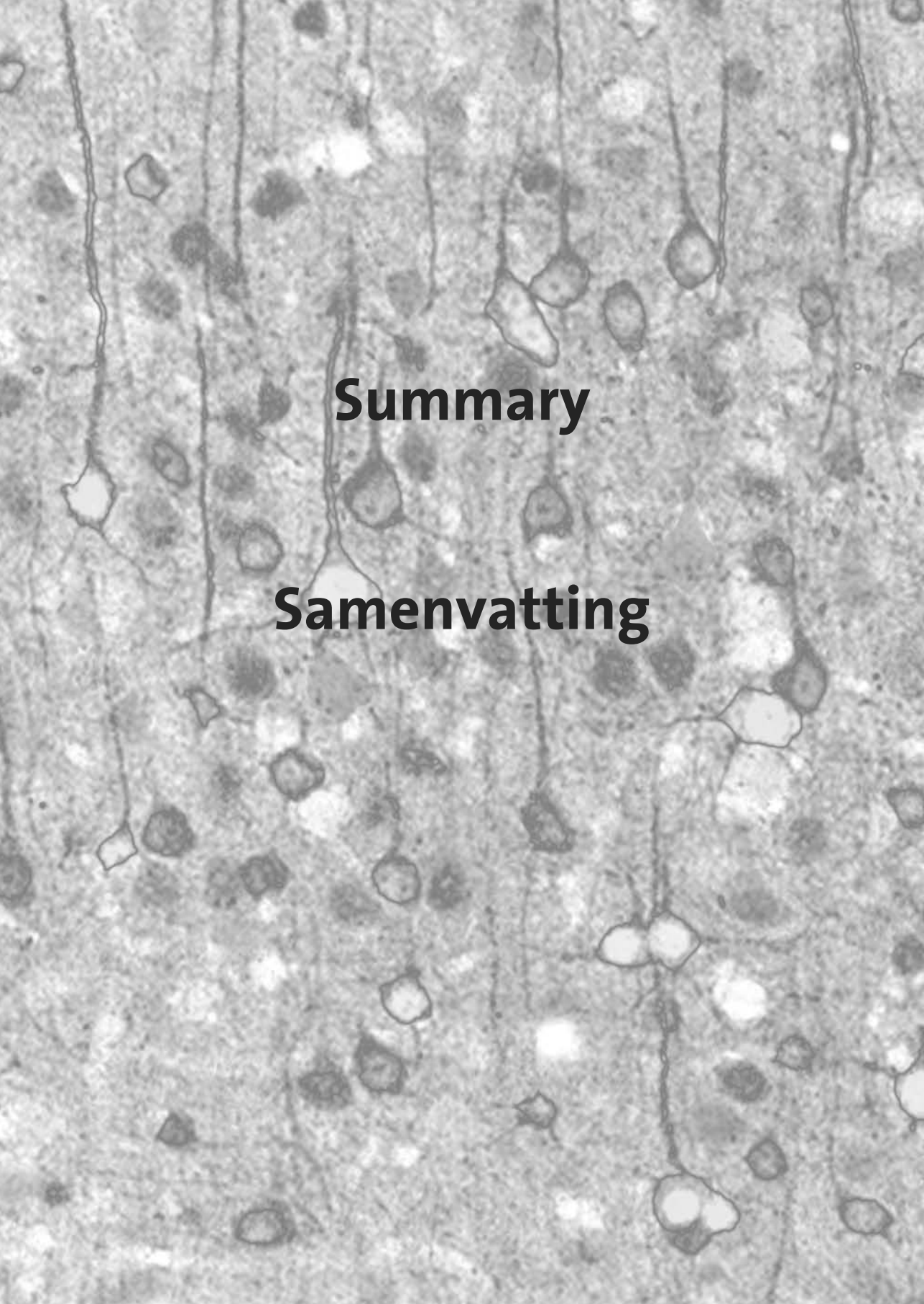

Summary

Samenvatting

SUMMARY

In **chapter 1**, the introduction, the current state of knowledge regarding clinical features, neuroimaging characteristics, pathology and causes of malformations of cortical development (MCD) is summarized.

Chapter 2 describes the consecutive cohort of 113 patients with MCD we investigated from 1992 to 2006. By combining clinical, radiological, and genetic classification; syndrome identification, and family studies with diagnostic molecular testing an etiological diagnosis could be made in 40% of the cases of MCD. This contributes to genetic counseling, to the possibility of prenatal diagnosis and improved patient treatment and disease management. We established an etiological diagnosis in 45 of 113 cases (40%). For 21 patients (19%), this included molecular and/or genetic confirmation (Miller-Dieker syndrome; *LIS1, DCX, FLNA, EIF2AK3* or *KIAA1279* mutations; or an inborn error of metabolism). In 17 patients (15%), a syndrome with an unknown genetic defect was diagnosed. In 7 patients (6%), we found evidence of a gestational insult. Of the remaining 68 patients, 34 probably have a yet-unknown genetic disorder based on the presence of multiple congenital anomalies (15 patients), a family history with multiple affected persons (12 patients), or consanguineous parents (7 patients).

