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RESEARCH PAPER

Ketogenic diet improves motor and anxiety-like behaviors plus cerebellar inflammation via axonal remyelination in the BTBR mouse model of autism

Raffaella Alò[a,*], Ilaria Olivito[a], Damiana Minervini[a], Ennio Avolio[a], Anna De Bartolo[b], Ida Perrotta[c], Maria Carmela Cerra[d], Tommaso Angelone[b,e], Carmine Rocca[b,e], Rosa Maria Facciolo[a]

[a] Comparative Neuroanatomy Laboratory, Department of Biology, Ecology, and Earth Sciences (DiBEST), University of Calabria, Cosenza, Italy
[b] Cellular and Molecular Cardio-vascular Physiology and Patho-Physiology, Department of Biology, Ecology and Earth Sciences (DiBEST), University of Calabria, Cosenza, Italy
[c] Centre for Microscopy and Microanalysis, Department of Biology, Ecology and Earth Sciences (DiBEST), University of Calabria, Cosenza, Italy
[d] Laboratory of Organ and System Physiology, Department of Biology, Ecology and Earth Sciences (DiBEST), University of Calabria, Cosenza, Italy
[e] National Institute of Cardiovascular Research (INRC), Bologna, Italy

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Abstract

Different evidence suggests that ketogenic diet (KD) ameliorates neurological dysfunctions including core symptoms of autism spectrum disorders (ASD). In view of this, we have hypothesized that KD may induce a positive effect even on some ASD comorbidities, such as motor impairment and anxiety, along with cerebellar inflammation and axonal demyelination. BTBR T+ Itpr3tf/J (BTBR) and C57BL/6 (C57) mice were fed with a standard chow diet (CD) or KD for 5 weeks. Modified beam walking (MBW) and open field (OFT) tests were used to examine locomotor activity and anxiety-related behaviors. At the same time, cerebellar pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 were evaluated together with g-ratio of myelinated axons, detected by TEM. The levels of different isoforms (21.5, 18.5/17, and 14-kDa) of the myelin basic protein (MBP) were also analyzed. As expected, KD improved motor deficits and anxiety in BTBR mice as displayed by significant increases ($P<.001$) in the number of peeking and laps during MBW test rather than BTBR treated with CD. Furthermore, reduced path speed and enhanced path length ($P<.001$) in OFT were reported for the same KD-fed mice strain. Contextually, some pro-inflammatory cytokines (TNF-α and IL-1β) were significantly ($P<.05$) reduced in the cerebellum of BTBRs exposed to KD. Such ameliorations were correlated to a reduction of g-ratio ($P<.001$), together with an upregulation ($P<.05$) of the MBP isoform 18.5/17-kDa. Overall, these findings support a promyelinating and anti-inflammatory action of KD, which very likely determined mitigation of motor disorder and anxiety-like behaviors.

Keywords: Autism spectrum disorders; Cytokines; Myelin basic protein; Myelin g-ratio; TEM; Cerebellum.

1. Introduction

Autism spectrum disorder (ASD) is a multisystem neurodevelopmental disorder that is featured by impaired social interaction and communication, coupled with repetitive patterns of behavior [1]. Aside from the core symptoms, ASD displays a higher burden of co-occurring medical conditions such as epilepsy, psychiatric/behavioral complaints, and gastrointestinal disorders, especially in subjects with intellectual disability [2,3]. Among the different factors, immune profile dysregulation during early life is considered a major factor contributing to ASD pathogenesis [4] as also supported by increased levels of several types of cytokines [5].

A growing body of current literature suggests that, in some cases, motor skill impairments can emerge before the onset of core diagnostic symptoms in ASD [6,7]. For this reason, motor abnormalities are indicated as an ASD behavioral biomarker, regardless of the level of symptom severity and age [8]. Recent findings have reported that ASD children suffer from higher levels of anxiety associated with alterations in amygdala subnuclei structure, which may in turn reflect morphological changes in the neurocircuitry of anxiety [9]. In addition, it has been shown that both motor deficits [10] and anxiety [11] are related to social communication and social motivation among individuals with ASD. While the cerebellum (Cb) has long been recognized for its role in motor coordination, emerging evidence suggests that it also contributes to a range of nonmotor functions, notably acting as a modulator of anxiety responses [12,13].

In the case of an abnormal cerebellar development, its atypical structure and altered functional connectivity may be a pri-

* Corresponding author at: Ponte Pietro Bucci 4B, Arcavacata di Rende, Cosenza 87030, Italy
E-mail address: raffaella.alo@unical.it (R. Alò).

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mary risk for ASD [14,15]. Indeed, increased excitability of presynaptic interneurons and decreased intrinsic excitability of postsynaptic Purkinje neurons result in low Purkinje cell firing rates in Cb of BTBRs [16]. Moreover, a marked disruption in the histological structure of the cerebellar cortex with significant decrease in the number and surface area of Purkinje cells have been revealed in both the adolescent and adult rats treated with valproic acid (VPA) [17]. Other studies on the same experimental model have documented signs of neuronal degeneration, such as vacuolations, irregularly shrunken with dark small heterochromatic nuclei and apoptotic blebs in the Purkinje and granule cells with vacuolated cerebellar glomeruli, alongside rarefaction of the myelinated nerve fibers and loss of their neurofilaments [18]. Moreover, hypomyelination in specific cortical and subcortical regions may affect brain connectivity, since white matter integrity correlates with the severity of the disorder, suggesting an important role for myelin in ASD [19].

