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Model organisms inform the search for the genes and developmental pathology underlying malformations of the human hindbrain

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Abstract

Congenital malformations the human hindbrain, including the cerebellum, are poorly understood largely because their recognition is a relatively recent advance for imaging diagnostics. Cerebellar malformations are the most obvious and best characterized hindbrain malformations due to their relative ease to view by MRI and the recent identification of several causative genes¹. Malformations of the pons and medulla have also been described both in isolation and in association with cerebellar malformations². Although little is understood regarding the specific developmental pathologies underlying hindbrain malformations in humans, much is known regarding the mechanisms and genes driving hindbrain development in vertebrate model organisms. Thus, studies in vertebrate models provide a developmental framework in which to categorize human hindbrain malformations and serve to inform our thinking regarding disrupted developmental processes and candidate genes. Here we survey the basic principles of vertebrate hindbrain development and integrate our current knowledge of human hindbrain malformations into this framework.

The hindbrain controls numerous important physiological processes including respiration, circulation, arousal and motor coordination. Understanding the pathology of human hindbrain malformations, and devising strategies to diagnose and treat them ultimately relies on identifying the causative genes and elucidating the molecular and cellular mechanisms underling the early events that form the nervous system. Studies from vertebrate model systems have already revealed much of the genetic control that underlies the establishment of hindbrain structures and circuits controlling these functions. Given the high degree of neuroanatomical and functional conservation across vertebrates, insights from the analyses of brain development in model organisms are essential to decipher the developmental and genetic bases of human hindbrain malformations.

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Overview of Early CNS Development and Patterning

The vertebrate central nervous system (CNS) derives from the neural plate, an epithelial sheet that arises from the dorsal ectoderm of the gastrula-stage embryo. Subsequently, the neural plate closes to form the neural tube. The neural tube is then patterned by secreted molecules from signaling centers, which regulate a hierarchy of localized transcription factors to establish a Cartesian-like coordinate system of positional information along the anterior/posterior (A/P) and dorsal/ventral (DV) axes of the CNS.

The vertebrate embryonic neuroepithelium is characterized morphologically by a series of swellings that appear transiently around neural tube closure. Ultimately the anterior portions give rise to the forebrain, midbrain and hindbrain, while the narrow posterior epithelium transforms into the spinal cord. These early morphological features are positioned and foreshadowed by localized expression of developmental control genes that orchestrate the regional plan of the CNS and indicate future functional specializations^{3,4}. As development proceeds, neurogenesis further leads to the formation of distinct neuronal groups that can be identified by morphology, molecular markers, axonal trajectory and neurotransmitter type. As neurons mature, they migrate from their birthplace to their final destination, where they form synaptic connections and integrate into functional circuits (reviewed in⁵). All of these features of nervous system development are largely conserved across vertebrates.

Hindbrain Segmentation: Development and Malformations

The hindbrain is the most evolutionarily ancient part of the vertebrate brain^{4,6}, and is fated to give rise to the medulla oblongata, the pons, and the cerebellum. As the hindbrain becomes distinguished from the midbrain and the spinal cord, around the time of neural tube closure, it is further subdivided into a series of seven A/P segments, or rhombomeres (r1-r7) (Fig. 1A,B). Rhombomeres are transient structures first distinguished by the expression of molecular markers and later as morphological segments. Recent fate mapping studies in mice, together with older chick/quail studies, show that dorsal r1 gives rise to the cerebellum and ventral r1 to the anterior pons, while more posterior rhombomeres form the remainder of the pons and the medulla⁷.

The hindbrain begins to show segmental gene expression patterns at the end of gastrulation, before the morphological appearance of rhombomere boundaries. The initial establishment of the rhombomeric pattern does not follow a strict rostro-caudal order^{8,9}, but rather boundaries form in stereotypical sequence. The presumptive r4 territory, in the center of the hindbrain is the first to be generated and it in turn acts as a local organizing center, signaling to adjacent territories and initiating a molecular cascade leading to the further partitioning of the r3, r5, and r6 segments^{10,11}. As the rhombomeres emerge, distinct physical boundaries form to separate the molecularly and neuroanatomically distinct segments^{12,13}. The process of compartmentalization first involves formation of an interface between adjacent segments, and this is followed by the induction of a specialized population of boundary cells at the interface. The initial formation of a clear interface between rhombomeres is concomitant with sharpening of initially indistinct segmental gene expression domains, such as those of *hox* genes and *krox-20*^{14,15}.

