Received: 1 May 2023 | Accepted: 17 January 2024

DOI: 10.1111/dmcn.15882

INVITED REVIEW

Diagnostic work-up in malformations of cortical development

Ellen Rijckmans[1,2] | **Katrien Stouffs**[2,3] | **Anna C. Jansen**[2,4,5]

[1]Pediatric Neurology Unit, Department of Pediatrics, KidZ Health Castle, UZ Brussel, Brussels, Belgium

[2]Neurogenetics Research Group, Vrije Universiteit Brussel, Brussels, Belgium

[3]Center for Medical Genetics, UZ Brussel, Brussels, Belgium

[4]Pediatric Neurology Unit, Department of Pediatrics, Antwerp University Hospital, Antwerp, Belgium

[5]Translational Neurosciences, University of Antwerp, Antwerp, Belgium

Correspondence
Anna Jansen, Pediatric Neurology Unit, Department of Pediatrics, Antwerp University Hospital, Drie Eikenstraat 655, 2650 Edegem, Belgium.
Email: anna.jansen@uza.be

Funding information
Fonds Wetenschappelijk Onderzoek, Grant/Award Number: FWO 1805321N; Marguerite-Marie Delacroix foundation, Grant/Award Number: Research Grant

Abstract

Malformations of cortical development (MCDs) represent a heterogeneous spectrum of disorders characterized by atypical development of the cerebral cortex. MCDs are most often diagnosed on the basis of imaging, although subtle lesions, such as focal cortical dysplasia, may only be revealed on neuropathology. Different subtypes have been defined, including lissencephaly, heterotopia, cobblestone malformation, polymicrogyria, and dysgyria. Many MCDs are of genetic origin, although acquired factors, such as congenital cytomegalovirus infections and twinning sequence, can lead to similar phenotypes. In this narrative review, we provide an overview of the diagnostic approach to MCDs, which is illustrated with clinical vignettes, on diagnostic pitfalls such as somatic mosaicism and consanguinity, and recognizable phenotypes on imaging, such as tubulinopathies, the lissencephaly spectrum, tuberous sclerosis complex, and *FLNA*-related periventricular nodular heterotopia.

Malformations of cortical development (MCDs) form a heterogeneous spectrum of disorders, which are characterized by atypical development of the cerebral cortex. MCDs are an important cause of intellectual disability, developmental delay, cerebral palsy (CP), and drug-resistant epilepsy.[1] In 2012, Barkovich et al.[2] proposed a classification system based on the genetic origin and the earliest step at which the developmental process is disturbed. Three main groups were identified, including malformations secondary to atypical neuronal and glial proliferation or apoptosis, malformations secondary to atypical neuronal migration, and malformations secondary to atypical postmigrational development (Table 1). Further updates to this classification are likely to emerge as information accumulates about the clinical, embryological, genetic, and molecular biology aspects of these disorders. Brain malformations can be focal or diffuse, unilateral or bilateral; associated malformations of, for example, the corpus callosum, basal ganglia, brainstem, or cerebellum are variably present. Severino et al.[3] reported a detailed description of the different neuroimaging features of MCD and made practical recommendations for both radiologists and neurologists. The broad variety of brain malformations in MCD highlights the need to use human phenotype ontology or human phenotype ontology terms. Human phenotype ontology terms for the different MCD subtypes were defined by the Neuro-MIG consortium and published by Oegema et al.[4] An overview is shown in Table 1. Human phenotype ontology terms favour uniform description in the literature and facilitate correspondence between different centres of expertise.

The causes of MCD are variable and include environmental factors, such as congenital infections, external factors, such as prenatal vascular events, and genetic causes.[4] By implementing next-generation sequencing techniques, the genetic origins of MCD have exponentially gained in importance. To date, more than 200 genes have been linked to different types of MCD.[5–9]

Abbreviations: MCD, malformation of cortical development; mTOR, mammalian target of rapamycin; OFC, occipitofrontal circumference; PMG, polymicrogyria; PVNH, periventricular nodular heterotopia; TORCH, toxoplasmosis, others (syphilis, hepatitis B), rubella, cytomegalovirus, herpes simplex; TSC, tuberous sclerosis complex; VOUS, variant of unknown significance.

Molecular and histological correlations have been reasonably well reported for some phenotypes, such as lissencephaly, cobblestone malformation, and some tubulinopathies.[10–12] Because focal cortical dysplasia represents the MCD subtype most amenable to surgery, its histopathological classification is most advanced, including neuroimaging and genetic features, as illustrated by a report produced by the ad hoc task force of the International League Against Epilepsy Diagnostic Methods Commission.[13] However, for many MCDs and most MCD-associated genes, pathological data are limited to non-existent.[14,15]

What this paper adds

- The basic genetic work-up for malformations of cortical development is best performed stepwise.
- When the clinical and genetic work-up in a patient remains negative, multiple options are available for further investigations.
- Neuroimaging cannot reliably distinguish genetic from non-genetic causes.
- Tubulinopathies, lissencephalies, tuberous sclerosis complex, and *FLNA*-related periventricular heterotopia are recognizable phenotypic entities on magnetic resonance imaging.

PRACTICAL RECOMMENDATIONS FOR THE WORK-UP OF PATIENTS WITH (NEWLY DIAGNOSED) MCD

The most common presenting features of MCD are abnormal head circumference, epilepsy, developmental delay, or motor abnormalities of tone, movement, and posture.[5] This practical work-up for patients with (newly diagnosed) MCD is based on the diagnostic MCD flow chart proposed by Oegema et al.[4] and the Neuro-MIG consortium. This flow chart can be divided into three major parts: (1) the initial work-up, including clinical and imaging phenotyping; (2) ruling out non-genetic causes; and (3) a stepwise approach to genetic testing (Figure 1). Options in case of a negative work-up are discussed in the 'What's next' section of the flow chart. Points of attention and potential challenges that may occur during the diagnostic process are illustrated in this article using clinical vignettes.

INITIAL WORK-UP

Personal and family history

A detailed personal history can provide precious clues to diagnosis. Special attention needs to be paid to potential infections, trauma, or medication use during pregnancy. A detailed family history should be obtained, spanning three generations where possible and with special attention to the presence of epilepsy, CP, and intellectual disability.

With the exception of X-chromosome genes, such as *ARX* and *DCX*, and genes encoding components of the GATOR1 complex, variants in MCD-associated genes seem to be fully penetrant as carrier probands always show characteristic structural changes in the brain. However, individuals with these variants might be clinically asymptomatic or present only minor features, such as learning difficulties, and therefore never undergo brain magnetic resonance imaging (MRI). In the case of inheritance of probably pathogenic variants from apparently unaffected parents, parental brain imaging is essential for accurate variant interpretation.[4,16]

Clinical vignette 1

It is important to keep an open mindset with respect to inheritance patterns, as illustrated by this clinical vignette.[17] The proband, born to second-degree cousins, presented with focal seizures at age 5 months. She later developed bilateral pyramidal signs and mild intellectual disability. Brain MRI showed a dysgyric cortex with areas of polymicrogyria (PMG), abnormally shaped basal ganglia, thin corpus callosum, and mild atrophy of the left pons (Figure 2a–c). Her younger sister presented with delayed motor milestones at age 9 months. She later developed bilateral pyramidal signs, mild dystonic posturing of the right hand with intermittent tremor, and limb ataxia with dysmetria. She drooled when performing complex motor tasks. She had mild intellectual disability; both receptive and expressive language development were severely delayed. MRI of the brain showed a dysgyric cortex, grey matter heterotopia, optic nerve hypoplasia, enlarged lateral ventricles with a hooked right frontal horn, dysmorphic basal ganglia, thin corpus callosum, hypoplasia of the pons and dentate nuclei with broad fourth ventricle, and vermian dysplasia (Figure 2d–f). Based on a family history of consanguinity, an autosomal recessive inheritance pattern was anticipated. However, the imaging features were highly suggestive of tubulinopathy, which is usually caused by a de novo heterozygous variant in one of the tubulin genes. A c.13A>C (p.Ile5Leu) missense variant in *TUBA1A* was identified in both siblings. The variant was present in 5.6% of total DNA in the peripheral blood of the clinically asymptomatic mother. Her brain MRI showed a thin corpus callosum, hypoplasia of the superior vermis, and a relatively thin medulla (Figure 2g–i). The variant was absent from the peripheral blood in the father.

Learning points for personal and familial history include the following: mildly affected parents are sometimes hard to diagnose, especially when a language barrier is present; parental germline mosaicism might confuse inheritance patterns.

Physical examination

Deep phenotyping is crucial for the diagnostic work-up of MCD. Points of attention include occipitofrontal circumference (OFC) (microcephaly/macrocephaly), (subtle) dysmorphic features, joint abnormalities, such as contractures or scoliosis, and skin abnormalities, such as hypomelanotic macules, fibrous cephalic plaques, or other pigmented lesions.

TABLE 1 Schematic overview of MCD with HPO terms adapted from Oegema et al.[4]

Pathogenic process	Timing during fetal development	Subtype (phenotype on MRI)	HPO ID	Description
Abnormal neuronal and glial proliferation or apoptosis	8–16 weeks of gestation	Microcephaly	HP:0000252	A significant reduction in OFC by ≥2 SD (compared with controls matched for age and sex)
		Megalencephaly/ macrocephaly	HP:0001355	A significant increase in OFC by ≥3 SD (compared with controls matched for age and sex)
Abnormal neuronal migration	12–20 weeks of gestation	PVNH	HP:0032388	Grey matter nodules along the ventricular walls
		Lissencephaly spectrum	HP:0001339	Includes agyria, pachygyria, and SBH
		Agyria, pachygyria	HP:0031882, HP:0001302	Abnormal gyration pattern with absent or broad gyri in combination with an abnormally thick cortex
		SBH	HP:0032409	A band of grey matter separated from the cortex and lateral ventricles according to zones of white matter
Abnormal postmigrational development	>24 weeks of gestation	COB	HP:0007260	An irregular and 'pebbled' cerebral surface with moderately thick cortex and jagged grey–white matter border with frequent vertical (perpendicular to the cortex–white matter border) striations
		PMG	HP:0002126	An excessive number of abnormally small cerebral gyri with cortical overfolding, irregular 'pebbled' cortical surface and a 'stippled' grey–white matter boundary
		Schizencephaly	HP:0010636	A full-thickness cerebral cleft lined with grey matter, which extends from the ventricular surface to the pial surface
		Dysgyria	HP:0032398	A cortex of variable thickness and a smooth grey–white boundary but with an abnormal gyral pattern characterized by irregularities of sulcal depth or orientation. This term is only used to characterize cortical malformations that do not meet the classic features of any of the aforementioned subtypes
		FCD	HP:0032046	Cortical dyslamination, with or without abnormal cell types (dysmorphic neurons and balloon cells). Other features can include gyral or sulcal irregularities; increased cortical thickness; blurring of the cortex–white matter junction; and white matter abnormalities, such as increased signal on T2-weighted images or a radially oriented 'transmantle sign' of T2-weighted hyperintensity extending from the abnormal cortex to the lateral ventricle

Abbreviations: COB, cobblestone malformation; FCD, focal cortical dysplasia; HPO, human phenotype ontology; MCD, malformation of cortical development; MRI, magnetic resonance imaging; OFC, occipitofrontal circumference; PMG, polymicrogyria; PVNH, periventricular nodular heterotopia; SBH, subcortical band heterotopia.