Recently, ketogenic diet (KD), a high-fat, adequate-protein, low-carbohydrate diet, is considered a valuable tool for balanced pro and antioxidant processes [20], since it forces the body to use fats and proteins as a source of energy thereby leading to diminished inflammatory events of numerous neurodegenerative syndromes [21]. Furthermore, KD can improve behaviors in ASD by modulating inflammation and by changing composition of the intestinal microbiota [22,23]. Evidence also indicates that KD is beneficial for individuals with ASD, especially for the mitigation of symptoms like anxiety, attention, cognition, language/communication, social interactions, and understanding [24]. However, the interplay between KD-mediated neuroprotection, Cb dysfunction, and their downstream effects on motor impairments, anxiety-like behaviors, neuroinflammation, and axonal myelination remains a critical knowledge gap.

On this basis, we have hypothesized that such dietary intervention could support cerebellar myelination and exert anti-inflammatory effects, thereby improving both motor and anxiety-like symptoms in ASD. Therefore, the aim of the current study was to investigate KD effects on neurobehavioral activities in two mouse strains, and namely BTBR-T+ Itpr3 tfl/J (BTBR), a validated model for idiopathic autism [25], and the highly social C57BL6/J (C57) strain. Specifically, locomotion, exploratory and anxiety-related behaviors were evaluated by using the modified beam-walking (MBW) and the open field (OFT) tests. In addition, the proinflammatory cytokines TNF-α, IL-6, and IL-1β were detected. Regarding axonal myelination, some ultrathin sections of Cb were analyzed by using transmission electron microscopy (TEM) and measuring myelin g-ratio (the ratio of the inner-to-outer diameter of myelinated axons). Such a parameter was correlated to the expression of some classical isoforms (21.5-, 18.5/17-, and 14-kDa) of the myelin basic protein (MBP), which is essential for elaboration and maintenance of myelin sheath in the central nervous system (CNS), and its reduced accumulation results in hypomyelination [26]. Overall, differences in behavioral/inflammatory profiles would help us to strengthen the effectiveness of KD on Cb in relation to motor skills and anxiety behaviors in ASD. Furthermore, studies on ultrastructural morphology could provide new insights into the pathogenetic mechanisms of ASD and a possible role of KD as an appropriate therapeutic approach to reduce hypomyelination in ASD.

2. Materials and methods

2.1. Animals and diet

Male offspring of BTBR breeding pairs and the social counterparts C57 (Jackson Laboratory, Bar Harbor, ME, USA) were housed in plastic cages (28 cm wide×17 cm long×12 cm high) at controlled humidity (60%) and temperature (22±2°C) with a cycle of 12-h light/dark (lights on at 07:30 AM) plus free access to food and water. The offspring were kept with the dam (who had free access to food and water) until weaning at postnatal day 22±1. Afterwards, mice of both strains (n=32) were grouped 3–5 per cage and assigned randomly to a standard chow diet (CD, 2019 Teklad Global Diet—9% fat, 19% protein, and 44.9% carbohydrate; Envigo RMS, Udine, Italy) or KD (PF4390 diet, 67.70% fat, 15.90% protein, and 1% carbohydrate; Mucedola, Milan, Italy) for 5 weeks, as recently reported [23]. Animal maintenance and experimental procedures were carried out in compliance with ethical provisions for care and use of Laboratory Animals reported in the legislative law no. 26 (04-03-2014) and authorized by the National Committee of the Italian Ministry of Health, approval number (n. 678/2019-PR, 08-10-2019). All measures were taken to minimize animal suffering and reduce the number of experiments.

2.2. Behavioral tests

At the end of 4 weeks of treatment, eight mice for each group were assessed for the different behavioral tests that lasted for 1 week until the end of KD treatment. Testing was conducted between 10:00 AM and 5:00 PM in an appropriate behavioral testing room in which mice were acclimatized for 60 min prior to each test. Motor activity and anxiety-like behaviors were evaluated by both MBW test and OFT. After each session, devices were cleaned with 70% ethanol and then water to prevent the influence of any lingering stimuli. Behavioral data were recorded and analyzed using ANY-maze software (version 7.20, Stoelting Co., Wood Dale, IL, USA).

2.2.1. MBW test

The apparatus consisted of a narrow beam measuring 120 cm in length and 5 cm in diameter, resting 50 cm above a tabletop on two poles. The beam contained dashed markings spaced 10 cm apart. At the end of the beam, a darkened box was placed as arrival point. The safe box had a circular opening of ~15 cm in diameter, which allowed the subject to enter easily. The black-colored safe box mimics the Elevated Plus Maze enclosed arms [27] thus, since the beam was narrow and elevated, MBW test was also anxiogenic [28].

Before the first trial, mice were acclimated to the behavioral room for 30 min and 1 day prior to testing, mice were trained to cross the beam in the same conditions as the testing (three trials per animal, with a 10 min interval between each trial according to Sweis et al. [29] with some modifications). During the test, mice were placed at the end of the beam opposite the safe box and the time required to cross to the escape box at the other end plus the number of times the animal travelled along the cylindrical axis for its entire length were measured. The stopwatch started when the nose of the mouse began to cross the beam and stopped when the animal reached the escape box. Mice were then left in the safe box for 5 min after crossing the beam examining peeking behaviors from the safe box.

