AFE BABALOLA UNIVERSITY

DEVELOPMENTAL GENETICS OF THE CEREBELLUM

NEUROANATOMY, ANA 303

Submitted by

ONOJI FAITH OGHENEVOVWERO

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DEPARTMENT OF MEDICINE AND SURGERY, 300L

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MR EDEM EDEM

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INTRODUCTION

The cerebellum lies in the posterior cranial fossa. It represents 10% of the total brain weight hence it is referred to as the 'little brain'. The cerebellum plays an essential role in the control of movement; ensuring that movement takes place smoothly, in the right direction and to the right extent. Cerebellar stimulation modifies movements produced by stimulation of motor areas of the cerebral cortex. The cerebellar cortex is also important for learning of movements. Although the cerebellum is one of the first structures of the brain to differentiate, it achieves its mature configuration only many months after birth. This lengthy formative period makes the cerebellum especially vulnerable to developmental irregularities. Over the past few years, genetic research has provided a great deal of information about the molecular events directing the formation of the cerebellum. Knowledge of these mechanisms addresses the nature of human diseases that have their root in developmental processes. In this review, these mechanisms as well as their clinical significance are outlined.

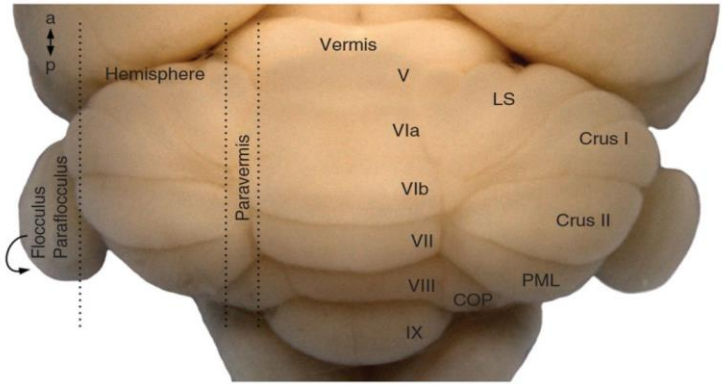

FIG. 1 the Cerebellum

OVERVIEW OF CEREBELLAR DEVELOPMENT

The brain is sometimes divided into the brain stem (mesencephalon, pons from the metencephalon, and myelencephalon) and the higher centres (cerebrum and cerebellum). The brain stem is a direct continuation of the spinal cord thus, distinct basal and alar plates. The dorsolateral parts of the alar plates bend medially and form the rhombic lips. As a result of a further deepening of the pontine flexure, the rhombic lips compress cephalocaudally and form the cerebellar plate. At week twelve, the plate shows a small midline portion, the vermis and two lateral portions, the hemispheres (FIG. 1). A transverse fissure soon separates the nodule from the vermis and the lateral flocculus from the hemispheres. Cells in the cerebellum arise from two different germinal matrices; the ventricular zone and the rhombic lips. From the ventricular zone, cells radiate laterally and evolve into the deep cerebellar nuclei and Purkinje cells of the cerebellar cortex. However, the cerebellar granule neurons arise from the rhombic lips (FIG. 4). Hence, the cerebellar cortex is made up of three layers which are the molecular layer, purkinje cell layer and granular layer. Cells found in these layers are purkinje cells, granule cells, stellate cells, basket cells and golgi cells.