Measuring OFC is one of the most helpful aspects in the work-up of MCD, especially in cases presenting with PMG,[18] as illustrated in the following clinical vignette.

Clinical vignette 2

A 14-year-old female presented for a second opinion concerning drug-resistant seizures, starting with interruption of activities with secondary tonic–clonic generalization. Seizures started when aged 6 months. She had a moderate developmental delay and prominent macrocephaly (weight 50th centile, height 50th centile, OFC > 97th centile). MRI showed bilateral perisylvian PMG and mildly dysplastic basal ganglia (Figure 3). Genetic testing showed a 12% somatic mosaicism for a class 5 c.1117G > A (p.Gly373Arg) missense variant in *PIK3R2*. *PIK3R2* is part of the mammalian target of rapamycin (mTOR) pathway and variants in this gene are associated with macrocephaly and MCD.

Other genes that are part of the mTOR pathway include *DEPDC5*, which together with *NPRL2* and *NPRL3* forms the GATOR1 complex, and *PIK3CA*, *PTEN*, *TSC1*, and

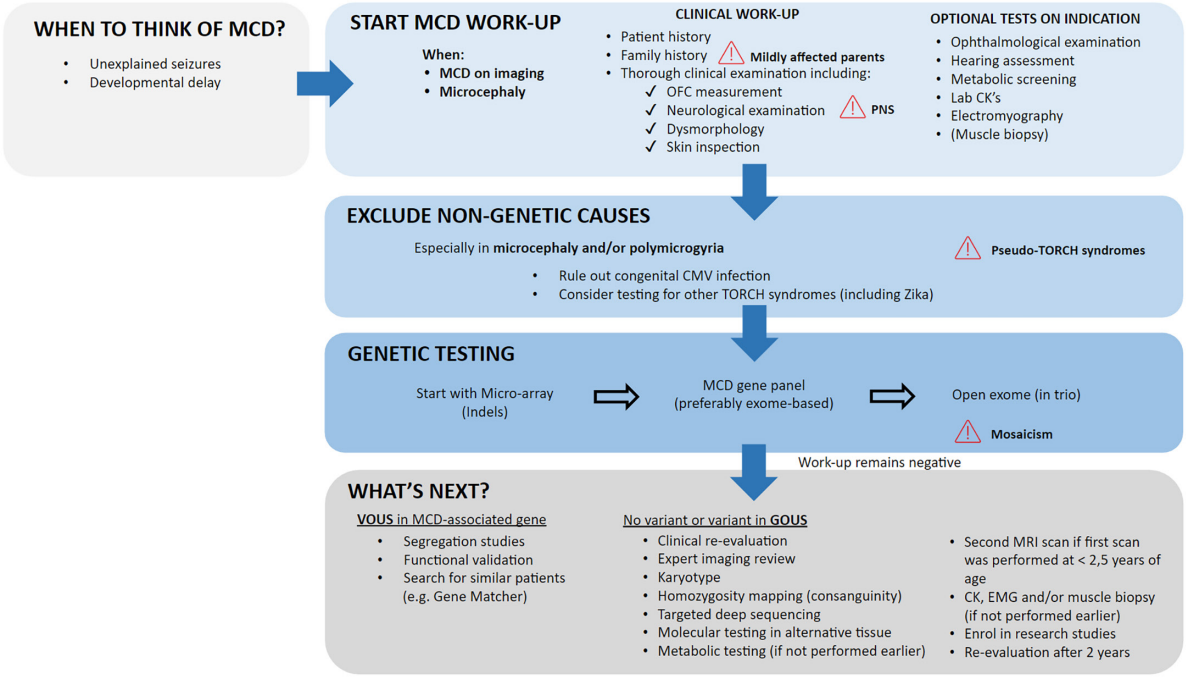

FIGURE 1 Diagnostic flow chart for malformations of cortical development (MCDs), adapted from Oegema et al.[4] This flow chart can be used as a step-by-step guide and checklist for the diagnostic work-up in individuals with MCD. When the underlying mechanism of MCD remains unknown, possible further steps are listed in the 'What's next' section of the article. Abbreviations: CMV, cytomegalovirus; MRI, magnetic resonance imaging; OFC, occipitofrontal circumference; PNS, peripheral nervous system, TORCH, toxoplasmosis, others (syphilis, hepatitis B), rubella, cytomegalovirus, herpes simplex.

TSC2. Loss-of-function variants in these genes result in a wide range of disorders collectively defined as mTORopathies.[19,20] mTORopathies are often associated with significant macrocephaly (ranging from +2.5 SD to +8 SD), global developmental delay, and autism together with other clinical features depending on the causative gene. Skin lesions can be subtle but highly informative, as demonstrated in vignette 3.

Clinical vignette 3

A male infant presented with clonic movements of the left arm and foot, increasing in frequency on day 3 of life and highly resistant to a wide range of antiseizure medications. Clinical and neurological examination showed axial hypotonia, OFC +2 SD, and a pigmented linear skin lesion on the right side of the forehead. The electroencephalogram (EEG) showed a burst suppression pattern. Brain MRI showed right-sided hemimegalencephaly with PMG and heterotopic grey matter alongside the right lateral ventricular wall (Figure 4). Seizures were eventually controlled with epilepsy surgery (hemispherotomy) at age 22 months. He consequently developed a left hemiparesis and had severe developmental delay. Genetic testing paying special attention to the mTOR pathway was negative. Replication of genetic testing on skin fibroblasts obtained from the hyperpigmented lesion on the forehead revealed a pathogenic variant in *PIK3CA* in 30% of the cells (somatic mosaicism). Pathogenic variants in *PIK3CA* are responsible for a range of overgrowth syndromes with recognizable malformations and dysmorphisms,[5] but can be hard to diagnose in the mosaic state. This case illustrates that genetic testing performed in skin fibroblasts can be valuable to identify the causative variant, compared to testing of peripheral blood.[21]

Learning points for the physical examination include the following: at clinical examination, attention should be paid to the OFC, height, and weight (failure to thrive), and (subtle) dysmorphisms; a skin examination should be performed routinely; joint abnormalities should be checked; in the mosaic state, genetic blood tests can remain negative; if possible, affected tissues (skin biopsy or other) should be sampled and genetic testing should be performed again.

Neurological examination

Central neurological manifestations of MCD are variable and can range from mild to severe intellectual disability and developmental delay over CP and drug-resistant epilepsy. It is estimated that 40% to 50% of drug-resistant epilepsies are caused by MCDs.[1]

Peripheral neurological examination can be a challenge, especially in individuals with severe CP, intellectual disability, or autism. Subtle signs of peripheral nervous system involvement can be overlooked or overshadowed by the global clinical presentation. It is important, however, to actively search for signs of neuropathy or myopathy because they can orient the selection of additional non-genetic tests (electromyography, creatine kinase levels) at an early stage in the

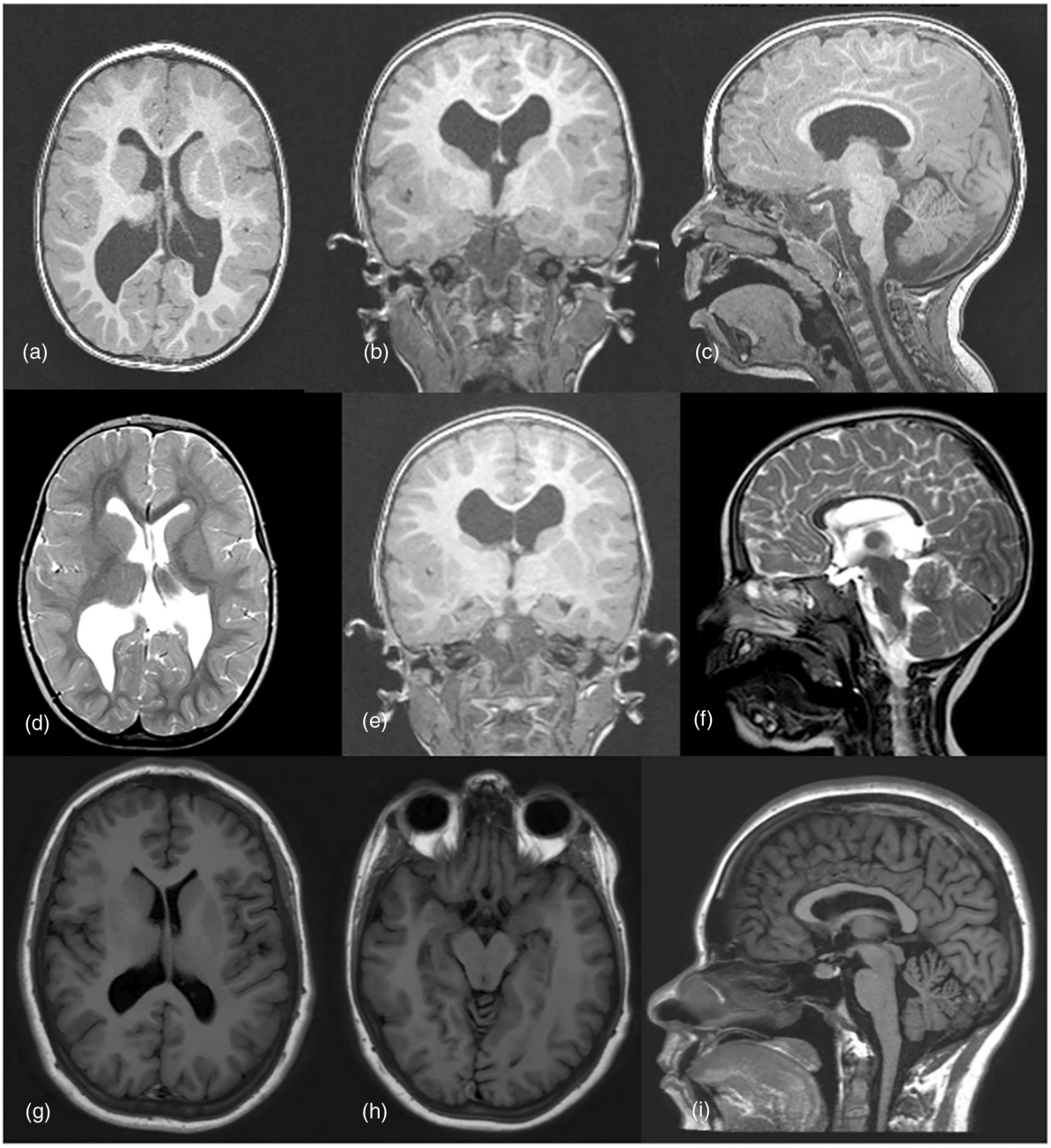

FIGURE 2 Clinical vignette 1: Magnetic resonance imaging (MRI) findings in familial *TUBA1A*. (a–c) Brain MRI of sibling 1 at 2 years 6 months of age. (a) Axial plane showing tubulin-related dysgyria with bilateral areas of polymicrogyria and large lateral ventricles with a hooked aspect of the frontal horns because of abnormally shaped basal ganglia related to dysgenesis of the anterior limb of the internal capsule. (b) Coronal plane showing the abnormal basal ganglia and the dysgyric cortex. (c) Sagittal plane illustrating the thin and dysplastic corpus callosum with dysmorphic aspect of the splenium and mild hypoplasia of the pons. (d–f) MRI of sibling 2 at age 2 years with the axial plane (d) showing bilateral frontal subcortical heterotopic grey matter, enlarged lateral ventricles, and dysmorphic basal ganglia with dysgenesis of the anterior arm of the internal capsule most pronounced on the right. (e) Coronal plane showing the dysgyric cortex. (f) Sagittal plane showing a thin corpus callosum with dysplastic splenium, hypoplasia of the pons and dentate nuclei, and dysplastic cerebellar vermis. (g–i) MRI of the mother showing a thin corpus callosum (g), hypoplasia of the superior vermis (h), and a relatively thin medulla (i).

diagnostic process, help delineate a particular clinical syndrome, or assist with variant interpretation, as reviewed by Rijckmans et al.[9]

Besides neurological features, non-specific or more subtle clinical symptoms, such as ophthalmological, cardiac, or gastrointestinal abnormalities may be present in association with different types of MCD. An ophthalmological or hearing assessment (or both) is useful when there are doubts about visual and hearing capacities.