Transplantation studies in chick have demonstrated that the process of boundary formation is regulated by a cell sorting mechanism^{16,17}, which requires Ca²⁺-dependent adhesion molecules¹⁸. Studies in zebrafish have shown that this cell sorting mechanism is controlled by the repulsive interaction between Eph receptors and Ephrin ligands in alternating segments, and possibly by differential adhesion within the same segment^{14,15,19–21} to ultimately prevent intermingling of cells across boundaries (Fig. 1C). Embryological studies in chick have also indicated that following the establishment of sharpened segmental gene expression, the

interactions between adjacent rhombomeres induce the formation of specialized boundary cells at the segment interface^{16,22}. Recent work in zebrafish showed that Notch activation in boundary cells regulates their formation²³; boundary expression of the Notch target gene *Hes1* in the mouse suggests that Notch may play a conserved role in rhombomere boundary establishment²⁴. In addition, in zebrafish the boundaries also function as sources of secreted Wnts, which help to pattern adjacent rhombomeres^{25,26}. Although Wnt expression has not been detected in boundaries of other vertebrates, another secreted signal, Fgf3, is expressed in chick rhombomere boundaries²⁷. In summary, the formation of rhombomere boundaries is required not only for prevention of cell mixing between adjacent segments, but also to establish local signaling centers that regulate hindbrain neurogenesis and thus to establish segmental neuronal organization of the hindbrain.

Interestingly, unlike the segmented nature of the hindbrain, the posterior part of the neural tube remains unsegmented during embryonic development. Recent work in zebrafish has identified Cdx transcription factors as key determinants of the unsegmented spinal cord region, demonstrating that repression of the hindbrain developmental program is required for the specification of the vertebrate spinal cord^{28,29}. This regulatory mechanism of establishing the boundary between hindbrain and spinal cord is most likely conserved across vertebrates, based on the similarity between patterns of Cdx gene expression during nervous system development of various vertebrate species²⁸.

At the same developmental stages that the hindbrain is undergoing morphological segmentation it is also become regionalized via segment-specific gene expression. In particular, the segmentally expressed *Hox* genes play a critical part in conferral of rhombomere identity. *Hox* genes code for a family of Antennapedia class homeodomain transcription factors that are well known for their conserved role in regionalization of the body plan. A defining feature of *Hox* genes is a clustered organization, with all vertebrates possessing multiple *Hox* clusters as a consequence of ancient genome duplication events³⁰. The *Hox* genes are further arranged in 13 paralog groups, with no one cluster retaining all 13 paralogs. The genes demonstrate an interesting property termed colinear expression, whereby the expression domains along the embryonic A/P axis mirror the locations of the genes along the chromosome. It is the most 3' located genes, members of paralog groups 1–4, that are expressed most anteriorly, within the developing hindbrain. Each rhombomere expresses a specific combination of *Hox* genes (with r1 being devoid of *Hox* expression), thus providing a mechanism to specify unique segment identities (Fig. 1A). Establishment of these segment-specific *Hox* expression domains is dependent on retinoic acid signaling^{31,32}.

The roles of several *Hox* genes in hindbrain patterning have been studied in the mouse via generation of knock-out alleles, and in chick and zebrafish via gain-of-function and knockdown approaches (reviewed in^{4,33}). Classic studies in *Drosophila* have established that *Hox* loss-of-function tends to cause anteriorizing homeotic transformations, in which posterior segments take on the identity of more anterior segments, whereas gain-of-function tends to cause posteriorizing homeotic transformations. Consistent with this model, when the r4 specific *Hoxb1* gene is misexpressed r2 takes on the identity of r4, and when *Hoxb1* function is disrupted r4 takes on the identity of r2^{34–36}. Interestingly, knock-out of the paralogous gene *Hoxa1*, which is the earliest expressed of the hindbrain *Hox* genes, causes an alteration not in segmental identity, but rather in the segmentation process itself^{37–39}, such that r5 is missing. Mutations in both of these *Hox* genes have been correlated with human malformations.

