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**ASSIGNMENT TITLE: DEVELOPMENTAL GENETICS OF THE CEREBELLUM AND HIGHLIGHTS OF GENETIC BASES OF CEREBELLAR DISORDERS.**

**ABSTRACT:**

The cerebellum is a pre-eminent model for the study of neurogenesis and circuit assembly. Increasing interest in the cerebellum as a participant in higher cognitive processes and as a locus for a range of disorders and diseases make this simple yet elusive structure an important model in a number of fields. In recent years, the understanding of some of the more familiar aspects of cerebellar growth, such as its territorial allocation and the origin of its various cell types, has undergone major recalibration. Furthermore, owing to its stereotyped circuitry across a range of species, insights from a variety of species have contributed to an increasingly rich picture of how this system develops.

**INTRODUCTION:**

The cerebellum (‘little brain’) resides at the anterior end of the hindbrain and is classically defined by its role in sensory-motor processing. In amniotes, it represents one of the most architecturally elaborate regions of the central nervous system (CNS), and in humans it contains over half of the mature neurons in the adult brain. This morphological complexity belies histological simplicity: the cerebellar cortex is composed of a very basic structure comprising a monolayer of inhibitory Purkinje cells sandwiched between a dense layer of excitatory granule cells and a sub-pial molecular layer of granule cell axons and Purkinje cell dendritic trees. Granule cells receive inputs from outside the cerebellum and project to the Purkinje cells, the majority of which then project to a variety of cerebellar nuclei in the white matter. A less well-defined complement of locally interacting inhibitory interneuron cell types and glutamatergic unipolar brush cells complete the circuit, which famously promised to be the first of any vertebrate neural network to be fully comprehended. Historically, studies of the cerebellum focused on its description through fate mapping, its induction via FGF signaling or its role as a locus for developmental cancer. However, in recent years, each of these perspectives has been subject to a more or less severe reworking, and this revision has generated important insights into the organization of neurogenesis, the cell lineages, temporal patterning and differentiation in the cerebellum. Collectively, this scrutiny has propelled the cerebellum into a pre-eminent model for neural development, an understanding of which impacts on a range of congenital and acquired disorders. The increasing recognition of the diversity of cerebellar-related syndromes reflects a growing understanding of the repertoire of brain regions influenced by cerebellar activity, as revealed by novel mapping techniques and implied from clinical studies. Most recently. the cerebellar circuit has achieved a new significance in the context of autistic spectrum disorder (ASD). Moreover, the recent explosion of genetic developmental techniques means that the cerebellum can perhaps fulfil its potential as a model in comparative approaches in biology.

**CEREBELLAR DEVELOPMENT:**

Although it is easiest to consider how developmental phases fit together in the mammal, it is important to recognise that, beyond the stereotyped neuronal Purkinje-granule cell circuit, evolutionary variability in cerebellum form reflects variability in how these phases are deployed in the embryo. Thus, the territory that will generate the cerebellum – its ‘anlage’ – is allocated during the early embryonic segmental phase of hindbrain development [in mouse, at approximately embryonic day (E) 8.5] close to the boundary (the ‘isthmus’) between the hindbrain and the midbrain. However, as we will describe, regulation of patterning in this earliest phase seems particularly important for the development of the uniquely mammalian midline expanded region of the cerebellum known as the ‘vermis’.

Lagging behind the establishment of rhombomere boundaries ([Simon et al., 1995](https://dev.biologists.org/content/141/21/4031#ref-131)), specific cell types are allocated along the dorsoventral axis. For glutamatergic cells of the cerebellum, this is a remarkably prolonged and, importantly, a dynamic process that takes place at the most dorsal interface between neural and non-neural ‘roof plate’ tissue: the rhombic lip (in mouse, at E10.5-E18.5). This phase generates the basic dichotomy between GABAergic and glutamatergic cell types that underlies the conserved Purkinje-granule cell circuit, but, as we will see, it is also responsible for the diversity of cerebellum output connectivity across species.