Clinical vignette 4

A first child (11 months old) of consanguineous parents presented at the paediatric neurology department with profound microcephaly, hypotonia, severe developmental

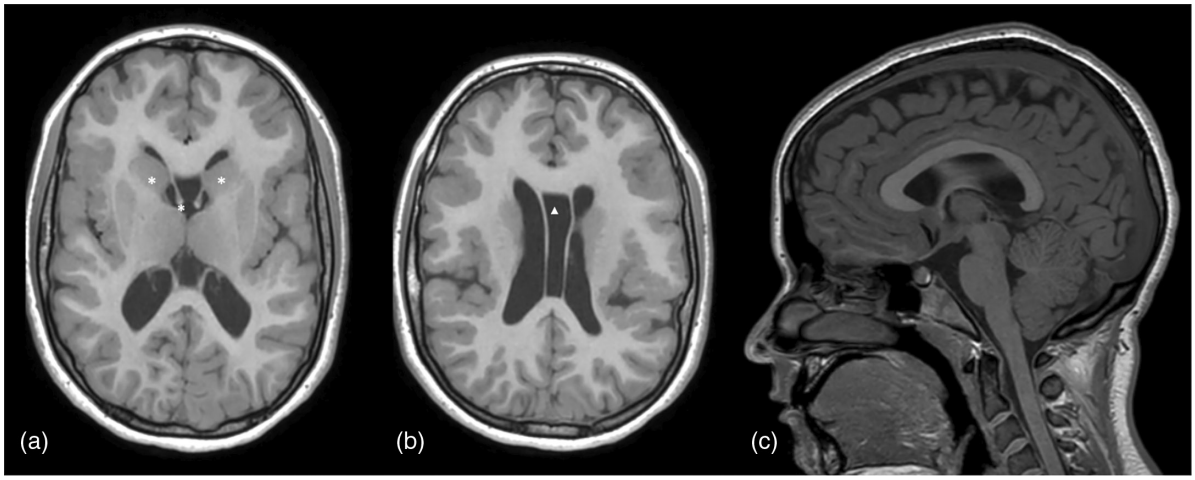

FIGURE 3 Clinical vignette 2: Magnetic resonance imaging (MRI) findings in *PIK3R2* (mosaic mutation). Brain MRI at 14 years of age. (a) Axial plane showing bilateral perisylvian polymicrogyria and mildly dysplastic basal ganglia with prominent caudate nucleus (asterisks) compared to the putamen. (b) Axial plane showing bilateral perisylvian polymicrogyria and a persistent cavum septum pellucidum (triangle). (c) Sagittal view showing normal corpus callosum, brainstem, and cerebellum.

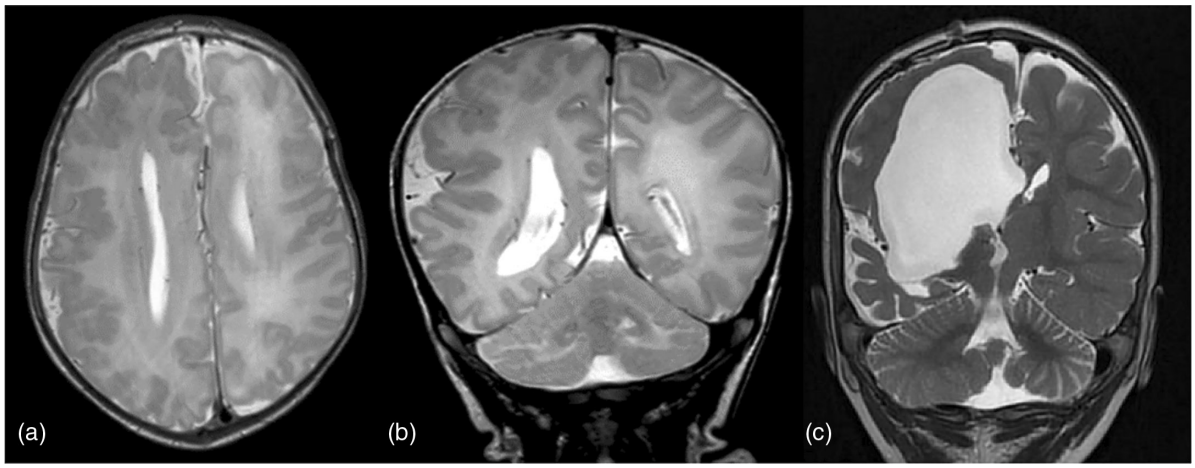

FIGURE 4 Clinical vignette 3: Magnetic resonance imaging (MRI) findings in *PIK3CA* (mosaic mutation). Brain MRI at 2 weeks of age. Axial (a) and coronal plane (b) showing right-sided hemimegalencephaly. (c) Coronal planes after hemispherotomy at age 22 months.

delay, facial hypertrichosis, cryptorchidism, and rocker-bottom feet. The eye examination showed microphthalmia, microcornea, and bilateral white cataract. MRI showed frontal predominant PMG (Figure 5). The combination of eye abnormalities, genital anomalies, developmental delay, and frontal PMG suggested a clinical diagnosis of Warburg Micro syndrome. This was confirmed by the identification of a homozygous c.52A > C (p.Thr18Pro) variant in *RAB3GAP1*.[22] Eye abnormalities are present in the neonatal period and provide a helpful clue to pinpoint the diagnosis.

Clinical vignette 5

A 14-year-old male was referred to our clinic for a genetic work-up. He had a moderate phenotype with bilateral sensorineural deafness, mild intellectual disability, and motor delay but with a complex brain malformation characterized by extensive frontal subcortical heterotopia and midline PMG, ventriculomegaly, partial agenesis of the corpus callosum, cerebellar dysplasia, and arachnoid cysts, as illustrated by Blauen et al.[23] The combination of sensorineural deafness and extensive brain lesions contrasting with the relatively mild disability pointed to Chudley–McCullough syndrome, which was confirmed by the identification of a homozygous c.742del variant in *GPSM2*. In addition to the sensorineural deafness, which provided an important clue to the diagnosis, this case also illustrates that the extent of the brain malformation does not always correlate well with the severity of the clinical phenotype and that the underlying aetiology most probably has a more important role in determining the clinical outcome.

Learning points for the neurological examination include the following: the disease course and long-term clinical outcome are often difficult to predict at an early stage, and medical management is rarely evidence-based; the severity of the clinical phenotype is impacted more by the underlying cause than by the extent of the abnormal cortex; when the

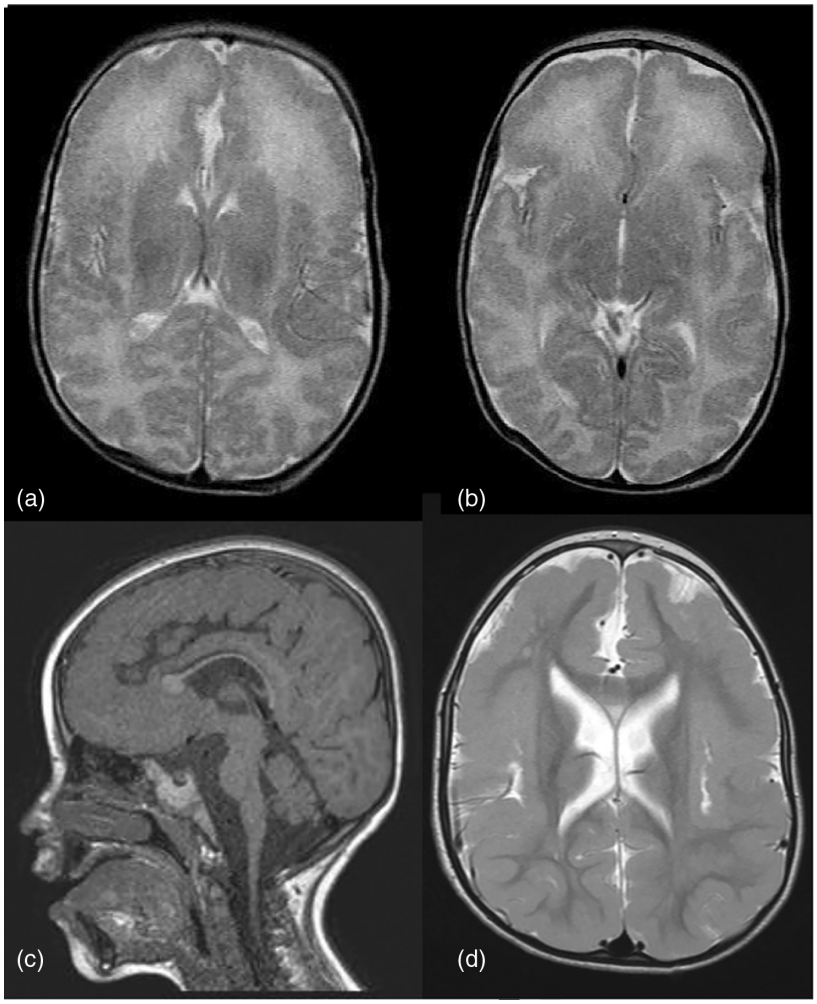

FIGURE 5 Clinical vignette 4: Magnetic resonance imaging (MRI) findings in Warburg Micro syndrome (*RAB3GAP*). (a,b) Brain MRI at age 2 weeks. Axial plane with bilateral frontally predominant polymicrogyria. (c,d) MRI of the same patient at 4 years of age. (c) Sagittal view showing hypoplastic corpus callosum. (d) Axial plane showing bilateral frontally predominant polymicrogyria.

clinical examination is suggestive, additional tests should be performed or specialized advice sought at an early stage in the diagnostic trajectory; signs of peripheral neurological symptoms should be actively sought because they might be overshadowed by the global presentation, especially in severely affected individuals.