The next chapters are based on specific observations of patients we investigated from the cohort and additional patients that were seen from 2006 to 2009.

Chapter 3 is focused on the natural history of patients with MCD with a known genetic cause. In **chapter 3.1** we describe the long term follow-up of a cohort of 24 patients with lissencephaly type 1 that were born the Netherland from 1972-1990. We reclassified the lissencephaly according to current views, and contacted these patients and their parents. We found that 19 of 24 patients were alive, which was longer than any of the parents had originally been told. Survival showed a significant relationship with the severity of the lissencephaly on neuroimaging. All patients showed severe mental retardation, intractable epilepsy and complete dependence on care. Molecular analysis of the *LIS1*-gene was not possible at the time of the original study and was now requested by 8 parents. This revealed a pathogenic mutation or large deletion of *LIS1* in 7 patients.
In **chapters 3.2 and 3.3** we focus on patients with *FLNA* related periventricular nodular heterotopia. First we describe a 71-year-old woman, who was known to have heart valve disease and bilateral periventricular nodular heterotopia when she died of a subarachnoid haemorrhage. Autopsy showed typical cerebral bilateral periventricular heterotopia and vascular abnormalities. Postmortally, the diagnosis of a *FLNA* mutation was confirmed. It is surprising that our patient never had seizures. It was already known that the phenotype associated with *FLNA* mutations include cardiac malformations, particularly aortic or mitral valve disease. We showed that other vascular abnormalities, including aneurysms, can be seen in these patients. We also confirmed the presences of glomeruloid microvascular anomalies in the brain, which had been described once before in a *FLNA*

patient. Recognition during life may have prevented cardiovascular problems and provided possibilities for genetic counseling. In the second part we further investigated the cardiac phenotype in our *FLNA* patients and found that these patients often present to a cardiologist first. A positive family history, skin of joint hyperlaxity or neurological problems may prompt earlier recognition.

Chapter 4 is concerned with new phenotypes associated with known genes. In **chapter 4.1** we report a girl with bilateral periventricular nodular heterotopia (BPNH) based on compound heterozygosity for two *ARFGEF2* mutations, the first reported patient since the discovery of the gene. This patient had a marked severe choreadystonic movement disorder, which was a new finding changing the limited knowledge about the phenotype. The brain MRI shows bilateral hyperintensity of the putamen, BPNH, and generalized atrophy. Loss of *ARFGEF2* function affects vesicle trafficking, proliferation/apoptosis, and neurotransmitter receptor function. This could explain BPNH and microcephaly.
We hypothesized that the movement disorder and the preferential damage to the basal ganglia, specifically to the putamen, was caused by an increased sensitivity to degeneration, a dynamic dysfunction due to neurotransmitter receptor mislocalization or a combination of both. In **chapter 4.2** we describe two families with a child with microcephaly with a simplified gyral pattern of the brain (SGP) and early onset insulin dependent diabetes mellitus (IDDM). The first patient was diagnosed with Wolcott–Rallison syndrome, and a homozygous mutation in the *EIF2AK3* gene was found. The patient from the second family had no mutation in this gene. SGP is considered to arise from decreased neuronal proliferation or increased apoptosis at an early stage of embryonic development, but insight into the pathways involved is minimal. *EIF2AK3* is one of the kinases involved in inhibition of protein translation under cell stress conditions. It has been proposed that loss of function mutations reduce the ability of the cell to respond to endoplasmic reticulum stress, resulting in apoptosis of pancreatic Langerhans cells. Our findings suggest that in some cases, early onset IDDM and SGP can arise from common mechanisms leading to increased apoptosis.

In **chapter 5** we report a girl with malonyl-CoA decarboxylase deficiency, a rare inborn error of metabolism, with diffuse pachygyria and periventricular heterotopia.

Chapter 6 is focused on syndromes that can be clinically delineated in the cohort of MCD patients. In **chapter 6.1** we describe the association between periventricular nodular heterotopia and transverse limb deficiency in three patients with a variable severity of the clinical and neuroradiological phenotype. These patients can be classified as having amniotic band sequence or Adams-Oliver syndrome (AOS), but there was no gene associated with this disorder. We hypothesized that this combination should be considered one syndrome, and suggested the involvement of vascular developmental pathways based on what is known about limb deficiency and PNH caused by mutations in known genes. In **chapter 6.2** we present two first cousins with a similar phenotype to the megalencephaly, perisylvian polymicrogyria, polydactyly and hydrocephalus (MPPH) syndrome. They both

had a submicroscopic chromosome 5q35 deletion as a result of an unbalanced der(5)t(5;20)(q35.2;q13.3) translocation. Five unrelated MPPH patients showed no submicroscopic chromosomal aberrations, indicating that this disorder is heterogeneous, but we conclude that a high-resolution array analysis should be part of the diagnostic workup for a patient with MPPH syndrome.

SAMENVATTING

In **hoofdstuk 1**, de introductie, wordt een samenvatting gegeven van de huidige kennis over de klinische verschijnselen, beeldvormende technieken, pathologie en oorzaken van aanlegstoornissen van de hersenschors.

