

Review

Microglia, cerebellar inflammation, and autism spectrum disorders: Developmental mechanisms and therapeutic perspectives

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ABSTRACT

Autism spectrum disorders (ASD) are neurodevelopmental conditions characterized by deficits in social interaction and communication, as well as restricted and repetitive behaviors. These conditions often co-occur with medical issues linked to synaptic alterations, which compromise synaptic integrity and are associated with brain circuit dysfunction. Both human and animal studies have identified cerebellar structural and functional alterations in subsets of ASD cases, with evidence of abnormal morphology and disrupted connectivity correlating with symptom severity. Numerous studies further suggest that neuroinflammation and microglia dysfunction may contribute to atypical cerebellar development during critical postnatal windows and may be associated with ASD-like phenotypes. However, the timing and mechanisms underlying the onset of these processes remain unclear, and a primary causal role in human ASD has not been established. This review synthesizes current knowledge on microglia in early stages of cerebellar development and discusses how cerebellar inflammation could be linked to ASD-related phenotypes. A better understanding of these pathological processes and their behavioral correlates may reveal opportunities for early-life intervention strategies.

Introduction

Autism spectrum disorders (ASD) are a heterogeneous group of neurodevelopmental conditions characterized by deficits in social interaction, verbal and nonverbal communication, and repetitive and stereotyped behaviors. ASD is often associated with cognitive deficits and epilepsy (American Psychiatric Association, 2013). Diagnosis typically occurs in early childhood, with symptoms (that can vary from one individual to another) often becoming apparent within the first two years of life; however, in several cases, symptoms may not be fully apparent or recognized until later (American Psychiatric Association, 2013). According to the most recent epidemiological estimates, the global prevalence is about 1 in 100 individuals, with an increasing trend

in recent years, and a 4:1 prevalence in males compared to females. The causes of this different prevalence are mostly unknown, but it has been hypothesized they might include exposure to prenatal steroid and social factors (Halladay et al., 2015; Ferri et al., 2018), a female protective effect (females show resilience to risk factors for ASD; Zwaigenbaum et al., 2012), as well as a female-specific tendency to mask or compensate for their autistic behaviors in social settings (“social camouflaging”; Cancino-Barros et al., 2025). The heterogeneity of the disorder makes it difficult to identify its precise causes, yet it offers numerous points of experimental study. ASD is considered a multifactorial condition, so several factors contribute to its pathogenesis. Over 1,000 genes have been associated with autism, mainly involved in transcription/translation control, synaptic plasticity, neural development, and neuronal

Abbreviations: ADHD, attention deficit-hyperactivity disorder; ASD, autism spectrum disorders; AST, astaxanthin; BBB, blood–brain barrier; CNS, central nervous system; *Cntnap2*, contactin associated protein-like 2; CSF1R, colony stimulating factor 1 receptor; E/I, excitation/inhibition; *En2*, Engrailed-2; Glx, glutamate/glutamine; GSH, glutathione; IL, interleukin; iPSC, induced pluripotent stem cell; MIA, maternal immune activation; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NAA, N-acetylaspartate; NAC, N-acetylcysteine; PET, positron emission tomography; SCZ, schizophrenia; *Shank3*, SH3 and multiple ankyrin repeat domains 3; TNF, tumor necrosis factor; VPA, valproic acid.

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connectivity (<https://gene.sfn.org>). Several ASD-associated genes are expressed in the neocortex (Parikshak et al., 2013; Willsey et al., 2013) and other brain structures implicated in ASD pathogenesis; for example, the amygdala expresses 80 ASD genes validated across species; this expression profile directly links to deficits in social-emotional processing (Herrero et al., 2020).

Growing evidence indicates that the cerebellum is also a crucial site for the expression of ASD-associated genes (Menashe et al., 2013). While traditionally recognized for its role in sensorimotor control (such as balance, coordination, and sensory integration), the cerebellum is increasingly understood to facilitate higher-order cognitive and affective functions, including language, attention, and social cognition (Fatemi et al., 2012; Stoodley, 2016). Thus, disruptions in cerebellar gene expression may contribute to both motor and social impairments characteristic of ASD. Recent studies have identified mutations in genes controlling cerebellar development (such as *EN2*, *SHANK3*, and *CNTNAP2*) in individuals with ASD, further supporting the hypothesis (emerging from mouse studies) that altered cerebellar structure and connectivity, whether arising from genetic or environmental insults, may impact on the behavioral symptoms observed in ASD (Provenzano et al., 2014; Wang et al., 2014; Gibson et al., 2022). Thus, the convergence of genetic, anatomical, and functional evidence from both human and animal studies highlights the importance of cerebellar pathways in ASD, warranting further research into how these molecular mechanisms contribute to the onset and progression of this condition.

Alongside a strong genetic component, several environmental factors (such as maternal infections, prenatal exposures to toxic substances, and subsequent immune alterations) have been associated with increased risk of ASD. Several animal studies indicate that immune dysfunction and neuroinflammation, often accompanied by altered states of microglia (the brain's resident immune cells) at prenatal and postnatal stages, are associated with ASD-relevant phenotypes, particularly in the cerebellum (Estes and McAllister, 2016; Stoodley and Tsai, 2021). The vulnerability of the cerebellum during early postnatal stages and the critical role of microglia in shaping synaptic refinement suggest that early-life inflammatory events might persistently alter cerebellar circuitry and contribute to ASD-like behaviors (Mastenbroek et al., 2024). Therefore, understanding how inflammation impacts cerebellar development represents a fundamental challenge in ASD research. In this review, we will summarize current knowledge of the role of microglia in early stages of cerebellar development and offer a novel perspective on the potential relationship between cerebellar inflammation and ASD pathogenesis.