Imaging

MRI is the preferred imaging modality in individuals with developmental delay, microcephaly, or epilepsy. Subtle anomalies, such as localized PMG, focal cortical dysplasia, and band heterotopia are sometimes difficult to distinguish. Diffusor tensor imaging and functional MRI can further characterize white matter organization and its perturbation in the case of MCD.[24] The combination of expert evaluation of MRI scans followed by targeted analysis of the most plausible causative variants can increase the diagnostic yield considerably.[4] Some MRI findings are pathognomonic for specific genetic causes and can be recognized by every neurologist. Examples include tubulinopathies, lissencephaly, and subcortical band heterotopia caused by pathogenic variants in *PAFAH1B1* and *DCX*, periventricular nodular heterotopia (PVNH) caused by pathogenic variants in *FLNA*, and tuberous sclerosis complex (TSC) caused by pathogenic variants in *TSC1* or *TSC2*. Knowledge of these recognizable phenotypes facilitates the genetic work-up.

Tubulinopathies (Figure 6) are characterized by an abnormal cortex that can range from dysgyria to classical lissencephaly. Dysgyria refers to a cortex of variable thickness and a smooth grey–white boundary but with an abnormal gyral pattern characterized by irregularities in sulcal depth or orientation.[25,26] Basal ganglia are often abnormal and have a bulbous caudate nucleus and a thalamus with diffuse, branched, or absent anterior limb of the internal capsule. Consequently, the lateral ventricles have an abnormal rounding of the frontal horns.[7] The corpus callosum in tubulinopathies can range from complete agenesis to completely normal. Cerebellar and brainstem hypoplasia are also a common finding in tubulinopathies.

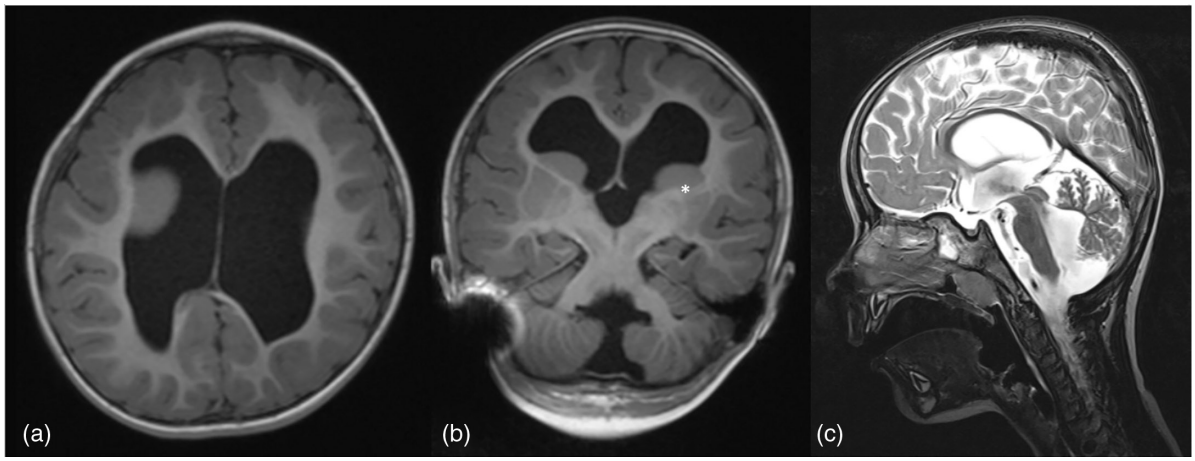

FIGURE 6 Magnetic resonance imaging (MRI) of tubulinopathy. Brain MRI at 3 years of age in an individual with a pathogenic *TUBB2B* variant. (a) Axial plane showing a dysgyric cortex most prominent in the right frontal region, and dysmorphic basal ganglia with hooked aspect of the frontal horns of the lateral ventricles. (b) Coronal plane showing dysgenesis of the anterior limb of the internal capsule (asterisk). (c) Sagittal plane showing a thin and dysplastic corpus callosum, and hypoplasia of the pons and cerebellar vermis.

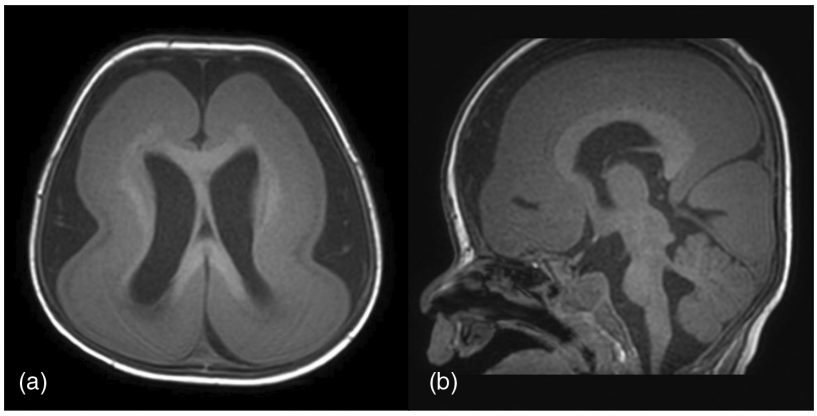

FIGURE 7 Magnetic resonance imaging (MRI) of lissencephaly (*PAFAH1B1*). Brain MRI at 7 months of age in an individual with a pathogenic *PAFAH1B1* variant. Axial (a) and sagittal (b) planes showing complete agyria with enlarged sylvian fissure ('figure of eight' configuration), intact corpus callosum, and mild brainstem hypoplasia.

In rare cases, imaging abnormalities can be limited to cerebellar vermis dysplasia and mild dysgyria (Figure 2g–i).[26] Genes associated with tubulinopathies include *TUBA1A*, *TUBB*, *TUBB2A*, *TUBB2B*, *TUBB3*, and *TUBG1*; each has subtle differences in phenotype.[27] Pathogenic variants in *TUBA1A* give rise to dysplasia of the basal ganglia and dysgyria, but a notable exception are the p.Arg402Cys and p.Arg402His variants, which result in (microcephaly) lissencephaly (approximately 40% of cases involving the *TUBA1A* gene).[7,28] *TUBB3* variants can show distinct symptoms, such as congenital fibrosis of the extraocular muscles, facial weakness, facial dysmorphism, and progressive peripheral neuropathy.[29] Pathogenic *TUBG1* variants result in 2 of 3 cases of pachygyria.[30] Differential diagnosis of tubulin phenotypes involve variants in genes encoding for molecular motors, such as kinesins (*KIF2A*, *KIF5C*, and *KIF1A*) and the heavy chain of dynein (*DYNC1H1*), which can mimic tubulinopathies.[7]

The lissencephaly spectrum includes classical lissencephaly, pachygyria, and subcortical band heterotopia. Lissencephaly is hallmarked by a thickened cortex with reduced or absent formation of cerebral convolutions. *PAFAH1B1* (*LIS1*) and *DCX* were discovered in the 1990s and were revealed as the two most common causes of lissencephaly. To date, more than 20 genes are associated with the lissencephaly spectrum.[6] Pathogenic variants in *PAFAH1B1* are associated with diffuse and posterior predominant forms of classical lissencephaly (Figure 7), while pathogenic variants in *DCX* result in anterior predominant lissencephaly in males and lead to subcortical band heterotopia in females (Figure 8).[3] Important differential diagnoses for the lissencephaly phenotype are variants in *TUBA1A* and *TUBG1*, although these are often associated with other features suggestive of a tubulinopathy.[30]

In PVNH, neurons fail to migrate from the ventricular zone to the developing cortex and accumulate as nodules

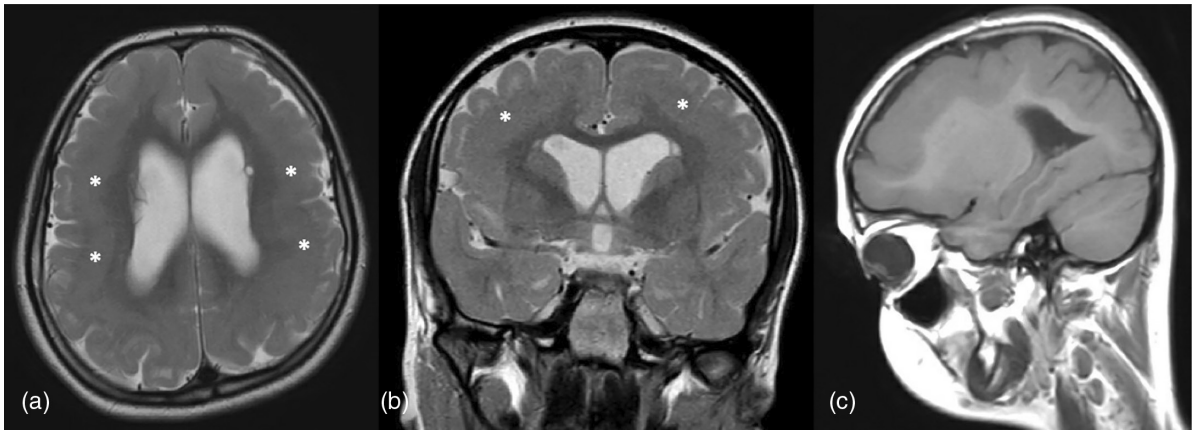

FIGURE 8 Magnetic resonance imaging (MRI) of lissencephaly (*DCX*). Brain MRI at 14 years of age in an individual with a pathogenic *DCX* variant. Subcortical band heterotopia in the axial (a) (asterisks), coronal (b) (asterisks), and sagittal (c) planes. (c) Anterior to posterior gradient.

