

Megalencephaly and Macrocephaly

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Abstract

Megalencephaly is a developmental disorder characterized by brain overgrowth secondary to increased size and/or numbers of neurons and glia. These disorders can be divided into metabolic and developmental categories based on their molecular etiologies. Metabolic megalencephalies are mostly caused by genetic defects in cellular metabolism, whereas developmental megalencephalies have recently been shown to be caused by alterations in signaling pathways that regulate neuronal replication, growth, and migration. These disorders often lead to epilepsy, developmental disabilities, and behavioral problems; specific disorders have associations with overgrowth or abnormalities in other tissues. The molecular underpinnings of many of these disorders are now understood, providing insight into how dysregulation of critical pathways leads to disease. The advances in molecular understanding are leading to improved diagnosis of these conditions, as well as providing new avenues for therapeutic interventions.

Keywords

- ▶ megalencephaly
- ▶ hemimegalencephaly
- ▶ macrocephaly
- ▶ somatic mutation

Megalencephaly is defined as a condition in which the size or weight of the brain is greater than two standard deviations above the age-related mean.¹ This is in contrast to macrocephaly, which is defined based on increased orbitofrontal head circumference for age. The head circumference can be influenced by many factors other than the size of the brain, including skull size, subdural fluid collections, and ventricular size. Therefore, megalencephaly is a more specific term related to dysfunction in neurons or glia causing an abnormal size or number of these cells. Another major distinction is that macrocephaly can be isolated and benign, such as in benign familial macrocephaly, whereas megalencephaly is more often syndromic and unlikely to be benign.¹ In particular, megalencephaly is often associated with developmental disabilities and epilepsy, which can be medically refractory. In clinical practice, the distinction between megalencephaly and macrocephaly relies on neuroimaging studies to identify enlarged cerebral structures or associated anomalies (▶ **Fig. 1**). However, distinguishing these conditions is important clinically in terms of diagnosis, further testing, and overall prognosis for the patient and family.²

Classically, megalencephaly has been divided into two categories: metabolic and anatomic.¹ The metabolic megalencephalies encompass multiple disorders featuring accumulation of abnormal metabolites, whereas anatomic megalencephaly was described as increased size and/or number of cells without an identifiable metabolic abnormality. Both forms of megalencephaly have underlying genetic etiologies, and identification of causative single gene defects in these disorders has largely supported the original classification system. These studies have clarified underlying mechanisms, as well as provided new concepts for further study. Metabolic defects leading to megalencephaly are often caused by germline mutations and generally cause diffuse abnormalities in the brain, although there are examples of asymmetric involvement.

We will refer to disorders previously characterized as anatomic megalencephaly as developmental megalencephaly because the current identified genetic causes involve signaling mediators that regulate cell growth, migration, and replication during development.^{3,4} Development of the cerebral cortex is characterized by massive proliferation,

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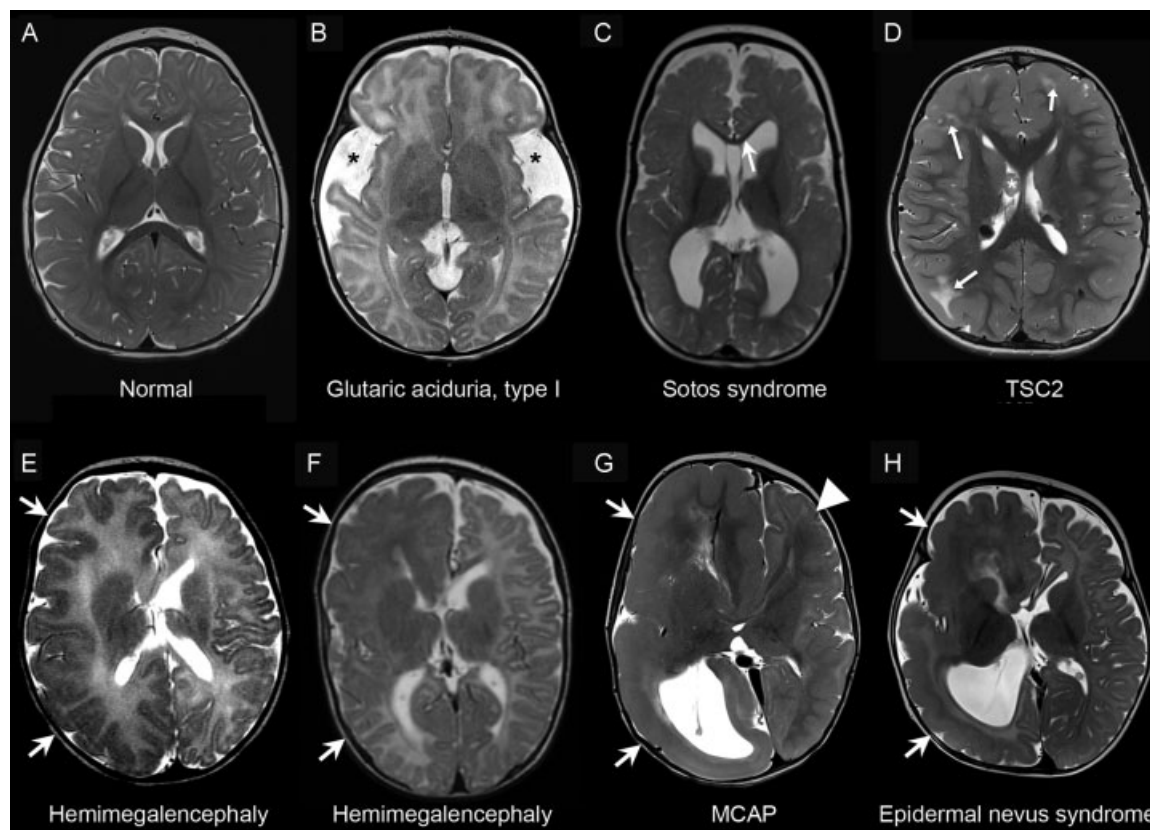