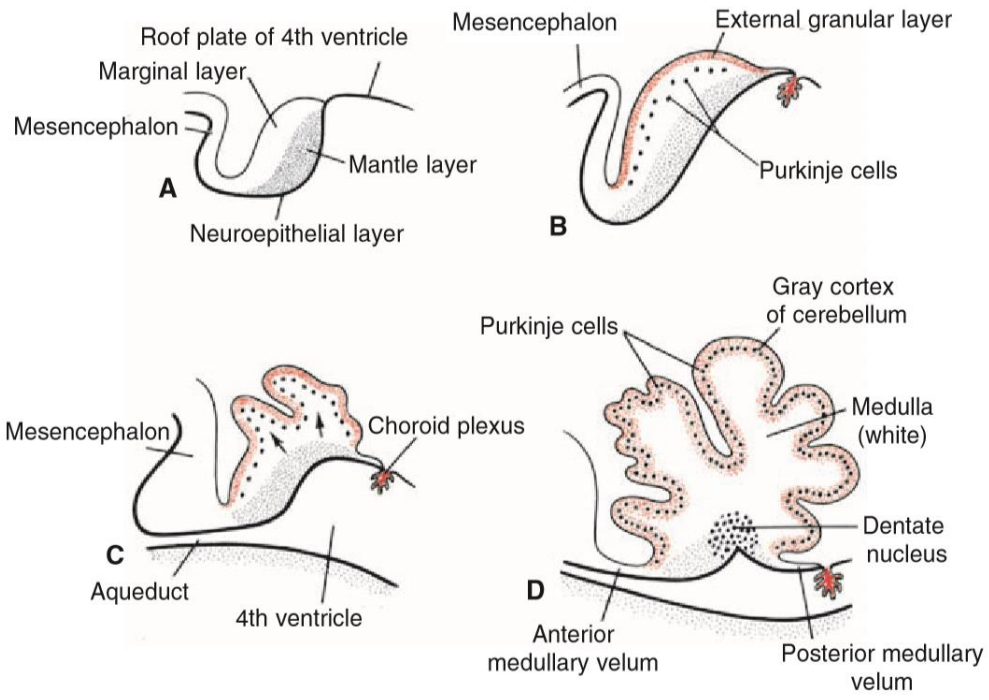

FIG. 2 development of the cerebellum

GENETICAL CONTROL OF CEREBELLAR DEVELOPMENT

The cerebellum is derived from rhombomere 1, which comprises the most anterior aspect of the rhombocephalon. A rhombomere is a transiently divided segment of the developing neural tube, within the hindbrain region. In humans, there are about eleven rhombomeres which gives rise to different structures. The proper patterning of the mesencephalon and the metencephalon is dependent on molecular signals released from the isthmus organizer (*IO*), which is located just caudal to the junction of these two regions. Morphologically, this region is marked by a sharp bend of the neural tube (FIG. 3). The *IO* is set up by the expression of a complex array of genes. Two, in particular, are central to its development: the homeobox genes *Otx2* and *Gbx2* (FIG. 3). *Otx2* is expressed in the mesencephalon, and it is bounded posteriorly by the rostral metencephalon, whereas *Gbx2* is expressed in the metencephalon and it is bounded anteriorly by the caudal mesencephalon. In addition to helping form the *IO* molecularly, *Gbx2* and *Otx2* also regulate the expression of *Fgf8* (fibroblast growth factor 8); *Otx2* negatively regulates *Fgf8* expression, whereas Gbx2 maintains it. Fgf8 is involved in regulating the various genes expressed in the mid- and hindbrain regions. *Fgf8* exerts its action partially by inducing the expression of wingless homologue 1 (*Wnt1*) through Lim homeobox 1b (*Lmx1b*). *Wnt1*, in turn, maintains the expression of Engrailed (*En1*), which then positively regulates *Fgf8* expression, completing the feedback regulatory loop. Several other genes that are not part of this pathway are also important in

patterning of the mid-/hindbrain region. The paired box genes *Pax2* and *Pax5* are expressed in the mid-/hindbrain region.

