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**Answer**

Developmental Genetics of The Cerebellum

**Cellular and molecular basis of cerebellar development**

Historically, the molecular and cellular mechanisms of cerebellar development were investigated through structural descriptions and studying spontaneous mutations in animal models and humans. Advances in experimental embryology, genetic engineering, and neuroimaging techniques render today the possibility to approach the analysis of molecular mechanisms underlying histogenesis and morphogenesis of the cerebellum by experimental designs. Several genes and molecules were identified to be involved in the cerebellar plate regionalization, specification, and differentiation of cerebellar neurons, as well as the establishment of cellular migratory routes and the subsequent neuronal connectivity. Indeed, pattern formation of the cerebellum requires the adequate orchestration of both key morphogenetic signals, arising from distinct brain regions, and local expression of specific transcription factors. Thus, the present review wants to revisit and discuss these morphogenetic and molecular mechanisms taking place during cerebellar development in order to understand causal processes regulating cerebellar cytoarchitecture, its highly topographically ordered circuitry and its role in brain function.

**Introduction**

The vertebrate brain is a remarkably complex anatomical structure that contains diverse subdivisions and neuronal subtypes with specific, sometimes prodigal, synaptic connections that contribute to the complexity of its function. During development the primordial brain (the neural tube) has to be progressively regionalized. A precise spatial and temporal arrangement of gene expression regulates intercellular and intracellular signals driving a proper molecular patterning that is required for this regionalization. Pioneering genoarchitectural studies and fate mapping experiments established correlations on how morphogens, transcription factors, and other signaling molecules modulate the specification of neuroepithelial territories, to generate the structural complexity and cellular diversity that characterizes the brain (revised in [Puelles and Rubenstein, 2003](https://www.frontiersin.org/articles/10.3389/fnana.2013.00018/full%22%20%5Cl%20%22B101); [Martínez et al., 2012](https://www.frontiersin.org/articles/10.3389/fnana.2013.00018/full%22%20%5Cl%20%22B72); [Puelles and Ferran, 2012](https://www.frontiersin.org/articles/10.3389/fnana.2013.00018/full%22%20%5Cl%20%22B99)). Thus, the combination of molecular genetics (gene expression maps) and modern neuroanatomy (based on histochemistry and highly sensitive neuroimaging) have led to an increased interest in describing the neurodevelopmental mechanisms underlying structural disorders and intellectual discapacities that we currently observe in congenital anomalies of the human brain.

Among the classical systems used to study the structure and function of the central nervous system the cerebellum has steadily gained popularity and has become one of the most experimentally tractable systems in the brain. Much of our knowledge about structure, function, and development of the mouse cerebellum was achieved by studying spontaneous mutations ([Sotelo, 2004](https://www.frontiersin.org/articles/10.3389/fnana.2013.00018/full#B118)), but also by using sophisticated genetic tools allowing a more precise and mechanistic level of analysis ([Joyner and Sudarov, 2012](https://www.frontiersin.org/articles/10.3389/fnana.2013.00018/full#B56); [Tvrdik and Capecchi, 2012](https://www.frontiersin.org/articles/10.3389/fnana.2013.00018/full%22%20%5Cl%20%22B125)).

This review focuses on the basic developmental biology of the cerebellum starting from morphological features in order to distinguish the origin and specification of the cerebellar neuroepithelial anlage, as well as to describe the molecular mechanisms implicated in the development of its architectural morphology, stereotyped cellular differentiation, and neuronal distribution. Finally, we summarize relevant works in correlations with those findings in developmental cerebellar disorders of the human cerebellum ([Barkovich, 2012](https://www.frontiersin.org/articles/10.3389/fnana.2013.00018/full%22%20%5Cl%20%22B7)).

CLINICAL FEATURES

Joubert syndrome (JS) is an autosomal recessive neurodevelopmental disorder, which is characterized by the molar tooth malformation (MTM), a complex brainstem malformation that reflects aplasia or marked hypoplasia of the cerebellar vermis, thickened and elongated superior cerebellar peduncles and a deepened interpeduncular fossa that is apparent on axial MRI at the midbrain–hindbrain junction. Clinically, classic JS is associated with neonatal hypotonia (loss of muscle tone), ataxia, developmental delay, mental retardation, and often neonatal apnea/hyperpnea (irregular breathing) and/or ocular motor apraxia (difficulties in initiating rapid horizontal eye movements—saccades). Autistic features have also been reported as a relatively common component of JS.