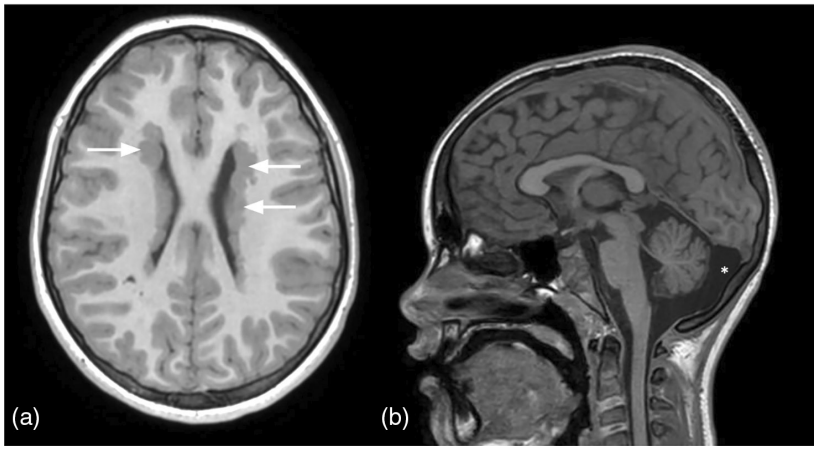

FIGURE 9 Magnetic resonance imaging (MRI) of periventricular nodular heterotopia (PVNH) (*FLNA*). Brain MRI at 10 years of age in an individual with a pathogenic *FLNA* variant. (a) Axial plane showing bilateral extended PVNH (arrows). (b) Sagittal plane showing mega cisterna magna (asterisk).

along the surface of the lateral ventricles.[31] Heterozygous pathogenic loss-of-function variants in *FLNA* are the most common cause of anterior predominant bilateral PVNH, especially when associated with mega cisterna magna and a hypoplastic or absent corpus callosum (Figure 9).[32,33] Variants in *FLNA* are frequently associated with extracerebral findings, including cardiac valve disease, thoracic aortic aneurysm, patent ductus arteriosus, joint hyperextensibility, chronic constipation, chronic obstructive lung disease, or coagulopathy.[32]

TSC is a genetic disorder that causes tumours to form in many different organs, primarily in the brain (Figure 10), eyes, heart, kidney, skin, and lungs. Clinical expression is age-dependent and highly variable. Aspects of TSC that most strongly impact quality of life are generally associated with the brain, including cortical tubers, which contribute to the development of epilepsy, subependymal giant cell astrocytoma, and TSC-associated neuropsychiatric disorders. TSC results from a pathogenic variant in *TSC1* or *TSC2* that can be inherited or de novo. TSC should be managed in multidisciplinary fashion. According to the International TSC Consensus Guidelines, special attention is required for epilepsy management (antiseizure medication, epilepsy surgery) and for surveillance of subependymal giant cell astrocytoma on brain MRI, renal cysts and angiomyolipomas on abdominal MRI, cardiac rhabdomyomas on echocardiography, lymphangioleiomyomatosis, and TSC-associated neuropsychiatric disorders.[34]

The aspect of the cortical malformation may change as myelination progresses, as illustrated in Figure 5. If imaging is performed before age 2 years and work-up remains negative, it might be helpful to repeat MRI after age 2 years 6 months, when most of the myelination is completed.[4]

Because MCDs are a frequent cause of drug-resistant focal epilepsy, the option of epilepsy surgery should be explored, especially for focal cortical dysplasia, hemimegalencephaly, and TSC-related epilepsy, and to a lesser extent for selected cases of PMG or heterotopia. Depending on the lesion, the

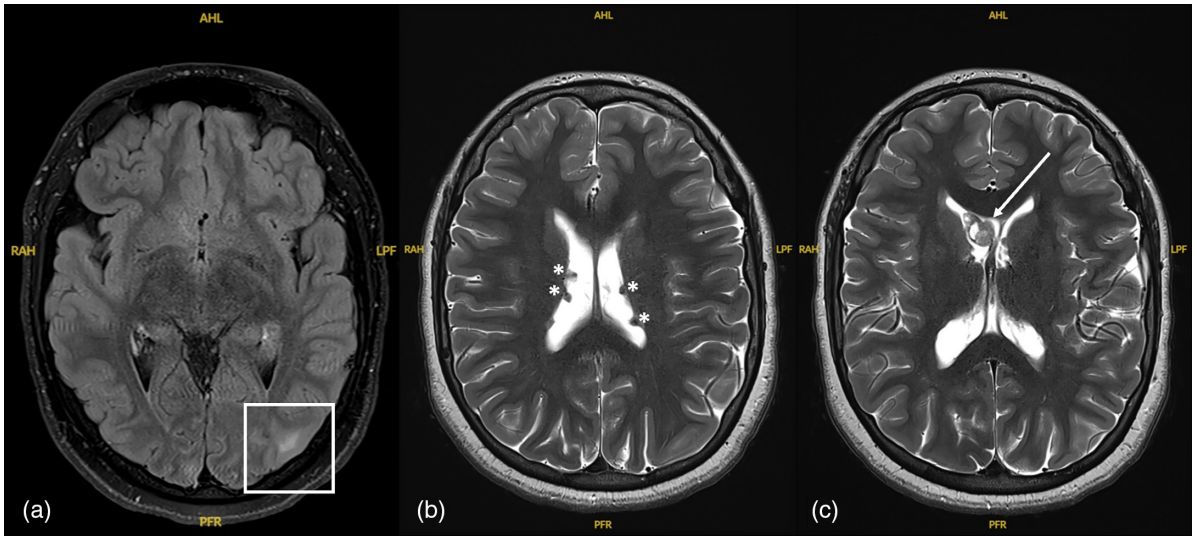

FIGURE 10 Magnetic resonance imaging (MRI) of tuberous sclerosis complex. Brain MRI at 15 years of age in a female with a mosaic contiguous gene deletion of *TSC2* and *PKD1*. (a) Axial plane with cortical tuber (square). (b) Axial plane with subependymal nodules (asterisks). (c) Axial plane with subependymal giant cell astrocytoma (arrow).

presurgical work-up can benefit from magnetoencephalography, fluorodeoxyglucose-positron emission tomography, single-photon emission computed tomography, electrocorticography, or invasive EEG monitoring.[35]

Learning points for imaging include the following: in a child with developmental delay, microcephaly, or epilepsy MRI is the first-tier brain imaging modality; subtle anomalies can be missed at the MRI review. When doubtful, an expert should be consulted; the diagnostic yield of targeted testing is determined to a large extent by the availability of a multidisciplinary evaluation with input from an expert neuroradiologist; some MCD imaging patterns are easily recognizable and may provide anchor points for genetic testing; the extent of the MCD on imaging does not always correlate directly with the severity of the clinical phenotype; imaging findings (generalized vs focal, bilateral vs unilateral malformations) cannot reliably distinguish genetic from nongenetic causes; the imaging aspects of MCD might change with age, especially between birth and 2 years 6 months of age. When the work-up remains negative, one might consider repeating the MRI after age 2 years 6 months.

EXCLUDING NON-GENETIC CAUSES OF MCD

Congenital viral infections interfere with several developmental processes, for example, myelination, migration, or cortical organization. Findings on neuroimaging are variable and depend on gestational age at the time of the initial infection and the selective affinity of the infectious agents for different brain structures. Cytomegalovirus, but also other members of the toxoplasmosis, others (syphilis, hepatitis B), rubella, cytomegalovirus, herpes simplex (TORCH) family, are important causes of MCD.

Cytomegalovirus is the most frequent TORCH infection, affecting 0.4% to 1% of all pregnancies, of which 10% are symptomatic at birth.[36,37] Common clinical features include jaundice, petechiae, poor feeding, hepatosplenomegaly, sensorineural hearing loss, and brain abnormalities on MRI. Microcephaly or calcifications (or both) are present in up to 70% of symptomatic cases. Cortical malformations depend on the timing of the infection; the earlier the infection, the more severe the phenotype.[36,37] Additional neuroimaging findings in congenital cytomegalovirus infection include ventriculomegaly, abnormal white matter signal intensity, which is located in the temporal lobes and represents delayed or deficient myelination, cysts in the anterior portion of the temporal lobes, and cerebellar hypoplasia[38] (Figure 11). The most important risk factor for seroconversion during pregnancy is contact with young children, especially those attending day care.[36,37]

TORCH infections are also spread by animals and insects, such as toxoplasmosis by feline feces and Zika virus by mosquitoes, the latter reflected by the dramatic epidemic in South America in 2015 to 2016.[39,40] More than 3500 children were born with profound microcephaly after congenital Zika virus infection. Guerrero et al.[41] recently published a systematic review on teratogenesis, congenital anomalies, and child mortality after congenital Zika virus infection. The most severe brain malformations are related to infections during the first trimester of gestation, including profound microcephaly, hydrocephalus, almost complete agyria, holoprosencephaly, and multiple calcifications in the cortex and subcortical white matter.[42]

In general, to exclude TORCH syndromes, MRI is more sensitive compared to ultrasonography.[38]

MCD in combination with white matter anomalies and calcifications are highly evocative of TORCH, although sometimes things are not what they seem to be.

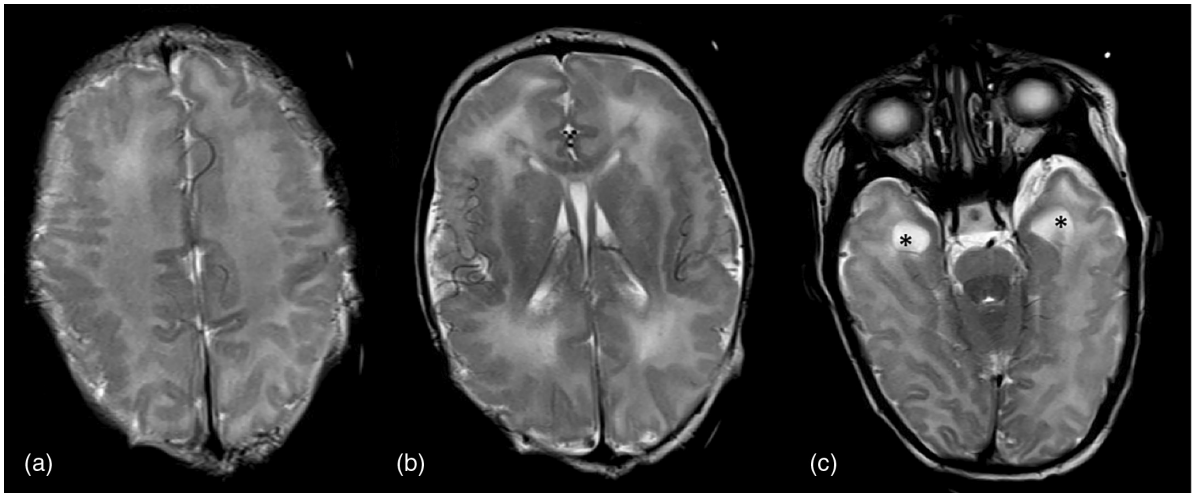

FIGURE 11 Magnetic resonance imaging (MRI) in an infant with cytomegalovirus (CMV). Brain MRI of an infant with congenital CMV infection at 3 days of age. (a,b) Axial planes showing bilateral diffuse polymicrogyria and T2-weighted white matter hyperintensity. (c) Axial plane showing cystic changes of the temporal poles (asterisks).