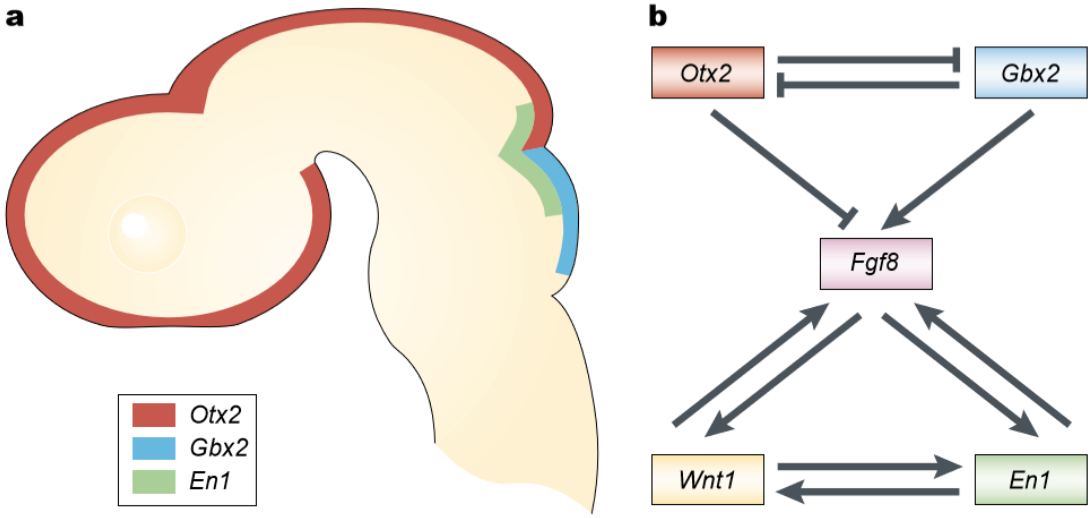

FIG. 3 the Homeobox Genes

The Purkinje, Golgi, stellate and basket cells all arise from the ventricular neuroepithelium. Relatively little is known about the specific factors that govern Purkinje cell differentiation. Shortly after their final mitosis, Purkinje cells begin to express the calcium-binding protein *calbindin*. *Calbindin* positive cells migrate in a radial direction over the already formed deep cerebellar nuclei. These Purkinje cells then settle and become suspended beneath the external granular layer (EGL), awaiting the inward migration of granule neurons. The timely arrest of migration is dependent on the reelin pathway. The external granular layer promotes Purkinje cell migration by secreting reelin (*RELN*), an extracellular matrix component attracting or repealing precursors and axons during development, acting as an extracellular signaling molecule. Mutations in the *Reln gene* or in components of its signalling pathway lead to various cerebellar defects. Although Purkinje cells depend on signals from the granule neuron precursors to migrate, their differentiation programme seems to be independent of granule neurons. *Math1*, a transcription factor, is critical for the proper development of the granular layer of the cerebellum. Mice deficient in *Math1* show a loss of external granular layer. In late embryogenesis, climbing fibres from the inferior olivary nucleus start to innervate Purkinje cells. Extensive interactions occur between the climbing fibres and the Purkinje cells, and these interactions are believed to influence Purkinje cell development. During their final maturation phase, Purkinje cells develop extensive dendritic arbours and synapse onto granule neurons. Throughout the course of development, various growth factors are important for Purkinje cell survival. Nerve growth factor, acetylcholine, brain-derived neurotrophic factor (*BDNF*) and ciliary neurotrophic factor have all been shown to have a positive effect on Purkinje cell number in vitro. Similarly, the *Rorα* (RAR-related orphan receptor α) gene is also important for the survival of Purkinje cells.

Table 1 | **Genes involved in various stages of cerebellar development**

Stages/areas of development	Genes, proteins and molecules
Cerebellar primordium	*Otx2, Gbx2, Fgf8, Wnt1, En1/2, Pax2/5,* Bmps*, Shh, Hoxa2*
Granule cell generation	*Math1, RU49/Zipro1, Zic1,2,3, Shh* pathway, *Ccnd2, p27, Neurod1, NSCL1*
Granule cell migration	Tag1, Tuj1*, Pax6,* Dcc/netrin pathway, *Unc5h2,3* GIRK2, astrotactin, thrombospondin, tenascin, neuregulin
Purkinje cell maintenance	Ngf, BDNF, ciliary neurotrophic factor, acetylcholine, Nt4/5, Rorα
Purkinje cell migration	Reelin pathway

The granule neurons are derive from a separate germinal epithelium known as the rhombic lip. The rhombic lip is located between the fourth ventricle and the roof plate in the metencephalon. Expression of the *Math1* gene governs the germinal epithelium of the rhombic lip. *Math1* is expressed in the mid-/hindbrain region and persists in the rhombic lip and many of its derivatives including the granule neurons of the cerebellum and the pontine nucleus of the precerebellar system. The rhombomere 1 region of the rhombic lip is the probable source of granule neuron precursors. Inside the rhombic lip, granule neuron precursors proliferate and then assume a unipolar morphology, with a single process that projects away from the rhombic lip. They begin to migrate out from the rhombic lip to populate the external granular layer (FIG. 2). As the cells continue to migrate, the external granular layer differentiates into outer and inner external granular layers (FIG. 4). From the outer EGL, granule neuron precursors migrate into the inner EGL. In the inner EGL, granule neuron precursors no longer express *Math1*; instead, they express two other basic transcription factors, *Neurod1* and *NSCL1*. *Neurod1* is important for the survival of inner EGL granule neuron precursors. The next stage of development for granule neuron precursors is the inward migration into the inner granule layer (IGL) under the guidance of radial glial fibres.

