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QUESTION1: CONCISE REVIEW ON THE GENTICAL BASIS OF CEREBELLAR DEVELOPMENT.

**ABSTRACT**

In humans, the cerebellum develops from the dorsal region of the posterior neural tube, and its cells arise from two germinal matrices. Most cells are derived from the ventricular zone, but the granule neurons come from a specialized germinal matrix called the rhombic lip. The cerebellum is derived from the dorsal part of the anterior hindbrain and contains two groups of cerebellar neurons: glutamatergic and gamma-aminobutyric acid (GABA)ergic neurons. Purkinje cells are GABAergic and granule cells are glutamatergic. Granule and Purkinje cells receive input from outside of the cerebellum from mossy and climbing fibers. During early neurogenesis, rostrocaudal patterning by intrinsic and extrinsic factors, such as Otx2, Gbx2 and Fgf8, plays an important role in the positioning and formation of the cerebellar primordium. The cerebellar glutamatergic neurons are derived from progenitors in the cerebellar rhombic lip, which express the proneural gene Atoh1. The GABAergic neurons are derived from progenitors in the ventricular zone, which express the proneural gene Ptf1a. The mossy and climbing fiber neurons originate from progenitors in the hindbrain rhombic lip that express Atoh1 or Ptf1a. Purkinje cells exhibit mediolateral compartmentalization determined on the birthdate of Purkinje cells, and linked to the precise neural circuitry formation.

***INTRODUCTION***

The cerebellum is derived from the dorsal part of the most anterior segment of the hindbrain, rhombomere 1. Thus, any developmental defect that results in the failure to specify the anterior hindbrain or r1itself, will inevitably result in cerebellar aplasia as might global defects in dorsal patterning mechanisms. Early steps in brain development include the speciﬁcation of neural tissue (neural induction), formation, and internalization of the neural tube (neurulation), and patterning of the neural tube. The latter process imparts positional identity to different compartments along the anterior–posterior neuraxis, a process primarily achieved through the formation of specialized signaling centers, also referred to as secondary organizers. Secondary organizers secrete growth factors that pattern the adjacent tissue through the induction of distinct patterns of gene expression on either side of the organizer, as a result of the presence of the differential expression of competence factors . The signaling center that divides and patterns the mesencephalon and r1 is the **mid-hindbrain or IsO**.

Classic studies in a number of model organisms have shown that the key organizing molecule secreted by the IsO is **ﬁbroblast growth factor 8(FGF8)**. The initiation ofFgf8 expression at the IsO is dependent upon **the transcription factor LMX1B** (Lim homeobox tr anscription factor 1 beta), whereas the position of the IsO at the mid-hindbrain boundary is determined the mutually repressive activities of **the homeobox genesOtx2 (orthodenticle homeobox 2) anteriorly**, and **Gbx2 (gastrula-tion brain homeobox 2)**, posteriorly. Once established, a stable transcriptional and signaling net-work maintains gene expression at the IsO. Critical componentsof this regulatory network include the transcription factors PAX2(paired box gene 2), EN1 (engrailed 1), EN2 (engrailed 2), andGLI3 (GLI-Kruppel family member 3) and sig naling moleculesFGF8, FGF17, WNT1 (wing less-type MMTV integration site fam-ily, member 1), and SHH (Sonic Hedgehog).

Detailed fate-mapping studies in the mouse have located the progenitors of the medial cerebellar vermis to anterior r1 of the early embryo. Conditional gene deletion experiments in the mouse have proven to be an extremely powerful approach to dissect different requirements of key signaling pathways during cerebellar development . The FGF and WNT signaling pathwaysare prime examples. Since the initial identiﬁcation of Fgf8 andWnt1 gene expression in cells at the IsO, various approaches to disrupt the function of these genes during cerebellar development have been employed. The germline deletion of Fgf8 revealed an early functioning gastrulation, such that the role of Fgf8 in cerebellar development could not be investigated in these mutants. The deletion of Fgf8 speciﬁcally from the early IsO was found to result in the rapid cell death of all progenitors of the mid-brain and cerebellum, identifying FGF as an essential survival factor cells in the mesencephalic(mes)/r1 region.

