**NAME**:OBUNADIKE CHINENYE BLESSING

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**ASSIGNMENT**

WRITE ON A CONCISE REVIEW OF THE DEVELOPMENTAL GENETICS OF THE CEREBELLUM AND HIGHLIGHT THE GENETIC BASES OF KNOWN CVEREBELLAR DISORDERS.

**INTRODUCTION**

The cerebellum is a vital component in the human brain as it plays a role in motor movement regulation and balance control. The cerebellum coordinates gait and maintains posture, controls muscle tone and voluntary muscle activity but is unable to initiate muscle contraction. Damage to this area in humans results in a loss in the ability to control fine movements, maintain posture, and motor learning.

In the last several decades, various approaches have contributed to our understanding of the molecular basis of cerebellar development. The study of spontaneous neurological mouse mutants aided many initial discoveries that are further reviewed below . Significant advances in mouse genetics have allowed for more targeted studies using engineered gene knockouts and transgenic mice. These mice have facilitated the examination of more subtle phenotypes such as mild behavioral abnormalities and small disruptions in cerebellar circuitry . Advances in brain imaging techniques and improvements in the classification of human cerebellar malformations have further aided the discovery of genes regulating cerebellar development. Genetics has recently enabled the identification of genes causing human pontocerebellar hypoplasia, Joubert syndrome, and Dandy–Walker malformation (DWM). When combined with studies in mouse, a variety of molecular mechanisms, including transcriptional regulation, mitochondrial function, and ciliary signaling have been implicated in homeostasis, patterning, and cell proliferation during cerebellar development. Concurrently, the application of new genomic techniques, which amass vast amounts of biological information, is just beginning to unravel the systems biology of the developing cerebellum. Here we discuss these issues and advocate the integrated use of human and mouse systems to further advance our knowledge of the molecular and developmental processes that form the cerebellum.

 

**EMBRYOLOGICAL DEVELOPMENT OF THE CEREBELLUM**

The following text description is from a study of 20 human embryos and fetuses between 6 weeks to 16 weeks from the Madrid Collection.

* 6 weeks (CRL 12–16 mm) - anlage of the cerebellum was first identified as a pair of thickenings on the lateral site of the alar plate that faced the fourth ventricle.
* 7-9 weeks (CRL 28 mm) - rhombic lip (a pair of thickenings of the alar plate) protruded dorsally, bent laterally, extended ventrolaterally and fused with the medially located midbrain. During that process, the primitive choroid plexus appeared to become involved in the cerebellar hemisphere to form a centrally located eosinophilic matrix. At that stage, the inferior olive had already developed in the thick medulla. Thus, the term 'bulbo-pontine extension' may represent an erroneous labeling of a caudal part of the rhombic lip. The cerebellar vermis developed much later than the hemisphere possibly from a midline dark cell cluster near the aqueduct.
* 11–12 weeks (CRL 70–90 mm) - cerebellar hemisphere became as thick as the mid- brain. In the hemisphere, a laminar configuration became evident but the central eosinophilic matrix remained pres- ent. Fissures of the future vermis appeared in the midline area (Fig. 6): the developing fissures provided island-like structures in horizontal sections. The hemisphere and ver- mis, including the surfaces of the fissures, were covered by the external germinal cell layer.
* 15–16 weeks (CRL 110–130 mm) - cerebellar hemisphere contained the primitive dentate nucleus. The nodule and flocculus were identified, vermis became as thick as the hemisphere and it accompanied several deep fissures.

