A developmental and genetic classification for midbrain-hindbrain malformations

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Advances in neuroimaging, developmental biology and molecular genetics have increased the understanding of developmental disorders affecting the midbrain and hindbrain, both as isolated anomalies and as part of larger malformation syndromes. However, the understanding of these malformations and their relationships with other malformations, within the central nervous system and in the rest of the body, remains limited. A new classification system is proposed, based wherever possible, upon embryology and genetics. Proposed categories include: (i) malformations secondary to early anteroposterior and dorsoventral patterning defects, or to misspecification of mid-hindbrain germinal zones; (ii) malformations associated with later generalized developmental disorders that significantly affect the brainstem and cerebellum (and have a pathogenesis that is at least partly understood); (iii) localized brain malformations that significantly affect the brain stem and cerebellum (pathogenesis partly or largely understood, includes local proliferation, cell specification, migration and axonal guidance); and (iv) combined hypoplasia and atrophy of putative prenatal onset degenerative disorders. Pertinent embryology is discussed and the classification is justified. This classification will prove useful for both physicians who diagnose and treat patients with these disorders and for clinical scientists who wish to understand better the perturbations of developmental processes that produce them. Importantly, both the classification and its framework remain flexible enough to be easily modified when new embryologic processes are described or new malformations discovered.

Keywords: cerebellum; brain stem; malformations; development
Abbreviations: CDG = congenital disorders of glycosylation; FOXC1 = Forkhead box C; GABA = gamma-aminobutyric acid; GPR = G protein-coupled receptor; JSRD = Joubert syndrome and related disorders; LCH = lissencephaly with cerebellar hypoplasia; MHB = midbrain-hindbrain boundary; OPHN = oligophrenin; PCH = pontocerebellar hypoplasias; Shh = sonic hedgehog signalling molecule

Introduction

Recent advances in developmental biology, molecular genetics and neuroimaging have led to an increased interest in and understanding of developmental disorders of the embryonic midbrain and hindbrain that grow into the adult brainstem and
cerebellum. Malformations of the brainstem and cerebellum often occur as the only recognized malformation in individuals with mental retardation or autism (Soto-Ares et al., 2003; Courchesne et al., 2005). However, they have also been increasingly recognized in patients with malformations of the cerebrum such as lissencephaly (Ross et al., 2001; Poirier et al., 2007), cobblestone malformations (Aida et al., 1994; Barkovich, 1998; Triki et al., 2003; van Reeuwijk et al., 2006) or callosal anomalies (Barkovich et al., 2007); and in patients with developmental disorders of other organ systems such as the kidneys or skin (Brocks et al., 2000; Gleeson et al., 2004; Tan et al., 2005; Valente et al., 2005).

The number and complexity of recognized malformations of the brainstem and cerebellum has been steadily increasing. While the practical ‘every day’ approach to a patient with a midbrain-hindbrain malformation is still based mainly on the neuroimaging ‘pattern recognition’ approach, a system by which these disorders can be clearly identified and compared is badly needed. A few classification systems have been proposed (Patel and Barkovich, 2002; Parisi and Dobyns, 2003), but none are comprehensive or widely used. Here we take advantage of a combination of large clinical practices and an expanding knowledge base regarding neuroembryology and developmental biology, structural imaging and molecular genetics to present a comprehensive, yet flexible, system of classification for these collectively common disorders.

This classification system (Table 1) relies most heavily on embryology and genetics, as these comprise the bodies of knowledge that most easily allow relationships among a large group of disorders to be clarified. A similar classification system for malformations of cortical development (Barkovich et al., 2005) has proven useful for both physicians who diagnose and treat patients with these disorders and for clinical scientists who wish to understand better the perturbations of developmental processes that produce them. Importantly, both the classification and its framework remain flexible enough to be easily modified when new embryologic processes are described or new malformations discovered (Barkovich et al., 2005).

**Overview of midbrain and hindbrain development**

The central nervous system derives from the dorsal epiblast of the vertebrate embryo, and is induced by a combination of signals originating in the region of Hensen’s node at the posterior margin of the early embryo (Wurst and Bally-Cuif, 2001). After many steps, a neural tube is formed that subsequently develops a series of vesicles at its anterior (rostral) end. These three vesicles are designated the prosencephalon or forebrain (which soon divides into diencephalon and telencephalon), the mesencephalon (midbrain), and the rhombencephalon (hindbrain), which divides into the rostral metencephalon (pons and cerebellum) and caudal myelencephalon (medulla oblongata). This differentiation along the anteroposterior axis (also called the rostral-caudal axis) is called patterning, a name given to the early differentiation of the neural tube (Lumsden and Krumlauf, 1996).

<table>
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<th>Table 1 Overview of developmental and genetic classification of mid-hindbrain malformations</th>
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<td>I. Malformations secondary to early anteroposterior and dorsoventral patterning defects, or to misspecification of mid-hindbrain germinal zones</td>
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<tr>
<td>A. Anteroposterior patterning defects</td>
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<tr>
<td>1. Gain, loss or transformation of the diencephalon and midbrain</td>
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<td>2. Gain, loss or transformation of the midbrain and rhombomere 1</td>
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<td>3. Gain, loss or transformation of lower hindbrain structures</td>
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<tr>
<td>B. Dorsoventral patterning defects</td>
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<td>1. Defects of alar and basal ventricular zones</td>
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<td>2. Defects of alar ventricular zones only</td>
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<td>3. Defects of basal ventricular zones only</td>
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<td>II. Malformations associated with later generalized developmental disorders that significantly affect the brainstem and cerebellum (and have pathogenesis at least partly understood)</td>
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<td>A. Developmental encephalopathies associated with mid-hindbrain malformations</td>
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<td>C. Malformations of neuronal and glial proliferation that prominently affect the brainstem and cerebellum</td>
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<td>D. Malformation of neuronal migration that prominently affect the brainstem and cerebellum</td>
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<tr>
<td>1. Lissencephaly with cerebellar hypoplasia</td>
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<td>2. Neuronal heterotopia with prominent brainstem and cerebellar hypoplasia</td>
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<td>3. Polymicrogyria with cerebellar hypoplasia</td>
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<td>4. Malformations with basement membrane and neuronal migration deficits</td>
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<tr>
<td>E. Diffuse molar tooth type dysplasias associated with defects in ciliary proteins</td>
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<td>1. Syndromes affecting the brain with low frequency involvement of the retina and kidney</td>
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<td>2. Syndromes affecting the brain, eyes, kidneys, liver and variable other systems</td>
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<tr>
<td>III. Localized brain malformations that significantly affect the brainstem and cerebellum (pathogenesis partly or largely understood, includes local proliferation, cell specification, migration and axonal guidance)</td>
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<tr>
<td>A. Multiple levels of mid-hindbrain</td>
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<td>B. Midbrain malformations</td>
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<td>C. Malformations of rhombomere 1 including cerebellar malformations</td>
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<td>D. Pons malformations</td>
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<td>E. Medulla malformations</td>
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<td>IV. Combined hypoplasia and atrophy in putative prenatal onset degenerative disorders</td>
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<td>A. Pontocerebellar hypoplasia</td>
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<td>B. Mid-hindbrain malformations with congenital disorders of glycosylation</td>
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<tr>
<td>C. Other metabolic disorders with cerebellar or brainstem hypoplasia or disruption</td>
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<tr>
<td>D. Cerebellar hemisphere hypoplasia (rare, more commonly acquired than genetic, often associated with clefts or cortical malformation)</td>
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The mechanisms that result in early anteroposterior patterning are partially understood (Chambers et al., 2009) and, other than the formation of the diencephalic-mesencephalic boundary and the midbrain-hindbrain boundary (MHB), are beyond the scope of this manuscript. In murine and chick models, the diencephalic-mesencephalic boundary appears to form, at least in part, from interactions between the Pax6, Pax2, En1 and En2 genes. Pax6 confers diencephalic fate by repressing both Pax2 and En1, while En1 represses Pax6 expression in the mesencephalon (Lim and Golden, 2007). Changes in expression of these genes will shift the diencephalic-mesencephalic boundary caudally (more Pax6) or rostrally (more Pax2/En1). Similarly, the location of the MHB is determined by the expression of Otx2 in the caudal midbrain and Gbx2 in the rostral hindbrain; increase or posterior shifts in the expression of Otx2 or decrease in Gbx2 shift the MHB caudally, while decrease in Otx2 or increase or anterior shift in Gbx2 shifts the MHB rostrally (Nakamura et al., 2005). The interaction of Otx2 and Gbx2 also specifies the location of the isthmus organizer (Fig. 1), a critical structure located at the MHB that functions via secreted Wnt and fibroblast growth factor signalling.
molecules to organize expression of genes and specify cell type (Broccoli et al., 1999; Wurst and Bally-Cuif, 2001): it is essential for normal brainstem and cerebellar development (Sotelo, 2004).

At the same time that anteroposterior patterning is taking place, an analogous process is occurring along the dorsoventral axis (Fig. 1). Dorsoventral patterning depends on the relative amounts of dorsalizing and ventralizing factors. The most important dorsalizing factors are proteins belonging to the bone morphogenetic protein family that are produced by the non-neural ectoderm of the prechordal plate and floor plate (Tanabe and Jessell, 1996; Wurst and Bally-Cuif, 2001). Along the dorsoventral axis, the mesencephalon is divided into the tegmentum (ventral region) and tectum (dorsal region) while the rostral hindbrain is divided into the pons (ventral region) and cerebellum (dorsal region). The neuronal subtypes produced in these regions are specified by expression of local Hox genes and other transcription factors (Gauffo et al., 2004) and their targets (Chambers et al., 2009), as well as graded doses of signalling molecules, such as Shh and bone morphogenetic protein from the floor and roof plates (Wurst and Bally-Cuif, 2001), all influenced by local organizers especially the isthmic organizer (Fig. 1) (Ye et al., 1998; Chizhikov et al., 2006b; Canning et al., 2007).

Although several of the genes involved in generation of mid- and hindbrain neurons have been discovered (Wang and Zoghbi, 2001; Wang et al., 2005; Sieber et al., 2007), the forces controlling neuronal progenitor proliferation are not as well understood as the timing and location of the proliferation. Many neurons in the posterior fossa are generated in the ventricular zone of the hindbrain, while far more are generated in the rhombic lips, the dorsal-most portion of the hindbrain proliferative neuroepithelium (Fig 1B) (Wingate and Hatten, 1999; Sotelo, 2004). The rhombic lips are separated into the upper (cerebellar) rhombic lip, located at the level of rhombomere 1, and the lower (hindbrain) rhombic lip, located at rhombomeres 2–8 (Fig. 1C) (Landsberg et al., 2005). Some of the neurons produced in the ventricular zone, such as the cerebellar Purkinje cells and other gamma-aminobutyric acid (GABA)-ergic cerebellar neurons, migrate radially in a relatively straightforward manner to their final location (Wang and Zoghbi, 2001). Many rhombic lip derivatives, however, such as the cerebellar granule cells and the so-called ‘precerebellar nuclei’ of the brain stem (i.e. inferior olive, lateral reticular and external cuneate nuclei) migrate along complex pathways, often tangential to the radial neuraxis and sometimes over considerable distances, guided by adhesion molecules, neurotrophins, and repulsive molecules that may be on the surface of cells or in the interstitium (Bourrat and Sotelo, 1990; Wingate and Hatten, 1999; Sotelo, 2004; Bloch-Gallego et al., 2005; Kawuuchi et al., 2006; Yamada et al., 2007). Of interest, specific cell types seem to originate from distinct neuroepithelial domains (Fig. 1C). For example, Ptf1a+ domains generate the GABAergic cerebellar Purkinje cells and mossy fibre neurons of the pontine nuclei, lateral reticular nuclei, and external cuneate nuclei (Bermingham et al., 2001), whereas Atoh1+ (also called Math1) domains produce the glutamatergic cerebellar granule cells and climbing fiber neurons of the inferior olivary nuclei (Yamada et al., 2007). It was accepted for many years that deep cerebellar nuclear projection neurons (from the dentate, fastigial, globiform, and emboliform nuclei) are produced in the ventricular zone along with Purkinje cells (for review, see Wang and Zoghbi, 2001), migrating first outward to form a nuclear transitory zone, where they start to differentiate, and then entering a phase of inward migration that takes them to their ultimate position (Altman and Bayer, 1978; 1985). However, recent work suggests that glutamatergic deep cerebellar nuclear projection neurons arise from the rhombic lip, and then migrate rostrally in a subpial stream to the nuclear transitory zone (Fig. 1C) (Wang et al., 2005; Fink et al., 2006). Moreover, recent analysis suggests that all glutamatergic cerebellar neurons (deep nuclear projection neurons, in addition to granule cells, and unipolar brush cells) are produced in the rhombic lips, whereas all GABAergic cerebellar neurons (Purkinje cells and inhibitory interneurons) are produced in the cerebellar ventricular zone (Englund et al., 2006; Fink et al., 2006).