2.2.2. OFT

OFT was used to assay spontaneous locomotor activity, as a general measure of motor function as well as anxious behavior. The apparatus consisted of a square arena (42×42×30 cm) polyvinyl chloride box. The floor was divided by white lines: it had an inner zone (25×25 cm) and an outer zone [30]. The OFT was lit by a neon tube that yielded about 180 lux in the center of the field. All mice were accustomed to the apparatus 2 days before the test. On

the test day, each mouse was placed in the central zone of the apparatus. Each animal's behavior was recorded for 5 min by a photo camera placed at the top of the apparatus. The following parameters were scored: time (s) spent in the central zone, distance travelled (cm), and average speed (cm/s). Higher levels of anxiety corresponded to less time in the center and more time at the periphery. Conversely, increased duration in the central zone was indicative of lower anxiety and greater exploratory behaviors.

2.3. Pro-inflammatory cytokine assessment

The proinflammatory profile of the Cb was assessed by quantifying the key cytokines TNF-α, IL-1β, and IL-6. Cb tissues of both hemispheres were dissected on ice from mice (n=6 per group) across each experimental condition, as also indicated by others [31]. Tissues were then processed for cytokine analysis, following manufacturers' protocols and as previously described [32]. Specifically, tissues were cut into ~ 0.5–1 mm pieces and homogenized by using a T10 basic IKA Ultra-Turrax in 1× PBS or in the lysis buffer provided with the respective assay kits—according to the recommended tissue-to-buffer (w/v) ratio—supplemented with a protease inhibitor cocktail, and stored overnight at ≤−20°C. Homogenates were then subjected to two freeze-thaw cycles, followed by centrifugation at 5,000×g for 5 min. The resulting supernatants were collected and assayed immediately. TNF-α levels were measured using the "Mouse TNF alpha ELISA Kit" (MBS825075; MyBioSource, San Diego, USA), IL-1β using the "Mouse IL-1β ELISA Kit" (RAB0275; Sigma Aldrich, St. Louis, MO, USA), and IL-6 using the "Mouse IL-6 ELISA Kit" (RAB0309; Sigma Aldrich). Absorbance readings were obtained using a microplate reader (Multiskan SkyHigh, Thermo Fisher Scientific Inc., Waltham, MA, USA).

2.4. Myelination analysis

For TEM myelination analysis, Cb samples (n=6 per group) were collected from all animals immediately after sacrifice. Tissue specimens (~1 mm^3) were fixed by immersion in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 2 h at 4°C.

Following primary fixation, samples were rinsed in the same buffer and postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 2 h at 4°C. After rinsing, tissues were dehydrated through a graded acetone series and infiltrated with increasing concentrations of epoxy resin prior to embedding in Epoxy resin (Sigma-Aldrich). Polymerization was performed at 60°C for 72 h. Semithin sections (0.5–1 μm) were cut and stained with toluidine blue for light microscopic evaluation and selection of representative areas. Ultrathin sections (~70–90 nm) were then obtained using a PT-PC PowerTome ultramicrotome (RMC Boeckeler) and mounted on copper grids. Importantly, ultrathin sections were not contrasted with uranyl acetate or lead citrate. Adequate image contrast was achieved by optimizing the objective aperture settings of TEM, allowing clear visualization of axons and myelin sheaths without additional staining.

Sections were examined with a JEOL JEM-1400 Plus TEM operating at 80 kV. Digital images were acquired at standardized magnifications for quantitative analysis.

The average number of g-ratios analyzed for each experimental group ranged from 60–81 measures. ImageJ software was used to manually measure g-ratio, which is conventionally defined as the ratio of the inner radius (representing the axon) to the outer radius (including the myelin sheath). The myelin g-ratio was calculated for each axon as the ratio between the inner (d) and the outer (D) diameter of the myelin sheath: g-ratio=d/D [33]. The g-ratio is widely utilized as a functional and structural index of myelin sheath thickness of individual axon fibers. The smaller the g-ratio, the thicker the myelin sheath layer.

2.5. Myelin basic protein isoforms

Cb tissues (n=6/group) were quickly removed and homogenized in ice-cold RIPA lysis buffer supplemented with a mixture of protease and phosphatase inhibitors and centrifuged at 15,000g for 20 min at 4°C, as reported in previous studies [34,35]. The supernatant was collected and used to determine the total protein concentration with a Bradford assay (Bio-Rad Protein Assay, Hercules, CA, USA) using bovine serum albumin as a standard. Equal amounts of proteins (40 μg) were separated on 15% SDS-PAGE gels for MBP and transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). After incubation, in a blocking solution (5% dry nonfat milk, Sigma-Aldrich), membranes were incubated overnight at 4°C shaking with the primary antibody anti-MBP (A85322, antibodies, Stockholm, Sweden). β-Actin antibody (1:1,000) (SC-47778 C4) was used as loading control. Subsequently, the membranes were washed in TBS with 0.1% Tween-20 and incubated with the peroxidase-linked secondary antibody at room temperature for 1 h (antimouse, diluted 1:1,000). Immunodetection was visualized by a Clarity Western ECL Substrate (Bio-rad) using an imaging system (ChemiDoc MP Imaging Systems, Bio-Rad Laboratories). Densitometric analysis was carried out by ImageJ software (National Institutes of Health, Bethesda, MD, USA), as previously reported [35–37].