**GENETICS OF CEREBELLUM DEVELOPMENT**

The mature cerebellum has exquisite, stereotypical morphology, foliation, and lamination, which are consistent between individuals and highly conserved across vertebrates. At the cellular level, unlike other regions of the CNS, the cerebellum is composed of very few neuronal types, each with distinct morphology, arranged in discrete lamina, and connected in stereotypical circuits . The cerebellum has essential roles in motor coordination, but is not essential for viability. Thus, compared with other regions of the central nervous system (CNS) the cerebellum has been more amenable to genetic studies since disruptions in development, which lead to abnormal morphology or function, are readily observed in obvious neurological and behavioral phenotypes. Because of this, it has been possible to obtain a precise understanding of cerebellar development. The mechanisms deciphered from the study of cerebellar development have broad applicability to other CNS regions such as the cerebral cortex. For example, while initial insights regarding the function of **the Reelin gene** were gleaned from studying the cerebella of **reeler mice**), recent studies have revealed that this gene is required for the emigration of dentate gyrus progenitors from a transient subpial zone and into the subgranular zone . Also, while Foxc1 controls normal cerebellar and posterior fossa development by regulating secreted growth factor signals from the mesenchyme , it is also required for the development of meningeal structures that in turn influence skull and cortical development.

Because the circuits of the cerebellum are unique in their morphology, the mechanisms of cerebellar neurogenesis are a subject of intense investigation. Neuronal/glial migrations as well as dendritogenesis are fundamental processes leading to functional cerebellar microcircuits being effective for plasticity and learning. Interestingly, the anatomy of the cerebellum with a midline vermis and two hemispheres located laterally is highly conserved from rodents to human, suggesting that the analysis of the development in rodents should provide direct relevant informations in human, including for cerebellar malformations.

The major features of cerebellar development can be briefly summarized as follows. Neuronal populations are generated in a sequential manner. The inhibitory interneurons emerge from the ventricular zone and the glutamatergic neurons are generated by the rhombic lip . In mouse, the glutamatergic and gabaergic neurons in nuclei are produced first, followed by Purkinje neurons. It is established that gabaergic interneurons of the cerebellar cortex originate from a ventricular zone progenitor . After generation of cerebellar nuclei, the external granular layer is formed from precursors of granule cells originating from the rhombic lip. Granule cells will migrate to form the internal granular layer. It is interesting to note that these events occur at the third trimester of development in human (see also below the impact of very premature birth upon cerebellar development). Survival and maintenance of Purkine neurons and granule cells is dependent on the antiapoptotic protein Lifeguard, which is highly expressed in the cerebellum and is strongly upregulated during postnatal brain development). Lifeguard antagonizes the FAS pathway. FAS receptors tune neuronal survival following trophic factors deprivation . Lifeguard affects cerebellar size, internal granular layer thickness, and Purkinje cell development, suggesting that lifeguard could participate in the pathogenesis of various human cerebellar disorders characterized by cerebellar atrophy. Glutamatergic unipolar brush cells migrate to the internal granular layer. Whereas the ventricular zone will lose its progenitors at late embryogenic stages, the rhombic lip remains active until postnatal period.

**CEREBELLAR DISORDERS**

**Pontocerebellar hypoplasia type 1 (PCH1**) is a genetic disease that affects the development of the brain. Babies and children with this disease have an unusually small and underdeveloped [cerebellum](https://medlineplus.gov/ency/imagepages/18008.htm), which is the part of the brain that coordinates movement. A region of the brain called the [pons](https://medlineplus.gov/ency/imagepages/19236.htm%22%20%5Ct%20%22_blank) also fails to develop properly. The pons, which is located at the base of the brain in an area called the [brainstem](https://medlineplus.gov/ency/imagepages/18007.htm), sends signals between the cerebellum and the rest of the brain Individuals with PCH1 also experience a degeneration of the [anterior horn cells](http://www.sciencedirect.com/topics/neuroscience/anterior-horn-of-spinal-cord), which are responsible for helping the [spinal cord](https://www.merckmanuals.com/home/brain%2C-spinal-cord%2C-and-nerve-disorders/biology-of-the-nervous-system/spinal-cord) send signals to the muscles. Problems with the anterior horn cells cause severe muscle weakness.
PCH1 is caused by mutations to [**EXOSC3**](https://ghr.nlm.nih.gov/gene/EXOSC3)**,**[**TSEN54**](https://ghr.nlm.nih.gov/gene/TSEN54)**,**[**RARS2**](https://ghr.nlm.nih.gov/gene/RARS2)**, and**[**VRK1**](https://ghr.nlm.nih.gov/gene/VRK1)**.**The disease is inherited in an **autosomal recessive manner**. Diagnosis of PCH1 is based on brain imaging and tests to rule out other causes of problems with brain development. Treatment for PCH1 is aimed at relieving the symptoms of the disease. Most children with PCH1 pass away in infancy or early childhood.



