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Neuroanatomy

1.

Developmental Genetics of Cerebellum

- The cerebellum represents 10% of the brain's total volume, but contains more than half of our neurons. It acts as a coordination centre, using sensory inputs from the periphery to fine-tune our movement and balance. It is one of the first structures in the brain to begin to differentiate, but one of the last to mature, and its cellular organization continues to change for many months after birth. The study of mouse homologues of *Drosophila* genes has provided valuable insights into the molecular basis of cerebellar development.
- 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 mesencephalon and metencephalon both contribute to the developing mouse cerebellum. The patterning of these two regions depends on signals from the isthmus organizer (IO), located just caudal to their junction. *Otx2* and *Gbx2* are central to IO development. *Otx2* is expressed in the mesencephalon, with a posterior boundary at the rostral metencephalon; *Gbx2* is expressed in the metencephalon, and its anterior boundary abuts the *Otx2* boundary. Reciprocal repression maintains a sharp boundary between these domains. *Otx2* and *Gbx2* form part of a regulatory loop that includes *Wnt1*, *En1* and *Fgf8*. Many other genes, including members of the Pax and Hox families, are also involved in patterning this region.
- Purkinje cells (PCs), Golgi neurons, stellate and basket cells all arise from the ventricular neuroepithelium. PCs are born around embryonic day 13, and they migrate along radial glial fibres into the cerebellar anlage. During their final maturation phase, PCs develop extensive dendritic arbors and synapse

onto granule neurons. This depends on granule neuron signals, probably including *Wnt3*. There are various growth factors are required for PC survival, including nerve growth factor, acetylcholine, neurotrophic 4/5, brain-derived neurotrophic factor and ciliary neurotrophic factor.

- The rhombic lip, located between the fourth ventricle and the metencephalic roof plate, gives rise to granule neurons. Proliferation in its germinal epithelium is governed by the *Math1* gene. Rhombic lip cells migrate to the cerebellar anlage and settle on its periphery to form the external granule layer, another zone of proliferation. As the cells begin to migrate, they express markers that include *RU49/Zipro1*, *Zic1* and *Zic3*. *RU49/Zipro1* and *Zic1* are thought to be involved in cell proliferation, which requires interaction with PCs. PCs might release a diffusible factor such as sonic hedgehog (Shh), and *Zic1* could control cell proliferation by indirectly regulating the S*hh* pathway. The final stage of granule neuron maturation occurs after precursor cell migration into the inner granule layer.
- Many genes, including *En1*, *En2*, *Pax2*, *Wnt7b*, and some of the ephrins and their receptors, show characteristic patterns of spatial expression in the cerebellum, but only *En2* has been studied specifically for its role in compartmentalization. In addition to the patterning genes, several other gene families, such as the heat shock proteins and proteins involved in neuronal migration, are also expressed in specific patterns. Spatial- and temporal-specific knockout strategies should yield more information about the roles of these genes in patterning the cerebellum.

Citation

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2.

Genetic Basis of Cerebellar Disorders

In the last decade, there has been considerable effort in defining the genetic basis of human cerebellar malformations. Causative genes include those involved in cerebellar patterning, cell fate specification, and other developmental processes.

List of genes and suspected cellular processes that have been implicated in human cerebellar malformations

Cerebellar malformations	Implicated human genes	Likely disrupted process
Cerebellar vermis hypoplasia (CVH)	*OPHN1*	Spine morphogenesis
Dandy–Walker malformation (DWM)	*ZIC1*, *ZIC4*, *FOXC1*	Granule cell differentiation
Joubert syndrome and related disorders (JSRD)	*AHI1*, *ARL13*, *CCD2A*, *CEP290*, *INPP5E*, *NPHP1*, *RPGRIP1L*, and *TMEM67*	Granule cell proliferation
Pontocerebellar hypoplasia (PCH)	*CASK*, *RARS2*, *TSEN54*, *TSEN34*, and *TSEN2*	Spine development, cell proliferation, tRNA splicing, cellular maintenance.

Pancreas specific transcription factor 1a (Ptf1a) was initially implicated as a basic helix–loop–helix transcription factor in pancreatic development since mice with a targeted deletion lacked pancreatic tissue. However, its role in brain development was not investigated until truncations of this gene were found to result in cerebellar agenesis in multiple families. Further investigations determined that loss of Ptf1a causes a failure to generate GABAergic cerebellar neurons in the embryonic cerebellar anlage in both human and mouse. Since Purkinje cells, which are GABAergic, are also required for the proliferation of cerebellar granule neurons, humans and mice lacking Ptf1a exhibit profound cerebellar agenesis.

Transcription factors have also been implicated in other types of cerebellar malformations. Heterozygous loss of the *ZIC1* and *ZIC4* genes encoding zinc finger transcription factors can cause DWM, a phenotype which is mimicked

in *Zic1* and *Zic4* double heterozygous mutant mice. Mutations in *FOXC1*, a transcription factor gene located in the 6p25.3 locus, have recently been shown to contribute to human DWM. Mouse models have demonstrated that *Foxc1* is developmentally expressed in the mesenchyme adjacent to the cerebellum, where it is critical for normal posterior fossa development. In addition to regulating skull development, Foxc1 controls mesenchymally expressed signaling molecules including Bmp2 and Bmp4. Loss of these signaling molecules causes the adjacent cerebellar rhombic lip to lose *Atoh1* (*Math1*) expression, a gene critical for normal granule cell differentiation. These findings, based on studies in both human and mice, have surprisingly implicated mesenchymal signaling as a critical regulator of early cerebellar anlage development.