Cell type allocation precedes a third, distinct temporal phase of development that extends into early prenatal life (postnatal day 21 in mouse and up to 2 years in humans). Here, the principal derivative of the rhombic lip, the granule cell precursor, accumulates over the surface of the cerebellum and undergoes further rounds of symmetric divisions in a process of transit amplification that exponentially expands its numbers. Growing evidence suggests that this most investigated phase of cerebellum development is substantially reduced or absent in aquatic vertebrate. The final form of the mammalian cerebellum is so much a product of the first and third phases of development.

**CEREBELLAR ANALAGE: MOLECULAR BOUNDARIES AND THE ROLE OF FGF8.**

A fundamental determinant of cerebellar morphology is the allocation of a territory in which its component cell types are specified. Despite the distinct structure and clear boundaries of the cerebellum, this simple problem has proved more enduring than might have been anticipated. Similarly, the association of cerebellar induction with the diffusible morphogen fibroblast growth factor 8 (Fgf8) has acquired a more nuanced perspective. It seems likely that FGF signalling has far-reaching evolutionary and developmental significance for other aspects of brain development, such as the allocation of isthmic territory and the origins of the mammalian vermis, tying embryonic events at the early stages of axial specification to surprisingly profound clinical consequences for higher cognitive function. The anlage of the cerebellum is a product of the mechanisms of segmentation that establish iterated rhombomeric subdivisions within the early hindbrain just after neural tube closure. The establishment and maintenance of the boundaries defining the territory of the cerebellum has been a subject of several recent studies. These have built our current understanding that all of the cells of the cerebellum arise from dorsal rhombomere 1 (r1), a region that is definitively characterised by an absence of the expression of Otx and Hox genes. Early studies concluded that the majority of the cerebellum arises from the metencephalic (rostral) hindbrain, but that as much as one-third of cerebellar granule cells originate from the mesencephalon, which is rostral to the midbrain-hindbrain constriction ([Hallonet et al., 1990](https://dev.biologists.org/content/141/21/4031%22%20%5Cl%20%22ref-64)). Later, this idea was overturned by instead mapping the molecular boundary between the midbrain and hindbrain as the caudal extent of Otx2 expression ([Millet et al., 1996](https://dev.biologists.org/content/141/21/4031#ref-99)), showing that all cerebellar cells are born from Otx2-negative tissue and also demonstrating a surprising degree of anisotropic growth proximal to the midbrain hindbrain-boundary (MHB). The caudal boundary of cerebellar territory has also been mapped by chimeric grafting to the r1/2 boundary, as marked by Hoxa2 expression ([Wingate and Hatten, 1999](https://dev.biologists.org/content/141/21/4031#ref-153)).

**TRANSIT ENLARGEMENT AND THE SIZE AND FOLIATION OF THE CEREBELLUM:**

Although early events can significantly bias patterns of cerebellar growth, the final shape and size of the cerebellum of mammals and birds (possibly all reptiles) is the product of a remarkable example of a discrete phase of transit amplification that occurs much later in development. This proliferative episode takes a small number of Atoh1-positive granule cell precursors and multiplies their numbers by many folds through multiple symmetrical mitoses of single fated germinal cells. The transient appearance of this population of granule cell precursors over the surface of the cerebellum was quickly identified as a key feature of cerebellum development (Ramón y [Cajal, 1894](https://dev.biologists.org/content/141/21/4031%22%20%5Cl%20%22ref-113)) and offered an intuitive explanation for the massive foliation of the cerebellar surface in mammals. More recently, the same logic has made the outermost layer of the cerebellum, the external germinal layer, an obvious candidate for medulloblastoma, a devastating childhood cancer. This has exemplified how insights from development both explain and offer therapeutic avenues for disease.