Fig. 1 Neuroimaging of metabolic and developmental megalencephaly. Representative axial T2-weighted magnetic resonance images: (A) A neurologically normal 1-year-old individual with symmetric hemispheres and normal myelination pattern for age. (B) An individual with glutaric aciduria, type I with typical features including enlarged extra-axial spaces and hypoplasia of the temporal lobes (*asterisks*), and delayed myelination pattern. (C) An individual with Sotos syndrome showing dolichocephaly and thinning of the corpus callosum (*arrow*). (D) An individual with tuberous sclerosis complex as a result of a *TSC2* mutation. Note typical features including the multiple cortical tubers in the bilateral frontal and right parietal lobes (*arrows*), and the subependymal giant cell astrocytoma (SEGA) (*asterisk*). (E) Hemimegalencephaly of unknown etiology, with symmetric enlargement of one hemisphere of the brain (*arrows*). (F) *AKT3* mutation resulting in asymmetric hemimegalencephaly, with *arrows* indicating enlargement of one hemisphere of the brain. (G) An individual with megalencephaly-capillary malformation–polymicrogyria syndrome (MCAP) showing hemimegalencephaly (*arrows*) and an abnormal gyration pattern on the contralateral side as well (*arrowhead*). (H) An individual with epidermal nevus syndrome that demonstrates asymmetric enlargement of one hemisphere (*arrows*).

differentiation, migration, and ultimately appropriate organization of neurons and glia. Neural progenitor proliferation, differentiation, and migration are controlled by multiple intrinsic and extrinsic signaling pathways that continually overlap during development. Classification of disorders is based on the earliest abnormal step, with the caveat that cells with proliferative defects often do not migrate or organize properly.⁵ Focal involvement is more common in the developmental megalencephalies; recently, somatic mosaicism has also been shown to play an important role in these diseases.^{6,7}

Metabolic Megalencephaly

Although metabolic diseases can be associated with both micro- and macrocephaly, there are several that are clearly associated with megalencephaly (► **Table 1**). However, not all patients with these disorders have megalencephaly, as there is often subsequent brain atrophy secondary to cell death.³ Despite the fact that these diseases arise from critical metabolic pathways, there is often a limited range

of cells that are affected. For example, leukodystrophies or disorders of the white matter, such as Canavan disease, Alexander disease, and megalencephalic leukoencephalopathy with subcortical cysts preferentially affect oligodendrocytes and astrocytes.

Canavan disease is a disorder of glial degeneration caused by mutations in the gene *ASPA* that encodes the enzyme aspartoacylase.⁸ Affected infants often appear normal until age 3 to 6 months, when they present with hypotonia and then rapidly progress to have limb spasticity with continued axial hypotonia and seizures.⁹ The aspartoacylase enzyme catalyzes the hydrolysis of N-acetylaspartic acid (NAA) to acetate. N-acetylaspartic acid is synthesized by and transported into the extracellular region by neurons, where it is internalized by oligodendrocytes and metabolized.¹⁰ Loss of function of aspartoacylase leads to accumulation of NAA in oligodendrocytes; elevated NAA excretion in urine is sufficient to diagnose the condition.¹¹ N-acetylaspartic acid accumulation leads to myelin vacuolization and astrocyte swelling, but neuronal cytoarchitecture is relatively well preserved.^{10,11}

Table 1 Metabolic megalencephalies and their genetic causes^a

Organic acid disorders	Glutaric aciduria, type I	<i>GCDH</i>
	L-2-Hydroxyglutaric aciduria	<i>L2HGDH</i>
	D-2-Hydroxyglutaric aciduria	<i>D2HGDH</i>
Lysosomal storage diseases	Hunter syndrome (mucopolysaccharidosis type II)	<i>IDS</i>
	Hurler syndrome (mucopolysaccharidosis type IH)	<i>IDUA</i>
	Sanfilippo syndrome (mucopolysaccharidosis III)	<i>SGSH</i> (IIIA), <i>HAGLU</i> (IIIB), <i>HGSNAT</i> (IIIC), <i>GNS</i> (IIID)
	Maroteaux-Lamy syndrome (mucopolysaccharidosis type VI)	<i>ARSB</i>
	Sly syndrome (mucopolysaccharidosis VII)	<i>GUSB</i>
	Tay–Sachs disease	<i>HEXA</i>
	Sandhoff disease	<i>HEXB</i>
	Krabbe disease	<i>GALC</i>
Leukoencephalopathies	Canavan disease (N-acetylaspartic aciduria)	<i>ASPA</i>
	Alexander disease	<i>GFAP</i>
	Megalencephalic leukoencephalopathy with subcortical cysts (MLC)	<i>MLC1</i> , <i>HEPACAM</i>
	Leukoencephalopathy with vanishing white matter	<i>EIF2B1</i> , <i>EIF2B2</i> , <i>EIF2B3</i> , <i>EIF2B4</i> , <i>EIF2B5</i>

Source: Adapted from Mirzaa GM, Poduri A. Megalencephaly and hemimegalencephaly: breakthroughs in molecular etiology. *Am J Med Genet C Semin Med Genet* 2014;166C(2):156–172.