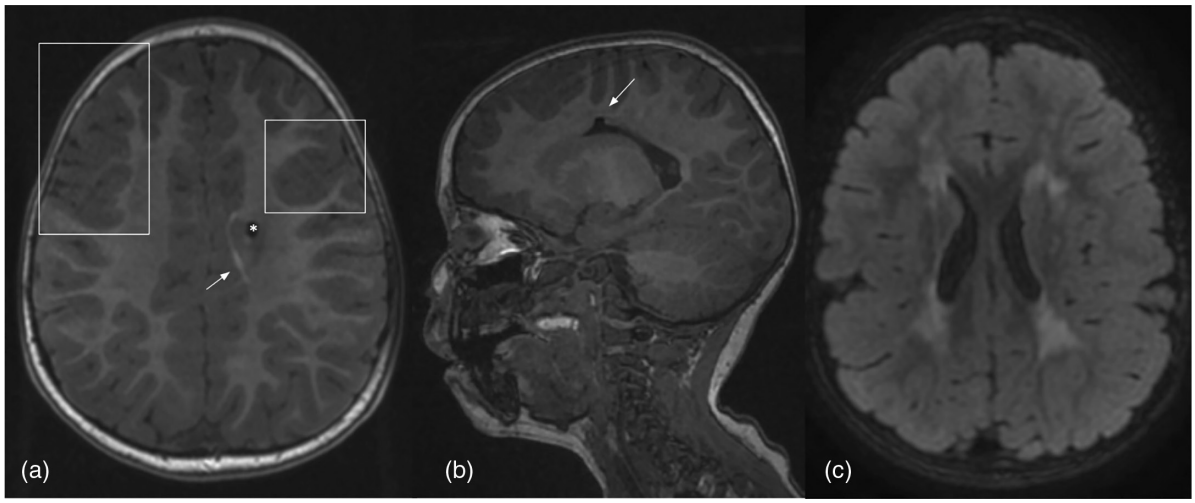

FIGURE 12 Clinical vignette 6: Magnetic resonance imaging (MRI) findings associated with a pathogenic variant in *COL4A1*. Brain MRI at age 2.5 years. Axial (a,c) and sagittal (b) planes showing a small porencephalic cyst (asterisk in [a], arrow in [b]) with adjacent linear calcification (arrow in [a]), bilateral frontal focal polymicrogyria (square), and diffuse T1-weighted hyperintense white matter (c).

Clinical vignette 6

A second child, born after an uneventful pregnancy and delivery, presented with hypotonia, bilateral cataracts, and a 32-cm OFC (–1.5 SD). Both parents were healthy and non-consanguineous. Transcranial ultrasound showed calcifications. MRI of the brain showed periventricular leukomalacia, a small porencephalic cyst with adjacent linear calcification, and areas of PMG in both frontal lobes (Figure 12). TORCH screening was negative. At the age of 18 months, the child developed drug-resistant focal seizures and had moderate developmental delay. A de novo c.3548G > T pathogenic missense variant was found in *COL4A1* and linked to the patient's phenotype.[43]

This case is an example of pseudo-TORCH syndrome. Pseudo-TORCH syndromes generally present with profound microcephaly, white matter changes, PMG, calcifications, and developmental delay, mimicking TORCH.[38] Another example is Aicardi–Goutières syndrome. Aicardi–Goutières syndrome typically presents in infancy with irritability, poor feeding, progressive microcephaly, spasticity, and dystonia; death often occurs in early childhood.[44] Unlike congenital viral infections, inherited disorders typically have a progressive clinical course.

Besides congenital infections, external factors, such as maternal drug ingestion and vascular events during pregnancy, can cause MCD, as illustrated in clinical vignette 7.

Clinical vignette 7

A monochorionic, diamniotic twin was born by Caesarean section at 32 weeks of gestation because of a twin anaemia polycythaemia sequence and distress of fetus 1. Both twins had a good start, with partial exchange transfusion for polycythaemia (haematocrit of 71%) in fetus 1. Brain MRI in the context of preterm birth showed unilateral perisylvian

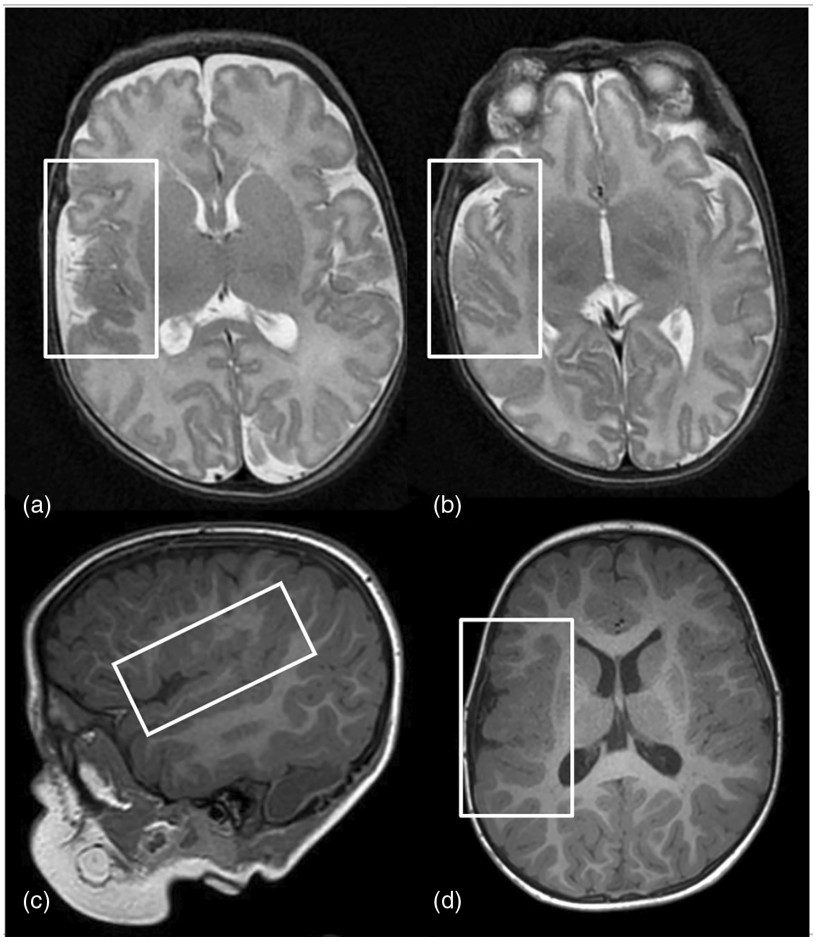

FIGURE 13 Clinical vignette 7: Magnetic resonance imaging (MRI) findings in a twin anaemia polycythaemia sequence. (a,b) MRI at age 6 weeks (2 weeks' corrected age). (c,d) MRI at 2 years 6 months of age in the same individual. The rectangles indicate limited asymmetrical, right-sided perisylvian polymicrogyria in the sagittal (c) and axial (d) planes.

PMG in fetus 1 (Figure 13). The brain MRI of fetus 2 was normal. Screening for cytomegalovirus and other TORCH was negative. Early vascular events in utero have been linked to PMG.[45] Other examples are fetal cerebral ischaemia from placental perfusion failure, twin–twin transfusion, loss of a twin in utero, and maternal drug ingestion.[46]

Learning points for non-genetic causes of MCD include the following: exclusion of congenital TORCH infection, especially in microcephaly or PMG; checking for other external factors during pregnancy (importance of a family history); remembering that pseudo-TORCH syndromes may mimic congenital infections and should be considered when MRI is evocative of TORCH and screening remains negative.

GENETIC TESTING

Conventional genetic testing can be divided into four types: (1) chromosomal microarray analysis; (2) targeted gene testing; (3) gene panels; and (4) open exome analysis.

Chromosomal microarray analysis is the first-tier genetic test in MCD.[4] MCDs have been linked to a wide range of copy number variations. Several copy number variations are consistently associated with MCD, the most common being the 22q11 and 1p36 deletions associated with PMG, the 17p13.3 deletion (encompassing *PAFAH1B1*, *YWHAE*, and other genes) causing Miller–Dieker syndrome and isolated lissencephaly, and the 6qter deletions associated with several brain malformations, including PMG and PVNH. Several MCD-related genes frequently harbour intragenic deletions or duplications, which might be identified using standard microarrays.[17]

Clinical vignette 8

A 6-week-old infant presented with prenatally confirmed intrauterine growth restriction and lissencephaly (complete agyria) with bilateral ventriculomegaly on fetal ultrasound. At birth, she had severe axial hypotonia and facial dysmorphism but further work-up was declined by the parents. At age 5 months, she developed epileptic spasms, and had severe developmental delay and increased muscle tone in the limbs. MRI confirmed the presence of lissencephaly (Figure 14). The combination of lissencephaly and facial dysmorphism was evocative of Miller–Dieker syndrome,[47,48] which was confirmed by the identification of a heterozygous 17p13.3 deletion involving the *YWHAE*, *HIC1*, *PAFAH1B1*, and *KIAA0664* genes.

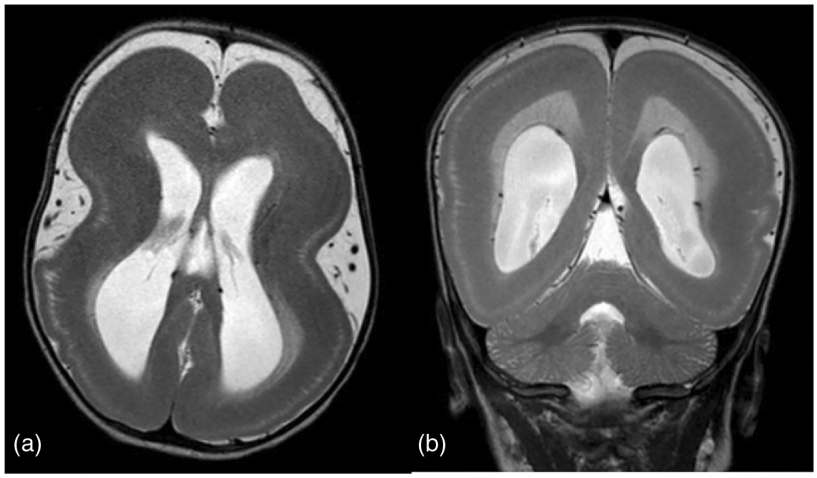

FIGURE 14 Clinical vignette 8: Magnetic resonance imaging (MRI) findings in Miller–Dieker syndrome. Brain MRI at 6 months of age in an individual with Miller–Dieker syndrome. Axial (a) and coronal (b) planes showing complete agyria with posterior predominant cell sparse layer. Note the normally formed cerebellum.

The parents did not agree with further genetic counselling; 2 years later, they had a second child with the same condition. Although Miller–Dieker syndrome usually occurs de novo, the deletion can be inherited from a parent with a balanced chromosomal translocation, as was the case in this family.

When clinical features are pathognomonic for a single-gene disorder, targeted testing by single-gene polymerase chain reaction or multiplex ligation-dependent probe amplification analysis can be used as a first-tier genetic test. Targeted testing relies on clinical experience and extensive knowledge of genetic phenotypes. When gene panels are available, targeted testing is usually not cost-efficient. However, in low-resource settings or when a variant is suspected in a gene that has not yet been included in the MCD panel, single-gene testing can remain relevant.

Clinical vignette 9

A child presented with severe neurodevelopmental delay, refractory seizures, bilateral PVNH, perisylvian PMG, and syndactyly. Three other family members with mild developmental delay, isolated PVNH, and syndactyly were identified, showing a high degree of intrafamilial phenotypic variability. The MCD panel testing was negative. PVNH and PMG in combination with syndactyly were evocative of *NEDD4L* syndrome. Because *NEDD4L* was not yet included in the MCD panel, targeted testing was performed and a heterozygous c.623G > A missense variant segregating with the phenotype was identified.[49]

When microarray analysis is negative, MCD panel analysis is the next step in the diagnostic process. MCD gene panels are compiled by local molecular and clinical geneticists and differences exist between genetic centres. For example, the MCD panel at the UZ Brussel consists of 235 different genes linked to MCD (https://www.brightcore.be/gene-panels). Preferentially, panels are exome-based because they can be more easily adjusted according to the latest literature and provide the possibility to convert to open whole-exome sequencing analysis when panel analysis is negative. Within the Neuro-MIG network, targeted gene panels resulted in diagnostic yields of 15% to 37%, although wide variability was observed among the different clinical MCD subtypes. The yield was highest for lissencephaly (75%–81%), followed by cobblestone malformation (75%), PVNH (30%–37%), PMG (20%), and microcephaly (18%–20%).[4]

When the MCD panel analysis is negative, open whole-exome sequencing analysis in trio is an option, when available. To date, there is little information on the diagnostic yield of open whole-exome sequencing analysis in MCD.