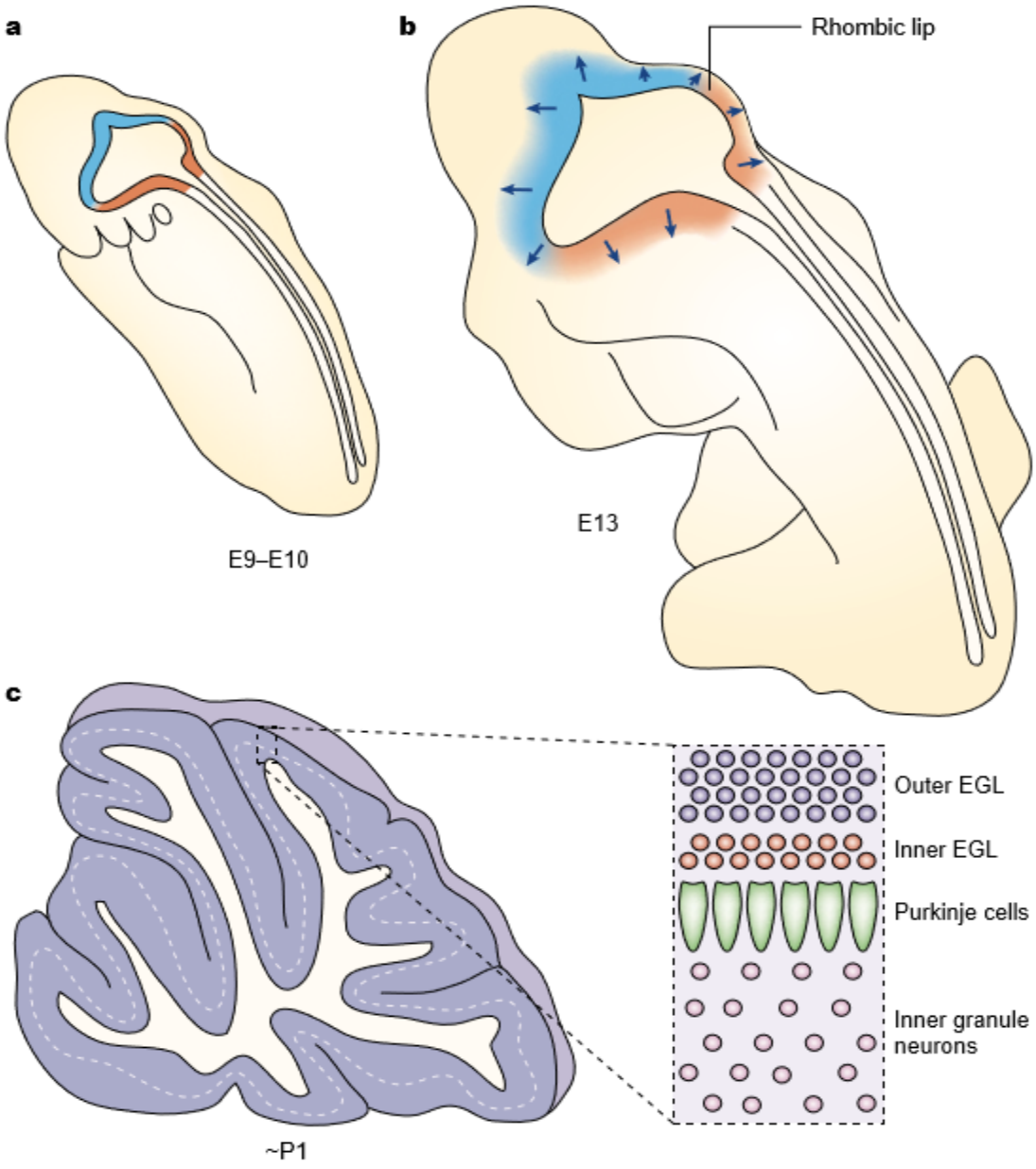

FIG. 4 the rhombic lips

Sonic hedgehog (*shh*) is highly expressed in the cerebellum. Sonic hedgehog is a morphogenetic factor which is a master player in cerebellar patterning and foliation. Indeed, sonic hedgehog controls the proliferation of progenitors in the cerebellum. Sonic hedgehog pathway involves the *GLI* family of transcription factors. Sonic hedgehog exerts critical mitogenic functions. For instance, sonic hedgehog stimulates very strongly the proliferation of cerebellar granular neuronal precursors through the induction and repression of cell cycle regulators genes.

