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Medicine and Surgery

ANA 303: Neuroanatomy

Cerebellum And Its Connections

**Question 1**

**Write a concise review on the developmental genetics of the cerebellum and highlight the genetic bases of known cerebellar disorders.**

Overview of cerebellar development

During the early stages of embryonic development, the brain starts to form in three distinct segments; the prosencephalon, mesencephalon, and rhombencephalon. The rhombencephalon is the most caudal segment of the embryonic brain; it is from this segment that the cerebellum develops. Along the embryonic rhombencephalic segment develop eight swellings, called rhombomeres. The cerebellum arises from two rhombomeres located in the alar plate of the neural tube, a structure that eventually forms the brain and spinal cord. The specific rhombomeres from which the cerebellum forms are rhombomere 1 (Rh.1) caudally and the isthmus rostrally. Two primary regions are thought to give rise to the neurons that make up the cerebellum. The first region is the ventricular zone in the roof of the fourth ventricle. This area produces Purkinje cells and deep cerebellar nuclear neurons. These cells are the primary output neurons of the cerebellar cortex and cerebellum. The second germinal zone (cellular birthplace) is known as the Rhombic lip, neurons then move by human embryonic week 27 to the external granular layer. This layer of cells—found on the exterior of the cerebellum—produces the granule neurons. The granule neurons migrate from this exterior layer to form an inner layer known as the internal granule layer.

The development of the cerebellum occurs in four basic stages:

1. Characterization of the cerebellar territory in the hindbrain.

Gene expression studies in chick and mice embryos have shown that the isthmus organizer, i.e. a transverse patterning centre at the midbrain–hindbrain boundary (MHB), regulates the early development of the mesencephalon and the rostral part of the rhombencephalon. MHB cells secrete fibroblast growth factors (FGFs) and Wnt (mouse homologues of the Drosophila gene wingless) proteins which are required for the differentiation and patterning of the midbrain and hindbrain. A number of homeobox-containing transcription factors are expressed across the isthmus, such as the homologues of the Drosophila gene engrailed En1 and En2. Mutations in these genes cause deletions of mesencephalic and cerebellar structures.

1. Formation of two compartments of cell proliferation, giving rise to the Purkinje cells and the granule cells, respectively.

The Purkinje cells, the deep cerebellar nuclei and later the Golgi, stellate and basket cells arise from the ventricular zone of the metencephalic alar plates. Purkinje cells migrate along radial glial cells to their future position, making use of a Reelin-dependent pathway. Towards the end of the embryonic period, granule cell precursors are added from the rhombic lip. The rhombic lip is the dorsolateral part of the alar plate, and it forms a proliferative zone along the length of the hindbrain. Cells from its rostral part reach the superficial part of the cerebellum, and form the external germinal or granular layer at the end of the embryonic period. The Math1 (mouse atonal homologue) gene is expressed in the rhombic lip. Migratory granule cell precursors exclusively maintain expression of Math1, Zic1 and Zipro1. Zipro1 (formerly known as the zinc-finger-containing factor RU49) marks the expression of cerebellar granule cells. Zipro1 expression is maintained at all stages of cerebellar granule cell differentiation.

1. Inward migration of the granule cells.

Granule cells are formed in the external germinal layer. The granule cells form axons, the parallel fibres, and migrate along the processes of Bergmann glia cells to their deeper, definitive site, the internal granular layer. Sonic hedgehog (Shh), a member of the hedgehog family of secreted signalling proteins, is expressed in migrating and settled Purkinje cells, and acts as a potent mitogenic signal to expand the granule cell progenitor population.

1. Differentiation of cerebellar neurons.

Granule cell differentiation is characterized by a prolonged period of clonal expansion that occurs after progenitors have been specified. In contrast, Purkinje cells cease proliferation within the ventricular zone and rapidly express numerous differentiation markers. Math1 is expressed in the rhombic lip and, subsequently, in the external granular layer, but its expression is downregulated at later stages of granule cell development. . Axon extension is characterized by the expression of the membrane proteins TAG1 and L1, and the migration of granule cells along Bergmann glia into the internal granular layer requires the formation of a cell-surface-neuronal glycoprotein, astrotactin.