^aIncluded are only syndromes associated with megalencephaly, and omitted are syndromes that might be associated with macrocephaly without brain enlargement.

Alexander disease is another disorder that preferentially affects glial cells. It is caused by mutations in the gene *GFAP*; this encodes glial fibrillary acidic protein, a structural component of astrocytes.⁸ Heterozygous gain-of-function mutations in *GFAP* cause accumulation of the GFAP protein within astrocytes into Rosenthal fibers, which are thought to be toxic to astrocytes and to cause cell death.¹² There is also abnormal myelination, possibly secondary to disrupted astrocyte–oligodendrocyte interaction.¹³

In contrast to the leukodystrophies, there are also metabolic disorders that primarily affect neurons and lead to megalencephaly. For example, Tay–Sachs disease is a classic metabolic disorder caused by a loss of function of the enzyme β -hexosaminidase. There are two forms of β -hexosaminidase: β -hexosaminidase A is a heterodimer comprised of an α and β subunits and β -hexosaminidase B is a homodimer comprised of two β subunits. The *HEXA* gene encodes the α subunit of the β -hexosaminidase A enzyme, which converts GM2-ganglioside to GM3-ganglioside. Patients with Tay–Sachs disease have loss-of-function alleles for both copies of the *HEXA* gene, which eliminates the activity of β -hexosaminidase A and leads to GM2 accumulation. These patients often present in infancy with hypotonia and failure to thrive, and progress to intractable epilepsy, spasticity, and death. GM2 specifically accumulates in neurons and forms of storage bodies that can be seen on electron microscopy.¹⁴ This metabolic defect results in progressive neuronal cell death and cerebral atrophy, although whether this is secondary to direct toxicity, secondary effects on neuronal development, or resultant

inflammation is unclear.¹⁵ Interestingly, the residual activity of the enzyme is correlated with age of onset and severity of the phenotype in Tay–Sachs disease.¹⁴

Glutaric aciduria type 1 is caused by loss of function of the glutaryl-CoA dehydrogenase enzyme, encoded by the *GCDH* gene; it is associated with macrocephaly present in the neonatal period (–Fig. 1). Infants often have subtle initial symptoms of hypotonia, poor feeding, and irritability, but untreated patients will develop encephalopathic crises caused by a catabolic state in the setting of an infection.¹⁶ These crises often lead to basal ganglia damage, resulting in an irreversible dystonic–dyskinetic movement disorder.¹⁷ *GCDH* is a mitochondrial matrix protein that is expressed in neurons and involved in the catabolism of tryptophan, lysine, and hydroxylysine. Lack of *GCDH* activity results mainly in accumulation of glutaric acid and 3-hydroxyglutaric acid, and these metabolites have been implicated in alterations in energy metabolism and oxidative stress, which is thought to explain the neuronal vulnerability.¹⁸

Despite the seemingly simple paradigm of the metabolic megalencephalies, these disorders encompass a relatively heterogeneous group of disorders (–Table 1). Many of the disorders do involve a loss-of-function mutation in an enzyme, but this is not the rule, as evidenced by Alexander disease. In addition, these disorders affect general biochemical pathways but cause initial dysfunction in a single cell type. The mechanism of brain enlargement observed is not well understood and ranges from cell autonomous mechanisms involving enlargement of certain cells to inflammatory

reactions to the presence of abnormal metabolites. Therefore, metabolic disorders represent several different pathophysiological mechanisms that contribute to brain enlargement and general dysfunction.

Developmental Megalencephaly

Developmental megalencephaly has also been referred to as anatomic megalencephaly or non-metabolic megalencephaly. Recent identification of mutations in genes regulating cell growth, migration, and replication has led to the recognition that developmental processes underlie these megalencephalies.^{3,4} Developmental megalencephalies are largely characterized by single gene mutations, with the most common mutations identified affecting the mTOR, Ras/MAPK, or SHH pathways (►Table 2). Asymmetric and focal lesions are more common in developmental megalencephalies, with the spectrum of abnormalities ranging from megalencephaly and hemimegalencephaly to the discrete tubers of tuberous sclerosis complex (TSC) (►Fig. 1). It is hypothesized that the variability in presentation may be in part due to somatic mosaicism.^{6,7}

Somatic Mutation

As opposed to the recessive mutations that commonly give rise to the metabolic megalencephalic disorders, *de novo* mutations have proven to be an important contributor to the developmental megalencephalies.^{3,6} As opposed to inherited mutations, *de novo* mutations are not found in both parent and offspring by standard genetic testing of DNA from leukocytes. A *de novo* mutation that arises after zygote formation is referred to as a somatic mutation, and will lead to mosaicism, a state in which various cells in an individual that can have different genotypes. These genetic alterations can happen at any point between gamete formation in the parent and organ development in the fetus, and can affect multiple organs or a single part of one organ, depending on the developmental stage at which they occur and the range of expression of the particular gene.