As in the cerebrum, the final destination of migrating neurons in the developing cerebellar cortex, and their specific neuronal cell fate, depend upon many factors: (i) genetic programming; (ii) disengagement signals at the end of migration; (iii) molecular signals received from the surrounding cellular milieu after termination of migration; and (iv) the establishment of distant and local axonal connections (Sotelo, 2004; Chizhikov et al., 2006b; Englund et al., 2006; Kawuuchi et al., 2006; Leto et al., 2006; Porcionatto, 2006; Weisheit et al., 2006). The later parts of this process, including final positioning within the cortex, development of (axons and) dendrites and synapses, and other changes to form a functionally mature neuron, are termed ‘cortical organization’; this process probably begins during neuronal migration, as the distances are shorter and the pathfinding easier in the less mature brain. Axons of the same pathways can later navigate more simply, by detecting signals emanating from these pioneer axons, a process known as fasciculation (Tessier-Lavigne and Goodman, 1996). As for neuronal migration, pathway selection by axons is oriented by a large variety of short and long range guidance cues distributed along the entire pathway, to which different axons respond differently (Richards et al., 2004). Indeed, the growth cone on the leading process of a migrating neuron in many ways resembles that of a pathfinding axon and the mechanisms of pathfinding are likely to be similar (Hatten, 2002; Gomez and Zheng, 2006; Round and Stein, 2007). Neurons of brain stem nuclei also migrate to their final location. With the exception of the oculomotor (third nerve) nuclei, which derive from the mesencephalon, cranial nerve nuclei are derived from rhombencephalic (hindbrain) neuronal precursors: the fourth nerve from rhombomere 1, fifth nerve from rhombomeres 2–3, sixth nerve from rhombomeres 5–6, and seventh nerve from rhombomeres 4–5 (Traboulsi, 2004). Due to their compartmental identity, the neuronal progenitors display programmed migratory behaviors and send axons along defined trajectories to their peripheral targets. While the position of the neuronal cell progenitors along the anteroposterior axis determines the identity of the nucleus, its sensory or motor function is determined by its position along the dorsoventral axis. Graded expression of Shh, together with Pax6 and Nkx2.2, along the dorsoventral axis appears to generate domains conducive to either motor (ventral) or sensory (dorsal) cell fate.
we place in the last group. On the basis of these considerations, evidence for both prenatal origin and disease progression, which and embryology progresses. This leaves a few rare disorders with current knowledge. The flexibility of the system allows the distinctions were classified in the most likely category according to our of many brainstem and cerebellar malformations, among which included and used as part of the classification process. Recognizing proteins, or their functions, were known, this information was underlying processes involved. When the associated genes and expected to, result from developmental aberrations during a particular. These groups differ substantially from those used in previously proposed classifications of cerebellar malformations (including ours), which were largely based on the anatomic regions involved (Paris and Dobyns, 2003) or the end result mor-
phologic appearance of the malformation (Patel and Barkovich, 2002). They also differ from classifications of cortical malforma-
tions based on embryology and genetics (Barkovich et al., 2005), largely because the embryology of the midbrain and hindbrain, and the morphologic consequences of disturbing the normal embryologic processes, are currently not as well defined. As with previous classifications based on embryology and genetics, this classification integrates previous and novel findings, provides a comprehensive view of all major midbrain and hindbrain structures, and has the possibility to expand to accommodate new discoveries. Additional strengths of this system are its flexibility and the understanding it renders to those using it. There is flexi-
bility both in the framework of the classification and in the distribution of malformations within each group: either can be changed as our knowledge of the malformation, its cause, or of the processes involved in midbrain-hindbrain development, change. Ultimately, as in a similar genetic/embryologic classification of malformations of cortical development (Barkovich et al., 2005), we expect that this classification will evolve into a system that almost exclusively uses embryology and genetics as the bases for classification, with clinical phenotypes as subcategories listed under the major categories that are the causative genes and the pathways or networks in which their protein products participate.

Framework of the classification

In constructing this classification, we used known embryologic, genetic, imaging, and pathophysiologic information from the literature plus information acquired from our own patients and laboratory work. Whenever the genetics/embryology of the disorder was well enough understood, we have classified disorders primarily by genotype (ultimately, we would hope that the entire classification will be arranged this way); when the genetics/embryology was incomplete, we classified by clinico-radiologic phenotype. Recognizing that humans have differences from other animals in all of these areas, we have specified when using chick, murine, or zebra fish-derived data in both our tables and in the text. The first step was to use fundamental embryology in order to separate localiz-
ed MH MHB malformations due to early defects in anteroposterior and dorsoventral patterning or mis-specification of cell prolif-
eration zones in the MHB, from malformations that result from later events such as axonal pathfinding and neuronal migration (or disruptions). We next considered existing knowledge regarding MHB malformations associated with more widespread development-
al disorders affecting forebrain structures and those restricted to regions derived from the midbrain or hindbrain; we separated these two large groups and then classified them according to the underlying processes involved. When the associated genes and proteins, or their functions, were known, this information was included and used as part of the classification process. Recognizing that we have only limited knowledge regarding the pathogenesis of many brainstem and cerebellar malformations, among which are some of the most common and best known, the malforma-
tions were classified in the most likely category according to our current knowledge. The flexibility of the system allows the disorders to be reclassified as our knowledge of underlying genetics and embryology progresses. This leaves a few rare disorders with evidence for both prenatal origin and disease progression, which we place in the last group. On the basis of these considerations, we propose to separate midbrain-hindbrain malformations into the following four major groups.

(i) Malformations secondary to early anteroposterior and dorsoventral patterning defects, or to misspecification of mid-hindbrain germinal zones.
(ii) Malformations associated with later generalized development-
al disorders that significantly affect the brainstem and cerebellum (and have a pathogenesis that is at least partly understood).
(iii) Localized brain malformations that significantly affect the brainstem and cerebellum (pathogenesis partly or largely understood, includes local proliferation, migration and axonal guidance).
(iv) Combined hypoplasia and atrophy in putative prenatal onset degenerative disorders.

These groups will form the framework of the new classification and, wherever possible, will contain those disorders known, or expected to, result from developmental aberrations during a particular process. These groups differ substantially from those used in previously proposed classifications of cerebellar malformations (including ours), which were largely based on the anatomic regions involved (Paris and Dobyns, 2003) or the end result morphologic appearance of the malformation (Patel and Barkovich, 2002). They also differ from classifications of cortical malformations based on embryology and genetics (Barkovich et al., 2005), largely because the embryology of the midbrain and hindbrain, and the morphologic consequences of disturbing the normal embryologic processes, are currently not as well defined. As with previous classifications based on embryology and genetics, this classification integrates previous and novel findings, provides a comprehensive view of all major midbrain and hindbrain structures, and has the possibility to expand to accommodate new discoveries. Additional strengths of this system are its flexibility and the understanding it renders to those using it. There is flexi-
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Justification of classification

Group I. Malformations secondary to early patterning defects

Malformations secondary to early patterning defects include those with abnormalities of anteroposterior or dorsoventral segmenta-
tion of the brainstem (Table 2), and are often associated with...
Table 2 Group I. Malformations secondary to early anteroposterior and dorsoventral patterning defects, or to misspecification of mid-hindbrain germinal zones