Image showing pontocerebellar syndrome

**Joubert syndrome** is disorder of brain development that may affect many parts of the body. It is characterized by the absence or underdevelopment of the cerebellar vermis (a part of the brain that controls balance and coordination) and a malformed brain stem (connection between the brain and spinal cord). Together, these cause the characteristic appearance of a [molar tooth sign](http://depts.washington.edu/joubert/joubertsyndrome.php) on MRI. Signs and symptoms can vary but commonly include weak muscle tone (hypotonia); abnormal breathing patterns; abnormal eye movements; ataxia; distinctive facial features; and intellectual disability. Various other abnormalities may also be present. Joubert syndrome may be caused by mutations in any of many genes. Inheritance is usually autosomal recessive, but rarely it may be X-linked recessive.Treatment is supportive and depends on the symptoms in each person. (NINDS Joubert Syndrome Information Page. NINDS. , 2016)



Diagram showing jouberts syndrome.

**Dandy-Walker malformation** affects brain development, primarily development of the cerebellum, which is the part of the brain that coordinates movement. In individuals with this condition, various parts of the cerebellum develop abnormally, resulting in malformations that can be observed with medical imaging. The central part of the cerebellum (the vermis) is absent or very small and may be abnormally positioned. The right and left sides of the cerebellum may be small as well. In affected individuals, a fluid-filled cavity between the brainstem and the cerebellum (the fourth ventricle) and the part of the skull that contains the cerebellum and the brainstem (the posterior fossa) are abnormally large. These abnormalities often result in problems with movement, coordination, intellect, mood, and other neurological functions. Researchers have found mutations in a few genes that are thought to cause Dandy-Walker malformation, but these mutations account for only a small number of cases. 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](https://ghr.nlm.nih.gov/condition/trisomy-18) (an extra copy of [chromosome 18](https://ghr.nlm.nih.gov/chromosome/18)), but can also occur in people with [trisomy 13](https://ghr.nlm.nih.gov/condition/trisomy-13), [trisomy 21](https://ghr.nlm.nih.gov/art/large/down-syndrome-karyotype.jpeg%22%20%5Co%20%22Image%22%20%5Ct%20%22_blank), or trisomy 9. This condition can also be associated with missing ([deletions](https://ghr.nlm.nih.gov/art/large/chromosomaldeletion.jpeg)) or copied ([duplications](https://ghr.nlm.nih.gov/art/large/chromosomalduplication.jpeg)) 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.

Research suggests that Dandy-Walker malformation could be caused by environmental factors that affect early development before birth. For example, exposure of the fetus to substances that cause birth defects (teratogens) may be involved in the development of this condition. In addition, a mother with diabetes is more likely than a healthy mother to have a child with Dandy-Walker malformation. Most cases of Dandy-Walker malformation are sporadic, which means they occur in people with no history of the disorder in their family. A small percentage of cases seem to run in families; however, Dandy-Walker malformation does not have a clear pattern of inheritance. Multiple genetic and environmental factors likely play a part in determining the risk of developing this disorder. [First-degree relatives](https://ghr.nlm.nih.gov/art/large/first-degree-relative.jpeg) (such as siblings and children) of people with Dandy-Walker malformation have an increased risk of developing the condition compared with people in the general population.