Hoofdstuk 2 beschrijft een cohort van 113 opeenvolgende patiënten met een aanlegstoornis van de hersenschors die onderzocht werden tussen 1992 en 2006. Door gegevens te combineren over de klinische verschijnselen, de beeldvorming van de hersenen, genetische classificatie, syndroom diagnoses en familie onderzoek met diagnostische moleculaire testen bleek het mogelijk om in 40% van de patiënten met een aanlegstoornis van de hersenschors een etiologische diagnose te stellen. Dit draagt bij aan erfelijkheidsvoorlichting, aan de mogelijkheid van prenatale diagnostiek en geeft een verbetering van behandeling van en zorg voor deze patiëntengroep. We stelden een etiologische diagnose in 45 van 113 casus (40%). Bij 21 patiënten (19%) betekende dit een moleculaire of genetisch bevestigde diagnose (Miller-Dieker syndroom, mutaties in *LIS1, DCX, FLNA, EIF2AK3* of *KIAA1279* of een erfelijke stofwisselingsziekte). Bij 17 (15%) was dit een syndroom diagnose, waarvan de onderliggende genetische oorzaak onbekend is. Bij 7 patiënten (6%) vonden we aanwijzingen voor een prenatale gebeurtenis die een aanlegstoornis kan veroorzaken. Van de overgebleven 68 patiënten hebben 34 patiënten waarschijnlijk een tot nu toe onbekende genetische aandoening, gezien de aanwezigheid van multipele aangeboren afwijkingen (15 patiënten), een familie anamnese met meerdere aangedane personen of ouders die bloedverwant zijn (7 patiënten).

De volgende hoofdstukken zijn gebaseerd op specifieke observaties van patiënten uit het bovengenoemde cohort en patiënten die we zagen van 2006 tot 2009.

Hoofdstuk 3 gaat over het natuurlijk beloop van patiënten met een aanlegstoornis van de hersenschors met een bekende genetische oorzaak. In **hoofdstuk 3.1** beschrijven we de lange termijn follow-up van een cohort van 24 patiënten met type 1 lissencephalie die in Nederland geboren werden tussen 1972 en 1990. We herclassificeerden de lissencephalie naar huidige inzichten en probeerden de patiënten en hun ouders opnieuw te bereiken. Van de 24 patiënten bleken er 19 nog in leven. Dit is een langere levensverwachting dan dat de ouders oorspronkelijk was verteld. De overleving toonde een significante correlatie met de ernst van de lissencephalie op de CT-scan. Alle patiënten hadden een ernstige mentale retardatie, moeilijk behandelbare epilepsie en volledige afhankelijkheid voor de functies van het dagelijks leven. In de tijd dat bij deze patiënten de diagnose gesteld werd, was moleculaire analyse van het *LIS1* gen nog niet mogelijk en 8 ouders wilden dit onderzoek nu alsnog laten doen. Dit toonde bij 7 patiënten een pathogene mutatie in het *LIS1* gen of een deletie van (een deel van) dit gen.

De **hoofdstukken 3.2 en 3.3** zijn gericht op patiënten met periventriculaire nodulaire heterotopieën ten gevolge van afwijkingen in het *FLNA* gen. Eerst beschrijven we een 71-

jarige vrouw die bekend was met een hartklepafwijking en bilaterale periventriculaire nodulaire heterotopieën toen zij overleed aan een subarachnoidale bloeding. Obductie toonde klassieke bilaterale periventriculaire nodulaire heterotopieën en vaatafwijkingen. Postmortem werd de diagnose *FLNA* mutatie bevestigd. Het is verrassend dat deze patiënte nooit epileptische aanvallen heeft gehad. Het is bekend dat bij het fenotype van *FLNA* mutaties ook hartafwijkingen horen, vooral aortaklep en mitralisklep afwijkingen. Onze casus liet zien dat ook andere vasculaire afwijkingen, zoals aneurysmata, gezien kunnen worden. We bevestigden ook de aanwezigheid van glomeruloïde microvasculaire afwijkingen in de hersenen die eenmaal eerder beschreven waren in een *FLNA* patiënt. Als deze genetische aandoening gedurende het leven herkend was, was er mogelijk meer aandacht voor het risico op cardiovasculaire problemen geweest en was er de mogelijkheid van erfelijkheidsvoorlichting geweest. In het derde deel van **hoofdstuk 3** bekijken we het cardiale fenotype van onze *FLNA* patiënten nader en beschrijven dat deze patiënten vaak eerst een cardioloog bezoeken. Een positieve familie anamnese, huid- of gewrichts-hyperlaxiteit of neurologische problemen kunnen voor de cardioloog een aanwijzing zijn om deze patiënten eerder te herkennen.