The mouse *Hoxb1* knock-out causes loss of the r4-derived VIIth (facial) motor nerve³⁵. This in turn causes paralysis of the muscles of facial expression, similar to the pathology of Bell's Palsy or Moebius Syndrome. While this disease has not been directly correlated with alterations in human *HOXB1*, changes in *HOXA1* have been linked with Bosley-Salih-Alorainy (BSAS)

syndrome. Linkage analysis and positional cloning in BSAS patients has indicated homozygous truncating mutations in the *HOXA1* gene⁴⁰. These patients exhibit horizontal gaze abnormalities, deafness, facial weakness, hypoventilation, vascular malformations of the internal carotid arteries and cardiac outflow tract and mental retardation. Interestingly, it was this spectrum of defects that suggested a potential *HOX* mutation, due to the similarities between the BSAS pathology and the phenotype of *Hoxa1* mutant mice^{37–39}, which show variable abnormalities of hindbrain segmentation, with a nearly complete loss of r5, partial loss of r4, and alterations to r3, 6, and 7. These mice also show reduced numbers of cell bodies and exiting cranial nerves of the abducens and facial nuclei; absence of the superior olive, and variable defects in cranial autonomic and sensory ganglia, the skull and the external and inner ears. Interestingly, the phenotypes of patients with BSAS also show that loss of *HOXA1* function segregates with cognitive impairment and suggest that brainstem dysgenesis may lead to higher cortical dysfunction⁴⁰.

Other human hindbrain malformations have also been suggested to arise through alterations in the segmentation process. For example, brain stem disconnection syndrome (BSDS) has been proposed to also be a segmentation disorder. In this very rare and very severe malformation, the junction of the midbrain and pons or the pons and the medulla are completely discontinuous^{41,42}, a feature not observed in model organisms. In all experimental situations in model vertebrates where hindbrain segments are missing, the remaining transformed hindbrain segments always remain contiguous with each other. This difference may suggest that an early catastrophic vascular accident is the cause of BSDS rather than a disruption in hindbrain segmentation.

A recent study in human patients has reported a reduced size of the basis pontis and an associated abnormally long and thick medulla with rounded ventral surfaces resembling the pons more than the normal medulla². These rare cases may represent true examples of abnormal human hindbrain segmentation, reflecting the hypothesis that they arose via posterior to anterior rhombomere transformations during early segmentation. Given the limited resolution of brain imaging studies it is likely that more subtle segmentation abnormalities exist but cannot be recognized as on MRI analysis as is the case with BSDS. It has been proposed that developmental mispatterning of neurons that regulate the specification of respiration control centers in r3 and r4 may underlie some forms of congenital respiratory failure⁴³. To fully prove that any human hindbrain malformation is the result of abnormal hindbrain segmentation, gene identification is required together with extensive neuropathological analysis using markers specific for the affected neurons and circuits, together with comparisons with appropriate animal models.

Cerebellar Development and Associated Malformations

The cerebellum is the most prominent derivative and best studied feature of the hindbrain. Thus cerebellar malformations are recognized with relative ease in both mice⁴⁴ and humans^{45,46}. However, confusion regarding the classification of human hindbrain and particularly human cerebellar malformations has been a major obstacle to progress in defining their causes. Despite this confusion, it is worth noting that many distinct malformations have been delineated based on very specific imaging criteria^{45,46} (Figure 2). For example, the relatively rare Joubert and related disorders (JSRD) and are defined by cerebellar vermis hypoplasia (CVH) and a complex midbrain clefting and cerebellar peduncle malformation that has a “molar tooth” appearance in axial brain images. CVH and Dandy-Walker malformation (DWM) are the most common human cerebellar phenotypes^{46,47}. Isolated CVH consists of vermis hypoplasia affecting the posterior more than the anterior vermis with normal vermis position and posterior fossa size. Classic DMW includes CVH, upward rotation of the vermis producing cystic enlargement of the 4th ventricle, and enlarged posterior fossa. While the “molar tooth” feature distinguishes

JRSD, the lack of similarly unique features and the overlap in appearance between CVH and DWM creates a significant source of diagnostic confusion.