Somatic mutation in the brain has been hypothesized as one of many mechanisms to increase neuronal diversity, but studies have demonstrated a wide range of rates of somatic mutation in the brain. Multiple studies have used fluorescent *in situ* hybridization (FISH) to examine aneuploidy in the developing and mature brain and found rates of aneuploidy ranging between 10 to 35%.¹⁹ However, using single cell sorting and whole genome sequencing, aneuploidy could not be detected in control brains, whereas it was reliably detected in an individual with known trisomy 18.²⁰ Efforts to determine the somatic mutation rate have led to relatively high estimates.²¹ However, there are disorders such as neurofibromatosis type 1 (NF1), in which somatic mutations in the skin are known to cause neurofibromas, and the burden of neurofibromas is much lower than what would be expected based on somatic mutation estimates alone, suggesting that there is additional complexity. Studies that have identified somatic mutations associated with brain malformations have obtained DNA directly from resected brain

tissue from epilepsy surgery or found disease-causing mutations in peripheral leukocytes, inferring their presence in the brain.^{22,23}

The mTOR Pathway

The mTOR protein is a kinase that functions within a protein complex to integrate cell signaling and coordinate cell growth and differentiation. There are two functionally independent protein complexes that utilize mTOR, mTORC1 involved in cellular metabolism and growth and mTORC2 involved in cytoskeletal organization.²⁴ Early studies in cortical tubers in TSC and some focal cortical dysplasias demonstrated that large ovoid cells with limited processes (giant cells or balloon cells) displayed selective activation of the mTOR pathway through increased amounts of phosphorylated ribosomal S6 kinase.^{25,26} Interestingly, some focal cortical dysplasias, cases of hemimegalencephaly, and TSC also demonstrate the presence of dysmorphic neurons, which have large soma and disorganized dendritic processes.²⁷ These disorders also demonstrate varying degrees of disruption of cortical laminar organization. The similarities between these disorders both molecularly and pathologically suggest shared mTOR dysregulation. A recent study identified a somatic mutation in *MTOR* in a pathologically typical case of hemimegalencephaly in which there was increased phosphorylation of ribosomal S6 kinase in this case, consistent with an increase in mTOR activity.²⁸ These data provide further evidence for the theory that dysregulation of mTOR is central to multiple forms of developmental megalencephaly.

There are many upstream molecules that regulate mTOR signaling, and many of these have been implicated in hemimegalencephaly (►Fig. 2). One key regulator of mTOR is the protein kinase AKT, which leads to activation of mTOR. Activating somatic mutations in *AKT1* cause Proteus syndrome, characterized by somatic overgrowth of connective tissue and associated with hemimegalencephaly.²⁹ *AKT2* is associated with hypoinsulinemic hypoglycemia with hemihypertrophy, but has not been associated with megalencephaly at this time.³⁰ *AKT3* is highly expressed in the developing brain, and one study identified an activating mutation in *AKT3* from dysplastic cortex of a patient with hemimegalencephaly, but not lymphocytes from the patient, demonstrating the presence of mosaicism.²² *AKT3* mutations have also been shown to cause megalencephaly–polymicrogyria–polydactyly–hydrocephalus (MPPH) syndrome.³¹ Interestingly, deletions that presumably lead to loss of function of *AKT3* are associated with microcephaly,³² demonstrating its key role in regulating proliferation and growth within the developing brain. Because there has been no target specificity associated with the AKT isoforms, the phenotypic variability seen within these disorders is likely due to a combination of the regulation of the gene leading to differential expression between tissues at different developmental time points and the exact population of cells affected by the mutation.

AKT is activated by recruitment to the cell membrane and binding to phosphatidylinositol-3,4,5-triphosphate (PIP₃), which allows phosphorylation and subsequent activation of