In **hoofdstuk 4** bespreken we nieuwe fenotypes bij bekende genen. In **hoofdstuk 4.1** beschrijven we een meisje met bilaterale periventriculaire nodulaire heterotopieën (BPNH) ten gevolge van een tweetal mutaties in het *ARFGEF2* gen (compound heterozygoot). Dit is de eerste patiënt gerapporteerd sinds de ontdekking van dit gen. De patiënt heeft een opvallend ernstige choreadystone bewegingsstoornis, wat nog niet eerder beschreven was als onderdeel van het fenotype. De MRI van de hersenen toont beiderzijds een hyperintensiteit van het putamen en gegeneraliseerd verlies van hersenstof, naast de BPNH. Een mutatie in het *ARFGEF2* gen veroorzaakt in de cel een stoornis in de functie van transportblaasjes, in de proliferatie en geprogrammeerde celdood en in de functie van receptoren voor neurotransmitters. Dit kan het optreden van BPNH en microcephalie verklaren. We denken dat de bewegingsstoornis en de schade aan de basale kernen, vooral het putamen, verklaard kan worden door een verhoogde gevoeligheid voor celafbraak, een stoornis in de receptoren voor neurotransmitters of een combinatie van beide. In **hoofdstuk 4.2** beschrijven we twee families met beide een kind met microcephalie met een vereenvoudigd gyraal patroon ('simplified gyral pattern': SGP) en jong ontstane insuline afhankelijke suikerziekte. De eerste patiënt bleek een Wolcott-Rallison syndroom te hebben op basis van een homozygote mutatie in het *EIF2AK3* gen. De patiënt van de tweede familie had geen mutatie in dit gen. Men denkt dat SGP veroorzaakt wordt door verminderde neuronale proliferatie en toegenomen apoptose in een vroege fase van de embryonale ontwikkeling, maar er is weinig bekend over de betrokken processen. EIF2AK3 is een van de kinasen die betrokken zijn bij het remmen van de eiwitaanmaak in de cel als de omstandigheden ongunstig zijn voor het verwerken van het eiwit. Er wordt gedacht dat verlies van functie van EIF2AK3 ervoor zorgt dat de cel slechter om kan gaan met problemen van het endoplasmatisch reticulum en dat daardoor de Langerhans cellen in de alvleesklier te gronde gaan. Onze casus suggereert dat in sommige gevallen, vroege

insuline afhankelijke suikerziekte en SGP kan ontstaan door eenzelfde mechanisme dat leidt tot geprogrammeerde celdood.

In **hoofdstuk 5** rapporteren we een meisje met een malonyl-CoA decarboxylase deficiëntie, een zeldzame aangeboren stofwisselingsziekte, die op MRI van de hersenen diffuse pachygyrie liet zien en een periventriculaire heterotopie.

Hoofdstuk 6 gaat over syndromale diagnoses in ons cohort. In **hoofdstuk 6.1** beschrijven we drie patiënten met allen een combinatie van periventriculaire nodulaire heterotopieën en ledemaatafwijkingen. Deze afwijkingen zijn in wisselende ernst aanwezig. De verschijnselen bij deze patiënten zouden zowel kunnen passen bij een amniotic band sequentie of Adams-Oliver syndroom, maar van deze aandoeningen is geen genetische oorzaak bekend. We suggereren dat op basis van algemene kennis over genetische oorzaken van heterotopieën en ledemaatafwijkingen dat deze combinatie een gezamenlijke oorzaak heeft, waarbij deze waarschijnlijk te vinden zal zijn in mechanismen van de bloedvatontwikkeling.
In **hoofdstuk 6.2** presenteren we twee meisjes waarvan de moeders zussen zijn, die beiden een fenotype hebben dat doet denken aan het MPPH syndroom ('megalencephalie, perisylvian polymicrogyrie, polydactylie en hydrocephalus'- syndroom). Zij hebben allebei een submicroscopische chromosoom 5q35 deletie door een ongebalanceerde translokatie der(5)t(5;20)(q35.2;q13.3). Bij 5 niet verwante MPPH patiënten konden we geen submicroscopische chromosomale afwijking vinden, wat betekent dat deze aandoening genetisch heterogeen is. We concluderen dat een chromosoom onderzoek met hoog-resolutie array bij patiënten met MPPH-syndroom geïndiceerd is.

LIST OF ABBREVIATIONS

CT: computed tomography
FISH: fluorescence in situ hybridization
FLAIR: fluid attenuated inversion recovery; type of MRI image
MCD: malformation of cortical development
MRI: magnetic resonance imaging
MSG: microcephaly with simplified gyral pattern
PET: positron emission tomography
PMG: polymicrogyria
PNH: periventricular nodular heterotopia

APPENDIX

Note: tables are updated up to 01-01-2010. Genes and syndromes associated with (isolated) cortical dysplasia are not included in this overview. References can be found on pages 34-44.