None of these features alone is diagnostic of JS, however, and in more recent years, it has become obvious that JS is a part of a spectrum of disorders involving vermis hypoplasia and the MTM. Some of these include COACH (OMIM 216360), referring to characteristic hallmarks of cerebellar vermis hypoplasia, oligophrenia (mental impairment), congenital ataxia, ocular coloboma and hepatic fibrosis; and Váradi Papp or orofaciodigital VI syndrome (OMIM 277170), defined by midline facial or hand abnormalities.

Varying degrees of extra-CNS involvement have further complicated diagnosis, including ocular colobomas, postaxial polydactyly, liver fibrosis, cystic dysplastic kidneys, retinopathy and/or nephronophthisis (NPHP) . These features significantly overlap with other disorders with cerebello-oculo-renal involvement, most notably NPHP; the significance of this relationship is strengthened by the identification of deletions of NPHP1, a gene commonly mutated in NPHP in a subset of JS patients.

NEUROPATHOLOGY

There have been few detailed studies of neuropathology in JS patients, but various abnormalities have been identified as common to JS, affecting a number of systems in the midbrain and hindbrain. The most striking is the absence of the cerebellar vermis, thought to be important for control of balance, regulation of muscle tone and saccadic (rapid) eye movements, although it should be noted that many lesions of the cerebellum could have this effect. The dentate nuclei, the major source of cerebellar output to the cerebral cortex, are fragmented into islands. Malformation of various pontine and medullary structures, including the basis pontis, reticular formation, inferior olivary, dorsal column and solitary tract nuclei, have been reported, which may explain the respiratory defects in JS. Heterotopias of Purkinje-like neurons have been described in some patients, suggesting a migration defect. Defects of proliferation are also likely, considering the absence of the vermis as well as the report of diminished density of granule neurons.

An interesting abnormality is the absence of decussation both of the superior cerebellar peduncles and of the corticospinal tracts at the medullary pyramids, which suggests that JS patients may have a defect of axon guidance. The pyramidal decussation is the site where the majority of corticospinal tracts cross at the midline, and defects in this event suggest that JS patients may display altered brain wiring. Indeed, a study on fMRI patterns reveals more bilateral activation in a JS patient versus a control, which is consistent with a defect of neural connectivity. In total, the pathology of JS seems to reflect abnormalities in a variety of events, suggesting that the genes involved are acting early in brain development.

OVERVIEW OF CEREBELLAR DEVELOPMENT

Understanding of cerebellar development provides some insights into the pathogenesis of JS. The cerebellum arises from both the mesencephalic and rhombencephalic vesicles of the neural tube and develops over a relatively long period of time between early embryogenesis and late childhood. Development of the cerebellum can be described in four basic stages.

In the first stage, characterization of cerebellar territory occurs at the midbrain–hindbrain boundary. Transplantation studies in chicken and mouse have found that the isthmus organizer (IsO), a region corresponding to the midbrain–hindbrain boundary expression, is crucial for specifying midbrain and cerebellar structures. At the isthmus, restricted expression of secreted factors, such as fibroblast growth factor 8, FGF8 and Wnt1, the mammalian homolog of Drosophila wingless gene , as well as homeobox proteins En1 and En2  and paired box genes Pax2 and Pax5 are required for early specification of midbrain and hindbrain structures. In the second stage, two compartments for cell proliferation are formed. Purkinje cells and cells of the deep cerebellar nuclei are generated in the roof of the fourth ventricle, and granule cell precursors, as well as cells of the precerebellar nuclei are formed in the rhombic lip. Development of Purkinje cells is not well understood, but they are known to secrete Sonic hedgehog which regulates proliferation of granule cells. By this time point, granule neuron precursors express a number of markers, Math1, nestin, zipro1/RU49 and Zic genes 1, 2 Purkinje cells migrate radially to their final positions, whereas granule neurons migrate over the surface of the developing cerebellum, forming the external granule layer (EGL). In the third stage, cells of the EGL migrate inward along the processes of Bergman glia to their final position in the internal granular layer (IGL). Finally, cerebellar circuitry is established and further differentiation occurs. The lower portion of the rhombic lip also gives rise to cells of the precerebellar nuclei such as the inferior olivary nuclei, which migrate to positions in the brainstem.