The analysis of embryos homozygous for hypomorphic alleles of Fgf8, suggested that the maintenance of normal levels of FGF8 signaling was particularly important for the formation of medial cerebellar tissue. Furthermore, the loss of vermis progenitors was found to be associated with roof plate expansion in anterior r1. A study by the Joyner lab has shown that the developmental stage at which Fgf8 expression is disrupted is a key determinant of the severity of vermis hypoplasia; **Fgf8 deletion from the early IsO cause severe vermishypoplasia**,. In the case of Wnt1, the germline deletion of Wnt1 resulted in a similar phenotype to the early mes/r1-deletion of Fgf8, namelythe absence of the midbrain and cerebellum by birth. A similar phenotype is observed upon the deletion of β-catenin using aWnt1-Cre line. Temporal requirements for WNT signalling have not been mapped as extensively as for FGF, but the deletion of β-catenin after E12.5 using Nestin-Cre, resulted in cerebellar vermis hypoplasia defects similar to Wnt1sw/sw mutants. This observation suggests that the requirement for WNT/β-catenin signaling during vermis development and midline “fusion” is later, or extends over a longer time window than the requirement for FGF signaling. Taken together, these studies indicate that the cerebellar vermis that develops from tissue in anterior r1 that is exposed to the highest levels of FGF and WNT for the longest time has the strictest requirement for these signals during development. In keeping with this general theme, mice deﬁcient in En1, the ﬁrst engrailed homeobox gene to be expressed during cerebellar development, results in cerebellar vermis aplasia. A substantial number of mice with conditional deletion ofEn1 after E9 exhibit normal cerebella, conﬁrming the importance of early En1 expression.

***Establishment of progenitor zones and neurogenesis***

After initial patterning and growth of r1 to form the cerebellar anlage, neurogenesis is initiated in two distinct germinal centers, the ventricular zone (VZ) and rhombic lip (RL). All cerebellar neurons and glia as well as progenitors that populate a number of extracerebellar nuclei are born within these germinal zones. Evidence that the production of different neuronal lineages is spatially restricted during cerebellar development comes from loss-of-function and lineage tracing experiments in the mouse. Ben-Arie et al. (1997) ﬁrst demonstrated that the loss of Atoh1 (atonal homolog 1), a gene speciﬁcally expressed in the RL, resulted in the failure to form an external germinal layer (EGL) and EGL-derived granule cells.

***The rhombic lip.***

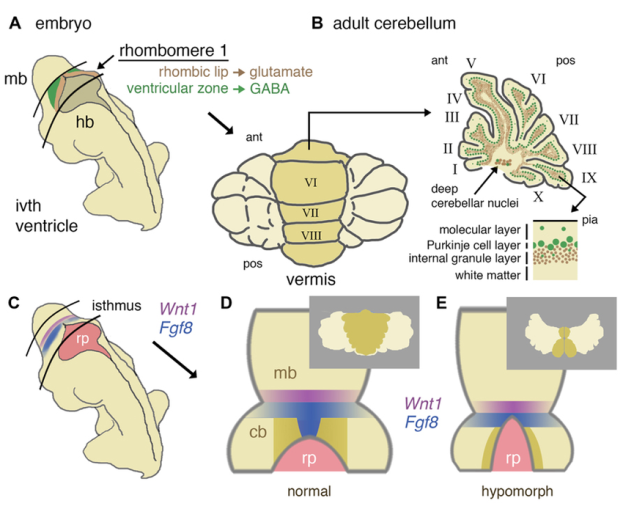
Genetic fate-mapping studies and Atoh1 loss-of-function studies have shown that progenitors of all excitatory glutamatergic neurons of the cerebellum are generated within the upper RL. Defects in the formation or induction of the RL or the speciﬁcation of granule cell progenitors (GCps) are predicted to result in severe cerebellar hypoplasia due to the absence of this rapidly proliferating transit amplifying cell population during postnatal development. The mechanisms required for the induction and functionality of the RL are being elucidated. A number of signaling pathways, including the TGFβ (transforming growth factor beta) and Notch pathways and signaling from the roof plate are implicated in the induction of the RL. Cell production from the RL appears to involve an iterative induction ofAtoh1 in successive waves of migratory derivatives. In addition to GCps, the Atoh1-positive RL also gives rise to neurons that populate the deep cerebellar and extracerebellarnuclei; these include both glutamatergic and cholinergic neurons. Moreover, the RL extends into the hindbrain where it generates neurons that participate in a number of deﬁned circuits including mossy ﬁber inputs to granule cells via the pons. This raises the possibility that defects across the extent of the cerebellar and hindbrain RL could be the cause of conditions, such as pontocerebellar hypoplasia where multiple distributed elements of the cerebellar systems are disrupted. Developmental defects affecting this progenitor zone and its descendants might have far reaching effects on cerebellar connectivity. In particular, these discoveries also point to a time window of sensitivity to developmental damage that might target deep cerebellar nuclei but leave later born granule cell derivatives untouched. Such a window of potential vulnerability to intrinsic or extrinsic damage to the embryo might have selective effects on cerebellar function (in particular connectivity) that are not necessarily correlated with substantial reduction in cerebellar size.