Genetic basis of known cerebellar disorders

1. Dandy–Walker malformation (DWM) is a heterogeneous disorder defined by a hypoplastic, upwardly rotated vermis, an enlarged fourth ventricle, and an enlarged posterior fossa with an elevated confluence of sinuses. Typically, the cerebellar hemispheres are less affected than the vermis, and the brainstem is normal to moderately hypoplastic. DWM can occur with additional brain abnormalities including agenesis of the corpus callosum (ACC) and hydrocephalus, but more often it occurs as an isolated brain-imaging finding. The clinical features and developmental outcomes vary widely. Patients may exhibit symptoms ranging from intellectual disability to autism or they may be completely unaware of any deficits until diagnosed as adults for unrelated reasons. The recurrence risk in isolated DWM is low at an estimated 1–5%, suggesting de-novo, somatic mosaic, or complex genetic causes. Few genes have been implicated in rare cases of DWM, including genomic imbalances that are part of a congenital syndrome and rare single gene disorders. *FOXC1*-related (Forkhead box C1) DWM is associated with multiple congenital anomalies, especially eye malformations consistent with Axenfeld–Rieger syndrome. Congenital anomalies associated with *FOXC1*-related DWM in severely affected patients overlap with Ritscher–Schinzel, or 3C (cranio-cerebello-cardiac) syndrome. Recently, mutations in *CCDC22* (Coiled-coil domain containing 22) were found in X-linked cases of 3C syndrome, suggesting that *CCDC22* mutations may be a new cause of DWM. Though these patients were noted to have DWM, limited neuroimaging data were reported to substantiate this diagnosis. *ZIC1/4*-related DWM is also associated with multiple congenital anomalies, including dysmorphic facial features and abnormal development of the eyelids. Recently, exome sequencing identified autosomal dominant mutations in *LAMC1* (Laminin, gamma 1) and *NID1* (Nidogen-1) as the cause of DWM with encephalocele in two families. Despite these genetic advances, the genetic cause remains unknown in the majority of DWM patients.
2. Cerebellar hyperplasia, or macrocerebellum, is a rare neuroimaging finding that occurs in isolation, or in coincident with a variety of neurodevelopmental disorders, including genomic imbalances and specific overgrowth syndromes, including megalencephaly–capillary malformation (MCAP) and megalencephaly–polydactyly–polymicrogyria–hydrocephalus (MPPH). Clinical features of MCAP/MPPH include seizures, capillary malformations, macrocephaly, and polydactyly, and diagnosis is often based on cerebral cortical overgrowth and polymicrogyria. Though cerebellar size is normal at birth, patients often develop a macrocerebellum with normal posterior fossa size. This may progress into cerebellar ectopia/Chiari I malformation, causing clinically associated symptoms (posterior headache, dysphagia, stridor) and hydrocephalus. It is unknown whether the cerebellar overgrowth is a feature of generalized brain overgrowth, or whether there are distinct mechanisms that specifically influence cerebellar overgrowth. Most MCAP and MPPH patients have activating de-novo mutations in *PIK3R2* (Phosphoinositide-3-Kinase Regulatory Subunit 2) and *PIK3CA* (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha), respectively, that result in increased cell growth. Mutations affecting the PI3K–AKT–mTOR pathway are found in a variety of cancers, opening the possibility of using drugs in development for cancer treatment to reduce brain overgrowth and neurological issues in patients with MCAP/MPPH.
3. Cerebellar dysplasia: Any part of the cerebellum can be dysplastic, from small focal regions within one hemisphere to abnormal foliation throughout the cerebellum. Hypoplastic cerebella are frequently also dysmorphic, as observed in tubulinopathies and cobblestone malformations. Chudley–McCullough syndrome (CMS) is an autosomal recessive disorder in which patients have striking disorganization of the inferior cerebellar hemisphere folia and additional brain abnormalities, including frontal polymicrogyria with subcortical heterotopia, corpus callosum hypogenesis, and arachnoid cysts. Clinical presentation includes severe neonatal sensorineural hearing loss and hydrocephalus that may require shunting in some patients. Surprisingly, these patients typically are not dysmorphic, lack additional congenital anomalies, and have relatively mild developmental problems. CMS is caused by biallelic truncating mutations in *GPSM2* (G-protein-signaling modulator 2) that encodes a GTPase regulator required for correct orientation of stem cell divisions in multiple tissues. The