The role of the cerebellum in ASD

The cerebellum is a brain structure located in the posterior cranial fossa under the cerebral hemispheres. Structurally, it consists of distinct lobules, each with specific functional properties and organized in a cerebellar cortex, which includes the molecular (external), Purkinje cell (intermediate), and granular (deep) layers; deep cerebellar nuclei are located within the white matter. Cerebro-cerebellar circuits, which link the cerebellum with prefrontal and limbic regions, serve as the primary neural substrate for cerebellar social processing (Fatemi et al., 2012; Stoodley, 2016).

The involvement of the cerebellum in ASD is supported by multiple pieces of evidence. Structural abnormalities including cerebellar hypoplasia, disrupted cerebellar cortical organization, volumetric differences, loss of Purkinje cells, aberrant synaptic connectivity, and gliosis have been consistently found in various cerebellar subregions in post-mortem samples from individuals with ASD (Kemper and Bauman, 1998; Bauman and Kemper, 2005; Fatemi et al., 2012; Stoodley et al., 2017; Mapelli et al., 2022; Sydnor and Aldinger, 2022; Mastenbroek et al., 2024). Damage to the posterior cerebellum in humans results in deficits in executive function, working memory, abstract reasoning, and emotional processing (Schmahmann and Sherman, 1998; Schmahmann

et al., 2021). In mice, cerebellar lesions can result in impaired attention, behavioral flexibility, and social interactions (Badura et al., 2018; Stoodley, 2016). Specifically, interfering with rodent *crus I* (a cerebellar region structurally altered in humans with ASD), results in repetitive behaviors as well as deficits in social interactions and cognitive flexibility.

Mutations in genes controlling cerebellar development have been reported in individuals with neurodevelopmental conditions, including ASD (Wang et al., 2014). Similarly, mice bearing mutations in genes that control cerebellar morphogenesis show altered formation of cerebellar circuits, accompanied by cognitive and behavioral difficulties resembling those observed in ASD (Provenzano et al., 2014; Gibson et al., 2022). Overall, these findings support an association between altered cerebellar structure and connectivity with ASD-related behavioral phenotypes. In the following paragraphs, we will elaborate on this hypothesis by addressing developmental mechanisms of cerebellar inflammation that may contribute to ASD pathogenesis.

Peripheral and central inflammation in ASD

Inflammation is a highly coordinated immune response initiated by various triggers, such as infection, physical trauma, or metabolic stress. In the periphery, this process involves the mobilization of immune cells and the release of cytokines, which collectively restore tissue homeostasis and support recovery from injury or disease (Medzhitov, 2008).

Immune dysfunction is often linked to high levels of systemic inflammation in individuals with ASD (Croonenberghs et al., 2002; Ashwood et al., 2011; Masi et al., 2017). Studies found elevated levels of pro-inflammatory cytokines, including interleukin (IL)-1, IL-6, IL-8, IL-12p40, tumor necrosis factor (TNF), and IL-17, in the plasma of children with ASD who were not taking medication (Ashwood et al., 2011). Further studies confirmed higher levels of peripheral inflammation markers (e.g., IL-1 β , IL-6, IL-17) in the plasma of children with ASD and interictal epileptiform activity, indicating the co-occurrence of immune system dysfunction and hyperexcitability in ASD (Inga J  come et al., 2016).

When inflammation occurs in the brain, it is called neuroinflammation. This form of inflammation typically engages specialized neural immune cells, namely microglia, which act to protect and maintain the neural environment. These cells coordinate the brain's immune response, highlighting both the similarities and unique aspects of inflammation in the central nervous system compared to the rest of the body. Within the brain, microglia constitute the predominant cell type of the innate immune system. Unlike the adaptive immune system, which generates antigen-specific responses, the innate immune system develops non-specific responses that recognize conserved structures expressed by pathogens. Microglia are estimated to account for approximately 80% of all brain immune cells, comprising 10%–15% of the total brain cell population (Pangrazzi et al., 2020; Pangrazzi et al., 2025b).

Neuroinflammation, even when mild or transient, can disrupt synaptic maturation, alter neurotransmitter homeostasis, and impair neuronal development (Thion et al., 2018). During sensitive developmental windows, especially the early postnatal period, these disruptions can have long-lasting consequences. An example that links neuroinflammation to ASD is maternal immune activation (MIA), a condition that refers to excessive immune system activity during pregnancy. Maternal infection and activation of the maternal immune system have been associated with an increased risk for neurodevelopmental disorders in human offspring (Mastenbroek et al., 2024); the occurrence of ASD (Jiang et al., 2016) and schizophrenia (SCZ) (Brown and Patterson, 2011; Khandaker et al., 2013) also correlates with MIA. Accordingly, prenatal inflammation in rodent models of MIA primes the offspring microglia for exaggerated responses, inducing ASD-like behaviors (Enayati et al., 2012). Postnatal studies in mouse models of MIA confirmed that elevated pro-inflammatory cytokines and microglial

reactivity correlate with behavioral deficits (Golan et al., 2006).

Importantly, MIA should be interpreted primarily as a model of early developmental vulnerability rather than as direct evidence that chronic neuroinflammation and persistent microglial activation are causally linked to ASD in humans. Epidemiological studies support an association between maternal infection during pregnancy and a modestly increased risk of ASD in offspring (with risk estimates varying according to the type, timing, and severity of the infectious exposure), but these data remain correlational and do not establish a causal inflammatory mechanism in the child's brain (Hall et al., 2023). Preclinical MIA paradigms are therefore most useful for investigating how transient prenatal or perinatal immune challenges can alter fetal brain development, microglial maturation, and later behavioral outcomes, while their translation to the heterogeneous human ASD phenotype remains uncertain and context-dependent. Accordingly, MIA provides a mechanistic framework for studying how early inflammatory perturbations may increase neurodevelopmental risk, while the extent to which these processes translate to the heterogeneous human ASD phenotype remains uncertain and likely context-dependent.