Table 2 Developmental megalencephalies and their genetic causes^a

PI3K-AKT-MTOR pathway	
<i>PTEN</i> *	Macrocephaly/autism syndrome
	Bannayan–Riley–Ruvalcaba syndrome (BRRS)
	Cowden syndrome
	Lhermitte–Duclos syndrome
<i>PI3KCA</i>	MCAP syndrome (megalencephaly-capillary malformation-polymicrogyria)
	Klippel–Weber–Trenaunay syndrome
	CLOVES (congenital lipomatous overgrowth, vascular malformations, and epidermal nevi)
<i>AKT3</i> *, <i>CCND2</i> , <i>PIK3R2</i>	MPPH syndrome (megalencephaly-polymicrogyria-polydactyly-hydrocephalus)
<i>AKT1</i> *	Proteus syndrome
	Cowden syndrome
<i>STRADA</i>	Pretzel syndrome or PMSE (polyhydramnios, megalencephaly, and symptomatic epilepsy)
<i>TSC1</i> *, <i>TSC2</i> *	Tuberous sclerosis complex
<i>TBC1D7</i>	Macrocephaly/megalencephaly syndrome, autosomal recessive
<i>MTOR</i> *	Hemimegalencephaly
Ras/mitogen-activated protein kinase (MAPK) pathway	
<i>NF1</i>	Neurofibromatosis 1
<i>SPRED1</i>	Legius syndrome
<i>HRAS</i>	Costello syndrome
<i>MAP2K2</i>	Cardiofaciocutaneous (CFC) syndrome
<i>NRAS</i> , <i>SOS1</i> , <i>RIT1</i> , <i>SHOC2</i>	Noonan syndrome
<i>KRAS</i> , <i>MAP2K1</i>	CFC syndrome, NS
<i>PTPN11</i>	LEOPARD syndrome, NS
<i>BRAF</i>	LEOPARD syndrome, CFC syndrome, NS
<i>RAF1</i>	LEOPARD syndrome, NS
<i>RIN2</i>	MACS syndrome (macrocephaly, alopecia, cutis laxa, and scoliosis)
SHH pathway	
<i>PTCH1</i>	Nevoid basal cell carcinoma (Gorlin) syndrome, 9q22.3 microdeletion
<i>KIF7</i>	Acrocallosal syndrome
<i>GLI3</i>	Greig cephalopolysyndactyly syndrome
Transcriptional regulators	
<i>NSD1</i>	Soto syndrome
<i>EZH2</i>	Weaver syndrome
<i>MED12</i>	Opitz–Kaveggia (FG) syndrome
	Lujan (Lujan–Fryns) syndrome
Signaling molecules and mitotic regulators	
<i>GPC3</i>	Simpson–Golabi–Behmel syndrome
<i>OFD1</i>	Simpson–Golabi–Behmel syndrome (type 2)
<i>DIS3L2</i>	Perlman syndrome

Source: Adapted from Mirzaa GM, Poduri A. Megalencephaly and hemimegalencephaly: breakthroughs in molecular etiology. *Am J Med Genet C Semin Med Genet* 2014;166C(2):156–172.

^aThe asterisks denote identified genes known to cause hemimegalencephaly to date.

the kinase. PIP3 is formed by phosphorylation of phosphatidylinositol-4,5-bisphosphate (PIP2) by PI3K, and this reaction is reversed by the phosphatase PTEN. PI3K is a heterodimer comprised of a catalytic and regulatory subunit, each of which

have multiple isoforms encoded in the genome. *PI3KCA* encodes a catalytic subunit of the PI3K complex, and activating somatic mutation has been shown to cause CLOVES syndrome (congenital lipomatous overgrowth, vascular

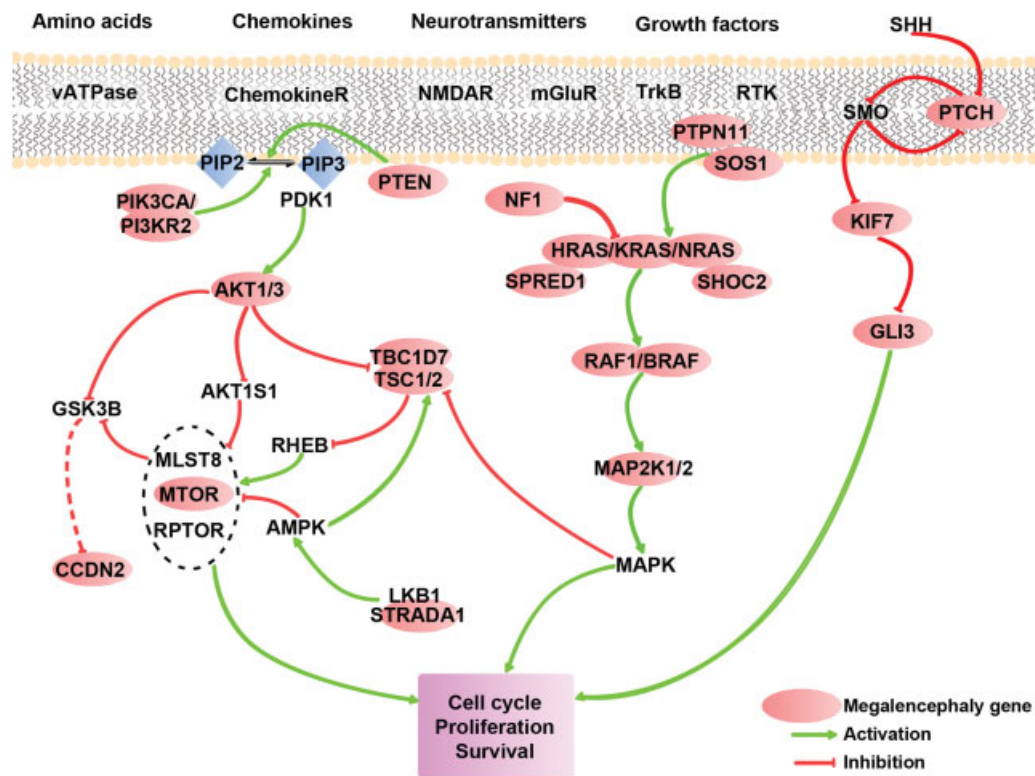


Fig. 2 Molecular signaling pathways whose dysregulation leads to developmental megalencephaly. The schematic shows key molecules in the mTOR, MAPK, and SHH signaling pathways. Categories of the extracellular signaling molecules are highlighted across the top with their receptors shown below. Specific interactions between the receptor categories and signaling mediators are not shown given the multitude and complexity of interactions. Interactions between signaling mediators are divided into activating (green arrows) and inhibitory (red blunt arrows). Signaling molecules whose dysregulation cause megalencephaly are highlighted by red circles. Some of the main downstream cellular effects are listed at the bottom.

