Seminars in Fetal & Neonatal Medicine 21 (2016) 349–355

Contents lists available at ScienceDirect

Seminars in Fetal & Neonatal Medicine

journal homepage: www.elsevier.com/locate/siny

Review

# Autism and cerebellar dysfunction: Evidence from animal models

Peter T. Tsai*

*University of Texas Southwestern Medical Center, Dallas, TX, USA*

*Keywords:*
Cerebellum
Autism spectrum disorders
Animal models
Critical periods
Circuitry

SUMMARY

Autism is a prevalent neurodevelopmental disorder whose origins are not well understood. Cerebellar involvement has been implicated in the pathogenesis of autism spectrum disorders with increasing evidence from both clinical studies and animal models supporting an important role for cerebellar dysfunction in autism spectrum disorders. This article discusses the various cerebellar contributions to autism spectrum disorders. Both clinical and preclinical studies are discussed and future research directions highlighted.



## 1. Autism and the cerebellum

Autism spectrum disorders are neurodevelopmental disorders characterized by impairment in social interaction and repetitive/inflexible/restricted behaviors and interests. The prevalence of this disorder is very high with studies documenting a prevalence of 1:68 children in the USA with a monetary cost of caring for individuals with ASD approaching 1.5% of the US gross domestic product [1]. However, despite the high prevalence and the economic impact of this disorder, the underlying pathogenesis remains poorly understood.

Neuroanatomically, many brain regions have been implicated in the disorder, but the most consistently implicated brain region is the cerebellum. Pathology studies have identified abnormalities in cerebellar postmortem samples taken from autistic donors with the most consistent finding in these studies being Purkinje neuronal loss [2]. Targets of the Purkinje neurons – the deep cerebellar nuclei – also demonstrate abnormalities in size and number in ASD [3]. In addition, the inferior olive, the source of olivo-cerebellar climbing fiber input to the cerebellum, displays ectopic neurons and disruption in nuclear organization [3]. Pathology studies also reveal evidence for oxidative stress/injury in the cerebellum of individuals with autism [4].

More recently, imaging studies have further reinforced a role for the cerebellum in autism. Initial studies demonstrated numerous alterations in cerebellar volume with initial studies pointing to alterations in posterior/inferior vermis volume most prominently [5]. However, subsequent volumetric studies have additionally implicated involvement of the cerebellar hemispheres, especially in the area of lobule VII, Crus I/II [6,7]. In addition, structural and functional connectivity studies in individuals with ASD revealed alterations in cerebellar white matter in addition to functional impairment in cerebellar neuronal activity [8–10].

Clinical evidence further supports a role for the cerebellum in ASD pathogenesis. Increasing evidence has revealed significantly elevated rates of ASDs and autistic behaviors in clinical disorders of the cerebellum. Children with neurodevelopmental disorders – including but not limited to cerebellar hypoplasias, cerebellar atrophies, Joubert's syndrome, rhomboencephalosynapsis – demonstrate elevated rates of autistic behavior [11,12]. Moreover, inflammatory disorders of the cerebellum in children have significant cognitive and behavioral sequelae including impaired social impairments. In addition, injuries to cerebellar tracts in posterior fossa syndrome is similarly associated with elevated rates of autistic behavior [13]. Finally, evidence from premature infants who are at particular risk for hemorrhagic cerebellar injuries demonstrated that isolated cerebellar hemorrhagic injury resulted in substantially increased rates of autistic behaviors [14]. These and other studies further support critical roles for the vermis in ASD pathogenesis with vermian involvement associated with increased rates of autistic behavior [14].

## 2. The cerebellum and non-motor function

Although significant evidence implicates cerebellar involvement in ASD, these findings would seem to contradict traditional cerebellar roles. Even as far back as three millennia but mostly with the work of Luciani, Babinski, and Holmes in the early twentieth century, a clear if

* Address: University of Texas Southwestern Medical Center, 6000 Harry Hines Blvd, Dallas, TX 75390, USA. Tel.: +1 214 648 3577.
*E-mail address:* peter.tsai@utsouthwestern.edu.

http://dx.doi.org/10.1016/j.siny.2016.04.009
1744-165X/

not exclusive role for the cerebellum in co-ordination of motor function has emerged. However, consistent with these cerebellar motor roles, children with ASD have significantly elevated rates of motor dysfunction. ASD patients present with prevalent alterations in tone, oculomotor abnormalities, and increased motor apraxia, consistent with the motor phenotypes seen in children with neurodevelopmental disorders of the cerebellum [15].

In addition, evidence has emerged that challenges the hypothesis of exclusive motor roles for the cerebellum. Over evolution, growth of the cerebellum has correlated with growth of the cerebral cortex in areas (in both cortex and cerebellum) that have non-motor functions [16]. Moreover, anatomic tracing studies further demonstrated extensive connections between the cerebellum and non-motor cortical areas [17,18]. These studies have since been corroborated by functional connectivity studies, which again support a role for the majority of the cerebellum in non-motor functions [19,20]. Additional support for these connections comes from children with isolated cerebellar injury of prematurity, who on later imaging demonstrated significant alterations in cortical domains involved in cognitive processes, suggesting a contribution of remote developmental diaschisis and alterations in distributed neuronal circuitry between the cortex and cerebellum that appears to contribute to developmental, cognitive, and behavioral sequelae [14,21]. Further supporting these findings, contributions of the cerebellum to neurodevelopment, behavior, and cognition have been identified in both clinical and preclinical studies [12,22,23]. A more in-depth discussion of these roles can be found in other articles within this issue of *Seminars*.