Further complicating diagnosis is the presentation of DWM in a mild form. Mild DWM is similar to classic DWM, except that the vermis is mildly rotated so that the inferior roof of the 4th ventricle is parallel to the brainstem (rather than being rotated up), and the posterior fossa is only mildly enlarged. Mega cisterna magna (MCM) is another phenotype that resembles aspects of both CVH and DWM. MCM as a true malformation includes CVH, normal position of the vermis with no rotation, and enlarged posterior fossa^{1,45,46}. An increased understanding of the developmental mechanisms regulating cerebellar development and the recent identification of a number of causative genes for several of these malformations is beginning to illuminate the previously confusing literature. There is also growing recognition that cerebellar development does not occur in isolation, rather it accompanies the development of the rest of the brain. Thus, many recognized cerebellar malformations do not exclusively affect the cerebellum, but also involve developmentally related structures, including the midbrain and pons.

The Isthmic organizer and mid/hindbrain segmentation

The cerebellum arises exclusively from dorsal r1 adjacent to the 4th ventricle. The developmental boundary at the junction of the midbrain and hindbrain does not form analogously to the boundaries between the other hindbrain rhombomeres. Instead, r1 segregates from the midbrain via formation of the mid/hindbrain boundary, which becomes the isthmic organizer (IsO) (Fig. 1A). The IsO is an important signaling center which secretes Wnt and Fgf molecules that promote proliferation and identity of the adjacent midbrain and hindbrain regions. The generation of the mid/hindbrain junction is the earliest segmentation event of the entire developing CNS, occurring at early neural plate stages. Its formation is coordinated through the activation of an extensive genetic network acting to juxtapose the expression of two transcription factors, *Otx2* and *Gbx2*, respectively located anterior and posterior to the mid-hindbrain junction, by signals from the underlying anterior mesoderm and dorsal non-neural ectoderm. These transcription factors together establish Wnt and Fgf8 expression in the resulting IsO signaling center^{48,49}.

Loss of either Wnt1 or Fgf8 results in complete loss of posterior midbrain and anterior hindbrain tissue causing an abnormal fusion of the tectum with the posterior cerebellum. This results in neonatal lethality in mice. An equivalent phenotype has never been reported in humans and is presumed to be incompatible with life. Alterations in the positioning of the IsO cause less severe phenotypes. For example, in mice, ectopic expression of *Otx2* in the presumptive anterior hindbrain results in nearly complete loss of the anterior cerebellar vermis and enlargement of the inferior colliculus, a posterior midbrain derivative⁵⁰. Potential IsO positioning phenotypes have recently been described in humans. For example, one patient with a substantially enlarged tectum accompanied by severe hypoplasia or aplasia of the cerebellar vermis suggests posterior displacement of the mid-hindbrain junction. The same authors also described a contrasting phenotype of a patient with a small tectum and an abnormally large anterior cerebellum². Confirmation of a developmental mispatterning of the IsO awaits identification of the molecular lesions in these patients. Notably, all of these patients were originally identified as DWM patients. However, awareness of the neurodevelopmental literature for model organism and careful evaluation of the imaging studies enabled reinterpretations of these particular images to recognize the nuanced specific features in these phenotypes. These examples illustrate that a clear understanding of the principles of developmental neurobiology in model organisms provides valuable paradigms with which to assess human hindbrain malformations and generate new hypotheses about their etiology.

Rhombomere 1 neurogenesis, migration and associated abnormalities of the pons

Cerebellar neurons arise from one of two germinal zones within the embryonic cerebellar anlage – the ventricular zone and the rhombic lip (Figure 3 **top**). The ventricular zone, expressing the bHLH factor *Ptf1a*, gives rise to the GABAergic neurons of the cerebellum⁵¹, including Purkinje cells. Post-mitotic neurons leave the VZ and migrate radially within the anlage. The rhombic lip, expressing bHLH factor *Atoh1* (*Math1*), is induced by Bmp-signals from the adjacent dorsal midline roof plate⁵² and gives rise to all cerebellar glutamatergic neurons in addition to several neuronal populations of the brain stem including the pontine nuclei^{7,53–55}. Neuronal progenitors leaving the rhombic lip continue to proliferate as they migrate anteriorly over the surface of the developing cerebellar anlage to form the external granule layer (EGL). Granule neuron progenitors within the EGL proliferate extensively, driven by Shh secreted by the underlying Purkinje cells^{56–58}. Once differentiated, inward radial migration of EGL granule neurons establishes the internal granule layer (IGL) underneath the monolayer of Purkinje cells to achieve the final laminar and folial structure of the mature cerebellum (Figure 3 **bottom**).