<table>
<thead>
<tr>
<th>Defects</th>
<th>Examples</th>
<th>Comments and references</th>
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<tbody>
<tr>
<td>Early patterning defects</td>
<td></td>
<td>These are predominately anteroposterior defects, but may have associated dorsoventral defects</td>
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<tr>
<td>I.A. Mid-hindbrain antero posterior pattern</td>
<td></td>
<td>This group is meant to include malformations associated with putative diencephalic–mesencephalic organizer disruption (Barkovich et al., 2007)</td>
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<tr>
<td>I.A.1 Gain, loss or transformation of the</td>
<td></td>
<td>(Barkovich et al., 2007)</td>
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<tr>
<td>diencephalon and midbrain</td>
<td></td>
<td>(Ericson et al., 1997)</td>
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<tr>
<td>I.A.1.a Gain of diencephalon or gain of</td>
<td></td>
<td>Barkovich, unpublished data</td>
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<tr>
<td>midbrain</td>
<td></td>
<td>(Barkovich et al., 2007)</td>
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<tr>
<td>I.A.1.b Loss of diencephalon or loss of</td>
<td></td>
<td>(Wurst and Bally-Cuif, 2001)</td>
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<tr>
<td>midbrain</td>
<td></td>
<td>(Poretti et al., 2007b)</td>
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<tr>
<td>I.A.1.c Gain of diencephalon and loss of</td>
<td></td>
<td>(Millet et al., 1999; Moog et al., 2005; Barkovich et al., 2007)</td>
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<tr>
<td>midbrain</td>
<td></td>
<td>(Broccoli et al., 1999; Barkovich et al., 2007)</td>
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<tr>
<td>I.A.1.d Loss of diencephalon and gain of</td>
<td></td>
<td>(Millet et al., 1999; Moog et al., 2005; Barkovich et al., 2007)</td>
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<tr>
<td>midbrain</td>
<td></td>
<td>(Broccoli et al., 1999; Barkovich et al., 2007)</td>
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<tr>
<td>I.A.2 Gain, loss or transformation of the</td>
<td></td>
<td>(Wurst and Bally-Cuif, 2001)</td>
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<tr>
<td>midbrain and rhombomere</td>
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<td>(Poretti et al., 2007b)</td>
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<tr>
<td>I.A.2.a Gain of midbrain or gain of</td>
<td></td>
<td>(Millet et al., 1999; Moog et al., 2005; Barkovich et al., 2007)</td>
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<tr>
<td>rhombomere 1</td>
<td></td>
<td>(Broccoli et al., 1999; Barkovich et al., 2007)</td>
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<tr>
<td>I.A.2.b Loss of midbrain or loss of</td>
<td></td>
<td>(Millet et al., 1999; Moog et al., 2005; Barkovich et al., 2007)</td>
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<tr>
<td>rhombomere 1</td>
<td></td>
<td>(Broccoli et al., 1999; Barkovich et al., 2007)</td>
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<tr>
<td>I.A.2.c Gain of midbrain and loss of</td>
<td></td>
<td>(Millet et al., 1999; Moog et al., 2005; Barkovich et al., 2007)</td>
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<tr>
<td>rhombomere 1</td>
<td></td>
<td>(Broccoli et al., 1999; Barkovich et al., 2007)</td>
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<tr>
<td>I.A.2.d Loss of midbrain and gain of</td>
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<td>(Millet et al., 1999; Moog et al., 2005; Barkovich et al., 2007)</td>
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<td>rhombomere 1</td>
<td></td>
<td>(Broccoli et al., 1999; Barkovich et al., 2007)</td>
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<tr>
<td>I.A.3 Gain, loss or transformation of lower</td>
<td></td>
<td>These structures are derived from hindbrain segments rhombomeres 2–7; the cerebellum</td>
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<td>hindbrain structures</td>
<td></td>
<td>should be less or not involved</td>
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<tr>
<td>I.A.3.a Gain of pons or medulla</td>
<td></td>
<td>We are looking for examples of elongated pons or medulla in humans</td>
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<tr>
<td>I.A.3.b Loss of pons or medulla</td>
<td></td>
<td>(Poretti et al., 2007b)</td>
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<tr>
<td>Defects</td>
<td>Examples</td>
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<td>I.A.3.c. Mixed gains and losses of pons or medulla</td>
<td>Mouse mutants  • Krox20&lt;sup&gt;-/-&lt;/sup&gt; with transformation of rhombomere 3 to rhombomeres 2/4 and rhombomeres 5 to rhombomeres 6 identities Human  • Short pons – long medulla malformation, some with ventral or dorsal midbrain clefts  • Enlarged ‘pons-like’ medulla</td>
<td>(Schneider-Maunoury et al., 1993; Barkovich et al., 2007)</td>
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<tr>
<td>I.A.3.d. Segmental shifts (A&gt;P or P&gt;A) of pons or medulla</td>
<td>Mouse mutants  • Hoxa1&lt;sup&gt;-/-&lt;/sup&gt;  • Hoxb1&lt;sup&gt;-/-&lt;/sup&gt;  • Hoxb2&lt;sup&gt;-/-&lt;/sup&gt;  • Hoxa1&lt;sup&gt;-/-&lt;/sup&gt;, Hoxb1&lt;sup&gt;-/-&lt;/sup&gt;, Hoxb2&lt;sup&gt;-/-&lt;/sup&gt; triple mutants  » These single, double and triple mutants have defects of hindbrain segments rhombomeres 4–6 Human by genotype  • HOXA1&lt;sup&gt;-/-&lt;/sup&gt;  » Athabaskan brainstem dysgenesis syndrome  » Bosley-Salih-Alorainy syndrome</td>
<td>Human HOXA1 mutations are associated with horizontal gaze abnormalities, hearing loss, facial weakness, hypoventilation, mental retardation and autism spectrum disorder (Gavalas et al., 1998, 2003; Studer et al., 1998, 2003; Tischfield et al., 2005; Bosley et al., 2008)</td>
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<tr>
<td>I.B. Mid-hindbrain dorsoventral patterning defects</td>
<td>Mouse mutants and humans  • Probably any widely expressed ventricular zone gene</td>
<td>Dorsoventral developmental defects mostly involving progenitor specification and proliferation</td>
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<tr>
<td>I.B.1a. Alar and basal ventricular zone defects involving all or uncharacterized dorsoventral sub-regions</td>
<td>Mouse  • Lbx1&lt;sup&gt;-/-&lt;/sup&gt; Human by phenotype  • Cerebellum agenesis with near normal development  • Rhombencephalosynapsis  • Gomez-Lopez-Hernandez syndrome</td>
<td>Disorders in this category will probably cause widespread CNS defects</td>
</tr>
<tr>
<td>I.B.1a. Alar and basal ventricular zone defects involving all or uncharacterized dorsoventral sub-regions</td>
<td>Mouse  • Atoh1&lt;sup&gt;-/-&lt;/sup&gt; (Math1&lt;sup&gt;-/-&lt;/sup&gt;)  • Lmx1a&lt;sup&gt;-/-&lt;/sup&gt;  • Itgb1&lt;sup&gt;-/-&lt;/sup&gt; in CNS only Human  • Diffuse granule cell hypoplasia of cerebellum Mouse mutants  • Ptf1a&lt;sup&gt;-/-&lt;/sup&gt; Human by genotype  • PTF1A&lt;sup&gt;-/-&lt;/sup&gt; » Pancreatic and cerebellar agenesis Mouse mutants  • Phox2b&lt;sup&gt;-/-&lt;/sup&gt; Human by genotype  • PHOX2B&lt;sup&gt;-/-&lt;/sup&gt; » Congenital central hypoventilation syndrome</td>
<td>Most known mutations affect multiple levels, but are best known in Rhombomere 1 We have placed human rhombencephalosynapsis in this group with some uncertainty. (Michaud et al., 1982; Schachenmayr and Friede 1982; Romanengo et al., 1997; Takanashi et al., 1999; Brooks et al., 2000; Toelle et al., 2002; Moog et al., 2005; Pascual-Castroviejo et al., 2005; Sieber et al., 2007; Schell-Apapick et al., 2008) This very old classification may correspond to the congenital disorders of glycosylation type 1a, which would be moved to group II.G.2. (Pascual-Castroviejo et al., 2006). (Ben-Arie et al., 1997; Millong et al., 2000; Blaess et al., 2004; Wang et al., 2005; Blazhikov et al., 2006a); (Hoveyda et al., 1999; Sellick et al., 2004; Glasgow et al., 2005; Hoshino et al., 2005)</td>
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<td>I.B.2a. Alar defects involving more than one dorsoventral sub-region</td>
<td>Mouse  • Atoh1&lt;sup&gt;-/-&lt;/sup&gt; (Math1&lt;sup&gt;-/-&lt;/sup&gt;)  • Lmx1a&lt;sup&gt;-/-&lt;/sup&gt;  • Itgb1&lt;sup&gt;-/-&lt;/sup&gt; in CNS only Human  • Diffuse granule cell hypoplasia of cerebellum Mouse mutants  • Ptf1a&lt;sup&gt;-/-&lt;/sup&gt; Human by genotype  • PTF1A&lt;sup&gt;-/-&lt;/sup&gt; » Pancreatic and cerebellar agenesis Mouse mutants  • Phox2b&lt;sup&gt;-/-&lt;/sup&gt; Human by genotype  • PHOX2B&lt;sup&gt;-/-&lt;/sup&gt; » Congenital central hypoventilation syndrome</td>
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<td>I.B.2b. Alar ventricular zone defects involving roof plate and rhombic lip derivatives including cerebellum granule cells, pontine nuclei, other cell types, choroid plexus</td>
<td>Mouse mutants  • Ptf1a&lt;sup&gt;-/-&lt;/sup&gt; Human by genotype  • PTF1A&lt;sup&gt;-/-&lt;/sup&gt; » Pancreatic and cerebellar agenesis Mouse mutants  • Phox2b&lt;sup&gt;-/-&lt;/sup&gt; Human by genotype  • PHOX2B&lt;sup&gt;-/-&lt;/sup&gt; » Congenital central hypoventilation syndrome</td>
<td></td>
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<tr>
<td>I.B.2c. Alar ventricular zone defects involving the cerebellum ventricular zone including cerebellum GABAergic neurons, inferior olives, other cell types</td>
<td>Mouse mutants  • Ptf1a&lt;sup&gt;-/-&lt;/sup&gt; Human by genotype  • PTF1A&lt;sup&gt;-/-&lt;/sup&gt; » Pancreatic and cerebellar agenesis Mouse mutants  • Phox2b&lt;sup&gt;-/-&lt;/sup&gt; Human by genotype  • PHOX2B&lt;sup&gt;-/-&lt;/sup&gt; » Congenital central hypoventilation syndrome</td>
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<tr>
<td>I.B.2d. Ventral alar ventricular zone defects involving multiple brainstem nuclei such as sensory cranial nerves, locus ceruleus (no cerebellum cells)</td>
<td>Mouse mutants  • Shh&lt;sup&gt;-/-&lt;/sup&gt;</td>
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<tr>
<td>I.B.3a. Basal ventricular zone defects involving more than one dorsoventral sub-region</td>
<td>Mouse mutants  • Shh&lt;sup&gt;-/-&lt;/sup&gt;</td>
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<tr>
<td>I.B.3b. Basal ventricular zone defects involving specific cranial motor nuclei</td>
<td>Human by phenotype  • Duane retraction syndrome (cranial nerve VI)</td>
<td>Also see 1.A.3.d. Segmental shifts of pons or medulla, which underlie some examples in mouse. For example, loss of mouse Hoxb1</td>
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</table>
cerebellar anomalies. Malformations isolated to the cerebellum are not included here, as (in concept) the malformations in this group involve processes that predate formation of the cerebellar anlage. Malformations of this type are well known in animal models, and have been suspected in humans. However, techniques of brain imaging have only recently advanced to a point where thin section, high resolution volumetric data can be acquired in clinically feasible time slots. This has allowed high quality images of the brainstem to be produced in multiple planes and greatly facilitates the identification of morphologic abnormalities. In addition, improvements in diffusion tensor imaging have allowed production of colour fractional anisotropy maps of the brainstem, giving information about the morphology and location of the larger axonal pathways (Sicotte et al., 2006; Widjaja et al., 2006; Jissendi-Tchofo et al., 2009). With the advantage of these tools, malformations are more easily identified; many were reviewed in a recent publication (Barkovich et al., 2007).

The first subgroup of Group I is composed of disorders of anteroposterior segmentation, in which there is gain, loss, or transformation of segments at boundaries between sections of the neural tube, such as the diencephalic-midbrain boundary (Group I.A.1) or midbrain-rhombomere 1 boundary (Group I.A.2). For example, the combination of a shortened midbrain and enlarged pons associated with enlarged anterior vermis (Fig. 2) presumably results from either loss of midbrain, gain of rhombomere 1, or both. Similar rostral displacement of the MHB results in increased Gbx2 expression or reduced Otx2 expression in mouse and chick models (Nakamura and Watanabe, 2005; Waters and Lewandoski 2006), producing an enlarged rhombomere 1, especially anteriorly, and consequently an enlarged anterior vermis (Sgaier et al., 2005). Elongation of the medulla with shortening of the pons (Fig. 3) is postulated to result from mixed gains and losses of pons or medulla (I.A.3.c) or a segmental shift (I.A.3.d). Similar abnormalities result from murine embryo exposure to retinoic acid, which causes a dose-dependent anterior to posterior transformation of cell fate in which the hindbrain is expanded at the expense of the midbrain and forebrain (Lumsden, 2004). Lesser changes in retinoic acid gradient or other regionalizing molecules could result in transformations of the middle rhombomeres from pontine to medullary fate.

The authors have observed several malformations in humans that suggest a posterior to anterior transformation at the diencephalon-mesencephalon junction. Shortening and thickening of the midbrain with midline (mesencephalic) cleft has been described as a malformation of unknown cause (Barkovich et al., 2007). But close inspection of imaging studies shows extension of the third ventricle and other diencephalic features into the upper part of the thickened midbrain (Fig. 4). This is interpreted as a putative posterior to anterior transformation of mesencephalon into diencephalon that results in caudal expansion of the diencephalon (I.A.1.c). A similar malformation has been described in mouse...
models with overexpression of Pax6 in the diencephalon and underexpression of En1/Pax2 in the anterior mesencephalon (Nakamura and Watanabe, 2005; Lim and Golden, 2007). Other patients have been described with elongated midbrain and medulla with short pons (Barkovich et al., 2007); classification is difficult in such cases. Further understanding of such patients awaits identification of genes and animal models.