## 3. Cerebellar models

With these emerging roles in non-motor function, Schmahmann postulated that cerebellar dysfunction would lead to a “dysmetria of thought” [24]. Because of the relatively consistent cerebellar anatomy and stereotyped organization of cerebellar anatomy, Schmahmann proposed that a “universal cerebellar transform” could explain the cerebellar role in both motor and non-motor functions, with recent studies suggesting that dysmetria may result from dysfunction in cerebellar-regulated timing mechanisms [25]. Ito and colleagues intertwined these models with previous models and have postulated that the cerebellum is a machine that generates and maintains internal models. These models for movement would consist of an internal representation of the dynamics of body movement [26]. These models require diverse neuronal connections with areas of instruction (premotor–motor cortices for movement) that provide instruction to the body part whereas the cerebellum provides instructive signals that predict and respond to error signals mediated by climbing fibers from the inferior olive. A similar model for the cerebellum in non-motor function and specifically social interaction would require a comparable neuronal circuit representation with prefrontal cortices, cingulate cortices, and temporal/parietal areas taking the places of premotor and body parts respectively [26]. Consistent with this model, cerebellar connectivity with these areas has been demonstrated [17,18]. Furthermore, in studies of ASD patients, connectivity and function have been demonstrated to be abnormal in these areas, raising the possibility that cerebellar disruption might be involved in aberrant function of these distributed circuits [27].

## 4. Preclinical studies of cerebellum and ASD

### 4.1. ASD genetics

Heritability studies point to a genetic contribution to ASD pathogenesis. With ongoing development in genomic advances, studies have revealed numerous genetic candidates. Not unexpectedly, many of these genes have pleiotropic effects and play roles in numerous organ systems. However, functions of these genes appear to cluster around several important biologic themes consistent with ASD phenotypes, including synaptic function, homeostasis, growth, and signaling. Many of these susceptibility genes demonstrate high expression in the cerebellum [28], and many demonstrate critical roles in cerebellar function and development in animal models (detailed further below).

In addition, defined genetic disorders associated with high rates of ASD have produced insights into ASD pathogenesis and specifically further supported cerebellar involvement in ASD pathogenesis. One such disorder is Fragile X syndrome which has rates of ASD approximating 30–50%, making it the largest monogenic contributor to ASD prevalence [29]. Fragile X deficits are diverse, but clear cerebellar abnormalities (especially vermian) differentiate Fragile X patients with ASD from those without ASD [3]. Furthermore, Fragile X patients demonstrated impaired eye-blink conditioning [30], a form of cerebellar-dependent associative learning that has previously been demonstrated to be abnormal in studies of patients with idiopathic ASD [31]. Another monogenic disorder, tuberous sclerosis complex (TSC), has similar rates of ASD – approximating 50% – and similarly supports a role for the cerebellum in the pathogenesis of ASD behavior. We have identified cerebellar abnormalities in patients with TSC, and cerebellar pathology in TSC is associated with higher rates of ASD [32]. Moreover, impaired cerebellar activity differentiates individuals with ASD from those without ASD in TSC, similar to findings seen in idiopathic ASD [10,33]. Patients with 22q13 deletions have elevated rates of ASD and have demonstrated cerebellar abnormalities (especially involving the vermis). The pathogenic gene associated with this deletion appears to be Shank3, which is expressed throughout the cerebellum [34].

Furthermore, numerous genes associated with neurodevelopmental disorders of the cerebellum have been implicated with increased autism risk. Joubert's syndrome is a ciliopathy marked by vermain hypoplasia, molar tooth appearance on magnetic resonance imaging (deep interpeduncular fossa with thickened/elongated superior cerebellar peduncles). Mutations in *Ahi1* are associated with certain forms of Joubert's syndrome and associated with elevated rates of ASD. Interestingly, even in the absence of Joubert's syndrome, *Ahi1* mutations are themselves associated with ASD [12,22,35]. Similarly, *Oligophrenin 1* (*OPHN1*) mutations are associated with cerebellar hypoplasia and increased rates of ASD; however, *OPHN1* mutations by themselves, even in the absence of cerebellar hypoplasia, result in social dysfunction and *OPHN1* is also an ASD susceptibility gene [36].

Although dysfunction in these ASD susceptibility genes results in marked cerebellar abnormalities, for most ASD susceptibility genes, data on clinical cerebellar involvement are unavailable. To better assess their function, preclinical models have been developed which have further supported contributions of cerebellar dysfunction to ASD. Although not a comprehensive list, several of these genes are discussed here.

#### 4.1.1. Engrailed 2

*Engrailed 2* (*En2*) is a homeobox transcription factor that has been identified as an autism candidate gene through numerous genetic studies. *En2* cerebellar expression is high, whereas disruption in these expression levels has been found in studies examining postmortem ASD subject brain samples [37]. To better understand *En2* function, transgenic mice were developed and found to have abnormal development of the cerebellum. *En2* knockout mice also displayed cerebellar phenotypes with reduction in cerebellar volumes and Purkinje cells in addition to marked