Cerebellar inflammation in ASD

Clinical and preclinical studies often highlight cerebellar dysfunction as a key factor in ASD, connecting structural and functional changes in the cerebellum with challenges in behavioral and cognitive regulation that are typical of ASD. Disruptions in the cerebellum's predictive and adaptive functions align with ASD symptoms; these disruptions may be influenced by immune responses and inflammation, which can affect cerebellar activity (Tsai, 2016; Kelly et al., 2021). Markers of neuroinflammation have been detected in ASD cerebella (Vargas et al., 2005; Wei et al., 2011; Suzuki et al., 2013; McCarthy and Wright, 2017; Kern et al., 2016). Additionally, patients with ASD frequently show imbalances in redox processes and their immune systems, including higher levels of pro-inflammatory molecules and evidence of oxidative stress in both the brain and blood (Shpyleva et al., 2014; Pangrazzi et al., 2020). Collectively, this research suggests that cerebellar inflammation is one aspect of a complex landscape in which genetic risk factors, oxidative stress, and dysfunctions of the peripheral and central immune systems combine to affect how ASD begins and progresses (Pangrazzi et al., 2020).

Studies performed in BTBR mice confirmed the presence of oxidative stress in the cerebellum of a widely used ASD model (Shpyleva et al., 2014). Moreover, rat and mouse studies suggest that, within the cerebellum, microglia dysfunction may contribute to ASD pathogenesis: failure of microglia activation during the development of cerebellar white matter, cortex, and other regions, may trigger the formation of impaired cerebellar circuits and neuronal activity associated with ASD (Yamamoto et al., 2019). More recent studies from our laboratory reported cerebellar neuroinflammation accompanied by microglia dysfunction in both *Cntnap2*^{-/-} (Pangrazzi et al., 2025a) and *Shank3b*^{-/-} (Pangrazzi et al., 2024; Madhavan et al., 2026) adult mice, two robust models of syndromic ASD. Our preliminary data also indicate that cerebellar inflammation is present in *Cntnap2*^{-/-} and *Shank3b*^{-/-} mice as early as the first postnatal week. Other studies performed in mice suggest that inflammatory signaling may interact directly with genetic factors linked to ASD during cerebellar development. For example, dysregulation of *Engrailed-2* (*En2*), a key transcription factor for Purkinje cell differentiation and cerebellar patterning, can heighten Purkinje cells' vulnerability to inflammation; experimental evidence indicates that pro-inflammatory stimuli worsen dendritic abnormalities in Purkinje cells depending on *En2*, possibly via microglia-driven TNF- α signaling (Bahaaeldin et al., 2024). These preclinical findings suggest that neuroinflammatory signaling can actively interact with developmental genetic mechanisms and potentially amplify cerebellar circuit alterations. These gene-immune interactions offer insight into how cerebellar susceptibility, altered neural connectivity, and behavioral

features typical of ASD are interconnected.

However, a note of caution is warranted when interpreting these findings: several factors indicate that a direct causal link between cerebellar inflammation and ASD pathogenesis remains elusive. First, neuroinflammation may not represent a primary driver of pathology but rather a downstream or compensatory response to excitation/inhibition (E/I) imbalance (resulting from altered neuronal activity, synaptic imbalance, or developmental miswiring), which has been proposed to contribute to ASD (Rubenstein and Merzenich, 2003; Nelson and Valakh, 2015). In this view, microglial activation could reflect attempts to restore homeostasis rather than the initiation of dysfunction. Similarly, disrupted cerebellar function might arise from intrinsic neuronal or circuit-level alterations, including Purkinje cell loss or synaptic deficits, independent of immune mechanisms (Fatemi et al., 2012; Wang et al., 2014). Second, monogenic mouse models (e.g., *Shank3*, *Cntnap2*, *Fmr1*, *Nlgn3*, *Tsc1/2* mutants, and others) provide valuable mechanistic insights, but capture only a small fraction of ASD cases, while ASD is predominantly idiopathic and highly heterogeneous in humans (Lord et al., 2020). In addition, environmental animal models of ASD (e.g., MIA, prenatal valproic acid exposure) and human-based systems such as induced pluripotent stem cell (iPSC)-derived neurons and brain organoids (Dixon and Moutri, 2023; Michels et al., 2025; Tenreiro and Muotri, 2026) clearly indicate that several different primary drivers may contribute to ASD. These models often recapitulate distinct, and sometimes non-overlapping, aspects of ASD pathophysiology, ranging from synaptic dysfunction and E/I imbalance to altered neurodevelopmental trajectories independent of inflammation (or that might involve inflammation only secondarily), underscoring that no single model fully captures the disorder.

Species-specific differences in microglial biology, immune responses, and cerebellar development further limit translational generalizability (Masuda et al., 2020). At present, the role of microglial responses in ASD and their involvement in pathogenic mechanisms remains unclear. Microglial activation within the CNS appears to play a dual role in brain inflammatory responses, acting both as a direct mediator of injury and as a neuroprotective factor (Nguyen et al., 2002). It is not yet well understood how or when microglia become activated in the brains of individuals with ASD; however, given that microglial activation represents the primary cellular response to CNS dysfunction, it is plausible that abnormal microglial activity is closely linked to ASD. Although a direct causal relationship has not been established, cerebellar inflammation and altered microglial activity may represent one developmental mechanism contributing to ASD-related phenotypes in a subset of cases, potentially through effects on synaptic pruning, neuronal maturation, and circuit refinement. In the following section, we will address the developmental contribution of microglia to cerebellar inflammation.