Defects in dorsoventral patterning are herein postulated to result in abnormal development or function of specific mid-hindbrain ventricular zones and structures derived from them, including abnormal formation of brain stem nuclei, cranial nerves, or any cerebellar structures (Section I.B). For example, abnormal development of the superior rhombic lip may cause diffuse granule cell hypoplasia (Group I.B.2.b) while abnormal development of the cerebellar ventricular zone due to mutation of the PTF1A gene causes cerebellar (and pancreatic) agenesis (Group I.B.2.c) (Sellick et al., 2004; Hoshino et al., 2005) and defects of the basal ventricular zone result in defects of specific cranial nerve nuclei such as the abducens and facial nerves (Section II.B.3.b) (Al-Baradie et al., 2002; Michielse et al., 2006). [Note that diffuse granule cell hypoplasia may, in fact be better classified as congenital disorder of glycosylation (CDG) type 1a (IV.B), as suggested by Pascual-Castroviejo et al. (2006). It is temporarily included in both categories.] The Ptf1a gene encodes a basic helix-loop-helix transcription factor that has been shown to be expressed in progenitor cells in the ventricular zone of the dorsal aspect or rhombomere 1; the protein product is required for the generation of GABAergic cells (Purkinje cells and interneurons) in the cerebellum (Hoshino et al., 2005), neurons of the inferior olivary nuclei (Yamada et al., 2007), and specification of dorsal interneurons in the spinal cord (Glasgow et al., 2005). [It is also necessary for the specification and formation of the pancreas (Hoshino et al., 2005).] The number of granule cells generated is extremely reduced when Purkinje cells are not located in their normal position and in normal numbers (Wetts and Herrup, 1982; Sotelo, 2004). In animal models, Purkinje cells regulate proliferation of granule cell precursors via secretion of Shh, perhaps by upregulation of Nmyc (Wallace, 1999; Kenney et al., 2003; Hoshino, 2006). Granule cells are reduced in number by any process that reduces the number of viable Purkinje cells. Thus, just as accentuated apoptosis can cause cerebral hypoplasia, it causes cerebellar hypoplasia, as well (Kaindl et al., 2006; Takano et al., 2006). In humans, mutations of PTF1A result in profound cerebellar hypoplasia (Fig. 5) (Sellick et al., 2004; Hoshino et al., 2005). It will probably take time for all of the precise causes of cerebellar hypoplasia to become fully elucidated; as these causes become better understood, this classification can be appropriately modified.

Several reports have described seven patients in whom the superior portion of the brain stem is connected to the inferior portion of the brain stem by a thin cord of tissue (Mamourian and Miller, 1994; Sarnat et al., 2002; Bednarek et al., 2005; McCann et al., 2005; Poretti et al., 2007b; Barth et al., 2008); these have been referred to as brain stem ‘disconnection syndromes’. In three of the patients, the disconnection was in the lower midbrain/upper pons (I.A.2.b) and in four it was in the lower pons/upper medulla (I.A.3.b, Fig. 6). Neuropathological analysis of two cases by Sarnat et al. (2002) showed a thin midline cord passing from the upper segment to the lower segment with hypoplasia of the cerebellar vermis and hemispheres and an anomalous basilar artery. Histological investigation revealed a poorly organized mixture of neurons in the tegmentum, but no evidence of any gliotic lesions to suggest hypoxia or ischaemia; this was interpreted as providing evidence in favour of a brain stem malformation, rather than a disruption (Sarnat et al., 2002). In contrast, Barth et al. (2008) found central cavitation that they interpreted as more of a syrinx and postulated a vascular cause. It is, indeed, possible that some ‘disconnection’ syndromes might be described as examples of segmental dysgenesis in which segments of the midbrain and hindbrain do not develop normally, perhaps as a result of malsexpression of the genes that are responsible for segmentation. One of the authors has seen a case of disconnection syndrome associated with periventricular nodular heterotopia, a finding that supports a genetic aetiology. In avian and murine models, the formation of the rhombomeres is closely related to expression of Hox genes, a set of chromosomally clustered genes whose close relatives are known to specify positional values along the main body axis of the fly embryo (Lumsden, 2004). In avian models, the loss of Hoxa1 function, for example, results in deletion of rhombomere 5, reduction of rhombomere 4, and loss of specific neuronal nuclei (I.A.3.c) (Mark et al., 1993). Another possibility is that disruption of the upstream modulators of Hox genes, such as Krox20 and Mathb, may be responsible for these disconnections (Lumsden, 2004). However, in animal models, deletion of a rhombomere results in a shortened brain stem, but not in a ‘gap’ within the brain stem (Lumsden, 2004). In addition, it is important to remember that early vascular
disruptions in the brain result in tissue liquefaction without glial response. Thus, gliosis would not be expected from an early segmental injury, and so an early vascular or toxic injury to the brain stem might be more likely. Further work with animal models or identification of families with these malformations may help to further elucidate these mechanisms.

In mouse models, absence of several cranial nerves has resulted from abnormal expression of anteroposterior patterning genes (I.A.3.c), including *Wnt1* (trigeminal nerve), *Gbx2* (trigeminal nerve), *Hoxb1* (loss of facial motoneurons, absent facial nerve), *Hoxb2* (absent facial nerve), and *Hoxa3* (hypoplasia of IXth cranial ganglia) (Cordes, 2001; ten Donkelaar et al., 2006). In *Krox20*−/− mice, rhombomeres 3 and 5 do not develop, the abducens nucleus and the visceromotor component of the facial nerve are absent, and the axons of trigeminal motoneurons join the facial nerve and enter the second pharyngeal arch (Schneider-Maunoury et al., 1997). These axons do not find the muscles of mastication (their proper targets), so the parent motoneurons undergo apoptosis (Schneider-Maunoury et al., 1997). It is likely that some mutations of the corresponding human genes will eventually be found in patients with congenital cranial neuropathies.

Segmental shifts in the brain stem are also present in humans with Athabaskan brainstem dysgenesis syndrome (seen in Native American tribes) and Bosley-Salih-Alorainy syndrome (observed in Saudi and Turkish families), both caused by homozygosity for mutations of *Hoxa1* (Bosley et al., 2008), resulting in horizontal gaze abnormalities, hearing loss, facial weakness, hypoventilation, mental retardation and autism spectrum disorder (Tischfield et al., 2005). Anomalies of the vascular system and the inner ear may be seen as well (Tischfield et al., 2005).
Group II. Generalized brain malformations that significantly affect the brain stem and cerebellum

Malformations in Group II are best classified as generalized brain disorders but involvement of the midbrain and hindbrain is so significant that they need to be included in this classification. Some of these disorders affect cell proliferation, others are believed to primarily affect cell migration, while still others are associated with defects in ciliary proteins and, therefore, probably affect cell migration, axon navigation, and possibly other aspects of brain development.

The first group in this section (Group II.A, Table 3) is mid-hindbrain malformations in association with developmental encephalopathies, a term used to describe mental retardation, autism-spectrum disorders, Rett syndrome, and other similar disorders. For example, a number of families with mental retardation or autism and nonprogressive cerebellar hypoplasia (Illarioshkin et al., 1996; Illarioshkin et al., 1999; Gardner et al., 2001; Tsao et al., 2006; Ventura et al., 2006) or isolated vermian hypoplasia (Courchesne et al., 1988; Carper and Courchesne, 2000; Bergmann et al., 2003; Philip et al., 2003; van Amelsvoort et al., 2004; Zinkstok and van Amelsvoort, 2005; Bish et al., 2006; Boland et al., 2007; Hill et al., 2007; Poot et al., 2007; van Bon et al., 2008; Webb et al., 2009) have been described, including some with mutations of oligophrenin 1 (OPHN1) (Zanni et al., 2005) and one found to have a locus in Xp11.21-q24 (Illarioshkin et al., 1999).

An important, and only recently described, group is mesenchymal-neuroepithelial signalling defects (Group II.B, Table 4). Work in Forkhead box C1 (Foxc1) knock-out mice has shown that, even though the gene is expressed only in the posterior fossa mesenchyme overlaying the cerebellum, absence of Foxc1 deficiency results in cerebellar hypoplasia (Aldinger et al., 2009). In humans, mutations of FOXC1 cause a range of posterior fossa hypoplasia.
Table 3  Group II.A. Developmental encephalopathies associated with mid-hindbrain malformations (these include mental retardation, autism spectrum disorders, schizophrenia, Rett-like disorders and others)

<table>
<thead>
<tr>
<th>Defects</th>
<th>Examples</th>
<th>Comments and references</th>
</tr>
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</table>
| II.A.1. Developmental encephalopathies associated with diffuse cerebellar hypoplasia | Mouse mutants  
- Grid2−/− (lurcher mouse)  
- Rora−/− (staggerger mouse)  
- Grik2−/− (weaver mouse)  
Human by phenotype  
- Isolated cerebellum agenesis  
- X-linked non-progressive cerebellar hypoplasia  
- Mental retardation, epilepsy with cerebellar hypoplasia  
- AD (or XL) cerebellar hypoplasia with improvement | This is most likely a heterogeneous group that needs further attention. (Illariohkin et al., 1996, 1999; Gardner et al., 2001; Chizhikov and Millen 2003; Tsao et al., 2006; Ventura et al., 2006; Gold et al., 2007; Vogel et al., 2007) |
| II.A.2. Developmental encephalopathies associated with cerebellum vermis hypoplasia | Human by phenotype  
- Mental retardation with cerebellar vermis hypoplasia  
  » OPHN1−/−  
  » del 1q44, del 22q11.2  
- Autism with cerebellum vermis hypoplasia | The link between autism and developmental defects of the cerebellum is now reasonably well established after years of controversy. The basis for the link is not understood. The oligophrenin 1 protein participates in morphogenesis and function of dendritic spines. (Courchesne et al., 1988; Carper and Courchesne 2000; Bergmann et al., 2003; Philip et al., 2003; van Amelsvoort et al., 2004; Zinkstok and van Amelsvoort 2005; Bish et al., 2006; Boland et al., 2007; Hill et al., 2007; Poot et al., 2007; van Bon et al., 2008; Webb et al., 2009) (Barkovich et al., 2007; Schmid et al., 2007) |
| II.A.3. Developmental encephalopathies associated with BS (especially pontine) hypoplasia | Mouse mutants  
- Nsc1/2−/− with pontine hypoplasia  
Human by phenotype  
- Pontine hypoplasia | |

Only limited data regarding pathogenesis are available for most disorders placed here. Some are probably due to defects in cell fate (downstream signalling) or cell maintenance.

Table 4  Group II.B. Mesenchymal-neurepithelial signalling defects associated with mid-hindbrain malformations

<table>
<thead>
<tr>
<th>Defects</th>
<th>Examples</th>
<th>Comments and references</th>
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</table>
| II.B.1. Combined cerebellar and posterior fossa malformations | Mouse mutants  
- Zic1+/−;Zic4+/−  
- Foxc1−/−  
Human by phenotype  
- Cerebellum vermis hypoplasia  
- Mega-cisterna magna with cerebellum vermis hypoplasia  
- Dandy-Walker malformation  
  » Foxc1+/−  
  » del 3q24 (loss of ZIC1-ZIC4)  
  » del 6p25.3 (loss of FOXC1), dup 9p, del 13q2, dup 17p13.3  
  » AD locus 2q36.1  
- Neurocutaneous melanosis  
- PHACES syndrome with Dandy-Walker malformation | Our data demonstrate a spectrum of cerebellar malformations including classic Dandy-Walker malformation in humans with mutations of FOXC1, which signals from mesenchyme to cerebellum, but is not expressed in the cerebellum itself (Aldinger et al., 2009). [Narayan et al., 1987; Kadonaga et al., 1992; Melaragno et al., 1992; Barkovich et al., 1994; Frieden et al., 1996; McCormack et al., 2002, 2003; Cazorla Calleja et al., 2003; Bhattacharya et al., 2004; Grinberg et al., 2004; Acosta Jr et al., 2005; Chen et al., 2005; Ballarati et al., 2007; Jalali et al., 2008; Aldinger et al., 2009 (in revision)] |
| II.B.2. Posterior fossa anomalies largely sparing the cerebellum | Human by phenotype  
- Arachnoid cysts of posterior fossa  
- Mega-cisterna magna, isolated | Mega-cisterna magna in this group consists of an enlarged posterior fossa with normal size of cerebellum (Barkovich et al., 1989; Siebert 2006) |

The rationale for this group comes from our data showing that loss of mesenchymal expression of FOXC1 leads to combined cerebellar and posterior fossa malformations (Aldinger et al., 2009).