2.6. Statistical analysis

Statistical evaluations were performed by using two-way ANOVA followed by *post hoc* Bonferroni's multiple range test, when P<.05. Data are expressed as mean±standard error of the mean (SEM) and P value less than .05 was considered statistically significant. The statistical analyses were all performed using the Software GraphPad Prism 5.

3. Results

3.1. KD effect on motor and anxiety-like behaviors

3.1.1. MBW test

In the present study, MBW test was used to evaluate both motor performances and anxiety-like behaviors. First of all, a significant interaction between strain and diet was reported for the time spent to travel along the cylindrical axis (Fig. 1A; $F_{(1, 28)}$=29.00, P<.001) and the number of laps in the full length of the cylindrical axis (Fig. 1B; $F_{(1, 28)}$=21.00, P<.001). Conversely, in the case of the number of peeking, only a main effect of diet (Fig. 1C; $F_{(1, 28)}$=19.80, P<.001) and strain ($F_{(1, 28)}$=8.515; P=.007) was detected.

Interestingly, KD was effective in reducing the time spent travelling along the cylindrical axis and reaching the safe box in BTBR as compared with the same mice strain fed with CD (P<.001; Fig. 1A). This was largely noticeable if we consider that this parameter turned out to be substantially higher (P<.001) in BTBR CD mice with respect to C57 CD. No difference was instead detected between C57 fed with CD and KD.

Concerning the number of laps along the cylindrical axis, a strong difference was observed between the two strains treated with a standard diet (Fig. 1B). Specifically, the value of BTBR CD appeared to be significantly lower (P<.001) than C57 CD. When BTBR mice received KD, this number of laps was significantly enhanced (P<.001).

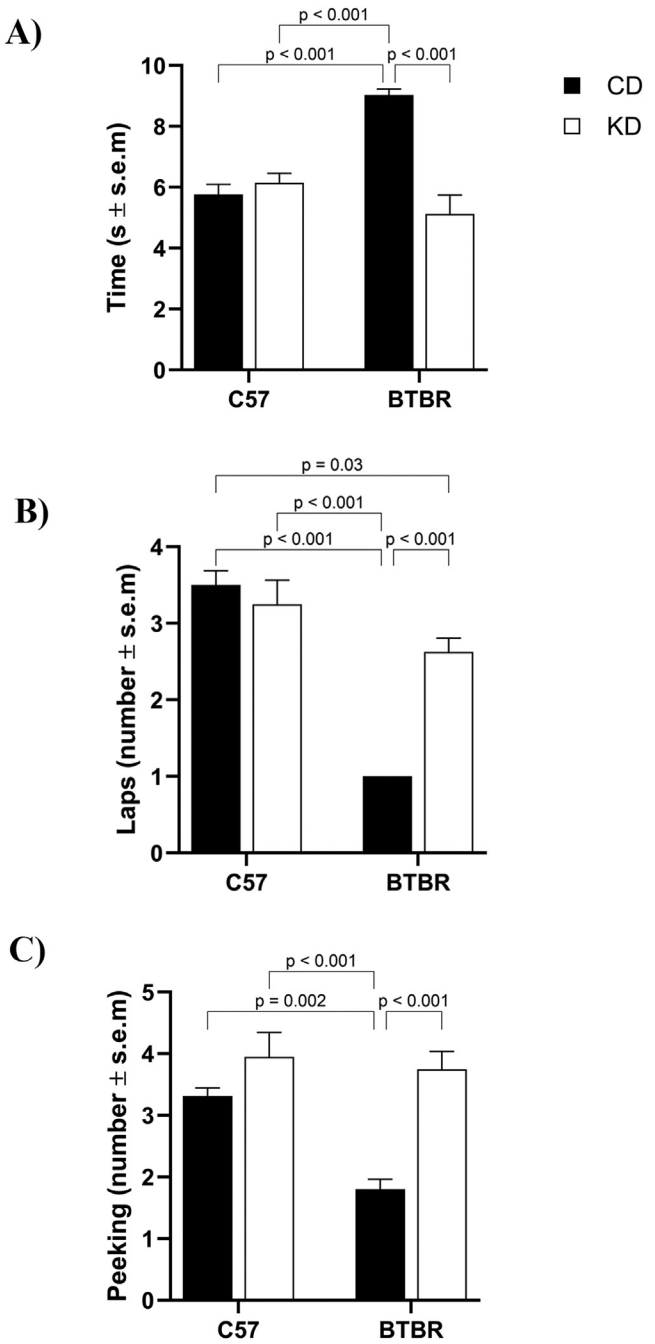

Fig. 1. Effect of ketogenic diet (KD) on motor and anxiety-like behaviors in the modified beam walking test. (A) Time (s±s.e.m.) spent in reaching the safe box and traveling the cylindrical axis, (B) number of laps (±s.e.m.) in the full length of the cylindrical axis and (C) number of peeks (±s.e.m.) were evaluated in both BTBR and C57 mice treated with a standard chow diet (CD) or KD (n=8/group). The data were statistically carried out by a two-way ANOVA with Bonferroni's multiple comparisons *post hoc* test to compare two variables when P<.05 as reported in Materials and Methods. Only statistically significant differences are indicated.