Microglia and cerebellar inflammation: A developmental perspective

Microglial ontogeny and cerebellar development. The cerebellum, including its microglial component, undergoes active development during both embryonic and postnatal stages. Microglia originate from yolk sac erythromyeloid progenitors and colonize the CNS early in embryogenesis. In the cerebellum, microglia are already present and active during early postnatal days, where they play essential roles in synaptic pruning, trophic signaling, and circuit refinement.

Mouse studies showed that microglia are critical regulators of CNS development, contributing to axonal sculpting, synaptic remodeling through the elimination of unused synapses, maintenance of neuronal homeostasis, and immune surveillance as brain-resident macrophages (Paolicelli et al., 2011). Their distribution, morphology, and transcriptional profile remain highly dynamic throughout early postnatal life: cerebellar microglia undergo a protracted maturation trajectory, with substantial transcriptional remodeling between postnatal days 3 and 21 in the mouse. Functionally, microglia continuously sense environmental signals via pattern-recognition receptors and shift between ramified

(surveying) and amoeboid (activated) states in response to developmental and physiological demands. When microglial maturation is impaired by genetic or environmental factors, or when their activation becomes excessive or persistently dysregulated, synaptic pruning, growth factor regulation, and cellular homeostasis can be disrupted. Several animal studies support the crucial role of microglia in shaping cerebellar circuitry (Stoessel and Majewska, 2021): as an example, impaired microglial maturation alters cerebellar synaptic refinement and leads to abnormal behavioral phenotypes (Pinto et al., 2024), while excessive early postnatal microglial activation creates an inflammatory environment that disrupts functional cerebellar network formation (Paolicelli and Ferretti, 2017). These data indicate that (at least in rodents) early-life microglial dysfunction alters cerebellar connectivity, subsequently affecting behavior.

Cerebellar development during the perinatal and early postnatal period. In both rodents and humans, cerebellar development occurs primarily during the perinatal and early postnatal periods and involves tightly coordinated processes, including neuronal migration, granule cell proliferation, synapse formation, Purkinje cell maturation, and the establishment of cerebellar circuitry. This developmental window represents a period of heightened vulnerability, during which environmental or biological insults can produce long-lasting alterations in cerebellar architecture and connectivity. Disruptions caused by inflammatory events, oxidative stress, metabolic stress, or exposure to toxins can interfere with neuronal differentiation, synaptogenesis, and long-range connectivity, ultimately compromising cerebellar contributions to motor and cognitive functions. Supporting this view, early-life oxidative stress has been shown to impair Purkinje cell maturation and synaptic density in rodents, while perinatal neuroimmune activation alters microglial maturation patterns within the cerebellum, leading to enduring deficits associated with ASD-like phenotypes (Belletti et al., 2022; Liu et al., 2023). Collectively, these findings highlight that early postnatal cerebellar development is highly sensitive to pathological insults, with long-lasting consequences for neurodevelopment.

Microglia, astrocytes, and cerebellar inflammation. Postnatal cerebellar inflammation can arise from a range of biological conditions, including perinatal infection, MIA, hypoxia-ischemia, environmental toxicity, and inherited vulnerability. When these events occur during early developmental stages, they can prematurely activate microglia, driving them toward a pro-inflammatory state characterized by excessive cytokine production, oxidative stress, and metabolic dysfunction. These processes negatively affect neuronal migration, neurogenesis, and the formation and refinement of cerebellar circuits. The newborn brain is particularly sensitive to inflammatory molecules.

Post-mortem studies of cerebellar tissue from individuals with ASD further reveal increased microglial activation and elevated pro-inflammatory cytokines (Vargas et al., 2005; Suzuki et al., 2013), while animal models consistently link excessive microglial activation to ASD-like behaviors (Zhan et al., 2014; Yuan et al., 2016; Coomey et al., 2020). Interestingly, mouse studies showed that cerebellar microglia exhibit a more pronounced immunogenic transcriptional profile as compared to their cortical counterparts, including elevated expression of genes involved in immune responses and pathogen detection (C-type lectin-like receptors Clec4e and Clec7a), interferon signaling (Stat1, Stat4, Irf7), antigen presentation (MHC I and II), and nucleic acid recognition (Zbp1) (Grabert et al., 2016). As a result, cerebellar microglia may be particularly sensitive to systemic inflammation, oxidative stress, and local insults. A large series of animal studies clearly indicates that aberrant microglial activation leads to the release of pro-inflammatory cytokines (e.g., TNF- α , IL-6, IL-4), reactive oxygen species, chemokines, and matrix-modifying enzymes, profoundly altering the local brain environment. This inflammatory milieu interferes with normal brain development, neuronal survival, synaptic pruning, and plasticity, ultimately leading to structural abnormalities and functional deficits (Haruwaka et al., 2019).