Anomalies ranging from vermis predominant cerebellar hypoplasia to mega cisterna magna to Dandy–Walker malformation (Aldinger et al., 2009). Similar ranges of posterior fossa anomalies (Fig. 7) have been described with deletion of 3q24 (loss of ZIC1-ZIC4) (Grinberg and Millen, 2005), duplication of 9p (Melaragno et al., 1992; Cazorla Calleja et al., 2003; Chen et al., 2005), deletion of 13q2 (McCormack et al., 2003; Ballarati et al., 2007), and deletion of 2q36.1 (Jalali et al., 2008), as well as in neurocutaneous
melanosis (Kadonaga et al., 1992; Barkovich et al., 1994; Acosta Jr et al., 2005) and PHACES (Posterior fossa malformations, Haemangioma, Arterial anomalies, Cardiac abnormalities/aortic coartation, Eye abnormalities, Sternal cleft defects) syndrome (Frieden et al., 1996; Metry et al., 2001), raising the possibility of significant effects of the developing leptomeninges upon MB-HB development. The finding of malformations of the leptomeninges (arachnoid cysts, mega cisternae magnae, meningoceles), which are derived from cranial mesenchyme, in some of the same families suggests that these malformations belong within the same group (II.B.2).

A number of malformations are proposed to result from abnormal cell proliferation (Group II.C, Table 5); these include decreased proliferation, increased proliferation, and proliferation of dysplastic cells. Increased proliferation (Group II.C.2) is very uncommon; it is mainly seen in the macrocephaly-capillary malformation syndrome (Conway et al., 2007), which has many similarities to the megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome and is likely to be closely related to it (Gripp et al., 2009). Both have overgrowth of cerebral and cerebellar hemispheres that often result in cerebellar tonsillar herniation and sometimes Chiari 1 malformation. Proliferation of abnormal cells (Group II.C.1) predominantly results in focal areas of overgrowth containing dysplastic cells. Dysplastic gangliocytoma of the cerebellum (Lhermitte-Duclos disease) and cerebellar cortical hamartomas (of tuberous sclerosis) are both mass-like disorders that are composed of dysplastic, rather than neoplastic, cells and are, therefore, included in this section. Lhermitte-Duclos is characterized pathologically by enlarged, circumscribed cerebellar folia containing large ganglion cells in the granular cell layer and prominent myelinated tracts in the outer molecular layer. However, the histology is variable, ranging from a recognizable

![Figure 7](image_url)

**Figure 7** Dandy–Walker malformations with multiple associated genetic/clinical disorders. All show a small cerebellum and a CSF containing structure that expands the posterior fossa; these seem to result from mutations of genes that affect both leptomeningeal and cerebellar development. Similar appearances are seen in patients with different gene mutations, while different appearances are seen in patients with mutations of the same gene. (A, B) Patients with deletion 3q24; note the markedly different severity of the hindbrain malformation. (C) Patient with deletion 6p25.3. (D) Patient with PHACES syndrome.
granular cell layer containing occasional large dysplastic neuronal cell bodies, to an unrecognizable granular layer occupied by a population of large nerve cell bodies between the molecular layer and internal white matter (Ambler et al., 1969). The hypertrophic granule cells express neurofilament protein in a manner similar to Purkinje cells, and it has been postulated that the increased expression of neurofilament proteins by the cerebellar granule cells may account for their hypertrophy and subsequent axonal enlargement leading to myelination within the molecular layer of the cerebellar cortex (Yachnis et al., 1988). Nearly 50% of cases are associated with Cowden syndrome, an autosomal dominant syndrome caused by mutations of the PTEN gene at 10q23.31, and characterized by multiple hamartomas throughout the body (Marsh et al., 1999). Cortical tubers of tuberous sclerosis, caused by mutations of either the TSC1 (at 9q34) or TSC2 (at 16p13) gene are composed of a coarse subpial gliosis, abnormal cortical lamination with many large, abnormal, often multinucleated cells, and multiple heterotopic subcortical neurons (Norman et al., 1995); the finding of cerebellar tubers is common (Eluvathingal et al., 2006). The effect of these cerebellar lesions upon outcome is not understood. Hemimegalencephaly is a poorly understood malformation of cerebral cortical development, composed of dysmorphic cells (both neuronal and glial) that are often in abnormal locations (Robain and Gelot, 1996; Flores-Sarnat, 2002; Flores-Sarnat et al., 2003). This most often occurs as an isolated malformation, but may be associated with epidermal nevus (linear nevus sebaceous of Jadassohn) or other neurocutaneous syndromes (Peserico et al., 1988; Pavone et al., 1991; Pelayo et al., 1994; Griffiths et al., 1994), or with tuberous sclerosis (Griffiths et al., 1998; Galluzzi et al., 2002) or other phakomatous (Cusmai et al., 1990; Dhamecha and Edwards-Brown, 2001). The reason for the presence of ipsilateral cerebellar hemispheric enlargement and dysplasia in some cases (Sener, 1997) is even more poorly understood.

Microcephalies with (disproportionately) decreased cerebellar cell proliferation (Group II.C.3) mostly have autosomal recessive inheritance (Albrecht et al., 1993; Sztriha et al., 1998; Hashimoto et al., 1998; Rajab et al., 2003) (although CASK mutations cause microcephaly with disproportionate cerebellar hypoplasia via X-linked inheritance (Najm et al., 2008)). Many patients with developmental microcephaly (in contrast to those with acquired microcephaly) have cerebellum that are proportionally small when compared to the cerebrum (Fig. 8) (Barkovich et al., 1998; Bellini et al., 2002; Kelley et al., 2002; Sheen et al., 2004; Chandler et al., 2006), suggesting that many of the same processes controlling cell proliferation or apoptosis apply in both the supra- and infratentorial compartments. Other patients with microcephaly (Hoveyda et al., 1999; Hoshino et al., 2005; Sztriha et al., 2005; Sztriha and Johansen, 2005) and some with normal head size (Patel and Barkovich, 2002) have disproportionately small cerebella, suggesting that developmental processes differ in the supra- and infratentorial compartments.

Many other malformations of cortical development are associated with MB-HB developmental abnormalities (Table 6), including lissencephalies (Group II.D.1), cerebral heterotopia (Group II.D.2), cerebral polymicrogyria (Group II.D.3), and cobblestone-like malformations with defects of the pial basement membrane (Group II.D.4). The association of cerebellar hypoplasia with cerebral heterotopia and polymicrogyria is not understood. The reason for cerebellar hypoplasia associated with lissencephaly (Ross et al., 2001), even when head size is normal, is not always known; as discussed in the previous section, some pathways and processes that are more involved in cerebellar than cerebral development are affected in these cases. Alternatively, the cerebellar hypoplasia may result from associated Purkinje cell involvement, or failure of connection of Purkinje cells with granule cells, causing subsequent apoptosis of the granule cells. In DCX and LIS1 mutations, the cerebellar hypoplasia is inconsistent and, when present, is rather mild (Ross et al., 2001). It is more consistently seen in DCX mutations rather than in LIS1 mutations (unpublished results), and is severe in a significant number of patients with TUBA1A mutations (Bahi-Buisson et al., 2008; Fallet-Bianco et al., 2008; Morris-Rosendahl et al., 2008). The mid-hindbrain is particularly severely affected in patients with RELN and VLDLR associated cortical malformations (Group II.D.1.b), in which the brain stem shows malpositioning of neurons (Nishikawa et al., 2003) and the cerebellum is extraordinarily small and smooth (nearly afoliar, Fig. 9) (Hong et al., 2000; Boycott et al., 2005). Reelin is a secreted glycoprotein that regulates neuronal positioning in cortical brain structures and the migration of neurons along the radial glial fibre network by binding to lipoprotein receptors VLDLR (very low density lipoprotein receptor) and APOER2 (apolipoprotein E receptor 2, or low density lipoprotein receptor-related protein 8) and the adapter protein disabled-1 (DAB1) (Hiesberger et al., 1999). In the cerebellum, Reelin regulates Purkinje cell alignment (Miyata et al., 1997) and granule cell proliferation (Wechsler-Reya and Scott, 1999), which are necessary for the formation of a normal sized cerebellum, as well as a well-defined cortical plate through which granule cells migrate to form the internal granular layer (Rakic and Sidman, 1970). Although both protein products function in the same pathway, RELN mutations seem to have a more severe effect than VLDLR mutations on
both the cerebral and cerebellar malformations. The reason for the difference is not known at this time, although it is probably related to the fact that reelin has multiple other receptors, including ApoER2, that result in different downstream effects and that these effects differ in the mid- and hindbrain compared to the forebrain (Gressens, 2006; Hack et al., 2007).

The so-called dystroglycanopathies (Group II.D.4), believed to be caused by impaired O-mannosylation of α-dystroglycan (Moore et al., 2002; Beltran-Valero de Bernabe et al., 2004; van Reeuwijk et al., 2005; Kanagawa and Toda, 2006; Saito et al., 2006; Martin, 2007), are associated with congenital muscular dystrophy and variable eye and brain anomalies. The brain abnormalities are sometimes called cobblestone malformation and involve the cerebrum, brain stem, and cerebellum. In these disorders, abnormal O-glycosylation of α-dystroglycan in the basal lamina of the pial basement membrane is postulated to result in abnormal fusion of the endfeet of radial glial cells with the basal lamina and gaps in the pial basement membrane; migrating neurons do not receive proper ‘stop’ signals and overmigrate into the subpial space (van Reeuwijk et al., 2005, 2006; Kanagawa and Toda, 2006; Saito et al., 2006; Martin, 2007). In the cerebellum, affected patients have variable degrees of dysmorphism, ranging from normal cortical foliation with a few cortical/subcortical cysts (Fig. 10) to profound cerebellar hypoplasia and dysmorphism with greater involvement of the vermis than the hemispheres (Fig. 11). The malformation may be related to disturbances in the external granule cell layer (Henion et al., 2003). The brain stem is affected in nearly all patients, manifesting enlarged quadrigeminal plates, fusion of the colliculi, and hypoplasia of the pons, often with a longitudinal ventral midline pontine cleft (Figs 10 and 11) (Barkovich et al., 2007). The small pons with midline cleft may be caused by hypoplasia of the middle cerebellar peduncles resulting in hypoplasia of their decussation; another component of the pontine hypoplasia may relate to impaired tangential migration of pontine nuclear neurons as shown in murine models of Large mutations (Qu et al., 2006). Thus, as in the cerebral hemispheres, the midbrain-hindbrain disorder appears to be the result of both abnormal neuronal migration and abnormal formation of white matter tracts. We know of several similar malformations associated with subtle differences in both the glycosylation defects and the clinical phenotype (Group II.D.4.b), including those due to mutations of GPR56 [often called bilateral frontoparietal polymicrogyria (Chang et al., 2003), ATP6V0A2 [associated with Debré type cutis laxa (Kornak et al., 2008; Van Maldergem et al., 2008)], and SNAP29 [which is associated with cerebral dysgenesis, neuropathy, ichthyosis, and palmoplantar keratoderma (CEDNIK) syndrome (Sprecher et al., 2005)]. Recent work shows that G protein-coupled receptor (GPR) 56 has a role in the organization of the pial basement membrane and in the regulation of anchorage of radial glial endfeet (Li et al., 2008).