When interpreting the results of any genetic test, one should always question whether the variant is compatible with the patient's phenotype. If not, the diagnostic process should be continued. Learning points for genetic testing include the following: genes included in MCD panels differ between genetic centres; panels need to be updated regularly to integrate the latest knowledge on the genetic basis of MCD; not all centres can call for indels when analysing an MCD panel; the diagnostic yield differs between MCD subtypes; the combination of expert evaluation of MRI scans followed by targeted analysis of the most plausible causative variants can considerably increase the diagnostic yield; when a variant is identified, its relevance should be checked in light of the clinical findings; deletions can be inherited from a parent with a balanced chromosomal translocation; genetic counselling is always indicated to discuss potential recurrence risk.

WHAT'S NEXT?

In some cases, no class 4 or 5 variant in an annotated MCD gene can be identified. In some cases, it is possible to identify a variant of unknown significance (VOUS) in a (candidate) MCD gene or to identify a pathogenic variant in a gene of

unknown significance. The possible options and next steps are discussed in the next sections.[4]

VOUS in a (candidate) MCD gene

Discuss if the VOUS could be responsible for the patient's phenotype during a multidisciplinary review, ideally attended by the treating physician, clinical and molecular geneticists, and the neuroradiologist. When the variant is a good candidate, segregation studies can be considered in the parents and other family members. For example, when a VOUS in a gene with a dominant expression is inherited from an unaffected parent, the pathogenicity and clinical relevance of this variant is less likely (unless the parent has a mild phenotype). When segregation studies are compatible, assess if the VOUS is a good candidate for further research. Search for additional patients with a variant in same gene. You can reach out to your international contacts or use international platforms such as GeneMatcher (https://genematcher.org/). Reach out to colleagues with expertise in research and collaborate where possible to perform functional studies that can evaluate the pathogenicity of variants and map the function of genes. The same strategy is applicable for pathogenic variants in genes of unknown significance.

Clinical vignette 10

A male with five healthy siblings presented with interhemispheric cysts, agenesis of the corpus callosum, and vermis hypoplasia on prenatal ultrasound. The postnatal MRI showed type 2C interhemispheric cysts, extensive subcortical heterotopia, PMG, complete agenesis of the corpus callosum, malrotation of the hippocampus, and hypoplasia of the brainstem and cerebellum (Figure 15). On examination at age 18 years, he had macrocephaly, had retrognathia, and a cleft in the left earlobe. He could express himself, count to 50, and read simple phrases. There were no behavioural challenges. He had dystonic bilateral (quadriplegia) CP, which was more pronounced on the left, but he was able to walk independently. Whole-exome sequencing showed a new homozygous c.607G > A class 3 missense variant in *MAN2C1*, previously linked to glycan catabolism and apoptotic signalling,[50] and was considered a potential candidate gene for MCD. Through GeneMatcher, three additional families with five affected individuals with biallelic variants in *MAN2C1* were identified. Phenotypes were compared and the effect of the different variants on protein stability and functionality was assessed. *MAN2C1* was proposed as an important factor in brain development and MCD and the variant was upgraded to class 5.[51]

When all previous strategies have failed

Helpful strategies include re-evaluation and expert discussion of clinical and MRI findings. Depending on the clinical presentation and if not performed before, consider karyotyping, metabolic screening, serum creatine kinase, electromyography, or muscle biopsy. When brain MRI is performed before the age of 2 years 6 months, it might be of value to repeat the MRI.[4]

Consider mosaicism and genetic testing in alternative tissues and targeted deep sequencing when the phenotype is suggestive for particular genes and syndromes. Mosaic (postzygotic somatic) mutations have been described in a wide range of MCDs (clinical vignettes 1, 2, and 3). Mosaic variants are frequently missed by standard coverage exon sequencing, especially in blood-derived DNA. Skin fibroblasts and saliva are more sensitive tissues for different neurological pathways. Ideally, multiple tissues from the same individual should be examined. When brain tissue is available (e.g. after surgery), then this is the first choice.[52] In consanguineous couples, consider homozygosity mapping using, for example, a single-nucleotide polymorphism-based chromosomal microarray analysis. Homozygosity mapping maps autosomal recessive traits in consanguineous families and can help to identify good candidate genes.[53,54]

Including the patient in a (local) research programme is another option. Patients without a diagnosis should be

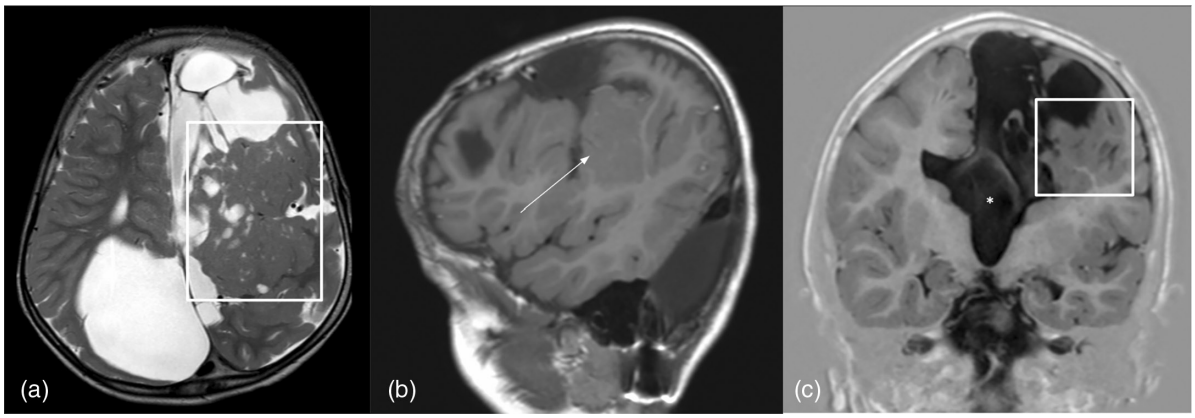

FIGURE 15 Clinical vignette 10: Magnetic resonance imaging (MRI) findings in *MAN2C1*. Brain MRI at 7 years of age showing complex brain malformation with extensive subcortical heterotopia (square) in the axial plane (a), polymicrogyria (arrow) in the sagittal plane (b), and complete agenesis of the corpus callosum and type 2C interhemispheric cysts (asterisk) (c).

considered for trio-based, whole-genome, and RNA sequencing, preferably within a large collaborative research network to allow rapid discovery of new causative variants, non-coding variants in regulatory elements, and epigenetic variations.[55–57]

In the years after the initial genetic work-up, new genes will be annotated as causes of MCD and re-analysis of the patient's exome data might uncover a causal variant in a gene with previously unknown function or not yet linked to MCD. A 2-year interval is suggested for re-analysis.[4]

CONCLUSION

In this review, we discussed the clinical work-up plan for individuals with MCD, highlighting the importance of a detailed (neurological) examination with special attention paid to dysmorphic features, skin abnormalities, and signs of potential peripheral nervous system involvement. Before proceeding with genetic testing, acquired causes of MCD should be ruled out, especially in the presence of microcephaly or PMG. We highlighted the value of expert neuroradiological evaluation to assist with variant interpretation. When a variant is identified, its relevance should be critically questioned in light of the clinical presentation. When a genetic diagnosis is established, genetic counselling is indicated and recurrence risk should be discussed with attention to potential germline mosaicism or balanced translocations. When results remain negative despite systematic and extensive work-up, reaching out for collaboration with clinicians and researchers is the best way forward.

ACKNOWLEDGEMENTS

Ellen Rijckmans is funded by a research fellowship from the Marguerite-Marie Delacroix Foundation. Anna Jansen is supported by a Senior Clinical Investigator fellowship of the Research Foundation Flanders (FWO 1805321 N).

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Ellen Rijckmans https://orcid.org/0000-0001-8258-5695
Anna C. Jansen https://orcid.org/0000-0002-3835-2824