A similar trend was also observed for the number of peeks, with BTBR CD mice performing fewer (P<.01) peeks than C57 CD whereas BTBR KD mice showed a strong increase (P<.001) of this behavior compared with BTBR CD, which was close to the level of the C57 group (Fig. 1C). No significant variation was observed in C57 following treatment with KD (P>.05).

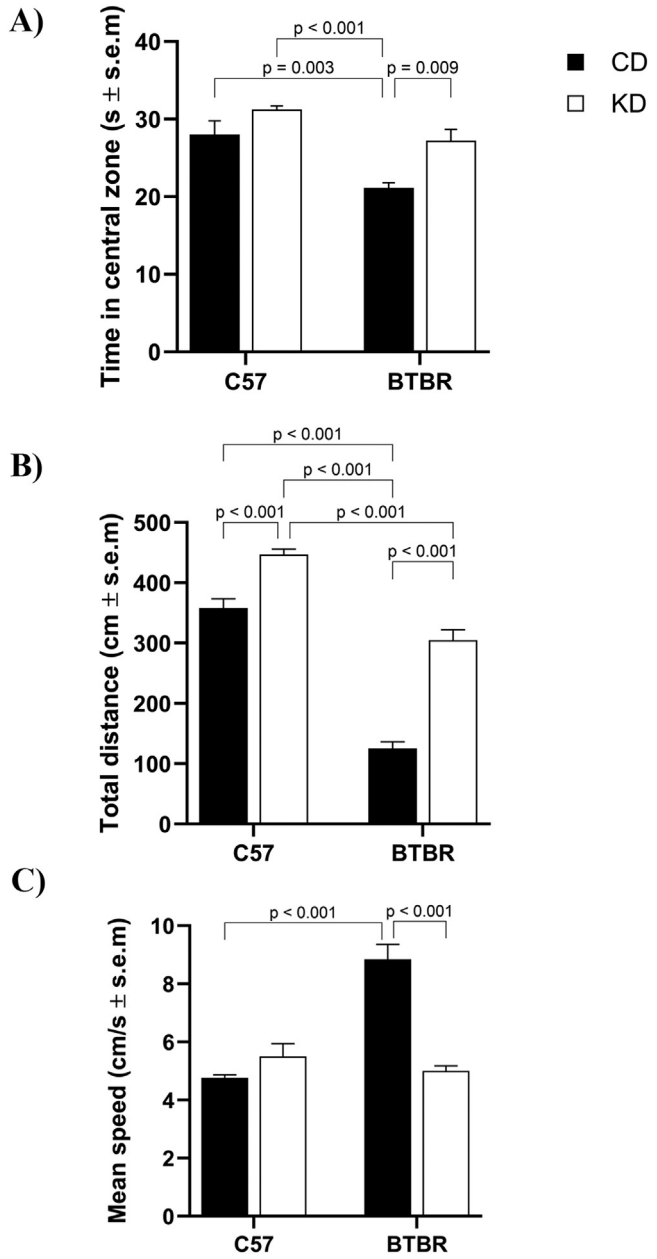

Fig. 2. Effect of ketogenic diet (KD) on motor and anxiety-like behaviors in the open field test. (A) Time spent (s±s.e.m.) in the central zone, (B) total distance traveled (cm±s.e.m.) and (C) mean speed (cm/s±s.e.m.) were evaluated in both BTBR and C57 mice treated with a standard chow diet (CD) or KD (n=8/group). The data were statistically carried out by a two-way ANOVA with Bonferroni's multiple comparisons *post hoc* test to compare two variables when P<.05 as reported in materials and methods. Only statistically significant differences are indicated.

3.1.2. OFT

Even OFT was used to assess the impact of KD on locomotor activity and anxiety-related behaviors of mice. An interaction between diet and strain was detected for the time spent in the central zone ($F_{(1, 28)}$=19.25; P<.001) and for the average speed ($F_{(1, 28)}$=42.17; P<.001). Conversely, only a main effect of the diet ($F_{(1, 28)}$=38.75; P<.001) and of the strain ($F_{(1, 28)}$=147.7; P<.001) was reported for the total distance traveled. *Post hoc* analysis demonstrated that BTBR CD mice spent less time in the central zone with respect to C57 CD (Fig. 2A; P<.01) while no significant differences were detected for the time spent in central zone in C57

KD mice compared with C57 CD ($P>.05$). On the other hand, BTBR mice treated with KD spent most of the time in the central zone with respect to BTBR CD (Fig. 2A; $P<.01$).

In the case of the total distance traveled, BTBR CD traveled a considerably shorter distance than C57 CD (Fig. 2B; $P<.001$). Even for this parameter, KD has proven to be significantly effective ($P<.001$) in increasing the total distance traveled by BTBR compared with its counterpart treated with CD (Fig. 2B).

Moreover, BTBR CD mice demonstrated significantly ($P<.001$) higher average speed than C57 mice treated with the same standard diet (Fig. 2C). However, statistical analyses showed that KD ameliorated motor behaviors in BTBR mice by also decreasing ($P<.001$) the average speed when compared to BTBR mice treated with CD.

3.2. KD effect on cerebellar pro-inflammatory cytokines

TNF-α, IL-1β, and IL-6 levels were analyzed in Cb of the different experimental groups. In the case of TNF-α, although no interaction effect was observed ($F_{(1, 20)}=3.069$; $P=10$), a significant main effect was recorded for both strain ($F_{(1, 20)}=26.33$; $P<.001$) and diet ($F_{(1, 20)}=5.218$, $P=.03$). Notably, TNF-α levels were markedly higher in BTBR CD mice compared with C57 ($P<.001$). Following KD exposure, this cytokine showed a significant reduction ($P<.05$) in BTBR mice but not at the same level of C57 ($P<.001$; Fig. 3A).