### Table 5 Group II.C. Malformations of neuronal and glial proliferation that prominently affect the brainstem and cerebellum

| Classification of mid-hindbrain malformations | Brain 2009: 132; 3199–3230 | 3213 |

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<th>Defects</th>
<th>Examples</th>
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<tr>
<td>II.C.1. Diffuse dysplasia with abnormal cell types, more severe in cerebellum</td>
<td>Mouse mutant: Pten&lt;sup&gt;−/−&lt;/sup&gt; Protein; Human by phenotype: Tuberous sclerosis (TSC1&lt;sup&gt;−/−&lt;/sup&gt;; TSC2&lt;sup&gt;−/−&lt;/sup&gt;; Cowden syndrome and Lhermitte-Duclos disease (PTEN&lt;sup&gt;−/−&lt;/sup&gt;; Hemimegalencephaly with ipsilateral cerebellaromegaly)</td>
<td>The abnormalities here are focal areas of overgrowth and dysplasia. (Eng et al., 1994; Nelen et al., 1996; Sener 1997; Backman et al., 2001; Eluvathingal et al., 2006)</td>
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<td>II.C.2. Megalencephaly associated with (probably) disproportionate cerebellomegaly and Chiari 1 malformation</td>
<td>Human by phenotype: Macrocephaly-capillary malformation syndrome including MPPH (megalencephaly-polymicrogyria-polydactyly-hydrocephalus); Costello syndrome: HRAS gain of function mutations</td>
<td>Macrocephaly-capillary malformation syndrome may be complicated by cerebellum tonsillar herniation and Chiari malformation (Conway et al., 2007). Our data suggest that macrocephaly-capillary malformation and MPPH syndromes represent either a single syndrome, or related disorders in the same pathway (Gripp et al., 2009). We have data on Costello syndrome showing cerebellum tonsillar herniation and Chiari similar to macrocephaly-capillary malformation. While CASK is X-linked, most disorders in this group have autosomal recessive inheritance. One of these was described as a form of pontocerebellar hypoplasia (Rajab et al., 2003), but we interpret this as diffuse cerebellum hypoplasia. (Albrecht et al., 1993; Hashimoto et al., 1998; Sztriha et al., 1998; Rajab et al., 2003, 2007; Najm et al., 2008)</td>
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<td>II.C.3. Microcephaly with severe and disproportionate brainstem and cerebellar hypoplasia</td>
<td>Mouse mutants: CASK−/− and CASK&lt;sup&gt;−/−&lt;/sup&gt; (male and female) Human by phenotype: Severe congenital microcephaly with disproportionate Brainstem and cerebellum hypoplasia and variable enlarged extra-axial spaces Postnatal microcephaly with disproportionate brainstem and cerebellar hypoplasia: CASK&lt;sup&gt;−/−&lt;/sup&gt;; CASK&lt;sup&gt;−/−&lt;/sup&gt; Postnatal microcephaly and diffuse cerebellum hypoplasia with spasticity, autosomal recessive: Locus in 7q11–q21</td>
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<td>Defects</td>
<td>Examples</td>
<td>Comments and references</td>
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<td>II.D.1. Lissencephaly with cerebellar hypoplasia (LCH)</td>
<td>Mouse mutants</td>
<td>A diagnosis of isolated lissencephaly sequence is used instead of LCH when the cerebellum appears normal on brain imaging studies, and is most common with DCX and LIS1 mutations. The LCH group includes classic or 4-layered lissencephaly in LCH group a, subcortical band heterotopia, and probably 2-layered lissencephaly in LCH groups c (which includes our prior group f) and d (see text and Ross et al., 2001). (Hirotsume et al., 1998; Ross et al., 2001; Cardoso et al., 2003; Toyo-oka et al., 2003; Forman et al., 2005; Keays et al., 2007; Morris-Rosendahl et al., 2008.)</td>
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<tr>
<td>II.D.1.a Lissencephaly with cerebellar hypoplasia (LCH), new mutation autosomal dominant and X-linked inheritance</td>
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<tr>
<td>Mouse mutants</td>
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<tr>
<td>• Lis1+/− and Lis1+/cko</td>
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<tr>
<td>• Tubat1a+/− (heterozygous partial loss of function)</td>
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<td>• Dcx−/− (obvious in rat, subtle in mouse)</td>
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<tr>
<td>Human by genotype</td>
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<tr>
<td>• Dcx−/−, Dcx−/− (males and females)</td>
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<tr>
<td>» LCH in males corresponding to Ross LCH group a, and subcortical band heterotopia in females. Most have subtle cerebellar hypoplasia; a few have mild or moderate cerebellum hypoplasia.</td>
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<td>Human by genotype</td>
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<tr>
<td>• Lis1−/−</td>
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<tr>
<td>» LCH in males corresponding to Ross LCH group a. Most have subtle cerebellum hypoplasia; a few have mild or moderate cerebellum hypoplasia.</td>
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<tr>
<td>• Lis1−/−; Ywhae+/−</td>
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<tr>
<td>» Miller-Dieker syndrome; most have subtle cerebellum hypoplasia; a few have mild or moderate cerebellum hypoplasia.</td>
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<td>• Tubat1a+/−</td>
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<td>» Most patients have Ross LCH group c with severe cerebellar and callosal hypoplasia, or group d with cerebellar hypoplasia only. Others have mild cerebellum hypoplasia (LCH group a), or lissencephaly with normal cerebellum by imaging.</td>
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<tr>
<td>II.D.1.b. LCH with hippocampal hypoplasia and nearly afoilary cerebellum, autosomal recessive inheritance</td>
<td>Mouse mutants</td>
<td>Mouse knockouts have inverted cortex, while humans have moderate frontal predominant lissencephaly in addition to severe cerebellum hypoplasia. (Trommsdorff et al., 1999; Hong et al., 2000; Ross et al., 2001; Boycott et al., 2005; Chang et al., 2007; Zaki et al., 2007)</td>
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<tr>
<td>Human by genotype</td>
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<tr>
<td>• Reln−/− (reeler mouse)</td>
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<td>• Dab1−/− (scrambler and yotari mice)</td>
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<td>• Vldlr−/−, Lrp8−/−</td>
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<tr>
<td>Human by genotype</td>
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<tr>
<td>• Reln−/−</td>
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<td>• Vldlr−/−</td>
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<tr>
<td>» Ross LCH group b with mild frontal lissencephaly, small dysplastic hippocampus and severe afoilary cerebellum hypoplasia; the RELN phenotype is more severe than the VLDLR phenotype</td>
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<tr>
<td>II.D.1.c. LCH, other types</td>
<td>Human by genotype</td>
<td>LCH group e consists of lissencephaly with sudden transition to simplified gyral pattern. (Ross et al., 2001; Forman et al., 2005)</td>
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<td>Human by genotype</td>
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<tr>
<td>• Ross LCH groups c and d not associated with TUBA1A mutations</td>
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<td>• Ross LCH group e</td>
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<tr>
<td>II.D.2. Neuronal heterotopia with prominent brainstem and cerebellum hypoplasia</td>
<td>Mouse mutants; Flna−/−</td>
<td>HET may be diffuse or regional, and are sometimes asymmetric. (Moro et al., 2002; Parrini et al., 2006)</td>
</tr>
<tr>
<td>Mouse</td>
<td></td>
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<tr>
<td>• Periventricular nodular heterotopia with cerebellum vermis hypoplasia</td>
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<td>» FLNA−/−, rarely FLNA−/Y</td>
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<td>Human</td>
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<tr>
<td>• Periventricular nodular heterotopia with cerebellum vermis hypoplasia</td>
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<tr>
<td>» FLNA−/−, rarely FLNA−/Y</td>
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<tr>
<td>II.D.2.b. Periventricular nodular heterotopia with overlying polymicrogyria and prominent brainstem and cerebellum hypoplasia</td>
<td>Mouse</td>
<td>(Wieck et al., 2005; Sarkisian et al., 2006)</td>
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<tr>
<td>Mouse</td>
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<tr>
<td>» Map3k4−/−</td>
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<tr>
<td>Human</td>
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<tr>
<td>• Frontal-perisylvian Periventricular nodular heterotopia-polymicrogyria with cerebellum hypoplasia</td>
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<td>• Posterior Periventricular nodular heterotopia-polymicrogyria with cerebellum hypoplasia</td>
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<tr>
<td>II.D.2.c. Subcortical nodular heterotopia with cerebellum hypoplasia</td>
<td>Human</td>
<td>(Dubeau et al., 1995; Barkovich 2000)</td>
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<tr>
<td>Human</td>
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<tr>
<td>• Subcortical nodular heterotopia with dysplastic cortex, ACC, cerebellum hypoplasia</td>
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**Table 6 Continued**