malformations, epidermal nevus, spinal/skeletal anomalies/scoliosis syndrome), characterized by focal overgrowth in various parts of the body (► Table 2).^{33,34} Within the brain, activating somatic mutation of *PI3KCA* can cause megalencephaly–capillary malformation–polymicrogyria syndrome (MCAP).³¹ This syndrome is characterized by megalencephaly with connective tissue and vascular involvement to a variable severity and extent (► Fig. 1).³⁵ In addition, somatic mutation of *PI3KCA* has been shown to be associated with isolated hemimegalencephaly with polymicrogyria.²⁸ One regulatory subunit isoform of the PI3K complex is encoded by *PI3KR2*, and mutations in this gene have been shown to be a common etiology for MPPH syndrome.^{31,36}

Germline mutations of *PTEN* have been shown to cause Cowden syndrome and Bannayan–Riley–Ruvalcaba syndrome. Both syndromes are associated with overgrowth of connective tissue and multiple hamartomas with a susceptibility to multiple cancers.^{37,38} Affected patients may demonstrate bilateral megalencephaly with preservation of cytoarchitecture, but more focal involvement has been described as well.³⁹ *PTEN* mutation is also one of the most common single gene mutations found in patients with autism and macrocephaly.^{40,41}

The genes *TSC1* and *TSC2* form a complex that regulates mTOR through inhibition of the mTOR-activator RHEB. Increased phosphorylated S6 kinase, a target of MTOR, has been shown as evidence of increased mTOR activity in cortical

tubers.²⁵ Tuberous sclerosis complex is an autosomal dominant disorder in which an individual carries only one functional copy of *TSC1* or *TSC2*. These patients often have discrete cortical malformations or cortical tubers and epilepsy, as well as multiple skin findings, including hypopigmented macules and angiofibromas.⁴² In addition, they are predisposed to cancerous growths such as subependymal giant cell astrocytomas (SEGAs) and renal angiomyolipomas. Although these patients carry one defective copy of *TSC1* or *TSC2* in all of their cells, it has been shown that SEGAs and angiomyolipomas arise through a second mutation leading to a cell population that is functionally null for *TSC1* or *TSC2*.⁴³ Given the discrete, dysplastic nature of the cortical tubers in the brain, it has been hypothesized that these malformations also arise from a second mutation in *TSC1* or *TSC2*. One study identified rare second hit mutations by sequencing bulk tissue from tubers.⁴⁴ However, another study isolated and sequenced cells with high levels of phosphorylated S6-kinase and found second hit mutations in five of six cortical tubers studied, further suggesting that some tubers result from a cell population functionally null for one of the TSC genes.⁴⁵ Interestingly, approximately 80% of TSC patients demonstrate cortical tubers on magnetic resonance imaging (MRI),⁴⁶ and the requirement of a second hit would provide one explanation for this phenotypic variability. Another member of the TS complex is *TBC1D7*, and loss of this subunit leads to decreased association between *TSC1* and *TSC2*.⁴⁷ Loss of function

mutations of this subunit have been described in patients with megalencephaly.⁴⁸ Another mechanism of regulation of the TS complex is through activity of the kinase AMPK, which is regulated through a complex containing STK11, STRADA, and CAB39. Loss of function mutations in *STRADA* have been shown to cause the syndrome polyhydramnios, megalencephaly, and symptomatic epilepsy (PMSE), also known as Pretzel syndrome.⁴⁹

mTOR is an important hub with multiple downstream effectors, regulating neuronal growth and development. However, mutations in the mTOR target *CCND2* can cause MPPH,⁵⁰ implicating the regulation of *CCND2* by mTOR as critical to the development of megalencephaly. *CCND2* is a cyclin that regulates exit from the G1 phase of the cell cycle, and one key mechanism of regulation of *CCND2* is phosphorylation by GSK3 β that leads to ubiquitination and degradation through the proteasome.⁵¹ However, mutations that cause MPPH abrogate this interaction, leading to accumulation of *CCND2* and neuronal overgrowth.⁵⁰ Interestingly, GSK3 β is inhibited by mTOR and AKT,^{52,53} suggesting that dysregulation in the AKT/mTOR pathway could lead to *CCND2* accumulation (► Fig. 2). Although each upstream component is part of a complex cellular signaling network, the developmental megalencephalies associated with alterations in mTOR signaling might share accumulation of *CCND2*, which would explain their pathological similarities.

Mutations in genes in the mTOR pathway that lead to increased mTOR signaling lead to disrupted cell growth and migration in the brain and other tissues. Although these disorders appear to be heterogeneous, the overgrowth abnormalities observed depend on the normal timing and location of gene expression. The normal expression of the gene determines the tissues that are vulnerable to mutation of the specific gene, illustrated by the differing phenotypes of *AKT* mutations. The tissues affected by the mutation are also critically dependent on the timing of the mutation in development, where earlier mutations in development will affect more tissues than later mutations.