Similarly, for IL-1β (Fig. 3B) no interaction effect was observed ($F_{(1, 20)}=3.323$; $P=0.08$), although a significant main effect was detected for both strain ($F_{(1, 20)} = 33.24$; $P<.001$) and diet ($F_{(1, 20)}=5.71$; $P=.03$). *Post hoc* analysis revealed that even this cytokine was upregulated in BTBR CD when compared with C57 CD and C57 KD ($P<.001$). On the contrary, there was a significant reduction of IL-1β levels in BTBR KD compared with BTBR CD ($P<.05$).

The evaluation of IL-6 (Fig. 3C) revealed only a main effect of strain, with no significant effects attributed to the diet or interaction for both (strain×diet: $F_{(1, 20)}=2.087$; $P=0.16$ Diet: $F_{(1, 20)}=2.694$, $P=.12$ Strain: $F_{(1, 20)}=54.03$; $P<.001$). Indeed, KD tended to reduce ($P<.05$) the elevated IL-6 levels observed in BTBR CD mice even when compared with C57 controls ($P<.001$).

3.3. Relationship among KD diet, myelin sheath thickness, and MBP

To assess whether KD was able to modulate the thickness of the myelin sheath, we evaluated the myelinated axons ultrastructure of C57 CD and C57 KD (Fig. 4A and B, respectively) as well as BTBR CD and BTBR KD (Fig. 4C and D), and calculated g-ratios (Fig. 4E). From the TEM analysis, it appeared that myelin thickness, as measured in ultrathin Cb sections, was influenced by KD ($F_{(1, 96)}=117.6$; $P<.001$). Indeed, BTBR CD mice had a significantly higher ($P<.001$) g-ratio compared to C57 CD (Fig. 4E). In contrast, a significant reduction in the g-ratio value occurred in C57 KD compared with C57 CD ($P<.01$) and, above all, in BTBR KD compared with BTBR CD ($P<.001$).

For this part, we also conducted a Western blot analysis to determine KD effects on the expression levels of the main MBP isoforms in Cb of all experimental groups (Fig. 5A). Such an evaluation revealed that BTBR mice exposed to CD were featured by a general downregulation ($P<.05$) of all MBP isoforms, *i.e.*, 21.5 kDa (Fig. 5B), 18.5/17-kDa (Fig. 5C), and 14 kDa (Fig. 5D) when compared with C57 CD mice. Noteworthy, an increase ($P<.05$) of 18.5/17-kDa isoform was observed in BTBR after KD treatment compared with BTBR CD mice.

No significant variation was observed for the other two MBP isoforms following exposure to KD.

4. Discussion

The present work correlates, for the first time, the impact of KD on motor and anxiety disorders to neuroinflammation and axonal myelination in Cb of an idiopathic model of ASD, the BTBR mouse. A growing number of reports highlighted the effects of KD on a variety of neurological and non-neurological disorders, including core symptoms and comorbidities of ASD [38,24], but until now little is known about KD effects on motor and anxiety behaviors. In view of this, 3-week-old BTBR mice were fed KD for 5 weeks, which overlaps with adolescence, a critical period for the maturation of neural circuits [39] and myelin plasticity [40]. The adolescence period is beginning to receive considerable attention since recently it has been reported that different motor assessments lead to differing patterns of age-related group differences in motor skills of autistic youth compared with nonautistic youth [41]. Additionally, since autism-relevant behavioral traits of the BTBR mouse model are already reliably detectable in early postweaning and adolescent stages [42], this time window may be considered ideal for evaluating environmental interventions.

Here, we demonstrated that a 5-week treatment of KD promotes motor function and modulates anxiety-related activities in BTBR mice. When fed CD, they displayed a tendency to take longer time to travel to the beam of the MBW and to reach the safe box, even though they did not show loss of balance (*i.e.*, absence of slips). Such feature may be correlated to poor sensorimotor integration of this experimental model as reflected by a shorter stride length causing a greater latency (*i.e.*, time spent to traverse the bar) on the balance beam task [43]. Other studies reported similar movement impairments when BTBR were subjected to the rotarod test. It was found that, instead of concentrating on the motor learning, animals ignored the unstable rotating rod exhibiting a latency to fall from the accelerated rod [42]. Additionally, our BTBR mice exhibited a fewer number of times in which they travelled the entire length of the cylindrical axis (laps) on the MBW test and similarly they peeked or fully emerged out of the safe box less. Such a behavioral feature may be considered a novel anxiety index that captures similar aspects to the standard anxiety index obtained from the elevated plus maze [44]. Moreover, both the time spent in the central zone and the total distance traveled in OFT were reduced in BTBR CD compared with C57 mice indicating that BTBR strain is more anxious than C57 and this is in accordance with other studies [45]. Contextually, the average speed was increased thus suggesting greater hyperactivity [46]. Nonetheless, others reported a lower level of anxiety in adolescence BTBRs since they are more prone to risk taking, impulsive behavior, and novelty seeking rather than juvenile and adult rodents [47,48]. Even though these parameters are somewhat different among research groups [49], possibly due to differences in experimental procedures, our behavioral data plus other indications [50] support an evident anxiogenic profile of BTBR that, in addition to ASD-like behaviors, make it a valid model for ASD with psychiatric comorbidity [3].