**REFERENCE**

(Carletti and Rossie, 2008) (NINDS Joubert Syndrome Information Page. NINDS. , 2016) (Pontocerebellar hypoplasia. Genetics Home Reference (GHR), 2014) (Rossi, 2011) (al., 2011)

 addition to oligodendrocytes, GABAergic interneurons and astrocytes were also

labeled with GFP in the mature cerebellum, indicating that the progenitors of

interneurons and glial cells are derived from the cerebellar parenchyma (Parmigiani

et al. 2015). Of interest, Grimaldi and colleagues observed that both parenchymal

electroporation and solid cerebellar grafts only gave rise to a minor faction of total

oligodendrocytes in the cerebellum, suggesting that oligodendrocytes have an extra-

cerebellar origin (Grimaldi et al. 2009). A later study conﬁrmed the minor contri-

bution (approximately 6%) of cerebellar oligodendrocytes being native to the

cerebellum and revealed that the rest originate from the Olig2

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neuroepithelial

domain in the ventral rhombomere1 (Hashimoto et al. 2016). The generation of

three different astrocyte subtypes (based upon location and morphology) in the

cerebellum has recently been investigated (Cerrato et al. 2018). In this study, Cerrato

and others tagged the developing radial glial cells that arise from the ventricular zone

(with the StarTrack cell tagging system) and examined the astrocyte types in adult

cerebellum. Their results revealed that radial glial cells are multipotent, in which a

particular radial glial progenitor can give rise to all three astrocyte subtypes, but this

multipotency is found to decrease over time (i.e., progenitors born at E12 have the

tendency to give rise to multiple types versus those born at E14 which tend to give

rise to a single type) (Cerrato et al. 2018). The mechanism that governs the

generation of astrocyte heterogeneity, whether they arise from a discrete subset of

progenitors or the consequence of stochastic events, however, remains unclear.

Bioinformatic Strategies to Identify Novel Genes

in the Specification of Cells During Cerebellar Development

Over the past decades, the studies of gene function in mutant mice, and other

organisms, have taught us greatly about the role of single genes in cerebellar

development. Recent advances in high-throughput sequencing and analysis provide

the opportunity to study the genetic underpinnings of cell fate and speciﬁcation at a

whole genome level. Here we describe two different whole genome approaches,

involving our lab, that utilize the latest technologies for whole transcriptome readout

in the cerebellum as a function of developmental time.

Cerebellar Gene Regulation in Time and Space (CbGRiTS)

In an effort to better understand cerebellar development from a molecular perspective,

we have undertaken a project aimed at assembling a microarray-based developmental

transcriptome for various mouse strains. The data collected for this project are publicly

available on our Cerebellar Gene Regulation in Time and Space website (www.cbgrits.

org). Several strains of mice were analyzed in the CbGRiTS project including two

standard mouse strains C57BL6/J and DBA and several mutant strains such as Pax6-

null Sey,Atoh1-null (also known as Math1-null), and meander tail mutant. Data were

collected for each strain across embryonic and postnatal development. For the wild-type

Genes and Cell Type Specification in Cerebellar Development 13

strains, RNA from cerebellar tissue was obtained each day during embryogenesis

(E12-birth) and every third day postnatally, currently until P9. Gene expression in

these samples was then analyzed using the Illumina microarray platform. The built-in

informatic tools found on the website allow the exploration of gene expression over time

as related to developmental events in the cerebellum by use of the DEM Transcriptome

Explorer (Ha et al. 2015). Several other informatic algorithms can be accessed such as

Paraclique, Helmert, and Polynomial analyses. In addition, there are visualization tools

that animate cerebellar development (see www.cbgrits.org/Visualization/

DevelopingCerebellum.aspx). Currently, we are processing these data using bioinfor-

matic tools in order to identify developmentally important genes (Ha et al. 2015). By

analyzing the transcriptome of the Pax6-null Sey mutants in this database, we identiﬁed

a novel molecule –Wntless –a key to cerebellar development (Yeung and Goldowitz

2017). The analysis of Sey transcriptome also revealed the dysregulation of Tbr1 and

Tbr2 in the Pax6-null mutant. This dysregulation led us to identify novel roles play by

Pax6 in the development of nuclear neuron and unipolar brush cells (Yeung et al. 2016).

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