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<th>Defects</th>
<th>Examples</th>
<th>Comments and references</th>
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<tr>
<td>II.D.3. Polymicrogyria with cerebellum hypoplasia</td>
<td>Mouse&lt;br&gt;• Tbr2−/−&lt;br&gt;• ACC-polymicrogyria-cerebellum hypoplasia&lt;br&gt;» TBR2&lt;br&gt;• Aicardi syndrome&lt;br&gt;• Dellemann syndrome (Oculocutaneouscerebral syndrome)</td>
<td>(Aicardi et al., 1965; Ferrer et al., 1986; Aicardi 2005; Baala et al., 2007a)</td>
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<tr>
<td>II.D.4. Malformations with basement membrane and neuronal migration deficits</td>
<td>Mouse mutants&lt;br&gt;• Dapko/+/&lt;br&gt;• Large−/−&lt;br&gt;» Both mutants have pial basement membrane disruption and other malformations similar to the human syndromes. Human by genotype&lt;br&gt;• POMGnT1−/−&lt;br&gt;» Muscle-eye-brain disease (MEB) and congenital muscular dystrophy (CMD) without recognized brain malformations&lt;br&gt;• FCMD−/−&lt;br&gt;» Walker-Warburg syndrome (WW5), Fukuyama congenital muscular dystrophy (FCMD) and congenital muscular dystrophy without brain malformations&lt;br&gt;• FKRP−/−&lt;br&gt;• LARGE−/−&lt;br&gt;• POMT1−/−&lt;br&gt;• POMT2−/−&lt;br&gt;» Walker-Warburg syndrome, muscle-eye-brain disease and congenital muscular dystrophy without brain malformations</td>
<td>Most of the cobblestone and cobblestone-like (due to lack of pathological confirmation) brain malformations are associated with defects in glycosylation. From most to least severe, the spectrum of cobblestone malformations-associated phenotypes includes Walker-Warburg syndrome, muscle-eye-brain disease, Fukuyama congenital muscular dystrophy and congenital muscular dystrophy without recognized brain malformations. All but POMGnT1 are associated with a wide spectrum of severity. (Kobayashi et al., 1998; Yoshida et al., 2001; Beltran-Valero de Bernabe et al., 2002, 2003, 2004; Holzfeind et al., 2002; Moore et al., 2002; Longman et al., 2003; Akasaka-Manya et al., 2004; Godfrey et al., 2007; Manzini et al., 2008)</td>
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<td>II.D.4.a. Cobblestone malformations with abnormal alpha-dystroglycan glycosylation</td>
<td>Mouse mutants&lt;br&gt;• Dapko/+/&lt;br&gt;• Large−/−&lt;br&gt;» Both mutants have pial basement membrane disruption and other malformations similar to the human syndromes. Human by genotype&lt;br&gt;• POMGnT1−/−&lt;br&gt;» Muscle-eye-brain disease (MEB) and congenital muscular dystrophy (CMD) without recognized brain malformations&lt;br&gt;• FCMD−/−&lt;br&gt;» Walker-Warburg syndrome (WW5), Fukuyama congenital muscular dystrophy (FCMD) and congenital muscular dystrophy without brain malformations&lt;br&gt;• FKRP−/−&lt;br&gt;• LARGE−/−&lt;br&gt;• POMT1−/−&lt;br&gt;• POMT2−/−&lt;br&gt;» Walker-Warburg syndrome, muscle-eye-brain disease and congenital muscular dystrophy without brain malformations</td>
<td>Brain imaging studies closely resemble those of classic muscle-eye-brain disease in all of these disorders, which thus differ from polymicrogyria. Two of them are classified as ‘congenital disorders of glycosylation’ (CDG). The Gpr56 knockout has pial basement membrane disruption. (Hansse et al., 2005; Peters et al., 2002; Piao et al., 2004, 2005; Morava et al., 2005; Sprecher et al., 2005; Kornak et al., 2008; Li et al., 2008; Van Maldergem et al., 2008; Koirala et al., 2009)</td>
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<tr>
<td>II.D.4.b. Cobblestone-like malformations with variable glycosylation defects</td>
<td>Mouse mutants&lt;br&gt;• Gpr56−/−&lt;br&gt;Human by genotype&lt;br&gt;• GPR56−/−&lt;br&gt;» Bilateral fronto-parietal cobblestone-like malformation&lt;br&gt;• ATP6V0A2−/−&lt;br&gt;» CDG type 2 with Debré type cutis laxa with cerebellum vermis hypoplasia or Dandy–Walker malformation&lt;br&gt;• B4GALT1−/−&lt;br&gt;» CDG type 2d with cerebellum vermis hypoplasia or Dandy–Walker malformation&lt;br&gt;• SNAP29−/−&lt;br&gt;» CEDNIK syndrome</td>
<td>The most common forms of lissencephaly have mild cerebellum dysplasia easily seen by pathology but not by imaging.</td>
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**GPR56** mutations affect cerebral cortical development by causing breaches in the pial basement membrane, thus allowing overmigration of neurons into the subpial space and resulting in a cobblestone-like malformation (Li et al., 2008). In the cerebellum, mouse models demonstrate that granule cells show loss of adhesion to extracellular matrix molecules of the pial basement membrane (Koirala et al., 2009). Both of these mechanisms are similar to what is seen in the dystroglycanopathies.

Group II.E consists of disorders known as Joubert syndrome and related disorders (JSRD; see Table 7 (Joubert et al., 1969; Boltshauser and Isler, 1977; Gleeson et al., 2004; Zaki et al., 2007), also called molar tooth malformations (Quisling et al., 1999)). These disorders have abnormalities of white matter tracts in the brain stem and abnormal superior cerebellar peduncles (Fig. 12) as well as dysplasia of the cerebellar vermis and, often, accompanying abnormalities of the eyes (retinal dysplasia or colobomas), kidneys (nephronophthisis), limbs (preaxial, mesial, or postaxial polydactyly), liver (fibrosis), orofacial deformities, and other central nervous system anomalies (including occipital encephaloceles and cerebral polymicrogyria).
Zaki et al. (2007). Zaki et al. (2008) have suggested a classification of these disorders based upon the associated anomalies, which we have adopted. JSRD seem to be caused by mutations of genes encoding ciliary and centrosomal proteins (Keeler et al., 2003; Valente et al., 2003, 2006a, b; Gleeson et al., 2004; Parisi et al., 2004; Louie and Gleeson, 2005; Badano et al., 2006; Sayer et al., 2006; Brancati et al., 2007; 2008; 2009; Baala et al., 2007b; Delous et al., 2007; Frank et al., 2008; Gorden et al., 2008). A consistent overlap between JSRD and Meckel-Gruber syndrome—an autosomal recessive and genetically heterogeneous lethal disorder characterized by a combination of renal cysts and other variable features including developmental anomalies of the central nervous system (typically occipital encephalocele), hepatic ductal dysplasia and cysts, and polydactyly (Baala et al., 2007b; Delous et al., 2007; Frank et al., 2008)—has been recognized, which is supported by discovery of mutations in several of the same genes. Thus, the two disorders represent different points along a single spectrum of malformations (Baala et al., 2007b; Delous et al., 2007; Frank et al., 2008). Although the precise mechanisms by which these mutations affect brain development are only starting to be elucidated (Arts et al., 2007; Chizhikov et al., 2007; Delous et al., 2007; Frank et al., 2008), it has been postulated that ciliary and centrosomal proteins may interact to respond to extracellular signalling or modulatory cues in renal and retinal homeostasis and in neuronal development (Louie and Gleeson, 2005; Badano et al., 2006; Valente et al., 2006b). Alteration of centrosomal dynamics can alter neuronal migration (Sapir et al., 2008), which may explain the severe vermian hypoplasia seen in affected patients (Quisling et al., 1999; Yachnis and Rorke, 1999). As growth cones of migrating neurons and pathfinding axons are similar, defective ciliary and centrosomal function could explain the aberrant axonal pathways in the midbrain and hindbrain in affected patients (Yachnis and Rorke, 1999; Widjaja et al., 2006; Poretti et al., 2007a).
Group III. Regional developmental defects (localized brain malformations that significantly affect the brainstem and cerebellum, pathogenesis partly or largely understood)

Patients in this group (Table 8) have malformations of the brain stem or cerebellum that are localized and manifest clinically with neurological signs that are attributable to one anatomofunctional system rather than diffuse. Most are present from the time of birth, although some may not become evident until childhood.

Developmental clefts are included in this group. These may be seen in the dorsal or ventral midline surface of the pons, particularly in patients with cerebellar hypoplasia or dysplasia, but also in patients with normal cerebella (Barkovich et al., 2007). These are believed to result from impaired pathfinding of axons in the developing brain stem. The most common clefts are ventral longitudinal and midline, involving the pons. These are likely to be due to absence of the decussation of the middle cerebellar peduncles and possibly the transverse pontine axons migrating from the cerebellar cortex to the pontine nuclei. They are often associated with cerebellar hypoplasia, although they are also reported as a manifestation of generalized axonal midline crossing defects; when the midline-crossing defect is more generalized, the corpus callosum is often abnormal (Barkovich et al., 2007). Midline dorsal clefts are thought to result from abnormal development of the median longitudinal fasciculus and the tectospinal tract (Barkovich et al., 2007). The best studied of these is the condition known as horizontal gaze palsy with progressive scoliosis (Group III.A) (Thomsen et al., 1996; Traboulsi, 2004; Bosley et al., 2005), a condition caused by mutations of the ROBO3 gene, which codes for a netrin receptor that is required for midline crossing of hindbrain axons (Jen et al., 2004). Affected patients have congenital horizontal gaze palsy and MRI shows quite characteristic brain-stem hypoplasia with absence of the facial colliculi, presence of a deep midline dorsal pontine cleft (split pons sign), and a ‘butterfly’ configuration of the medulla (Fig. 13) (Rossi et al., 2004). Diffusion tensor tractography shows more extensive white matter abnormalities including absence of major pontine crossing axons and absence of decussation of the superior cerebellar peduncles in addition to reduced volume of dorsal longitudinal tracts in the pontine tegmentum (Sicotte et al., 2006), the latter being consistent with reduced volume or absence of the medial lemniscus and median longitudinal fasciculus. A dorsal longitudinal cleft in the midbrain (Group III.B) has been seen by one of the authors in a patient with trisomy 14 (AJB, unpublished observation). Several other brain stem disorders purportedly secondary to abnormal axonal pathfinding have been described (Barkovich et al., 2007). These include the recently reported pontine tegmental cap dysplasia, a malformation in which the ventral pons is hypoplastic due to absence of normal ventral decussation of the middle cerebellar peduncles while a band of horizontally oriented axons is present, instead, along the dorsal surface of the pons (Fig. 14) (Barth et al., 2007b; Jissendi-Tchofo et al., 2009). Other disorders that are presumably due the white matter guidance disruptions in the brain stem have been described recently (Barkovich et al., 2007) and the authors continue to find more, as yet unpublished, brain stem malformations. It is likely that an increasing number will be discovered as the quality of brain imaging improves, with higher field strength magnetic resonance scanners and as diffusion tensor tractographic methods become more robust.

Also included in this group are disorders caused by localized abnormalities of cell specification, such as the Duane retraction syndrome. These include disorders caused by localized abnormalities of cell specification, such as the Duane retraction syndrome. Figure 11 shows examples of such disorders.

Figure 11 Midbrain and hindbrain malformations in severe dystroglycanopathy (Walker-Warburg phenotype). (A) Sagittal T1-weighted image shows massive hydrocephalus, a very small, dysplastic vermis (small arrowheads), large, round tectum (small arrows) and small, ventrally kinked pons (large arrowhead). (B) Axial T2-weighted image shows the extremely small, dysmorphic cerebellar hemispheres and the small pons with ventral midline cleft.

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Table 7 Group II.E. Diffuse molar tooth type dysplasias associated with defects in ciliary proteins

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<tr>
<td>II.E.1. Syndromes affecting the brain with low frequency involvement of the retina and kidney</td>
<td>Human by genotype&lt;br&gt;• JBT51 locus chr9&lt;br&gt;• AHI1+/−/− (JBT53 locus)&lt;br&gt;• AR1L138−/− (JBT59 locus)&lt;br&gt;• CC2D2A−/− (JBT59 locus)&lt;br&gt;» Mutations of these genes cause classic Joubert syndrome with the typical JSRD neurological phenotype, molar tooth malformation, and occasional retinal and renal disease</td>
<td>(Saar et al., 1999; Dixon-Salazar et al., 2004; Ferland et al., 2004; Gleeson et al., 2004; Cantagrel et al., 2008)</td>
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<td>II.E.2. Syndromes affecting the brain, eyes, kidneys, liver and variable other systems</td>
<td>• NPHP1+/−/− (JBT54 locus)&lt;br&gt;» Mutations of this gene usually cause renal cystic disease only, but rarely may cause a mild cerebello-oculo-renal syndrome phenotype&lt;br&gt;• JBT52 locus chr11&lt;br&gt;• CEP290+/−/− (JBT55 locus)&lt;br&gt;• MKS1+/−/− (MKS1 locus)&lt;br&gt;• TMEM67+/−/− (MKS3, JBT56 locus)&lt;br&gt;• RPGRIP1+/−/− (JBT57 locus)&lt;br&gt;» Mutations of these genes cause a wide spectrum of disease including (i) Meckel-Gruber syndrome (Meckel syndrome); (ii) the cerebello-oculo-renal syndrome variant with the JSRD neurological phenotype, molar tooth malformation, and often retinal, renal and liver disease; (iii) the COACH syndrome; (iv) classic Joubert syndrome; and (v) less common disorders without neurological problems such as Leber amaurosis.&lt;br&gt;Human by phenotype&lt;br&gt;• Oro-facio-digital syndrome type 6 (Varadi)</td>
<td>(Keeler et al., 2003; Valente et al., 2003, 2006a, b; Gleeson et al., 2004; Parisi et al., 2004; Sayer et al., 2006; Baala et al., 2007b; Brancati et al., 2007, 2008, 2009; Delous et al., 2007; Frank et al., 2008; Gorden et al., 2008)</td>
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The syndromes in this group are collectively referred to as Joubert syndrome and related disorders (JSRD), and are associated with the striking molar tooth malformation of the midbrain and hindbrain. The neurological phenotype includes cognitive and behavior problems, congenital oculomotor apraxia, ataxia and alternating hypopnea-apnea. All syndromes in this group have autosomal recessive inheritance, and all genes so far identified code for ciliary proteins.