Interestingly, marked improvements occurred after KD administration in BTBR mice compared with BTBR CD and C57 in both MBW test as well as in OFT. In particular, KD accounted for a reduction in anxiety and improvements in motor performance. This should not be so surprising since it has been widely demonstrated in both preclinical and clinical studies that KD has evident effects on mood, cognitive function and depression severity [51] with fewer adverse effects than traditional psychiatric/seizure medication [24]. Recently, in a pilot study, mediterranean diet plus KD accounted for reduced pain frequency and intensity in patients with chronic migraine [52]. Moreover, our results are also

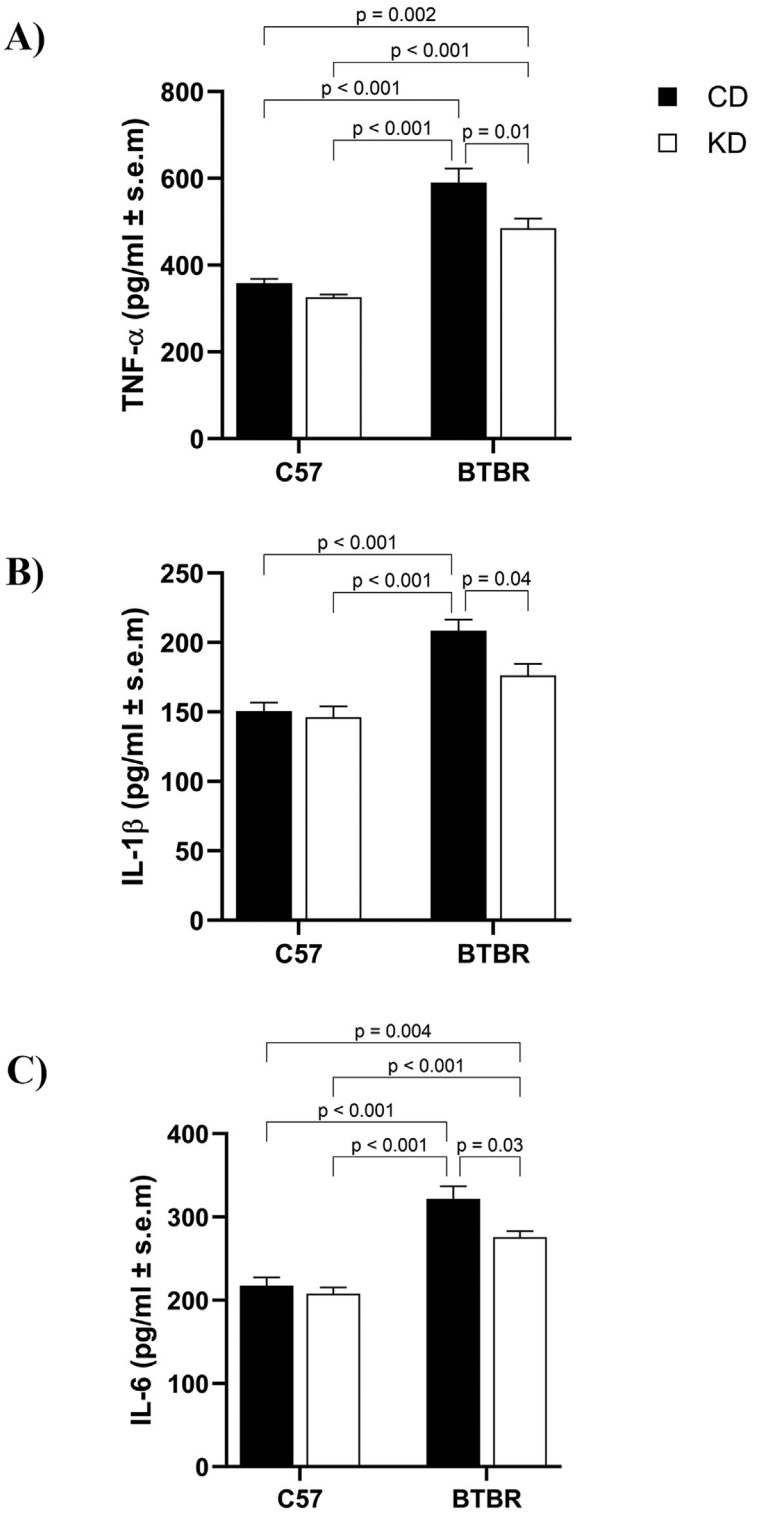

Fig. 3. Effect of ketogenic diet (KD) on the levels of key pro-inflammatory cytokines. Data were expressed as pg/mL (±s.e.m.) of TNF-α (A), IL-1β (B), and IL-6 (C) in the Cb of both BTBR and C57 mice fed a standard chow diet (CD) or KD (n=6/group). The data were statistically carried out by a two-way ANOVA with Bonferroni's multiple comparisons *post hoc* test to compare two variables when P<.05 as reported in materials and methods. Only statistically significant differences are indicated.

in line with KD being able to restore aberrant cortical motor maps and excitation-to-inhibition imbalance in BTBR mice [53]. Parallelly, administration of a ketone ester-enriched diet ameliorates motor deficits in a mouse model of Parkinson's disease [54].