FUNCTIONAL GENE CANDIDATES

Because the pathology of JS suggests defects of early cerebellar development, particularly of structures derived from the primitive isthmus, genes involved in cerebellar patterning at the IsO have been tested as candidate genes, especially those with comparable phenotypes in mouse models. The swaying mouse, which was shown to have a truncation mutation of Wnt1, has a phenotype reminiscent of JS, i.e. ataxia, and agenesis of cerebellar structures. Mutant alleles of Fgf8, En1 and En2 also result in cerebellar abnormalities. A hypomorphic allele of Fgf8 was shown to cause deletion of midbrain and hindbrain structures. En1 and En2 mice also manifest absent or abnormal cerebellar structures. Mutations in these genes, however, could not be identified in JS patients screened thus far, suggesting that they are not likely to be a common cause of JS.

The ZIC (zinc fingers in the cerebellum) family of transcription factors were named for their exclusively cerebellar expression in adults. Embryonically, they are more widely distributed and have been implicated in a variety of developmental functions. Zic1 mutant mice have hypoplastic cerebella that are missing anterior lobules of the vermis. They also have behavioral deficits of ataxia and hypotonia, as in patients with JS, and were thought to be a good model for the disease. However, in an analysis of 35 JS pedigrees, ZIC1 was also excluded as a causative gene. In fact, heterozygous mutations in ZIC1 and ZIC4 have been reported to be a cause of the related, but distinct Dandy–Walker malformation, characterized by hypoplasia and upward rotation of the vermis, cystic enlargement of the fourth ventricle and often hydrocephalus.

POSITIONAL CLONING

The lack of success with functional candidate screening suggests that novel genes are involved in JS and that linkage mapping may be more fruitful. Linkage studies for JS genes have relied on homozygosity mapping in consanguineous families, a more powerful method for detecting linkage for rare disease genes where large families may not be available.

JS is genetically heterogeneous and so far three genetic loci have been mapped to 9q34.3 (JBTS1: OMIM 213300), 11p12–q13.3 (JBTS2: OMIM 608091) and 6q23 (JBTS3: 608629). Because there are families that do not map to any of these loci, there are likely to be more genes involved. Genotype–phenotype analysis reveals differences at the mapped loci. JBTS1 appears to represent the classic Joubert phenotype of pure cerebellar and midbrain–hindbrain junction involvement, although the JBTS3 gene is also associated with cerebral cortical abnormalities, most notably polymicrogyria. JBTS2, in addition to classical JS features, is associated with a variety of other organ systems, involving kidney, eye and liver. Of the known loci, only JBTS3 has been cloned, with mutations identified in AHI1. AHI1, the Abelson helper integration 1 gene, was initially identified as a common helper provirus integration site and only more recently found to encode a protein. Jouberin, encoded by AHI1, contains seven WD40 repeats, an SH3 domain, potential SH3 binding sites and an N-terminal coiled–coiled domain. Numerous putative casein kinase, tyrosine kinase and protein kinase C phosphorylation sites can also be detected in the sequence. The mRNA is widely expressed in the brain at a range of time points. The domain structure suggests that Jouberin functions in signal transduction, perhaps as an adaptor molecule, but little is known about AHI1 and how it might be involved in the pathogenesis of JS. WD40 domains have been found in proteins involved in a variety of functions including signal transduction, RNA processing, transcriptional regulation, cytoskeleton assembly, vesicle trafficking and cell division. Similarly, SH3 domains are a common feature on signaling molecules, involved in numerous pathways.

RELATED DISORDERS

The range of the JS phenotype often involves systems outside the CNS and significantly overlaps with other disorders, for some of which, genes have been identified. The similarities in phenotype suggest that shared or similar pathways are affected in these disorders. Studying the functions of these genes may contribute to our understanding of JS. Interestingly, most of these genes have been shown to function in the cilia and/or intraflagellar transport (IFT).