The Ras/MAPK Pathway

The Ras/MAPK pathway transduces extracellular signals and regulates cell cycle, development, and senescence. There are germline mutations for multiple members of this signaling pathway that lead to multiple phenotypically overlapping syndromes, which demonstrate absolute or relative macrocephaly. These disorders are often associated with severe failure to thrive, and relative macrocephaly describes the situation in which a child's head circumference grows at an appropriate rate while length fails to keep up with the growth curve and crosses centiles. Imaging studies have also shown brain growth abnormalities in syndromes associated with increased Ras/MAPK signaling.⁵⁴

The Ras family of genes encodes GTPases that interact with cellular receptors and signal through the intermediary proteins Raf and MAP2K/MEK leading to activation of MAPK/ERK (► Fig. 2). MAPK/MEK is a kinase that has multiple downstream targets that influence cellular functions through transcriptional and nontranscriptional mechanisms. Activat-

ing mutations in Ras/MAPK pathway members have been shown to cause multiple overlapping syndromes, including Noonan syndrome, Noonan syndrome with multiple lentigines, Costello syndrome, cardiofaciocutaneous syndrome, NF1, and Legius syndrome.⁵⁵ Noonan syndrome can be caused by heterozygous activating mutations in *PTPN11*, *SOS1*, *NRAS*, *KRAS*, *RAF1*, *BRAF*, *SHOC2*, or *RIT1*.^{56,57} but *PTPN11* mutations are to date the most prevalent in this syndrome.⁵⁸ *PTPN11* and *SOS1* lead to activation of the Ras proteins, whereas *SHOC2* and *RIT1* modify Raf signaling. Costello syndrome is caused by *HRAS* mutations, and cardiofaciocutaneous syndrome is caused by *KRAS*, *BRAF*, *MAP2K1*, and *MAP2K2* mutations.⁵⁵ However, patients affected with Noonan, Costello, and cardiofaciocutaneous syndromes share many common features besides brain abnormalities, including failure to thrive, developmental delay, craniofacial dysmorphologies, cardiac defects, and skin abnormalities. Developmental delay in cardiofaciocutaneous syndrome is more prevalent and severe than in Noonan syndrome, and Costello syndrome has characteristic skin findings, including excessive wrinkling and redundancy over the dorsum of the hands and feet.⁵⁵ Noonan syndrome with multiple lentigines is phenotypically similar to Noonan syndrome, but there are abnormal genitalia and multiple lentigines. Interestingly, this syndrome is also caused by *PTPN11* mutations, but these mutations occur in the phosphotyrosine phosphatase domain.⁵⁹ NF1 is characterized by skin and eye findings, including cafe-au-lait macules, intertriginous freckling, neurofibromas, hamartomas of the iris (Lisch nodules), and optic pathway gliomas. Affected patients less commonly have developmental delays and craniofacial features similar to Noonan syndrome. This disorder is caused by germline gain of function mutations in the *NF1* gene, and the NF1 protein (neurofibromin) inhibits Ras signaling.⁶⁰ Legius syndrome is similar to NF1, but patients do not have neurofibromas or Lisch nodules and there is no increased risk of optic pathway gliomas. Heterozygous inactivating mutations in *SPRED1* lead to Legius syndrome by altering Raf signaling.⁶¹

Ras/MAPK signaling in the developing telencephalon has been shown to promote the generation of neurons.⁶² In vivo and in vitro studies have demonstrated that reduction of *PTPN11* decreases the number of neurons generated during development.^{63,64} However, a murine model of Noonan syndrome carrying a heterozygous activating mutation of *PTPN11* showed a slight increase in neurons, but a substantial reduction in glial cells.⁶⁵ It is difficult to reconcile these findings with the human syndrome, in which a substantial proportion of patients demonstrate megalencephaly. Another potential mechanism underlying the development of megalencephaly in these patients is an interaction with the mTOR pathway. ERK directly phosphorylates and inhibits TSC2 in tumor cell lines, leading to mTOR activation.⁶⁶ Therefore, overactivation of Ras/MAPK signaling could allow unchecked mTOR signaling, leading to atypical cellular and cortical growth. However, patients with these Ras/MAPK signaling disorders rarely present with focal cortical dysplasia as seen in cases with mTOR signaling defects.⁶⁷ In addition, an animal model of Noonan syndrome with a gain of function mutation

of *PTPN11* did not have increased phosphorylated S6, arguing against contributions of increased mTOR signaling in the model.⁶⁵

Gliogenesis is also affected by aberrant Ras/MAPK signaling. Conditional transgenic animals carrying a gain of function mutation in *PTPN11* in *Olig2*⁺ cells demonstrated increased oligodendroglial progenitors,⁶⁵ seemingly contradictory to prior studies demonstrating reductions in glial cells.⁶⁴ The *Olig2* driver used in the conditional transgenic animals preferentially targets ventral telencephalon, whereas the prior study had used in utero electroporation, suggesting regional or temporal differences in Ras/MAPK signaling leading to the observed differences. Animal models of NF1 inactivation or *MAP2K1*/*MAP2K2* gain of function also show expansion of glial and glioprogenitor populations,^{68–70} reinforcing the role of Ras/MAPK signaling in gliogenesis. Patients with NF1 also have increased white matter volume with an abnormally thick corpus callosum.⁷¹ Therefore, alterations in glial progenitor proliferation and development may underlie some of the brain abnormalities seen in the Ras/MAPK signaling disorders.