In addition, activated microglia can induce astrocytes to adopt a

neurotoxic phenotype, further amplifying inflammation and perpetuating a self-sustaining cycle of neuroinflammatory damage (Luo and Wang, 2024). Indeed, under inflammatory conditions, reactive astrocytes undergo phenotypic changes (astrogliosis) and release pro-inflammatory mediators, including cytokines, nitric oxide, glutamate, and matrix metalloproteinases, which disrupt tight junctions and increase blood–brain barrier (BBB) permeability (Gullotta et al., 2023). This highlights the significant role of astrocytes and the BBB as central regulators of cerebellar inflammatory processes, acting through tightly coordinated mechanisms within the neurovascular unit (Heithoff et al., 2021). Astrocytic branches envelop cerebellar microvessels and actively maintain BBB integrity by promoting tight junction formation, regulating transporters, and releasing trophic factors such as sonic hedgehog (SH) and retinoic acid that stabilize endothelial function (Heithoff et al., 2021). During neuroinflammation, astrogliosis may cause breakdown of the BBB, facilitating the infiltration of peripheral immune cells into the cerebellum and amplifying local neuroinflammation. Concurrently, BBB endothelial cells themselves contribute to inflammation by producing immune signaling molecules and regulating leukocyte trafficking, acting as an active interface rather than a passive barrier (Acioglu and Elkabes, 2025). In the cerebellum (where precise microenvironmental control is essential for sensorimotor and cognitive functions), these alterations can profoundly affect neuronal circuits. Importantly, astrocytes exhibit a dual role: they can form secondary barrier-like structures and limit immune cell entry when endothelial integrity is compromised, highlighting their context-dependent protective versus detrimental effects during neuroinflammation (Gullotta et al., 2023). Together, astrocyte–BBB interactions represent a dynamic system that both regulates and propagates cerebellar inflammatory responses, with dysfunction in this crosstalk being a key contributor to neurodevelopmental and neurodegenerative disorders.

Early detection of inflammatory signatures: opportunities and limitations

Collectively, evidence from both animal and human studies supports the hypothesis that early postnatal or even embryonic cerebellar inflammation may occur in ASD, underscoring the need for early diagnostic strategies capable of detecting neuroimmune activation during the earliest stages of development and for proposing therapeutic interventions at very early time points in life. Early detection could enable therapeutic intervention during periods of heightened neuroplasticity, thereby potentially reducing the severity or likelihood of ASD symptom emergence.

Although research in this area remains at an early stage, one promising approach is to identify, in both preclinical and clinical settings, early-life time points of inflammation onset and to determine how inflammation influences neurobiological and behavioral phenotypes. Within this framework, the following considerations should be interpreted as key hypotheses and future research directions rather than conclusions supported by current evidence. In particular, experimental strategies aimed at inducing inflammation or depleting microglia in young rodents represent a promising but still largely untested avenue. The systemic injection of lipopolysaccharide (LPS, a component of Gram-positive bacterial membranes and a powerful activator of the innate immune system; Alexander and Rietschel, 2001) enables the simulation of an acute inflammatory insult and the study of its effects at the brain and behavioral levels. While LPS has been widely used to model maternal immune activation and induce neuroinflammation, relatively few studies have specifically investigated its effects on immune cell activation and behavior in established genetic or environmental ASD models, and the available evidence remains limited and sometimes conflicting (Manjeese et al., 2021; Zhang et al., 2023). Similarly, no study has yet systematically and comparatively examined, in rodent models of ASD, the effects of pharmacological microglial depletion via inhibition of the CSF1R receptor (essential for microglial and macrophage survival, proliferation, and differentiation; Meng et al.,

Antioxidant and anti-inflammatory effects of different treatments in rodent models of ASD