MONOGENETIC CAUSES OF MCD

Microcephaly with simplified gyral pattern (MSG)

Established genes

Gene	Chrom.	OMIM	Inh.	Protein	Function	Phenotype	Refs
MCPH1	8p23	607117	AR	Microcephalin	Initiation of chromosome condensation	MSG <-3SD, mental retardation and normal neurological examination	78, 79
CDK5RAP2	9q33	608201	AR	CDK5 regulatory subunit-associated protein 2	Centrosomal mechanism during mitosis	MSG <-3SD, mental retardation and normal neurological examination	80
ASPM	1q31	605481	AR	Abnormal spindle-like microcephaly-associated	Mitotic spindle cleavage plane orientation	MSG <-3SD, mental retardation and normal neurological examination	81
CENPJ	13q12.2	609279	AR	Centromeric protein J	Centrosomal mechanism during mitosis	MSG <-3SD, mental retardation and normal neurological examination	80
STIL	1p32	18590	AR	SCL-TAL1 interrupting locus	Mitotic spindle organization	MSG <-3SD, mental retardation and normal neurological examination	83
SCL25A19	17q25.3	606521	AR	Mitochondrial deoxynucleotide carrier	Mitochondrial DNA synthesis disorder	'Amish lethal microcephaly'; simplified gyration, early death, 2-ketoglutaric aciduria	84
EMG1	12p13.3	211180	AR	Ribosome assembly protein	Ribosome assembly	'Bowen conradi syndrome' simplified gyration, early death	81
NBS1	8q21	251260	AR	P95 protein/nibrin	DNA repair	Nijmegen Breakage Syndrome: MSG, other brain abnormalities, growth retardation, immunodeficiency, predisposition to cancer.	85, 86
CASK	Xp11.4	300172	XL	calcium/calmodulin-dependent serine protein kinase	Scaffolding protein	MSG with hypoplasia of the brainstem and cerebellum	87

Probable genes

Gene	Chrom.	OMIM	Inh.	Protein	Function	Phenotype	Refs
EIF2AK3	2p12	226980	AR	EIF2-alpha kinase 3	Protein translation initiation regulation	Wolcott Rallison syndrome: neonatal insulin dependent diabetes mellitus with or without MSG (1 case)	88

Lissencephaly/pachygyria/subcortical band heterotopia spectrum

Established genes

Gene	Chrom.	OMIM	Inh.	Protein	Function	Phenotype	Refs
LIS1	17p13.3	601545	AD	platelet-activating factor acetylhydrolase, isoform 1b, alpha subunit	Microtubule organization	Pachygyria with a posterior to anterior gradient	89
DCX	Xq22-23	300121	XL	Doublecortin	Microtubule organization	Males: lissencephaly. Females: subcortical band heterotopia.	90
RELN	7q22	600514	AR	Reelin	Microtubule organization and signalling	Lissencephaly with cerebellar and brainstem hypoplasia ('Norman-Roberts type lissencephaly')	91
VLDLR	9p24	192977	AR	Very low density lipoprotein receptor	Microtubule organization (receptor for RELN)	Pachygyria with cerebellar hypoplasia	92
TUBA1A	12q12	602529	AD	Alpha tubulin 1A	Component of microtubule	Pachygyria, corpus callosumdysgenesis, cerebellar hypoplasia.	93
ARX	Xp22.13	300382	XL	Aristaless related homeobox protein	Homeobox gene	Diverse phenotypes from lissencephaly, abnormal genitalia and corpus callosum agenesis to X-linked mental retardation	52

Cobblestone lissencephaly

Established genes:

Gene	Chrom.	OMIM	Inh	Protein	Function	Phenotype	Refs
FKTN	9q31	607440	AR	Fukutin	O-glycosylation of alpha dystroglycans (extracellular matrix protein)	Walker-Warburg syndrome, Fukuyama congenital muscular dystrophy.	94,95,96
FKRP	19q13.3	606596	AR	Fukutin related protein	O-glycosylation of alpha dystroglycans	Cobblestone lissencephaly, muscular dystrophy, eye abnormalities.	97
POMT1	9q34.1	607423	AR	O-mannosyl transferase-1	O-glycosylation of alpha dystroglycans	Walker-Warburg syndrome.	98
POMT2	14q24.3	607439	AR	O-mannosyl transferase-2	O-glycosylation of alpha dystroglycans	Walker-Warburg syndrome.	99
POMGnT1	1p34-33	606822	AR	O-mannose beta-1,2-N-acetylglucosaminyltransferase	O-glycosylation of alpha dystroglycans	Muscle-Eye-Brain disease.	100
LARGE	22q12.3-13.1	603590	AR	Acetylglucosaminyl transferase-like protein	O-glycosylation of alpha dystroglycans	Walker-Warburg syndrome.	101
RAB3GAP1	2q21.3	602536	AR	RAB3 GTPase-activating protein	Exocytosis	Warburg micro syndrome: pachygyria, microcephaly, microphthalmia, congenital cataract, hypogonadism.	102
GPR56	16q13	604110	AR	G-protein coupled receptor 56	Pial membrane integrity regulation	Bilateral frontoparietal cobblestone malformation, white matter abnormalities. Sometimes considered to be polymicrogyria.	103, 77
SNAP29	22q11.2	604202	AR	Synaptosomal-associated protein	Vesicle trafficking/fusion	With neuropathy, ichthyosis, and keratoderma.	104
TUBB2B	6p25.2	612850	AD	Beta tubulin	Microtubuli function	Cobblestone malformation (polymicrogyria like), (partial) CCA, cerebellar abnormalities.	56
ATP6V0A2	12q24.3	219200	AR	alpha-2 subunit of the V-type H+ ATPase	N-Glycosylation defect	Debré type – cutis laxa with fronto-parietal cobblestone malformation and cerebellar malformation.	105, 106