REFERENCES

1. Pasquier B, Peoc HM, Fabre-Bocquentin B, Bensaadi L, Pasquier D, Hoffmann D, et al. Surgical pathology of drug-resistant partial epilepsy. A 10-year-experience with a series of 327 consecutive resections. Epileptic Disord. 2002;4(2):99–119.
2. Barkovich AJ, Guerrini R, Kuzniecky RI, Jackson GD, Dobyns WB. A developmental and genetic classification for malformations of cortical development: update 2012. Brain. 2012;135(Pt 5):1348–69.
3. Severino M, Geraldo AF, Utz N, Tortora D, Pogledic I, Klonowski W, et al. Definitions and classification of malformations of cortical development: practical guidelines. Brain. 2020;143(10):2874–94.
4. Oegema R, Barakat TS, Wilke M, Stouffs K, Amrom D, Aronica E, et al. International consensus recommendations on the diagnostic work-up for malformations of cortical development. Nat Rev Neurol. 2020;16(11):618–35.
5. Guerrini R, Dobyns WB. Malformations of cortical development: clinical features and genetic causes. Lancet Neurol. 2014;13(7):710–26.
6. Koenig M, Dobyns WB, Di Donato N. Lissencephaly: Update on diagnostics and clinical management. Eur J Paediatr Neurol. 2021;35:147–52.
7. Maillard C, Roux CJ, Charbit-Henrion F, Steffann J, Laquerriere A, Quazza F, et al. Tubulin mutations in human neurodevelopmental disorders. Semin Cell Dev Biol. 2023;137:87–95.
8. Straka B, Hermanovska B, Krskova L, Zamecnik J, Vlckova M, Balascakova M, et al. Genetic Testing for Malformations of Cortical Development: A Clinical Diagnostic Study. Neurol Genet. 2022;8(5):e200032.
9. Rijckmans E, Stouffs K, Jansen AC, Brock S. Malformations of cerebral development and clues from the peripheral nervous system: A systematic literature review. Eur J Paediatr Neurol. 2022;37:155–64.
10. Devisme L, Bouchet C, Gonzales M, Alanio E, Bazin A, Bessieres B, et al. Cobblestone lissencephaly: neuropathological subtypes and correlations with genes of dystroglycanopathies. Brain. 2012;135(Pt 2):469–82.
11. Fallet-Bianco C, Laquerriere A, Poirier K, Razavi F, Guimiot F, Dias P, et al. Mutations in tubulin genes are frequent causes of various foetal malformations of cortical development including microlissencephaly. Acta Neuropathol Commun. 2014;2:69.
12. Friocourt G, Marcorelles P, Saugier-Veber P, Quille ML, Marret S, Laquerriere A. Role of cytoskeletal abnormalities in the neuropathology and pathophysiology of type I lissencephaly. Acta Neuropathol. 2011;121(2):149–70.
13. Najm I, Lal D, Alonso Vanegas M, Cendes F, Lopes-Cendes I, Palmini A, et al. The ILAE consensus classification of focal cortical dysplasia: An update proposed by an ad hoc task force of the ILAE diagnostic methods commission. Epilepsia. 2022;63(8):1899–919.
14. Brock S, Cools F, Jansen AC. Neuropathology of genetically defined malformations of cortical development-A systematic literature review. Neuropathol Appl Neurobiol. 2021;47(5):585–602.
15. Jansen AC, Robitaille Y, Honavar M, Mullatti N, Leventer RJ, Andermann E, et al. The histopathology of polymicrogyria: a series of 71 brain autopsy studies. Dev Med Child Neurol. 2016;58(1):39–48.
16. Zillhardt JL, Poirier K, Broix L, Lebrun N, Elmorjani A, Martinovic J, et al. Mosaic parental germline mutations causing recurrent forms of malformations of cortical development. Eur J Hum Genet. 2016;24(4):611–4.
17. Jansen AC, Oostra A, Desprechins B, De Vlaeminck Y, Verhelst H, Regal L, et al. TUBA1A mutations: from isolated lissencephaly to familial polymicrogyria. Neurology. 2011;76(11):988–92.
18. Stutterd CA, Dobyns WB, Jansen A, Mirzaa G, Leventer RJ. Polymicrogyria Overview. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, et al., editors. GeneReviews(®). Seattle (WA) 1993.
19. Dobyns WB, Mirzaa GM. Megalencephaly syndromes associated with mutations of core components of the PI3K-AKT-MTOR pathway: PIK3CA, PIK3R2, AKT3, and MTOR. Am J Med Genet C Semin Med Genet. 2019;181(4):582–90.
20. Grasby KL, Jahanshad N, Painter JN, Colodro-Conde L, Bralten J, Hibar DP, et al. The genetic architecture of the human cerebral cortex. Science. 2020;367(6484).
21. Bizzotto S, Walsh CA. Genetic mosaicism in the human brain: from lineage tracing to neuropsychiatric disorders. Nat Rev Neurosci. 2022;23(5):275–86.
22. Bem D, Yoshimura S, Nunes-Bastos R, Bond FC, Kurian MA, Rahman F, et al. Loss-of-function mutations in RAB18 cause Warburg micro syndrome. Am J Hum Genet. 2011;88(4):499–507.
23. Blauen A, Stutterd CA, Stouffs K, Dumitriu D, Deggouj N, Lockhart PJ, et al. Chudley-McCullough Syndrome: A Recognizable Clinical Entity Characterized by Deafness and Typical Brain Malformations. J Child Neurol. 2021;36(2):152–8.

24. Le Bihan D, Mangin JF, Poupon C, Clark CA, Pappata S, Molko N, et al. Diffusion tensor imaging: concepts and applications. J Magn Reson Imaging. 2001;13(4):534–46.
25. Mutch CA, Poduri A, Sahin M, Barry B, Walsh CA, Barkovich AJ. Disorders of Microtubule Function in Neurons: Imaging Correlates. AJNR Am J Neuroradiol. 2016;37(3):528–35.
26. Oegema R, Cushion TD, Phelps IG, Chung SK, Dempsey JC, Collins S, et al. Recognizable cerebellar dysplasia associated with mutations in multiple tubulin genes. Hum Mol Genet. 2015;24(18):5313–25.
27. Bahi-Buisson N, Maillard C. Tubulinopathies Overview. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, et al., editors. GeneRevie®(R)). Seattle (WA) 1993.
28. Aiken J, Moore JK, Bates EA. TUBA1A mutations identified in lissencephaly patients dominantly disrupt neuronal migration and impair dynein activity. Hum Mol Genet. 2019;28(8):1227–43.
29. Whitman MC, Barry BJ, Robson CD, Facio FM, Van Ryzin C, Chan WM, et al. TUBB3 Arg262His causes a recognizable syndrome including CFEOM3, facial palsy, joint contractures, and early-onset peripheral neuropathy. Hum Genet. 2021;140(12):1709–31.
30. Brock S, Stouffs K, Scalai' E, D'Hooghe M, Keymolen K, Guerrini R, et al. Tubulinopathies continued: refining the phenotypic spectrum associated with variants in TUBG1. Eur J Hum Genet. 2018;26(8):1132–42.
31. Manent JB, Wang Y, Chang Y, Paramasivam M, LoTurco JJ. Dcx reexpression reduces subcortical band heterotopia and seizure threshold in an animal model of neuronal migration disorder. Nat Med. 2009;15(1):84–90.
32. Lange M, Kasper B, Bohring A, Rutsch F, Kluger G, Hoffjan S, et al. 47 patients with FLNA associated periventricular nodular heterotopia. Orphanet J Rare Dis. 2015;10:134.
33. Lu YT, Hsu CY, Liu YT, Chan CK, Chuang YC, Lin CH, et al. The clinical and imaging features of FLNA positive and negative periventricular nodular heterotopia. Biomed J. 2022;45(3):542–8.
34. Northrup H, Aronow ME, Bebin EM, Bissler J, Darling TN, de Vries PJ, et al. Updated International Tuberous Sclerosis Complex Diagnostic Criteria and Surveillance and Management Recommendations. Pediatr Neurol. 2021;123:50–66.
35. Jayakar P, Gaillard WD, Tripathi M, Libenson MH, Mathern GW, Cross JH, et al. Diagnostic test utilization in evaluation for resective epilepsy surgery in children. Epilepsia. 2014;55(4):507–18.
36. Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. Rev Med Virol. 2007;17(5):355–63.
37. Leruez-Ville M, Magny JF, Couderc S, Pichon C, Parodi M, Bussieres L, et al. Risk Factors for Congenital Cytomegalovirus Infection Following Primary and Nonprimary Maternal Infection: A Prospective Neonatal Screening Study Using Polymerase Chain Reaction in Saliva. Clin Infect Dis. 2017;65(3):398–404.
38. Nickerson JP, Richner B, Santy K, Lequin MH, Poretti A, Filippi CG, et al. Neuroimaging of pediatric intracranial inf—tion--part 2: TORCH, viral, fungal, and parasitic infections. J Neuroimaging. 2012;22(2):e52-63.
39. Kornacka-Stackonis A. Toxoplasma gondii infection in wild omnivorous and carnivorous animals in Central E—rope - A brief overview. Vet Parasitol. 2022;304:109701.
40. Santos LLM, de Aquino EC, Fernandes SM, Ternes YMF, Feres VCR. Dengue, chikungunya, and Zika virus infections in Latin America and the Caribbean: a systematic review. Rev Panam Salud Publica. 2023;47:e34.
41. Guerrero Saldivia SE, Unnikrishnan S, Chavarria YY, Akindele AO, Jalkh AP, Eastmond AK, et al. Zika Virus: A Systematic Review of Teratogenesis, Congenital Anomalies, and Child Mortality. Cureus. 2023;15(2):e34735.
42. Alvarado MG, Schwartz DA. Zika Virus Infection in Pregnancy, Microcephaly, and Maternal and Fetal Health: What We Think, What We Know, and What We Think We Know. Arch Pathol Lab Med. 2017;141(1):26–32.
43. Straka B, Vlckova M, Liba Z, Hermanovska B, Kyncl M, Dornakova J, et al. COL4A1 mutation-related disorder presenting as fetal intracranial bleeding, hydrocephalus, and polymicrogyria. Epilepsia Open. 2023;8(1):211–6.
44. Livingston JH, Crow YJ. Neurologic Phenotypes Associated with Mutations in TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR1, and IFIH1: Aicardi-Goutieres Syndrome and Beyond. Neuropediatrics. 2016;47(6):355–60.
45. Squier W, Jansen A. Polymicrogyria: pathology, fetal origins and mechanisms. Acta Neuropathol Commun. 2014;2:80.
46. Mittelbronn M, Beschorner R, Schittenhelm J, Capper D, Goeppert B, Meyermann R, et al. Multiple thromboembolic events in fetofetal transfusion syndrome in triplets contributing to the understanding of pathogenesis of hydranencephaly in combination with polymicrogyria. Hum Pathol. 2006;37(11):1503–7.
47. Dobyns WB, Stratton RF, Parke JT, Greenberg F, Nussbaum RL, Ledbetter DH. Miller-Dieker syndrome: lissencephaly and monosomy 17p. J Pediatr. 1983;102(4):552–8.
48. Blazejewski SM, Bennison SA, Smith TH, Toyo-Oka K. Neurodevelopmental Genetic Diseases Associated With Microdeletions and Microduplications of Chromosome 17p13.3. Front Genet. 2018;9:80.
49. Stouffs K, Verloo P, Brock S, Regal L, Beysen D, Ceulemans B, et al. Recurrent NEDD4L Variant in Periventricular Nodular Heterotopia, Polymicrogyria and Syndactyly. Front Genet. 2020;11:26.
50. Wang L, Suzuki T. Dual functions for cytosolic alpha-mannosidase (Man2C1): its down-regulation causes mitochondria-dependent apoptosis independently of its alpha-mannosidase activity. J Biol Chem. 2013;288(17):11887–96.
51. Maia N, Potelle S, Yildirim H, Duvet S, Akula SK, Schulz C, et al. Impaired catabolism of free oligosaccharides due to MAN2C1 variants causes a neurodevelopmental disorder. Am J Hum Genet. 2022;109(2):345–60.
52. Mirzaa G, Graham JM, Jr., Keppler-Noreuil K. PIK3CA-Related Overgrowth Spectrum. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, et al., editors. GeneRe®ws((R)). Seattle (WA) 1993.
53. Lander ES, Botstein D. Homozygosity mapping: a way to map human recessive traits with the DNA of inbred children. Science. 1987;236(4808):1567–70.
54. Seelow D, Schuelke M, Hildebrandt F, Nurnberg P. Homozygos—yMapper--an interactive approach to homozygosity mapping. Nucleic Acids Res. 2009;37(Web Server issue):W593-9.
55. Murdock DR, Dai H, Burrage LC, Rosenfeld JA, Ketkar S, Muller MF, et al. Transcriptome-directed analysis for Mendelian disease diagnosis overcomes limitations of conventional genomic testing. J Clin Invest. 2021;131(1).
56. Kremer LS, Bader DM, Mertes C, Kopajtich R, Pichler G, Iuso A, et al. Genetic diagnosis of Mendelian disorders via RNA sequencing. Nat Commun. 2017;8:15824.
57. Perenthaler E, Yousefi S, Niggl E, Barakat TS. Beyond the Exome: The Non-coding Genome and Enhancers in Neurodevelopmental Disorders and Malformations of Cortical Development. Front Cell Neurosci. 2019;13:352.

How to cite this article: Rijckmans E, Stouffs K, Jansen AC. Diagnostic work-up in malformations of cortical development. Dev Med Child Neurol. 2024;00:1–16. https://doi.org/10.1111/dmcn.15882