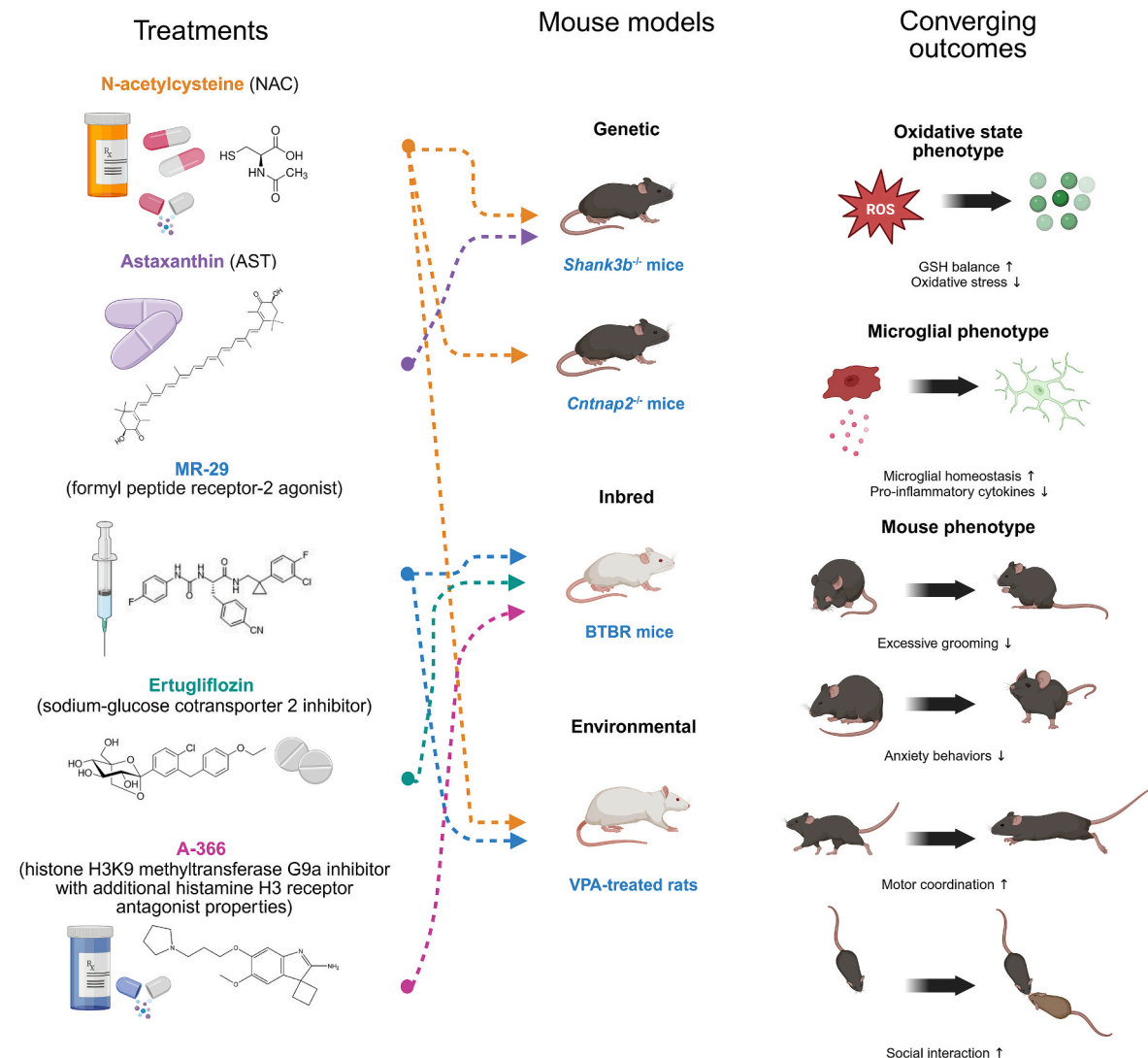


Fig. 1. Antioxidant and anti-inflammatory treatments successfully mitigate neuroinflammation and associated behavioral traits in genetic, inbred, and environmental rodent models of ASD (see Table 1 for references). Abbreviations are as in the text. Figure created in BioRender. Casarosa, S. (2026) <https://BioRender.com/4wqec64>.

2024) using PLX5622; thus, whether microglia play an active role in determining behavioral phenotypes or whether they represent a secondary response to pre-existing disease processes remains to be elucidated.

Likewise, the use of a wide range of techniques to identify early diagnostic markers of cerebellar inflammation in human subjects also opens promising but still largely untested avenues. Screening the expression of inflammatory cytokines within the cerebellum could help determine whether and when inflammatory processes occur. In addition, biomarkers derived from blood, cerebrospinal fluid, or neuroimaging approaches are increasingly investigated as potential indicators of microglial activation or oxidative stress in infants (Van Camp et al., 2022). For instance, elevated inflammatory cytokines in neonatal plasma have been reported to correlate with altered cerebellar morphology as early as one year of age (as detected by magnetic resonance imaging, MRI), and positron emission tomography (PET) tracers have been highlighted as useful tools for identifying microglial activation in pediatric neurological disorders (Evans et al., 2022; Martínez-Sosa et al., 2023).

Among available neuroimaging techniques, *in vivo* magnetic

resonance spectroscopy (MRS) is a complementary, non-invasive modality for examining neurochemical changes associated with neuroinflammation in the developing brain. Although current MRS studies in ASD are limited and somewhat heterogeneous, they have identified alterations in metabolites related to neuronal integrity and glial function, such as decreased N-acetylaspartate (NAA) and fluctuations in glutamate/glutamine (Glx) levels, indicating potential E/I imbalance and glial-related metabolism (Page et al., 2006; Kubas et al., 2012; Horder et al., 2018; Oya et al., 2023). While these observations are not universally consistent across studies due to differences in developmental stage and the brain regions examined, they offer valuable *in vivo* links between peripheral immune markers and postmortem findings. Integrating MRS with other biomarker methodologies may enhance early detection strategies and facilitate identification of neuroinflammatory signatures during critical periods of cerebellar development.

Therapeutic perspectives: Current treatments to reduce cerebellar inflammation and improve autistic symptoms

At present, no standardized treatment specifically targeting

Table 1
Antioxidant and anti-inflammatory effects of different treatments in monogenic, inbred, and environmental rodent models of ASD.

Treatment	Model(s)	Primary outcomes & mechanisms	References
N-acetylcysteine (NAC)	<i>Shank3b</i> ^{-/-} mice	Rescues cerebellar and peripheral inflammation; improves social interaction deficits	Pangrazzi et al., 2024
	<i>Cntnap2</i> ^{-/-} mice	Normalizes cerebellar oxidative stress and microglial dysfunction; reverses motor and social impairments	Pangrazzi et al., 2025a
	VPA-treated rats	Ameliorates social deficits and anxiety; restores glutathione (GSH) balance and synaptic gene expression; restores E/I imbalance by metabotropic glutamate receptor-dependent mechanisms	Chen et al., 2014Zhang et al., 2017Schiavi et al., 2022
Astaxanthin (AST)	<i>Shank3b</i> ^{-/-} mice	Improves social interaction, repetitive grooming, and motor coordination; reduces cerebellar pro-inflammatory cytokines and microglial hyperactivation	Pangrazzi et al., 2024
MR-39 (formyl peptide receptor-2 agonist)	BTBR & VPA mice	Reduces hippocampal inflammatory markers and restores neuronal plasticity; improves social phenotypes	Cristiano et al., 2022
Ertugliflozin (sodium-glucose cotransporter 2 inhibitor)	BTBR mice	Reduces repetitive behaviors and anxiety; inhibits microglial activation and oxidative stress in the hippocampus and prefrontal cortex	Wang et al., 2026
A-366 (histone H3K9 methyltransferase G9a inhibitor with additional histamine H3 receptor antagonist properties)	BTBR mice	Reduces pro-inflammatory cytokines; restores social behaviors and reduces repetitive behaviors	Hajar et al., 2026

cerebellar inflammation and microglial dysfunction in ASD has been established. Consequently, there is a pressing need to identify more effective anti-inflammatory and microglia-targeting therapeutic compounds. Emerging therapeutic strategies largely rely on anti-inflammatory and antioxidant agents, immune modulators, and behavioral or neuro-modulatory interventions, most of which remain at the preclinical stage.