***Ventricular zone.***

Genetic fate-mapping of cells in the cerebellar VZ, demonstrated that all GABA-ergic neurons, including Purkinje, Golgi, basket, and stellate cells, as well as small GABA-ergicneurons of the deep cerebellar nuclei are derived from this region. Compared to the number of glutamatergic granule neurons in the adult cerebellum, the contribution of GABA-ergic neurons to the overall size of the cerebellum is relatively minor. Thus, defects in the generation of GABA-ergic neurons are not expected to result directly in signiﬁcant cerebellar hypoplasia. However, VZ-derived Purkinje cell progenitors are the primary source of mitogen to GCps in the EGL. Thus, the absence or mislocalization of Purkinje cells due to VZ defects could be responsible for cerebellar hypoplasia owing to a deﬁcit in GCp proliferation and postnatal cerebellar growth. It is important to note that Bergmann glia are also derived from the VZ. As these cells form the scaffold that guides the radial migration of neuronal progenitors, defects in the generation or differentiation of these cells could also be responsible for the failure of Purkinje cell migration. Finally, evidence for interaction between progenitor zones comes from the analysis of cell fate upon the deletion of Ptf1aand Atoh1. Defects in cross-regulation, or in the formation or maintenance of cerebellar germinal zones may result in cerebellar hypoplasia by directly disrupting the formation of cerebellar neurons, or by undermining subsequent interactions that lead to the massive expansion of the granule cell precursor pool in the EGL.

***Progenitor cell migration, proliferation, and differentiation***

Tissue growth in the developing embryo has to be tightly regulated to allow the coordinated expansion of different cell types. Coordinated growth requires communication between two or more closely apposed tissue or cell layers. Perhaps the best known example is the orchestration of epithelial growth and morphogenesis through epithelial mesenchymal interactions. Postnatal cerebellar growth is regulated in a similar manner. Rapid cerebellar growth is primarily driven by the proliferation of GCps in theEGL, a process largely coordinated by a layer of Purkinje neurons under the surface of the cerebellum. As we have discussed, the failure to specify Purkinje neurons is associated with severe cerebellar hypoplasia. After their birth in the VZ, Purkinje neuron progenitors migrate along radial glia toward the pial surface of the cerebellar anlage. Genetic defects that disrupt the glial scaffold, or the production of signals and cell intrinsic mechanisms that control Purkinje cell migration result in various degrees of cerebellar hypoplasia. In addition, cell migration defects resulting in the ectopic localization of Purkinje cells are likely to underlie many examples of cerebellar heterotopias. One of the central pathways linked to GCp proliferation and differentiation is the SHH pathway. Immature Purkinje cells secrete SHH and that the proliferation of GCps is critically dependent on SHH signalling. In mouse, conditional deletion of Shh from PCs (Purkinje cells) or SHH signal transduction components like Smo (smoothened), Gli1, and Gli2 from GCps have all been shown to result in defects in GCp proliferation and cerebellar hypoplasia. These observations suggest that the primary cause of cerebellar hypoplasia associated with defects in Reelin signaling is the failure of SHH-expressing PCs to reach their appropriate position underneath the EGL where they provide a proliferative SHH signal.It is important to note that cerebellar hypoplasia caused by defects in SHH signaling affects the (primarily postnatal) proliferation of GCps in the vermis and hemispheres equally, resulting in a phenotype that differs signiﬁcantly from early IsO defects with disproportionally hypoplastic vermis. Conditional manipulation of signaling pathways that function during early cerebellar development have revealed additional functions during later stages of cerebellar development. Again, the WNT and FGF pathways provide good examples of this principle. Reduced WNT signaling during early development result in cerebellar defects typical of reduced IsO function, i.e., vermis aplasia (see The Isthmus Organizer). WNT/β-catenin signaling is also active at later stages of cerebellar development, particularly in the germinal zones and Bergmann glia. The role of WNT/β-catenin signaling at later developmental stages has been investigated more recently. Several groups have reported that increased β-catenin signaling can alter the proliferation and differentiation of neuronal progenitors in the developing cerebellum.



QUESTION 2: GENETIC BASIS FOR GENETIC DISORDERS.

Cerebellar disorders have numerous causes, including congenital malformations, hereditary ataxias, and acquired conditions. Symptoms vary with the cause but typically include ataxia (impaired muscle coordination). Diagnosis is clinical and often by imaging and sometimes genetic testing. Treatment is usually supportive unless the cause is acquired and reversible.