cerebellar dysplasia present in CMS is likely due to abnormal cell division, but the precise mechanism remains unknown. Poretti–Boltshauser syndrome (PBS) is characterized by cerebellar dysplasia, cysts, and vermis hypoplasia with and without retinal dystrophy and is caused by mutations in *LAMA1* (Laminin Subunit Alpha 1). The superior cerebellar peduncles are long in some patients, though their appearance differs from the classic molar-tooth appearance that defines Joubert syndrome. The clinical features of PBS include motor and speech delay with variable cognitive impact. Finally, cerebellar dysplasia with cysts is a prominent feature in cobblestone malformations and *GPR56*-related brain malformations
4. Joubert syndrome/molar tooth malformation (JS) is defined by a characteristic brain malformation including: cerebellar vermis hypoplasia or dysplasia; long, thick, elevated superior cerebellar peduncles; a thin midbrain–hindbrain junction; and a deep interpeduncular fossa producing the “molar tooth sign”. Patients present with neonatal hypotonia, abnormal eye movements, and alternating apnea and tachypnea. Polydactyly is present in a few patients, and subsets of patients develop retinal dystrophy, nephronophthisis, and liver fibrosis. Additional brain malformations may be present, including polymicrogyria, brainstem and cortical heterotopia, agenesis of the corpus callosum, and/or cephalocele . Diffusion tensor imaging can further show laterally displaced and dysmorphic deep cerebellar nuclei, hypoplastic medial lemnisci, and absent transverse fibers in the central vermis and deficient superior cerebellar peduncle decussation. JS is thought to be a frequent cause of congenital ataxia, with a prevalence of ~1/80,000 in Northern Europeans. The >30 genes implicated in JS encode proteins that function in and around the primary cilium, the cellular antenna that mediates a variety of signaling processes. JS is one of a new class of disorders called ciliopathies, named for their overlapping clinical features and shared pathophysiology involving cilium dysfunction. Brain malformations seen in JS may result from defects in midline fusion of the developing vermis, defects in sonic hedgehog-mediated neural tube patterning and cerebellar granule cell proliferation, and abnormal cilium-dependent neuronal migration and axon guidance. Diagnosing JS is important because it is recessive and carries a 25% recurrence risk. Additionally, patients are at risk for progressive retinal, kidney and liver disease, which requires surveillance and treatment to prevent additional complications.
5. Pontocerebellar hypoplasia (PCH) is a heterogeneous group of rare neurodegenerative disorders caused by genetic mutations and characterized by progressive atrophy of various parts of the brain such as the cerebellum or brainstem, particularly the pons. Where known, these disorders are inherited in an autosomal recessive fashion. There is no known cure for PCH. PCH type 1 associated with mutations in *EXOSC3* (Exosome component 3) and *VRK1* (VRK Serine/Threonine Kinase 1), is characterized by moderate PCH on neuroimaging in combination with spinal muscular atrophy, resulting in substantial global weakness and decreased or absent reflexes. PCH types 2, 4, and 5 have more severe hypoplasia and are caused predominantly by mutations in genes that encode tRNA splicing endonucleases (*TSEN54*, *TSEN34* and *TSEN2*). Patients with PCH type 2 represent the less severe end of the spectrum with early hyperreflexia, developmental delay, and feeding problems, eventually developing spasticity and involuntary movements in childhood, whereas patients with PCH type 4 represent the severe end of the spectrum characterized by polyhydramnios, severe hyperreflexia, contractures, and early death due to central respiratory failure. Microcephaly is present at birth in patients with PCH4, whereas microcephaly develops over time in PCH2. Seizures are frequent in both groups. A typical feature of *TSEN*-related PCH is more severe involvement of the cerebellar hemispheres versus the vermis. PCH type 6 is associated with elevated Cerebrospinal fluid lactate and caused by mutations in *RARS2* (Arginyl-TRNA Synthetase 2, Mitochondrial). The genes associated with PCH3 and PCH5 have not yet been identified. The mutated genes in PCH are autosomal recessive, which means that parents of an affected child each carry only one copy of the damaged gene. In each parent the other copy performs its proper function and they display no signs of PCH. A child inheriting two damaged copies of the gene will be affected by PCH

References

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