Malformations caused by abnormal cerebellar neurogenesis have been described. For example, loss of *PTF1A* function in humans and mouse causes a failure in the initial generation of all cerebellar GABAergic neurons. This secondarily causes loss of newly born cerebellar glutamatergic cells since their trophic support and targets are now missing, resulting in complete cerebellar atrophy or agenesis at birth^{51,59}. We have recently demonstrated that defects in rhombic lip neurogenesis likely underlie some forms of human CVH. This was based on the observation that most human cerebellar malformations preferentially affect the posterior cerebellar vermis⁴⁵. Only one mouse mutant has been shown to model this specific phenotype of posterior CVH. These mice harbor mutations in the gene encoding the transcription factor *Lmx1a*⁶⁰. Specifically, *Lmx1a* is required to define posteriorly fated vermis granule cell progenitors within the early rhombic lip⁶¹. Loss of *Lmx1a* causes these cells to adopt more anterior fates and early regression of the rhombic lip so that posterior vermis progenitors are completely lost. This is the first developmental insight into the cause of specific posterior cerebellar vermis hypoplasia. Though it is unlikely that human *LMX1A* mutations are compatible with life due to the other roles of this important transcription factor throughout the developing embryo, this data raise the possibility that disruption of rhombic lip neurogenesis is central to the pathology of some forms of human cerebellar malformations.

Not all cerebellar hypoplasia is caused by rhombic lip abnormalities. For example, the cerebellar vermis is profoundly hypoplastic in JSRD. Currently, nine genetic loci have been mapped for the various subtypes of Joubert syndrome and most genes have been identified. JSRD genes include: *AH11*, *NPHP1*, *CEP290*, *TMEM67*, *RPGRIP1L*, *ARL13B* and *CC2D2A*^{1,62}. Although the function of JSRD proteins remains largely unknown, several of the JSRD proteins localize to the basal body or cilium, and recent evidence suggests roles in either mediating the assembly/stability of cilia or mediating cargo transport within cilia. Analysis of cerebellar development in mice mutant for homologues of these specific genes has not been published. However, in mouse models that lack functional cilia in the developing brain, a primary defect in cerebellar granule cell progenitor proliferation is observed once granule cell progenitors leave the rhombic lip^{63,64}. Failure to proliferate precludes the dramatic expansion of cerebellar size. Instead, the newly born progenitors differentiate early and form a small IGL. Proliferative failure is caused the inability of granule cell progenitors to respond to the Purkinje-cell secreted Shh mitogenic signal, since the Shh receptor, smoothened, must be located on cilia to transmit the signal⁶⁵.

Severe CVH is also observed in an X-linked mental retardation (XLMR) syndrome that is caused by loss of function of the *OPHN1* gene⁶⁶. *OPHN1* is a rhoGAP protein expressed in neurons and glia of the developing and adult brain⁶⁷. *In vitro* experiments have demonstrated

a role for *Ophn1* in the dendritic spine morphogenesis of hippocampal neurons in mice and rats, though no obvious cerebellar morphological abnormalities were observed in *Ophn1* mutant mice^{68,69}. Thus, OPHN1 impacts cerebellar morphology through a currently unknown mechanism.

Many cerebellar malformations also involve the pons². This is not particularly surprising given the developmental relationship of cerebellar granule cells and pontine nuclei which are both derived from the cerebellar rhombic lip. Any disruption of rhombic lip neurogenesis is likely to disrupt the neurogenesis of pontine neurons. Developmental degenerations of the cerebellum and pons also occur. Pontocerebellar hypoplasias (PCH) are rare, heterogeneous disorders characterized primarily by hypoplasia of the cerebellum and ventral pons. Presently, four genes, *RARS2*, *TSEN2*, *TSEN34* and *TSEN54*, encoding components of tRNA charging or splicing machinery have been identified for subtypes of PCH using linkage or candidate gene approaches^{70,71}. In the human fetus, *TMEM54* is strongly expressed in the neurons of the pons, cerebellar dentate and olivary nuclei⁷⁰. However, the PCH genes have not been studied in vertebrate model organisms, thus their mechanisms of action within the hindbrain are unknown.