**Timing and diversity: how cells become specified**

Although Shh-dependent late-born populations represent the last stages of cell production in the cerebellum, a clear temporal order of cell production precedes this stage. This temporal pattern is superimposed onto dynamically maintained progenitor zones. Thus, in the rhombic lip, the production of granule cell precursors proceeds alongside that of a population of small unipolar brush cells ([Kita et al., 2013](https://dev.biologists.org/content/141/21/4031#ref-79)) that also express the T-box gene Tbr2 ([Englund et al., 2006](https://dev.biologists.org/content/141/21/4031%22%20%5Cl%20%22ref-43)). This represents the final phase in a sequence of cell specification. Granule cell precursor production is preceded by the generation of glutamatergic cerebellar nuclei, which briefly express Atoh1 but do not undergo transit amplification. The number of cerebellar nuclei varies between major amniote orders, with two in reptiles and between three and five divisions in mammals ([Nieuwenhuys et al., 1998](https://dev.biologists.org/content/141/21/4031%22%20%5Cl%20%22ref-106)). These accumulate in a sequence with the most lateral being born first.

As cerebellar nuclei represent the output connection of the cerebellum, this diversity is functionally significant. For example, birds lack the most lateral of the mammalian nuclei, the Lhx9-positive dentate nucleus, which in mammals targets the thalamus. This connection allows the cerebellum to participate in regulating cortical functions and its absence in birds marks a major difference in brain organisation.

Cerebellar nucleus neurons are the first cerebellar cells to be generated, but are not the earliest Atoh1 cells to be generated in rhombomere 1. At pre-cerebellar stages, the rhombic lip is patterned by FGF signalling from the isthmus and generates Lhx9-positive neurons that migrate into ventral and isthmic r1 ([Machold and Fishell, 2005](https://dev.biologists.org/content/141/21/4031%22%20%5Cl%20%22ref-94); [Wang et al., 2005](https://dev.biologists.org/content/141/21/4031#ref-147); [Green et al., 2014](https://dev.biologists.org/content/141/21/4031#ref-60)), contributing cells to multiple nuclei that form part of a wider hindbrain network of nuclei controlling proprioception, interoception and arousal ([Rose et al., 2009](https://dev.biologists.org/content/141/21/4031#ref-119)).

The switch from the production of cerebellar neurons to granule cells at E12.5 in mouse ([Machold and Fishell, 2005](https://dev.biologists.org/content/141/21/4031%22%20%5Cl%20%22ref-94); [Wang et al., 2005](https://dev.biologists.org/content/141/21/4031#ref-147)) is paralleled by a switch in the production of GABAergic neurons in the ventricular zone from Purkinje cells to other types of interneurons and glia ([Sudarov et al., 2011](https://dev.biologists.org/content/141/21/4031%22%20%5Cl%20%22ref-136)). This correlates with the changing patterns of Olig2 and Gsx1 expression between E12.5 and E14.5. The expression of Gsx1, which marks interneuron progenitors, gradually expands dorsally and into the Olig2 lineage that, before E12.5, gives rise to only cerebellar nucleus and Purkinje cells ([Seto et al., 2014](https://dev.biologists.org/content/141/21/4031%22%20%5Cl%20%22ref-129)). In contrast to the rhombic lip, where the outcome of a single inductive interaction changes over time, temporal patterning in the rest of the ventricular zone may reflect dynamic reorganisation of variously identified dorsoventral regions ([Chizhikov et al., 2006](https://dev.biologists.org/content/141/21/4031%22%20%5Cl%20%22ref-29); [Zordan et al., 2008](https://dev.biologists.org/content/141/21/4031%22%20%5Cl%20%22ref-163); [Grimaldi et al., 2009](https://dev.biologists.org/content/141/21/4031%22%20%5Cl%20%22ref-61); [Mizuhara et al., 2010](https://dev.biologists.org/content/141/21/4031%22%20%5Cl%20%22ref-102); [Florio et al., 2012](https://dev.biologists.org/content/141/21/4031#ref-51)).