Periventricular nodular heterotopia

Established genes:

Gene	Chrom.	OMIM	Inh.	Protein	Function	Phenotype	Refs
FLNA	Xq28	300017	XL	Filamin A	Anchoring of cytoskeleton to extracellular matrix	Loss of function mutations: bilateral PNH. Sometimes cardiac malformation, Ehlers-Danlos or other skeletal disorder.	59
ARFGEF2	20q13.13	605371	AR	brefeldin A-inhibited guanine nucleotide exchange protein 2	Vesicle trafficking	Periventricular nodular heterotopia, congenital microcephaly, severe developmental delay.	61

Polymicrogyria

Established genes:

Gene	Chrom.	OMIM	Inh.	Protein	Function	Phenotype	Refs
LAMA2	6q22-q23	156225	AR	laminin 2	extracellular matrix protein	Merosin negative muscular dystrophy. May have PMG, white matter changes, possibly cobblestone.	107, 108
TUBA8	22q11	605742	AR	Alpha tubulin 8	Unknown	Generalised PMG with optic nerve hypoplasia.	109
KIAA1279	10q22.1	609367	AR	Kif1-binding protein	Regulation of microtubule function	Diffuse PMG, M. Hirschsprung, dysmorphic features.	110

Probable genes (sporadic reports):

Gene	Chrom.	OMIM	Inh.	Protein	Function	Phenotype	Refs
TBR2 (EOMES)	3p21	604615	AR	Eomesodermin	Neurogenesis regulation	Congenital microcephaly, corpus callosum agenesis, bilateral PMG, small cerebellum. Multiple affected in one family.	111
MTTL1	Mt	590050	Mt	mitochondrial tRNA for leucine	Protein synthesis	MELAS syndrome. Single case with bilateral PMG.	112
PAX6	11p13	607108	AR	Paired box domain gene	Transcriptional regulator	Aniridia, absent pineal gland and commisura anterior. Single case with unilateral PMG.	113
SOX2	3q26.3-q27	184429	AD	SOX2 protein	Neurogenesis and differentiation	Microphtalmia, pituary dysfunction. Single case with bilateral schizencephaly.	114
AHI1	6q23.3	608894	AR	Jouberin	Ciliary function	Joubert syndrome with or without PMG.	115
SRPX2	Xq21.33-q23	300642	XL	Sushi repeat-containing protein	Protein folding	Rolandic epilepsy, speech disorder with or without perisylvian polymicrogyria.	116
COL18A1	21q22.3	1230328	AR	Collagen 18 alpha-1	Angiogenesis inhibition, basal membrane function	Knobloch syndrome: eye abnormalities, occipital encephalocele. Can have PMG, PNH and cerebellar anomalies.	117, 118

Note: Cobblestone malformation can have a similar presentation to polymicrogyria on neuroimaging. The cobblestone genes *POMT2, POMT1, LARGE, POMgnT1, TUBB2B, GPR56* are also described in patients with an MRI diagnosis of polymicrogyria [56, 103, 77]. In *LAMA2* mutations polymicrogyria is described, but the phenotype is probably also cobblestone-like.

CHROMOSOMAL LOCI (MICRODELETION OR DUPLICATION SYNDROMES AND LINKAGE DATA)

Microcephaly with simplified gyral pattern (MSG)

Locus	OMIM	Inh.	Phenotype	Refs
2p16	-	AR	MSG <-3SD, white matter cysts, corpus callosum hypoplasia, early death	120
15q15-21	604321	AR	MSG <-3SD, mental retardation and normal neurological examination	121
19q13	604217	AR	MSG <-3SD, mental retardation and normal neurological examination	122

Lissencephaly/pachygyria/subcortical band heterotopia spectrum

Locus	OMIM	Inh.	Phenotype	Refs
Del17p-	247200	AD	Miller-Dieker syndrome: lissencephaly with dysmorphic features (continuous gene syndrome wih *LIS1*.)	123

Periventricular nodular heterotopia (PNH)

Locus	OMIM	Inh.	Phenotype	Refs
5p15dup	608098	AD	Epilepsy, MR, bilateral PNH.	124
Del 5q14.3-15	-	AD	Bilateral temporal periventricular heterotopia and perisylvian polymicrogyria. Severe MR and epilepsy.	125
Del 7q11	194050	AD	Bilateral PNH associated with Williams syndrome.	126

Polymicrogyria, possibly with other MCD

Locus	OMIM	Inh.	Phenotype	Refs
Del 1p36	607872	AD	Psychomotor delay, facial dysmorphic features, cardiac malformation. May have perisylvian PMG with or without PNH, abnormal white matter signal or enlarged lateral ventricles.	69, 127, 128, 129
Del 6q26-qter	-	AD	Extensive PMG, one case with additional PNH.	69, 130, 131

Polymicrogyria (PMG)