Group IV. Defects secondary to combined hypoplasia and atrophy in putative prenatal onset degenerative disorders

The final group of defects is composed of progressive disorders in which the cerebellum is already small at birth and subsequently undergoes further atrophy (Table 9). The two best known disorders that fall into this category are the pontocerebellar hypoplasias (PCH) (Barth et al., 1990, 1993; Rajab et al., 2003; Patel et al., 2006; Barth 2007a; Hevner, 2007; Leroy et al., 2007; ) and the congenital disorders of glycosylation (CDG), especially CDG type 1a (CDG1a, formerly known as carbohydrate deficient glycoprotein syndrome) (Kier et al., 1999; de Lonlay et al., 2001; Drouin-Garraud et al., 2001; Freeze, 2001; Miossec-Chauvet et al., 2003; Giurgea et al., 2005). Five types of PCH have been described in the literature, although it now appears that types 2 and 4 may lie along the same continuum, with type 4 having more serious clinical and pathological manifestations (Barth et al., 2007a; Hevner,
All have a small brain stem and cerebellum from birth (Fig. 15), with the vermis relatively less affected than cerebellar hemispheres. Type 1 has spinal motor neuron loss; type 2 is characterized pathologically by normal spinal motor neurons and clinically by chorea/dystonia; type 3 has absence of dyskinesias, optic atrophy, and linkage to chromosome 7q11-21; types 4 and 5 have C-shaped inferior olivary nuclei with relative vermian sparing in type 4 (Hevner, 2007). Although the cerebellar malformation in both PCH and CDG1a are commonly referred to as ‘hypoplasia’, pathologic studies have shown the cerebellum to exhibit a combination of hypoplasia and atrophy (Norman et al., 1995; Pascual-Castroviejo et al., 2006; Barth et al., 2007a). This observation suggests that the causative gene(s) are important both for cerebellar neuronal development and for postmitotic neuronal survival (Hevner, 2007). In support of this concept, the authors have seen sequential MRI studies of several affected patients with PCH and CDG1a in whom the cerebellum was small at birth and underwent further atrophy postnatally. Thus, these disorders are classified in group V.

The other major disorders in this group are unilateral cerebellar hypoplasia and cerebellar cortical dysplasia [also called cerebellar polymicrogyria and cerebellar heterotaxia (Friede, 1989; Norman et al., 1995; Soto-Ares et al., 2002; 2004)]. Both disorders are most often detected incidentally on neuroimaging studies for patients with unrelated complaints (Fig. 16) (Boltshauser et al., 1996; Patel and Barkovich, 2002; Kilickesmez et al., 2004; Poretti et al., 2009). If assessed carefully, these patients typically have abnormal foliation (Soto-Ares et al., 2004) or clefts (Poretti et al., 2008) in the affected hemisphere; thus these conditions are considered together. Affected patients are typically asymptomatic or minimally symptomatic and, typically, no associated abnormalities are found elsewhere in the brain (Friede, 1989; Norman et al., 1995; Soto-Ares et al., 2004; Poretti et al., 2008). Familial cases have not been reported and some patients have been found to have associated destructive lesions such as schizencephaly (Poretti et al., 2008). Many authors, therefore, have postulated that these
are the result of prenatal injury (Friede, 1989; Norman et al., 1995; Boltshauser et al., 1996; Kilickesmez et al., 2004; Poretti et al., 2008, 2009). In support of this concept, the authors have been referred several cases in which focal cerebellar cortical dysplasia, usually associated with hypoplasia of the affected hemisphere, developed after a second trimester or early third trimester prenatal cerebellar haemorrhage that was detected on routine obstetrical sonography and confirmed by foetal MRI.

**Discussion**

This classification organizes malformations of the midbrain and hindbrain into a logical system based, as much as possible, upon known embryological events and genetic mutations from work in humans and animal models. As the genetics and embryology of mid-hindbrain development are still being elucidated, this classification is far from complete. Nonetheless, it brings some order to a very difficult and confusing group of malformations and can continue to be used as a framework as our knowledge of developmental process and genetics evolves.

This classification might be criticized for some of the assumptions that have been made in the categories selected. Why are some groups based upon known embryologic processes while others are based upon whether the processes are well localized or not? The answer is that this method of grouping gives maximum flexibility to the classification. As understanding of general
processes in hindbrain development increases, the categories can be modified, and as understanding of the pathophysiology of individual disorders increases, those disorders can be moved to more appropriate groups. Why propose a classification now, instead of waiting until the processes are better understood? We contend that the presence of a logical classification is essential to the investigation of disorders. Classifications, even early ones, bring groups of disorders from the realm of chaos, where every case differs from every other case, to science, where the complexity of nature is reduced to a more comprehensible form. Placing a malformation in a certain group will elicit testing to see whether it belongs in that category; if not, the classification is flexible enough that it can be moved to a more appropriate one.

This classification may also be criticized for some of its details, such as the categories to which certain malformations are assigned. Why are some cerebellar hypoplasias included in Group I, while others are in Groups IV? Cerebellar hypoplasia can be the result of many different processes, starting with patterning of the developing neural tube and progressing all the way to increased apoptosis due to abnormal late migration of granule cells (granule cells undergo apoptosis if Purkinje cells have not migrated normally to their end location (Wetts and Herrup, 1982; Wallace, 1999; Kenney et al., 2003; Hoshino, 2006)). In order to treat the potential causes, malformations with cerebellar hypoplasia need to be ‘split’ based upon the pathophysiology of the hypoplasia. By separating the different types of this disorder, we take the first step in making this complex diagnosis more comprehensible.

How can the authors justify the assumptions they have made in creating this classification? Any useful model is based upon some assumptions. Indeed, Sarnat uses a number of assumptions in assigning malformations to his molecular genetic classification of malformations (Sarnat, 2000). The authors do not claim that this version of the classification system is the last. Undoubtedly, new discoveries in the future will show that some of the disorders included in this classification should be reclassified into another group or, perhaps, a new group. Indeed, 5 years ago no one would have suggested classifying Joubert syndrome as one of

<table>
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<tr>
<th>Defects</th>
<th>Examples</th>
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<tr>
<td>III.A. Multiple levels of mid-hindbrain</td>
<td>Mouse Mouse mutants • Sall4−/− - Hoxb1−/− Human by genotype • CHN1−/− Duane retraction syndrome • SALL4−/− » Duane radial ray syndrome, Okihiro syndrome • PHOX2A4/− » Congenital fibrosis of extra-ocular muscles • ROBO3−/− » Horizontal gaze palsy with progressive scoliosis Human by phenotype • Diffuse brainstem hypoplasia</td>
<td>(Nakano et al., 2001; Al-Baradie et al., 2002; Kohlhase et al., 2002, 2005; Holve et al., 2003; Jen et al., 2004; Bosley et al., 2006, 2008; Chan et al., 2006; Michielse et al., 2006; Sakaki-Yumoto et al., 2006; Warren et al., 2007)</td>
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<td>III.B. Midbrain malformations</td>
<td>Human by phenotype • Midbrain dysgenesis with open clefts (ventral, dorsal with dorsoventral shortening)</td>
<td>Barkovich, unpublished</td>
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<td>III.C. Rhombomere 1 including cerebellar malformations</td>
<td></td>
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<tr>
<td>III.C.1. Cerebellar HET</td>
<td>Cb white matter heterotopia, usually associated with overlying Cb cortical dysgenesis</td>
<td>(Rorke et al., 1968; Patel and Barkovich 2002)</td>
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<td>III.C.2. Cerebellar foliation anomalies</td>
<td>Human by phenotype • Refer to recent classification</td>
<td>(Demaerel 2002)</td>
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<td>III.C.3. Cerebellar cell specification anomalies</td>
<td>Human by phenotype • Lhermitte Duclos syndrome</td>
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<td>III.C.4. Cerebellar hemispheric duplication</td>
<td>Human by phenotype • Duplication of cerebellar hemisphere and ipsilateral ear</td>
<td>(Jackson et al., 1990)</td>
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<td>III.D. Pons malformations</td>
<td>Human by phenotype • Pontine tegmental cap dysplasia • Pontine dysgenesis with open clefts (ventral, dorsal with dorsoventral shortening)</td>
<td>(Barth et al., 2007b; Jissendi-Tchofo et al., 2009)</td>
</tr>
<tr>
<td>III.E. Medulla malformations</td>
<td>Human by phenotype • Medullary tegmental cap, always associated with cerebral callosal agenesis/hypogenesis. Suspected axonal midline crossing defects.</td>
<td>Barkovich, unpublished</td>
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</table>
a group of multisystemic disorders caused by defects in ciliary proteins. Some disorders that are listed separately may have to be combined, while others that are listed as a single disorder may have to be divided into multiple groups. Discoveries may result in the creation of new groups and elimination of others. Indeed, the framework of the classification will probably need modification as new aspects of mid-hindbrain development are discovered. The strength of this classification system is that it has to flexibility to allow changes in both its framework and its listings with periodic updates as new discoveries necessitate change.

Why do the authors classify some disorders by genotype and others by phenotype? Ultimately, we hope that all malformations will be classified by genotype or disrupted embryologic step. Currently, however, our understanding of the genetic/embryologic causes of many of these disorders is not advanced enough to create such a sophisticated classification. Therefore, we have classified by genotype/embryology those malformations for which the cause is adequately understood; the more poorly understood malformations are classified by clinicoradiologic phenotype. We anticipate that number classified by genotype will increase in subsequent revisions.

Other classification systems of mid-hindbrain or cerebellar malformations have been proposed (Patel and Barkovich, 2002; Parisi and Dobyns, 2003), but have not been widely accepted by practicing neurologists and geneticists, possibly because there was no unifying thread tying together disorders within the same group. This classification attempts to rectify that problem by classifying the malformations according to the underlying processes affected. This allows the classification to grow with the knowledge of embryology and genetics that is the source of its structure. The multitude of recent advances in this understanding has brought the state of the art to the point where this genetic-embryological classification is now feasible.

This classification is not restricted to malformations restricted to the midbrain-hindbrain. To do so would be unrealistic as the developmental processes in the forebrain and mid-hindbrain share so many genes and gene products that it is nearly inevitable that the supratentorial compartment or other organs will be affected in some way when an infratentorial structures develops abnormally. Indeed, in many of these disorders, the supratentorial malformation may be the first one discovered, with the infratentorial malformation only being identified later. Discovery of the infratentorial malformation may allow a more refined classification of the overall malformation complex, however. Indeed, discovery of cerebellar abnormalities similar to those in dystroglycanopathies in patients with GPR56 mutations led to the suggestion that the mutant protein is associated with glycosylation defects (Ke et al., 2008).

In summary, a developmentally based classification of midbrain-hindbrain malformations is proposed in this manuscript in an attempt to organize these disorders for better clinical understanding and guidance of future research. It is hoped that this classification helps to clarify what is known (and what is not) about
normal and abnormal development of these structures and that it may help to guide future studies.

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