The correlation in the timing of fate switches and the observation that this occurs even when Atoh1 or Ptf1a are mis expressed in the ventricular zone or rhombic lip, respectively ([Yamada et al., 2014](https://dev.biologists.org/content/141/21/4031#ref-155)), suggest that a common, non-autonomous factor regulates the overall temporal development of the cerebellum and support the idea that progenitor populations share common features. Transplantation studies of both GABAergic ([Leto et al., 2006](https://dev.biologists.org/content/141/21/4031%22%20%5Cl%20%22ref-85), [2009](https://dev.biologists.org/content/141/21/4031#ref-86)) and glutamatergic rhombic lip progenitors ([Wilson and Wingate, 2006](https://dev.biologists.org/content/141/21/4031#ref-150)) support the concept of an extrinsic cue for developmental timing. The choroid plexus, which is generated from the roof plate lineage and whose development is at least partially regulated by the rhombic lip ([Broom et al., 2012](https://dev.biologists.org/content/141/21/4031#ref-19)), is an attractive candidate for orchestrating coordinated changes in cell fate through the secretion of a range of factors, including Shh ([Huang et al., 2010](https://dev.biologists.org/content/141/21/4031#ref-70)), Igf2 ([Lehtinen et al., 2011](https://dev.biologists.org/content/141/21/4031%22%20%5Cl%20%22ref-84)), retinoic acid ([Yamamoto et al., 1996](https://dev.biologists.org/content/141/21/4031#ref-156); [Wilson et al., 2007](https://dev.biologists.org/content/141/21/4031#ref-151)) and thyroid hormone. The choroid plexus may thus prove to be a factor in early cerebellar dysgenesis and offer a locus for understanding the coordinated diversification of glutamatergic and GABAergic neuronal subtypes during cerebellar evolution.

**GENETIC BASES OF CEREBELLAR DISORDERS**

The past 20 years have seen an increasing awareness of the role of the cerebellum in non-motor functions (Schmahmann, 2010). These functions are reflected in the higher cognitive function defects that accompany motor dysfunction following cerebellar damage (Schmahmann and Sherman, 1998). Pre-term damage to the developing cerebellum also predicates long-term cognitive deficits (Limperopoulos et al., 2007). Furthermore, congenital deficits, in particular vermal agenesis, lead to later communicative and affective relational disorders (Tavano et al., 2007). Accordingly, a study of structural brain abnormalities in mouse models of autistic spectrum disorder (ASD) revealed cerebellar-specific disruptions (Steadman et al., 2013). Although this variety of sources suggests that early patterning defects might generate significant cognitive impairment, the most compelling evidence in support of this hypothesis is a recent analysis of a mouse model of human CHARGE syndrome. CHARGE syndrome is reflected in a cluster of congenital abnormalities including ASD-like behavioural problems in humans. In mice, mutation in the chromatin modifier Chd7 leads to a vermal hypoplasia that can be directly linked to changes in Otx2 repression at the midbrain-rhombomere 1 boundary (Yu et al., 2013). This raises the possibility that other unrecognised early patterning defects may underlie a range of human cognitive deficit syndromes (Haldipur and Millen, 2013).

**Medulloblastoma and the EGL**

Medulloblastoma is a devastating paediatric cancer of the cerebellum. In recent years, whole genome and transcriptome sequencing of clinical samples has revealed a number of molecularly distinct subtypes of medulloblastoma (Jones et al., 2012; Pugh et al., 2012; Robinson et al., 2012) that frequently involve activation of the Shh and Wnt pathways. Disruption of transit amplification remains a compelling model for the Shh subgroup of tumours, based on experimental disruption of Shh signalling (Goodrich et al., 1997), and more recent developmental studies show that commitment to the granule cell lineage is a prerequisite for tumour formation (Schuller et al., 2008; Yang et al., 2008; Li et al., 2013). Although Wnt signalling also affects cerebellar proliferation, its effects are restricted to non-granule cells (Pei et al., 2012; Selvadurai and Mason, 2012) and accordingly the Wnt-dependent subgroup of tumours, along with some Shh subgroup tumours (Grammel et al., 2012), appears to have a hindbrain origin (Gibson et al., 2010). Pathways that might supress transit amplification, such as BMP signalling (SMAD) (Aref et al., 2013), or promote differentiation (Barhl1) (Li et al., 2004) are thus associated with improved patient prognosis (Poschl et al., 2011), in contrast to those associated with regulating granule cell precursor identity (Atoh1) (Schuller et al., 2008; Yang et al., 2008) or proliferation (Foxm1) (Schuller et al., 2007; Priller et al., 2011). Recent studies have also shown that activation of the FGF (Emmenegger et al., 2013) and Wnt pathways (Anne et al., 2013) has tumour-supressing actions. This raises the possibility that other genes that antagonise granule cell proliferation during development, such as Neurod1 (Butts et al., 2014a), may also provide a potential route to therapy.