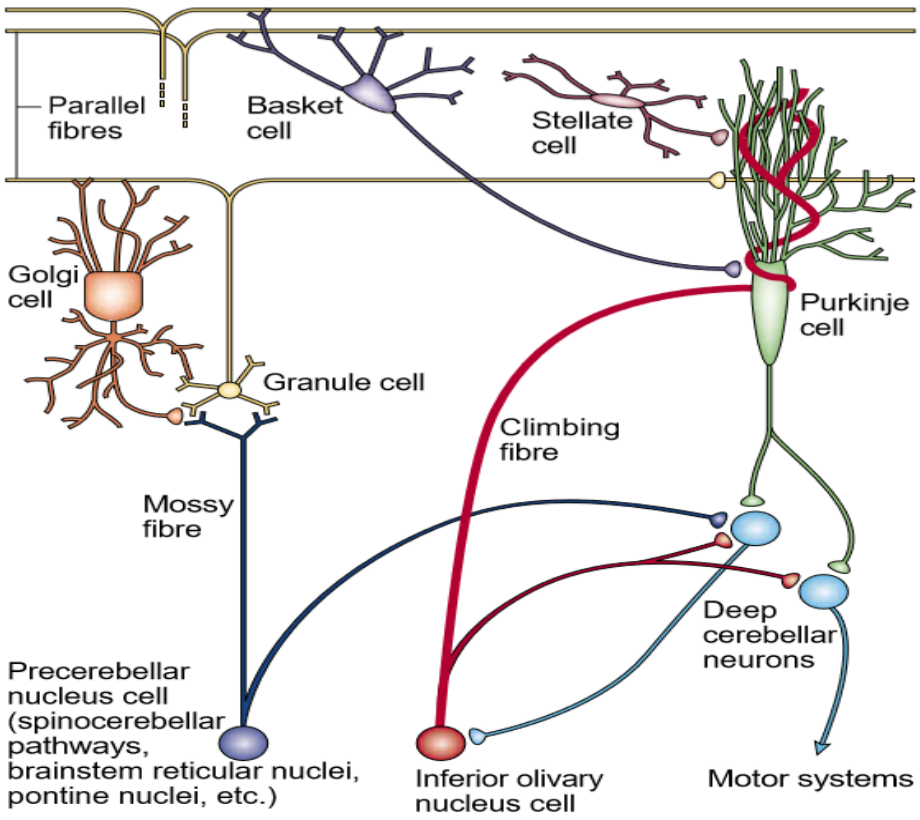

FIG. 5 connections of the cerebellum

CLINICAL SIGNIFICANCE

Joubert Syndrome

The disorder presents with developmental delay, hypotonia, impaired respiration, abnormal eye movements, and ataxia. Joubert syndrome is associated with mutations of genes encoding components of the primary cilia (they are cell surface microtubule-based organelles that dynamically extend from cells to receive and process molecular and mechanical signaling cues). Interestingly, primary cilia are determinant for sonic hedgehog signal transduction. Disruption of primary cilia formation blocks the proliferation of neural progenitors of granule cells mediated by sonic hedgehog.

Rhombencephalosynapsis

This is a malformation of the hindbrain characterized by fusion of the cerebellar hemispheres and dentate nuclei. It is assumed that the disorder is due to a failure of dorsal patterning at the midbrain-hindbrain boundary.

Dandy–Walker malformation

Sonic hedgehog might also be involved in the pathogenesis of Dandy–Walker malformation through a contribution of Zinc finger transcription factors which modulate the sonic hedgehog pathway.

Autism

Autism spectrum disorders are characterized by difficulties in communication, social skills, and repetitive behaviour. Cerebellar networks might be critically involved in the pathogenesis of autism. An immune dysfunction with local inflammation contributes to the pathogenesis of autism. It is plausible that En1 are implicated in neurodevelopmental disorders such as autism spectrum disorder.

Cerebellar Hypoplasia

Blocking GLI2 causes a failure in the development of cerebellar granular neuronal precursors, ending in cerebellar hypoplasia.

CONCLUSION

In this review, the recent advances in the understanding of the molecular mechanism governing cerebellar development were summarized. These discoveries are now bringing hope in a field which has often remained neglected because of a lack of understanding of the molecular events leading to the malformations. Mapping this developmental terrain in greater detail does not only give an understanding of malformations that involve the cerebellum, but also allow the understanding of problems of cell differentiation and migration.

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