Locus	OMIM	Inh.	Phenotype	Refs
Del 1q44-qter	-	AD	Bilateral perisylvian PMG.	69, 132
Del 2p15–p16.1	612513	AD	PMG, dysmorphic features.	133
Dup 2p16.1-p23.1	-	AD	Bilateral perisylvian PMG, with/without hydrocephalus.	69
Del 4q21.21-22.1	-	AD	Bilateral perisylvian PMG, with/without hydrocephalus. Dysmorphic features.	69, 134
Del 13q14.1–q31.2	-	AD	Bilateral PMG.	135
Del 18pter	-	AD	Bilateral perisylvian PMG.	69
Del 21q2	-	AD	Bilateral perisylvian PMG, partial agenesis of the corpus callosum.	69
Del 21q21.3-q22.1	-	AD	Bilateral perisylvian PMG.	69, 136
Del 22q11.2	192430	AD	Velocardiofacial/Shprintzen syndrome. Bilateral or unilateral PMG.	69

SOME ESTABLISHED SYNDROMES AND/OR REPEATED ASSOCIATIONS WITH MCD

Syndrome	OMIM	Inh	MCD	Phenotype	Refs
Baraitser Winter	-	AR?	Pachygyria	Iris coloboma, ptosis, hypertelorism, mental retardation.	1, 137, 138 ,139
PNH with limb deficiency	-	-	PNH	PNH with transverse limb deficiency.	60, 140, 141
PNH with hippocampal and cerebellar abnormality	-	-	PNH	Bilateral posterior PNH, with hippocampal malformation and cerebellar hypoplasia.	60
Oculocerebrocutaneous (Delleman)	164180	-	PMG and/or PNH	Frontal PMG and PNH, hydrocephalus, CCA, interhemispheric cysts, and mid-hindbrain malformation. Microphthalmia, hypoplastic skin defects.	142
Frontonasal dysplasia	136760	-	PNH and CD	Periventricular nodular heterotopia, cortical dysplasia, CCA, CC lipoma, hypertelorism, broadening of nasal root, facial cleft.	50, 143
Adams-Oliver	100300	AD AR	PMG and/or PNH	Distal limb defects, scalp/skull defect.	1, 144, 145, 146
MPPH/CM	-	-	PMG	Megalencephaly, polydactyly, PMG, hydrocephalus syndrome, macrocephaly capillary malformation.	34, 147
Kabuki make-up	147920	-	PMG and/or PNH	Facial dysmorphic features, growth deficiency, mental retardation, skeletal anomalies, congenital cardiac defects.	148, 149, 150
Aicardi	225750	XL?	PMG, PNH	Chorioretinal lacunae, corpus callosum dysgenesis.	151, 152
Galloway-Mowat	251300	AR	PNH and 'gyral anomalies' (MSG/PG-like)	Microcephaly, hiatus hernia, nephrotic syndrome, cerebellar atrophy.	153, 154, 155
Joubert	213300	AR	PMG	Atrophy cerebellar vermis ('molar tooth'), dysregulation of breathing, renal and eye abnormalities. Some with mutation in *AHI1* gene. Includes Arima syndrome (OMIM 243910).	156, 157
(Warburg) Micro	600118	AR	Cobbelstone malformation	Cobblestone pachygyria, microcephaly, microphthalmia, congenital cataract, hypogonadism. Some associated with *RAB3GAP1* gene.	102, 158
Proteus	176920	-	PMG and/or PNH, hemimegalencepahly	Hamartoneoplastic syndrome with overgrowth, epidermal nevi, hyperostosis. Some with *PTEN* mutations..	159, 160
CLOVE	612918	-	PMG and/or hemimegalencephaly	Overgrowth, cutaneous vascular malformation, epidermal nevi.	161, 162
Meningomyelocele	182940	-	PNH and/or PMG	Meningomyelocele usually with Chiari II malformation and hydrocephalus.	1, 30,31, 163
Shprintzen	192430	-	PMG	Most with 22q11 deletion.	70
Septo-optic dysplasia	182230	-	PNH	Clinically heterogeneous: optic nerve hypoplasia, pituitary abnormalities, midline abnormalities.	164, 165

METABOLIC DISORDERS

Disease	OMIM	Inh.	Phenotype	Refs
Zellweger syndrome	214100	AR	Peroxisomal biogenesis defect: large fontanel, facial dysmorphic features, hepatomegaly, PMG/PNH.	166
D-bifunctional protein deficiency	261515	AR	Inborn error of peroxisomal fatty acid oxidation with bilateral perisylvian PMG and occasional PNH.	167
Congenital disorder of glycosylation II	-	AR	PMG or probably cobblestone malformation with white matter abnormalities, dysmorphic features, cutis laxa.	106, 168
Fumarase deficiency	606812	AR	PMG, wide ventricles, periventricular cysts. Also hypotonia, failure to thrive, seizures, MR.	169
CPT2 deficiency	608836	AR	Neonatal form with PMG, heart failure, liver and kidney involvement.	170
SLO	270400	AR	Smith-Lemli-Opitz syndrome; rarely PMG.	171, 172
Malonyl-CoA decarboxylase deficiency	248360	AR	Brain atrophy, white matter loss, thickened cortex, and nodular heterotopia.	173
Multiple acyl-CoA-dehydrogenase deficiency	231680	AR	Glutaric aciduria type II, vermis hypoplasia, temporal lobe hypoplasia, PMG, heterotopia.	174