**ASD and cerebellar cell types**

The heterogeneous nature of autistic spectrum disorder (ASD) is reflected in the range of its different, potential developmental causes. Perhaps surprisingly, the most consistent pathological correlates of ASD are found in the cerebellum ([Courchesne, 1997](https://dev.biologists.org/content/141/21/4031%22%20%5Cl%20%22ref-33)). Furthermore, a recent meta-analysis suggests that a signature constellation of anatomical deficits makes cerebellar damage in ASD distinct from that in either ADHD or developmental dyslexia ([Stoodley, 2014](https://dev.biologists.org/content/141/21/4031%22%20%5Cl%20%22ref-133)). These include localised folia hypoplasia ([Courchesne et al., 1988](https://dev.biologists.org/content/141/21/4031%22%20%5Cl%20%22ref-34)) or the specific loss or alteration of Purkinje cells ([Ritvo et al., 1986](https://dev.biologists.org/content/141/21/4031%22%20%5Cl%20%22ref-115); [Fatemi et al., 2002](https://dev.biologists.org/content/141/21/4031%22%20%5Cl%20%22ref-45)). Specific disruption to white matter in the superior cerebellar peduncle might be associated with a loss of cerebellar output to the thalamus ([Brito et al., 2009](https://dev.biologists.org/content/141/21/4031#ref-17)). The dentate nucleus, which supplies this projection, is a crucial link in the cortico-cerebellar close loop circuits that potentially modulate higher cognitive functions in primates ([Kelly and Strick, 2003](https://dev.biologists.org/content/141/21/4031#ref-77); [Strick et al., 2009](https://dev.biologists.org/content/141/21/4031#ref-134)) and humans ([Kipping et al., 2013](https://dev.biologists.org/content/141/21/4031#ref-78)). The highly complex and enlarged dentate nucleus in humans shows a pronounced left-right asymmetry ([Baizer, 2014](https://dev.biologists.org/content/141/21/4031%22%20%5Cl%20%22ref-8)) and, correspondingly, consistent unilateral reduction in dentate projections is inferred from a study of individuals with Asperger's ([Catani et al., 2008](https://dev.biologists.org/content/141/21/4031#ref-24)). Finally, a recent transgenic study in which mutation of the tuberous sclerosis gene associated with human ASD was targeted specifically to Purkinje cells resulted in an ASD-like mouse phenotype ([Tsai et al., 2012](https://dev.biologists.org/content/141/21/4031#ref-143)). Collectively, these observations suggest that, by virtue of cortico-cerebellar connectivity, selective cerebellar cell loss can mimic the effects of what are more readily perceived as ‘cortical’ syndromes ([Schmahmann and Pandya, 2008](https://dev.biologists.org/content/141/21/4031%22%20%5Cl%20%22ref-124)).

 **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 atataxia

**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.

**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.

**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.

**CONCLUSION**

As with the study of many parts of the developing brain, specialised interests in specific phases of growth of subtypes of cells sometimes obscure a larger picture of coordinated programmes of development. These grander designs are often most apparent in the patterns of adaptation in evolution, where coherent regulation of developmental processes across time and scale are absolutely aligned. For this reason, the exploration of cerebellar development across a range of species will continue to be a valuable resource for generating perspective on development problems. For the cerebellum, the promise of a broad overview of how components of development are orchestrated seems particularly close due to its relatively simple circuit and small number of component cell types.

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