Studies of JSRD patients have also provided surprising insights into new developmental mechanisms. Of the nine loci linked to JSRD, eight have been cloned and the following causative genes identified: *AHI1*, *ARL13B*, *CC2D2A*, *CEP290*, *INPP5E*, *NPHP1*, *RPGRIP1L*, and *TMEM67*. Many of these genes are implicated in normal ciliary function and their protein products localize to the cilia or basal bodies. One such cilia-related protein is Nephrocystin, the product of *NPHP1*, which interacts with beta-tubulin and localizes to primary cilia. In cell culture, CEP290, centrosomal protein 290, is involved in ciliogenesis, localizes to centrioles in a microtubule-dependent manner, and regulates the microtubule network, as shown through RNAi. Furthermore, CEP290 interacts with the protein product of *CCD2A* both genetically and physically. Most recently, mutations in the *INPP5E* gene, which codes for inositol polyphosphate-5-phosphatase E, were found in patients with Joubert syndrome. While it was known that this enzyme hydrolyzes phosphatidylinositols, INPP5E was found to be localized to cilia and mutations resulted in premature destabilization of cilia after stimulation. Thus, examination of human patients led to a novel role for INPP5E in both cilia signaling and Joubert syndrome. Mutations in many components of this single biological pathway result in similar cerebellar defects. The actual purpose of cilia in the cerebellum is likely to be linked to SHH signaling. Significantly, loss-of-function mutations in murine *Kif3a* and *Ift88*—genes encoding intraflagellar transport proteins for the formation and maintenance of cilia—cause SHH-dependent proliferation defects of granule cell progenitors. This loss of SHH signaling results in cerebellar phenotypes resembling those seen in JSRD. JSRD now provide a model for how studies of human cerebellar

malformations can lead to the discovery of causative genes and expand our knowledge of the pathways involved in cerebellar development.

Additional molecules have been implicated in human cerebellar malformations, which are certain to illuminate new cerebellar developmental mechanisms. Deletions of the Rho-GAP protein encoding gene *Oliogphrenin-1* (*OPHN1*) have been found in multiple families with X-linked CVH. While Ophn1 is required for the stabilization of glutamatergic spines, it has not been implicated in regulating earlier developmental events such as cell division. Interestingly, mice with a targeted deletion of *Ophn1* exhibit learning deficits and have dilated lateral and third ventricles, but their cerebellar size and morphology are normal. This suggests that the mental retardation (MR) seen in human patients may not be due to cerebellar defects. However, until the connectivity and plasticity of the mutant mouse cerebellum are examined in detail this only remains a speculation. Recently Ophn1 has been shown to facilitate clathrin-mediated endocytosis of post-synaptic vesicles, including the AMPA receptor, by repressing the RhoA/ROCK pathway. Because of this, mutant mice lack LTD in the hippocampus. Cerebellar LTD still remains to be examined.

Mutations of another molecule with a known role in synapse development have also been seen in PCH. CASK is a calcium/calmodulin-dependent serine/threonine kinase localized to synapses via membrane-associated molecules, including Neurexin. CASK also regulates gene transcription during cell proliferation. Although mouse *Cask* mutants have cerebellar hypoplasia, the developmental basis for this pathology has not yet been studied. Genes from the tRNA splicing pathway have also been observed to cause PCH when mutated in humans. One family has been found with three members containing mutations in the *RARS2* gene, which encodes mitochondrial arginine-transfer RNA synthase. Individuals with PCH have also been found to have mutations in *TSEN54*, *TSEN34*, and *TSEN2*, which all encode tRNA splicing proteins. The study of mouse models will be essential to determine why developing cerebellar and pontine cells are particularly sensitive to the loss of these genes even though they are ubiquitously expressed.

Human studies have demonstrated that patient clinical phenotypes associated with severe congenital cerebellar malformations described here can be highly variable. Less severe cerebellar malformations have been reported in patients with non-syndromic MR, Autism Spectrum Disorders, and schizophrenia. Evidence of Purkinje cell dysfunction in cerebella from autistic patients has been demonstrated by reduced levels of *glutamate decarboxylase* (*GAD67*),

which codes for a GABA-synthesizing enzyme. In addition, levels of various gene transcripts involved in GABAergic transmission are altered in lateral cerebellar hemispheres of schizophrenic patients.
Specifically, *GAD67*, *GAD65*, *GAT-1*, *MGLUR2*, and *NOS1* were downregulated whereas *$GABA_A$-alpha6*, *$GABA_A$-delta*, *GLUR6*, and *GRIK5* were upregulated. Thus, it is likely that the genes underlying these more common and genetically complex neurodevelopmental disorders also influence cerebellar development. Notably, most patients with MR, autism, and other neurodevelopmental disorders rarely undergo brain imaging. Therefore, the coincidence of these disorders with cerebellar malformation is often missed. In order to fully and accurately delineate clinical phenotypes, we strongly advocate routine brain imaging of all human neurodevelopmental disorders. Further, given the extremely fine resolution with which cerebellar phenotypes can now be characterized in mice at the molecular, cellular, and systems level, mouse models for these common neurodevelopmental disorders are certain to be highly informative regarding their underlying pathology.

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 (1). 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)

They 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.

SCA type 3, formerly known as Machado-Joseph disease, may be the most common dominantly inherited SCA worldwide. Symptoms include ataxia, parkinsonism, and possibly dystonia, facial twitching, ophthalmoplegia, and peculiar bulging eyes.

Reference

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