An XLMR PCH phenotype that includes microcephaly is caused by *CASK* loss of function⁶⁶. *CASK* encodes a multidomain calcium/calmodulin-dependent serine protein kinase that is targeted to neuronal synapses by anchoring to membrane-associated proteins such as neuroligin⁷². *In vitro* experiments demonstrate a role for *Cask* in presynaptic neurotransmitter release, while hypomorphic *Cask* mutant mice exhibit cerebellar hypoplasia in addition to partially penetrant cleft palate^{73,74}. Interestingly, Tarpey et al. (2009) recently identified four missense variants in *CASK* by sequencing X-chromosome coding exons in families with XLMR. These variants were associated with mild to moderate mental retardation and nystagmus, which is not usually associated with XLMR⁷⁵ and imaging studies for these patients were not published. These recent findings serve to emphasize that the full spectrum of genotype-phenotype associations are not known. Rarely have developmental outcomes been rigorously correlated with specific structural malformations and in most instances, extensive phenotype-genotype correlations have not been conducted since the numbers of patients with identified molecular lesions remains low.

Once born in the rhombic lip, pontine nuclei neurons must undergo extensive migration around and through the tegmentum of the brain stem to arrive at their final ventral position. In mice, deciphering the developmental cues that specifically regulate this precise migration is in its infancy^{76,77}, but it is likely that many mechanisms controlling these migrations are similar to mechanisms that control neuronal migration throughout the brain. It is also very likely that these mechanisms are conserved in humans. For example, in mice, loss of the *Large glycosyltransferase* gene causes absence of the pons due to abnormal migration of pontine progenitors⁷⁸. Pontine hypoplasia and CVH are also features of congenital disorders of glycosylation in humans⁷⁹.

Pontine deficiency may not only be caused by cell migration abnormalities. It is important to note that model organism studies have demonstrated that many of the same molecules that regulate neuronal migration also regulate axon guidance. These molecules include ephrins, slits, netrins, semaphorins and Wnts⁸⁰. Axonal tract malformations of both the pons and medulla have been described both with cerebellar malformations or in isolation^{2,81}, though few causative genes have been identified. Model organisms provide interesting candidate genes and also can be used to test specific disease-relevant hypotheses. For example, in JSRD, a variety of major tract abnormalities are evident by MRI. Since JSRD are now identified as ciliopathies⁶⁵, it will be interesting learn whether cilia are required for long range cell migration and axon guidance in the developing brain stem in cilia deficient mouse models.

Dandy-Walker malformation – a new developmental perspective on a long recognized cerebellar malformation

In the last few years, identification of genes for JSRD and PCH has significantly clarified the developmental basis of these important, yet rare hindbrain malformations. There has also been major progress towards gene identification for DWM, the most common human cerebellar malformation. The first DWM causative genes, *ZIC1* and *ZIC4*, were chosen as strong candidate genes within the first DWM locus on chromosome 3q24 due to their clear expression within the mouse cerebellum during development. Mice with heterozygous deletion of the homologues of these two linked transcription factors further display a phenotype that recapitulates human DWM⁸². Thus, the first paradigm for identifying DWM-causative genes held that the aberrant genes must normally be expressed in the cerebellum in order for their alteration to impact cerebellar development.

We recently characterized a second DWM locus on chromosome 6p25.3⁸³. Within this locus no clear DWM-candidate genes emerged based on strong cerebellar expression. However, mice with null mutations in *Foxc1* displayed a striking, early embryonic cerebellar defect. *Foxc1* is a forkhead box transcription factor that is never expressed with the developing brain. Instead, *Foxc1* is expressed in the adjacent head mesenchyme and is required for normal skull development⁸⁴. We demonstrated that *Foxc1* in the early posterior fossa mesenchyme is also required for the expression of growth factors, including *Tfgeb1* and several *Bmps*. Without these growth factors, the adjacent cerebellum does not maintain cerebellar *Atoh1* expression which is required for normal EGL development⁸³. Thus *Foxc1* regulates both posterior fossa development and cerebellar development, two defining features of abnormal DWM pathology. Based on these studies, we are now reexamining our *Zic1/4* mutant mouse models to determine whether the *Zic* genes have additional roles in posterior fossa mesenchyme development.