1. **Hereditary ataxias**

Hereditary ataxias may be autosomal recessive or autosomal dominant. Autosomal recessive ataxias include Friedreich ataxia (the most prevalent), ataxia-telangiectasia, abetalipoproteinemia, ataxia with isolated vitamin E deficiency, and cerebrotendinous xanthomatosis.

* ***Friedreich ataxia***: results from a gene mutation causing abnormal repetition of the DNA sequence GAA in the FXN gene on the long arm of chromosome 9; the FXN gene codes for the mitochondrial protein frataxin. The GAA sequence is repeated 5 to 38 times within the FXN gene in people who do not have Friedreich ataxia; however, in people with Friedreich ataxia, the GAA sequence may be repeated 70 to > 1000 times. Inheritance is autosomal recessive. Decreased frataxin levels lead to mitochondrial iron overload and impaired mitochondrial function. In Friedreich ataxia, gait unsteadiness begins between ages 5 and 15; it is followed by upper-extremity ataxia, dysarthria, and paresis, particularly of the lower extremities. Mental function often declines. Tremor, if present, is slight. Reflexes and vibration and position senses are lost. Talipes equinovarus (clubfoot), scoliosis, and progressive cardiomyopathy are common. By their late 20s, patients may be confined to a wheelchair. Death, often due to arrhythmia or heart failure, usually occurs by middle age.
* ***Spinocerebellar ataxias (SCAs)***: are the main autosomal dominant ataxias. Classification of these ataxias has been revised many times recently as knowledge about genetics increases. Currently, at least 43 different gene loci are recognized; about 10 involve expanded DNA sequence repeats. Some involve a repetition of the DNA sequence CAG that codes for the amino acid glutamine, similar to that in Huntington disease. Manifestations of SCAs vary. Some of the most common SCAs affect multiple areas in the central and peripheral nervous systems; neuropathy, pyramidal signs, and restless leg syndrome, as well as ataxia, are common. Some SCAs usually cause only cerebellar ataxia.

1. **Dandy Walker’s Malformation**

Dandy–Walker malformation (DWM), also known as Dandy–Walker syndrome (DWS), is a rare congenital brain malformation in which the part joining the two hemispheres of the cerebellum (the cerebellar vermis) does not fully form, and the fourth ventricle and space behind the cerebellum (the posterior fossa) are enlarged with cerebrospinal fluid. Most of those affected develop hydrocephalus within the first year of life, which can present as increasing head size, vomiting, excessive sleepiness, irritability, downward deviation of the eyes and seizures. Dandy-Walker malformation has also been associated with many chromosomal abnormalities. This condition can be a feature of some conditions in which there is an extra copy of one chromosome in each cell (trisomy). Dandy-Walker malformation most often occurs in people with trisomy 18 (an extra copy of chromosome 18), but can also occur in people with trisomy 13, trisomy 21 or trisomy 9. This condition can also be associated with missing (delitions) or copied (duplications) pieces of certain chromosomes. Dandy-Walker malformation can also be a feature of genetic syndromes that are caused by mutations in specific genes. However, the brain malformations associated with Dandy-Walker malformation often occur as an isolated feature (not associated with other health problems), and in these cases the cause is frequently unknown.

1. **Joubert Syndrome**

Joubert syndrome is a rare brain malformation characterized by the absence or underdevelopment of the cerebellar vermis - an area of the brain that controls balance and coordination -- as well as a malformed brain stem. Many cases of Joubert syndrome appear to be sporadic (not inherited). In most other cases, Joubert syndrome is inherited in an autosomal recessive manner (meaning both parents must have a copy of the mutation) via mutation in at least 10 different genes, including NPHP1, AHI1, and CEP290.

1. **Cerebellar Hypoplasia**

Cerebellar hypoplasia is characterised by a reduced cerebellar volume due to the maldevelopment of one or both hemispheres and a small but normally shaped vermis. This heterogeneous condition is associated with trisomies 9, 13 and 18, congenital disorders of glycosylation, anticonvulsant drugs (valproic acid) or cocaine.

REFERENCES

1. Frontiers in Neuroanatomy pdf. Congenital hypoplasia of the cerebellum: developmental causes and behavioral consequences M. Albert Basson and Richard J. Wingate.
2. Pandolfo M: Friedreich ataxia. Arch Neurol. 65 (10):1296–1303, 2008. Doi:10.1001/archneur.65.10.1296.
3. <https://www.nlm.nih.gov/>
4. Wikipedia.org
5. Voogd, J. & Glickstein, M. The anatomy of the cerebellum. Trends Neurosci. 21, 370–375 (1998).