Other Pathways and Indirect Regulators

The Sonic Hedgehog pathway is involved in early pattern formation in the developing nervous system. The Sonic Hedgehog ligand (SHH) binds to its receptor PTCH1 removing the inhibition of PTCH1 on SMO, and SMO then is able to activate the GLI transcription factors (GLI1, GLI2, and GLI3) (→ Fig. 2). Grieg cephalopolysyndactyly syndrome (GCPS) can be caused by mutations in *GLI3* and is characterized by macrocephaly, craniofacial dysmorphism, and limb malformations.⁷² Mutations identified thus far have led to loss of one functional allele; therefore, haploinsufficiency of this transcription factor is thought to cause this syndrome.⁷² However, animal models with loss of function of *GLI3* have severely reduced telencephalon,⁷³ whereas animals with a *GLI3* hypermorphic allele have larger forebrains.⁷⁴ Interestingly, the increased forebrain size was thought to be mediated by a shorter cell cycle in progenitors caused by upregulation of the *GLI3* target *CCND1*.⁷⁴ Mutations in the SHH receptor PTCH1 leading to haploinsufficiency can cause nevoid basal cell carcinoma syndrome. This syndrome is characterized by multiple basal cell carcinomas, as well as skeletal and facial features.⁷⁵ These disorders highlight the importance of dysregulation of the SHH signaling pathway in contributing to brain growth abnormalities.

There are many other genes associated with developmental megalencephaly; many of them can affect either or both mTOR or Ras/MAPK signaling. Sotos and Weaver syndromes have characteristic craniofacial dysmorphologies, including high broad forehead and prominent chin, overgrowth with tallness being more common than macrocephaly, and intellectual disability (→ Fig. 1).⁷⁶ Sotos and Weaver syndromes are associated with *NSD1* and *EZH2* mutations, respectively; both genes encode histone methyltransferases involved in epigenetic regulation. Sotos syndrome is associated with haploinsufficiency of *NSD1*, and Weaver syndrome is associated with missense mutations in *EZH2*, suggesting loss of

function of these genes as the pathological mechanism underlying these disorders. *NSD1* and *EZH2* have been suggested to activate both mTOR and MAPK signaling through modulating expression of upstream molecules. For example, *EZH2* has been shown to suppress PTEN and lead to increased proliferation in neuroprogenitors.⁷⁷ *EZH2* and *NSD1* have been shown to activate MAPK signaling through *CXXC4* and *RASIP1*, respectively.^{78,79} Therefore, loss of function of these genes likely has complex effects on the mTOR and Ras/MAPK pathways, leading to brain growth abnormalities.

Although there are many genes with varying functions associated with megalencephaly, many appear to modify mTOR and/or MAPK signaling pathways. This suggests that these genes may cause brain enlargement by converging onto known mechanisms. However, this is not surprising given the central role of mTOR and MAPK in cell-signaling networks. The phenotypic variability seen between these disorders is likely due to either variable penetrance or the degree that specific pathways are modulated by these mutations.

Treatment

Most of the metabolic and developmental megalencephalies are associated with developmental delay and epilepsy, as well as other disease-specific manifestations. Developmental delay and regression in certain cases may be due to neuronal cell death, but in some disorders, they appear to be related to medically intractable epilepsy. In some cases with focal abnormalities of cerebral development, epilepsy surgery can be an effective treatment.⁸⁰ In addition, epilepsy surgery in TSC can help in terms of both seizure freedom and developmental improvements.⁸¹ However, for patients who are not surgical candidates, the mainstay of treatment is antiepileptic medication.

Given the important role of mTOR in the development of several different forms of megalencephaly, there has been increasing interest in using mTOR inhibitors as medical therapy. Some studies in animal models suggest that mTOR inhibition with rapamycin may help to prevent epileptogenesis, although this model was not related to the disorders of mTOR signaling.⁸² In a small study of patients with TSC, another mTOR inhibitor, everolimus, was suggested to be beneficial as an antiepileptic medication for some patients.^{83,84} However, more study is required to determine whether this effect is reproducible. Ultimately, there may be a role for specific mTOR inhibitors applicable to a broader range of developmental epilepsy associated with megalencephaly.

Conclusions

Syndromes associated with brain growth abnormalities comprise a diverse set of disorders. We propose refining the characterization of these disorders by dividing them into metabolic and developmental causes of megalencephaly. Metabolic megalencephalies have been well characterized, and these disorders mostly involve defects in cellular metabolic pathways, leading to accumulation of abnormal metabolites. There is a diverse range of pathways and

pathophysiological mechanisms that contribute to these disorders, but most result in progressive cellular dysfunction and death. Developmental megalencephalies are caused by defects in signaling pathways that alter neuronal replication, growth, or migration leading to abnormal development of the brain. The mTOR, Ras/MAPK, and SHH pathways represent the most commonly affected pathways (→ Fig. 2), and mutations in some of these molecules at any point between gamete formation and organogenesis can lead to disease. Postzygotic mutations that can affect only part of the brain explain the ability of a genetic disorder to cause asymmetric abnormalities. At this point, it is unclear whether the seizures, developmental disabilities, or behavioral symptoms observed are secondary to an ongoing molecular dysregulation or a product of an abnormally developed brain. However, the understanding of the molecular etiology and pathophysiological mechanisms of these disorders is providing new avenues for investigation and intervention.

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