Natural antioxidants and anti-inflammatory molecules have emerged as promising candidates for counteracting core ASD symptoms both in

humans and rodents. Such treatments may involve the administration of compounds such as vitamin C, vitamin E, astaxanthin (AST), glutathione, and N-acetylcysteine (NAC), either by intraperitoneal injection, oral supplementation, or dietary intake of nutraceutical-rich plant, fish, and crustacean-derived foods that target inflammation and oxidative stress (Pangrazzi et al., 2020). Evidence from both animal and human studies indicates that these molecules, in addition to their anti-inflammatory properties, exhibit neuroprotective potential by preserving cognitive function (Suswidianoro et al., 2026).

Recent studies from our laboratory showed that treatment with NAC was able to lower cerebellar neuroinflammation, correct microglial dysfunction, and improve ASD-like social behaviors in adult *Cntnap2*^{-/-} mice (Pangrazzi et al., 2025a). Similarly, AST treatment markedly reduced cerebellar neuroinflammation, microglial dysfunction, and motor and social behaviors in adult *Shank3b*^{-/-} mice (Madhavan et al., 2026). Antioxidant and anti-inflammatory drugs also showed efficacy in lowering inflammation, oxidative stress, and ASD-like deficits in inbred and environmental models of ASD in rodents (Chen et al., 2014; Zhang et al., 2017; Schiavi et al., 2022; Cristiano et al., 2022; Hajar et al., 2026; Wang et al., 2026). Fig. 1 and Table 1 summarize the major outcomes of antioxidant and anti-inflammatory treatments in monogenic, inbred, and environmental rodent models of ASD.

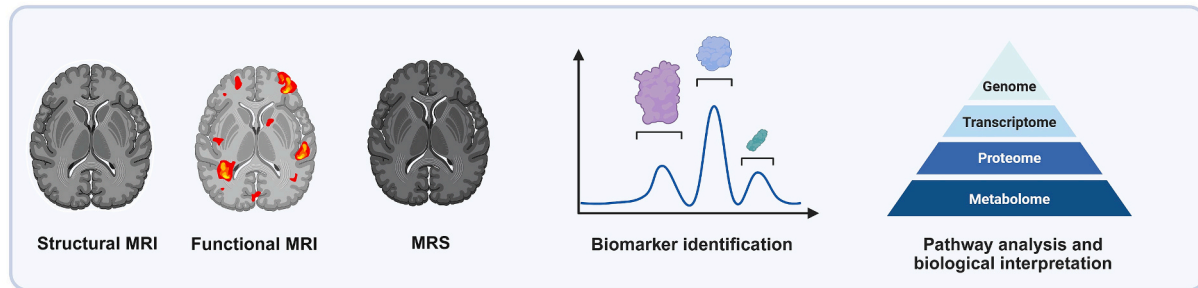
In humans, clinical studies suggest that immunomodulatory and anti-inflammatory treatments (e.g., prednisolone, pregnenolone, celecoxib, minocycline, NAC, sulforaphane, and/or omega-3 fatty acids) may be beneficial for the management of core (e.g., stereotypies) and associated (e.g., irritability, hyperactivity, lethargy) symptoms in individuals with ASD (Lee et al., 2021; Arteaga-Henríquez et al., 2023). Nevertheless, well-designed randomized clinical trials are still required to evaluate the efficacy and long-term outcomes of these interventions.

Although these interventions showed therapeutic promise, their safety profiles and long-term effects are not yet fully understood. Antioxidants are generally regarded as safe when used appropriately; however, excessive doses may result in serious adverse effects. For example, NAC is well tolerated and has shown improvements in irritability and repetitive behaviors among individuals with ASD, though side effects such as gastrointestinal disturbances and variable efficacy have been reported (Wink et al., 2016). Likewise, antioxidant agents including AST and vitamins C and E are considered safe at standard dosages, but concerns persist regarding chronic administration, optimal dosing, and the possibility of off-target effects, such as disruption of physiological redox signalling (Bjelakovic et al., 2012; Pangrazzi et al., 2020). Additionally, immunomodulating therapies may entail risks of excessive immunosuppression or alterations in neurodevelopment, particularly when administered during critical periods of brain maturation (Estes and McAllister, 2016). In the near future, it will be therefore important to conduct longitudinal studies and clinical trials to evaluate the efficacy, safety, tolerability, and developmental outcomes of antioxidant and anti-inflammatory treatments, thereby determining the potential for these interventions to be translated into safe and effective clinical strategies for ASD.

Unresolved questions

Despite growing evidence linking cerebellar inflammation to ASD, it is still unclear whether inflammatory features described in the cerebellum are specific to ASD pathogenesis or instead reflect a broader vulnerability shared across neurodevelopmental disorders. Disruptions in cerebellar function, possibly caused by inflammation, are observed across a range of human neurodevelopmental conditions: postmortem and imaging studies indicate that cerebellar neuroinflammation (characterized by microglial activation and cytokine dysregulation) is a common feature across ASD, attention deficit-hyperactivity disorder (ADHD), and SCZ, raising the possibility that immune alterations are not disorder-specific but rather indicative of a common response to developmental perturbations (Vargas et al., 2005; Stoodley, 2016; Estes &

Neuroimaging & biomarkers - early detection of microglial activation



Anti-inflammatory & antioxidant treatments to shift microglial state and modify developmental trajectories