The complementary analyses between human and model organisms not only recognized a new role for *FOXC1* in cerebellar development, but also highlighted a novel mechanism for early cerebellum development through neuroepithelium-mesenchyme interactions. These reciprocal analyses also informed our understanding of human cerebellar phenotypes and led us to revise our paradigm for selecting future candidate genes. Interestingly, patients with deletions or duplications encompassing *FOXC1* exhibit a wide range of cerebellar phenotypes, including classic DWM, mild DWM, MCM and isolated CVH (listed in order of severity), suggesting a single spectrum with shared etiology in these patients⁸³. In contrast, mutations in genes such as *OPHN1* and *CASK* are associated with CVH or diffuse cerebellar hypoplasia, but not MCM or DWM^{66,74}. Together these observations lead us now to propose that genes with expression in the developing cerebellum will be associated with CVH or diffuse cerebellar hypoplasia, while genes with posterior fossa mesenchymal expression will be associated with the complete CVH-MCM-DWM spectrum of malformations.

Conclusion

Hindbrain developmental neurobiology has been well described in vertebrate model organisms, though it is not completely understood. Easy manipulation of model organisms further provides a conduit for unraveling the specific genes and mechanisms underlying hindbrain development. In turn, these hindbrain developmental programs provide valuable insights for interpreting the origin of human hindbrain phenotypes and suggesting functionally relevant candidate genes. Future synergistic analyses of human and model organism hindbrain phenotypes are the keys to improvements in diagnoses and potential treatments for these disorders.

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Figure 1. Segmental organization of the vertebrate hindbrain

Anterior is to the left in all panels (A) Schematic representation of the expression of Hox Paralog (PG) 1–4 genes in mouse. Paralog groups are color-coded: PG1, red; PG2, blue; PG3, green; PG4, yellow. IsO: isthmus organizer; MHB; midhindbrain boundary (B) *in situ* hybridization with *rfn3* mRNA showing expression in rhombomere boundaries of 24 hpf zebrafish embryos. (C) Hindbrain segmentation is detected at the molecular level (anti-EphA4 antibody, red, labels r3 and r5), and neuronal organization level (anti-Hu antibody, green, labels post-mitotic neurons). Neurons in the hindbrain are clustered in the center of rhombomeres and absent from rhombomere boundaries. Longitudinal confocal sections through the dorsal zebrafish hindbrain at 24 hours post fertilization (hpf) of double Hu immunolabeling and EphA4 immunolabeling.

Figure 2. Distinct human cerebellar malformations

T1-weighted sagittal (or axial) MR images. Joubert syndrome is shown at the level of the isthmus in both images. The 'molar tooth' is noted in sagittal (white arrow) and axial (black arrows) views. Midline sagittal images are shown for all other phenotypes. The lower limit of the posterior cerebellum (vertical line), distance between the brainstem and cerebellum (arrowhead), and enlarged posterior fossa (asterisk) are indicated. All phenotypes have a hypoplastic cerebellum; pontocerebellar hypoplasia also has a hypoplastic pons (arrow).

Figure 3. Mouse cerebellar development

Top panel: Diagram of the mouse e12.5 neural tube showing a parasagittal section through the mid-hindbrain region. Signals from both the isthmus organizer (ISO) and fourth ventricle (4th) roof plate (rp) transform the dorsal region of rhombomere 1 (r1) into the cerebellum (CB). *Atoh1* and *Ptf1a* transcription factors mark the rhombic lip (RL) and ventricular zone (VZ) progenitor regions, respectively, within the developing cerebellar anlage. *Atoh1* and rhombic lip progenitors induced by roof plate Bmp signaling give rise to glutamatergic derivatives that migrate over the surface of the cerebellar anlage in successive waves and include pontine nuclei (PN), deep cerebellar nuclei and granule cells. *Ptf1a* progenitors in the ventricular zone exit the cell cycle, migrate radially into the cerebellar anlage and give rise to glutamatergic deep cerebellar nuclei and cerebellar interneurons. M: midbrain. Bottom panel: Midsagittal diagram of the newborn mouse cerebellum. The Purkinje cell layer (PCL) secretes Shh, inducing extensive proliferation of cells within the external granule cell layer (EGL). Once EGL cells differentiate, they migrate through the PCL to form the inner granule cell layer (IGL) of the adult cerebellum. IGL cell axons form the molecular layer (ML), while Purkinje cell axons project into the cerebellar white matter (WM). Drawings are not to scale.