NON-GENETIC CAUSES

Established non-genetic causes: multiple reports, pathogenic mechanism understood

Cause	Mechanism	Phenotype	Refs
Irradiation	Loss of progenitors	MSG, mental retardation, epilepsy	175, 176
Intrauterine CMV infection	Infection	PMG	72, 177
Twin-twin transfusion syndrome	Ischemia/infarction	PMG	74, 74
Cocaine	Vasoconstriction	PMG	75, 178
Fetal alcohol syndrome	Ethanol toxicity	PMG; rare complication, 2 cases reported	179

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CURRICULUM VITAE

The author was born on 18-02-1973 in Amsterdam. She graduated from the Eerste Christelijk Lyceum (gymnasium ß) in 1991 cum laude, and started medical school at the Rijksuniversiteit Groningen after a gap year spent studying at a community college in Florida, USA.
In 1999 she graduated from medical school cum laude and started working as a resident in Neurology in the Erasmus MC, Rotterdam. From 2000 to 2008 she was trained as a neurologist under prof.dr. F.G.A. van der Meché and prof.dr. P.A.E. Sillevis Smitt, including a one year residency in Pediatrics under dr. M. de Hoog. During 2008 she worked as a fellow in Pediatric Neurology in the Erasmus MC-Sophia Children's Hospital under prof. dr. W.F.M. Arts and became a pediatric neurologist on February 1, 2009.
During her neurology training she was a member of the junior neurologist board of the Dutch Neurology Society, a member of the visitation committee and the plenary consilium of the Dutch Neurology Society, and president of the Erasmus MC resident association. She participated in several committees to implement the electronic medical record, and organized the annual department ski-trip. Since 2009 she works as a pediatric neurologist in the Erasmus MC Sophia Children's Hospital. For this thesis she was awarded the Jacobus Willemse Award of the Dutch Society for Pediatric Neurology in April 2010.

LIST OF PUBLICATIONS

de Wit MCY, de Rijk-van Andel JF, Halley DJJ, Poddighe PJ, Arts WFM, de Coo IFM, Mancini GMS. Long term follow-up of lissencephaly [submitted].

Roodbol J, de Wit MCY, Walgaard C, Catsman-Berrevoets CE, Jacobs BC. Recognizing Guillain-Barré syndrome in preschool children [submitted].

de Wit MCY, de Coo IFM, Lequin MH, Halley DJJ, Roos-Hesselink JW, Mancini GMS. Combined cardiological and neurological abnormalities due to filamin A gene mutation. Clin Res Cardiol (2010), accepted.

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PhD Portfolio: summary of PhD training and teaching

1. PhD training	Year	Workload (ECTS)
General courses		
ESP03 Introduction to data-analysis	2006	0.9
CC02 Classical methods for data-analysis	2006	5.7
SNP's and human diseases (Molmed course)	2007	1.0
Specific courses (e.g. Research school, Medical Training)		
Biemond courses 2004, 2005, 2006, 2007, 2008, 2009	-	2.4
EPNS training courses 2005, 2007, 2009	-	2.1
Boerhave course on neuromuscular disorders	2007	0.2
Advanced Pediatric Life Support	2008	1.5
Seminars and workshops		
Voorjaarsvergadering NvKN 2005, 2006, 2007, 2008, 2009, 2010	-	1.0
Werkgroep neuroneonatologie	2006	0.2
Presentations		
Zellweger syndrome; voorjaarsvergadering NvKN	2005	1.0
Referring children for epilepsy surgery; EPNS Sweden	2005	1.0
Diabetes mellitus&simplified gyral pattern; voorjaarsvergadering NvKN	2006	1.0
Early onset diabetes mellitus and symplified gyral pattern. Doorwerth conference	2006	1.0
Genetische oorzaken van migratiestoornissen; SEIN Heemstede	2006	1.0
Microcephaly and simplified gyral pattern with early onset diabetes mellitus; ESMRN	2007	1.0
Normal and abnormal cortical development; Werkgroep neuro-neonatologie	2007	1.0
Cortical brain malformations; EPNS Turkey	2007	1.0
Long term follow up of classic lissencephaly; voorjaarsvergadering NvKN	2008	1.0
Migratiestoornissen door het leven heen; najaarssymposium NvKN	2009	1.0
(Inter)national conferences		
European Neurological Society, Barcelona	2004	1.0
EPNS Congress 2005 Göteborg Sweden, 2007 Kusadasi Turkey	-	2.0
ISHN, St Andrews, Schotland	2006	1.0
International Doorwerth conference, UMC Radboud	2007	1.0
ESMRN 2004 Genoa Italy, 2007 Tübingen Germany, 2009 Zürich Switserland	-	3.0
2. Teaching		
Teacher pediatric nursing degree 2004-2008	-	1.0
Cerebral cortical development for pediatricians (PAO-Sophia)	2008	0.5
Total		**34.5**