Longitudinal studies to assess developmental impact of therapies & early intervention

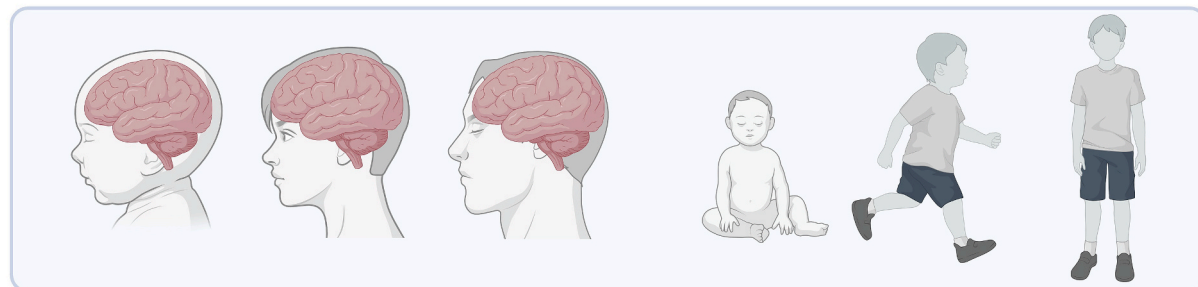


Fig. 2. Emerging diagnostic and microglia-targeted therapeutic strategies for early intervention in ASD. Abbreviations are as in the text. Figure created in BioRender. Casarosa, S. (2026) <https://BioRender.com/4wqec64>.

McAllister, 2016). Comparative studies across disorders, particularly at early developmental stages, will be essential to disentangle specificity from shared pathology.

Another unresolved issue concerns the functional role of microglial activation in the cerebellum, as it remains uncertain whether it is primarily pathogenic or represents a compensatory response to underlying circuit abnormalities. Classical studies in mice showed that microglia play a critical role in synaptic pruning and circuit refinement during development, and that their dysregulation can directly contribute to altered connectivity (Paolicelli et al., 2011; Schafer et al., 2012). However, microglial activation may also arise in response to neuronal dysfunction or environmental insults, potentially contributing to protective or homeostatic functions (Salter and Stevens, 2017). Distinguishing between these possibilities will require longitudinal, cell-type-specific approaches capable of establishing causality.

Finally, the temporal dynamics of cerebellar inflammation remain poorly defined in humans. It is not yet established whether immune alterations emerge early in development and contribute to ASD onset, or whether they arise later as a consequence of ongoing neural dysfunction. Evidence from the MIA mouse model suggests that early-life

inflammatory events can have lasting effects on cerebellar development and ASD-like behaviors (Shi et al., 2009; Hsiao et al., 2012), but direct translation to human ASD remains limited. Addressing this gap will require integrating human longitudinal data with mechanistic insights from animal models.

Clarifying these unresolved questions will be critical for defining the precise role of cerebellar inflammation in ASD and for determining whether targeting neuroimmune pathways represents a viable therapeutic strategy.

Conclusions and future perspectives

Evidence reported in this review supports the view that ASD is a multifactorial condition in which inflammation and microglial dysfunction may contribute to the pathophysiology, at least in a specific subset of cases (Bozzi, 2025). While the cerebellum has been traditionally associated with sensorimotor control, it is now recognized as playing a key role in cognition and social behavior (Fatemi et al., 2012; Stoodley, 2016), making cerebellar pathology particularly relevant to the study of ASD.

Postmortem studies and animal models have reported glial activation and cerebellar inflammatory signatures (altered cytokine profiles) in association with ASD-related phenotypes, often occurring alongside Purkinje cell loss and disrupted synaptic architecture (Fatemi et al., 2012; Mastenbroek et al., 2024). Because the cerebellum continues to develop during the neonatal period, inflammatory insults during this stage could alter neuronal networks and behavioral trajectories; however, the extent to which this contributes to human ASD remains uncertain, as these signatures are present only in a proportion of individuals. Consequently, cerebellar inflammation should be viewed as a contributing factor in specific cases rather than a universal underlying mechanism.

Future research efforts should focus on improving diagnostic precision and expanding therapeutic strategies that target these neuro-immune pathways. Advances in neuroimaging techniques (such as PET, MRI, and MRS), along with plasma biomarker profiling, may help identify markers of microglial activation early in life, though their reliability and specificity are still being established. There is also growing interest in developing microglia-targeted therapies and utilizing antioxidant or anti-inflammatory interventions (e.g., NAC and AST) to shift microglial phenotypes from pro-inflammatory to neuro-protective states.

The success of these approaches will depend on preclinical and clinical longitudinal studies designed to identify the appropriate therapeutic windows and the specific subpopulations most likely to benefit (Fig. 2). Furthermore, current research must address the limitations of many preclinical models, which often fail to account for the multifactorial nature of ASD, including gene-environment interactions and sex differences. An integrative approach (combining human-derived systems, diverse model organisms, and comprehensive phenotyping) is essential to link early neuroimmune alterations with later behavioral outcomes. By clarifying whether, when, and in which ASD subgroups neuroinflammation contributes to disease-relevant mechanisms, researchers can move these strategies from theoretical potential to clinically actionable interventions.

Declaration of generative AI in scientific writing

Generative artificial intelligence (AI) and AI-assisted technologies (Chat-GPT, Google Gemini) were used in the writing process solely to improve readability and language of the manuscript. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

CRediT authorship contribution statement

Martina Schiano-Visconte: Writing – original draft. **Luigi Balasco:** Writing – review & editing, Writing – original draft. **Simona Casarosa:** Writing – review & editing, Funding acquisition. **Yuri Bozzi:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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