As is widely known, the relationship between KD and ASD may be mediated in part by inflammatory markers [55,56], influencing neurogenesis, synaptogenesis and neuronal physiology related to behavioral alterations in different neurological conditions [57]. The delicate balance between pro and anti-inflammatory signals is

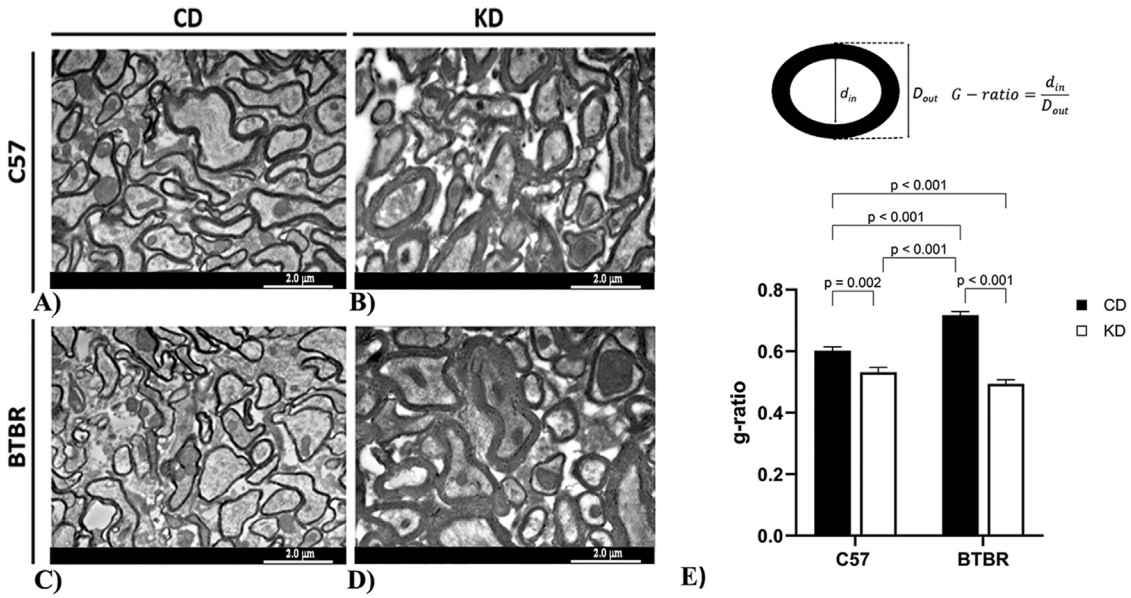

Fig. 4. Effect of ketogenic diet (KD) on the myelination of cerebellar axons. Representative TEM photograms of coronal sections (×6,000 and scale bar 2 µm) show cerebellar axons for both C57 (A and B) and BTBR (C and D) mice fed a standard chow diet (CD; A and C) or KD (B and D) (n=6/group). g-ratio (±s.e.m.) was calculated as the ratio of the inner (din) to outer (dout) diameter of myelinated axons (E) in all experimental groups. The data were statistically carried out by a two-way ANOVA with Bonferroni's multiple comparisons *post hoc* test to compare two variables when P<.05 as reported in materials and methods. Only statistically significant differences are indicated.

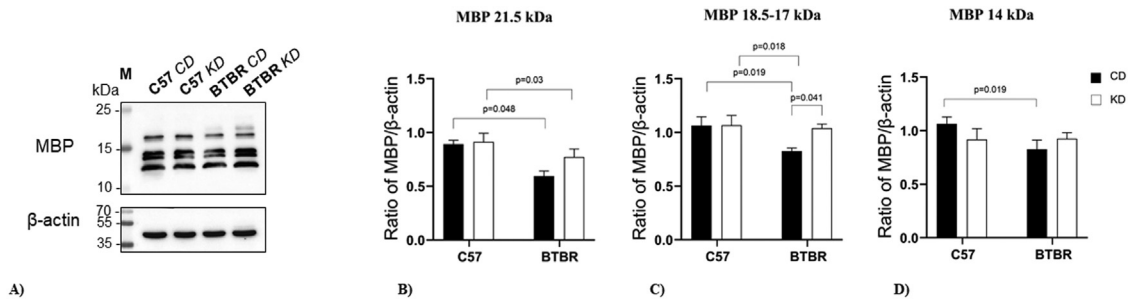

Fig. 5. Effect of ketogenic diet (KD) on different myelin basic protein (MBP) isoforms. (A) Representative immunoblots of MBP isoforms along with the loading control (β-actin) of both C57 and BTBR fed a standard chow diet (CD) or KD. Densitometric analysis of 21.5-kDa (B), 18.5/17-kDa, (C) and 14-kDa (D) MBP isoforms. Data are expressed as the mean±s.e.m. (n=6/group). The data were statistically carried out by a two-way ANOVA with Bonferroni's multiple comparisons *post hoc* test to compare two variables when P<.05 as reported in materials and methods. Only statistically significant differences are indicated.

fundamental for the correct proliferation, differentiation, and myelination of the oligodendrocyte lineage cells. Microglia affect oligodendrocyte development through cytokines, growth factors, and phagocytosis. Environmental factors may disrupt these regulatory processes, thus altering white matter development so leading to ASD symptomatology in social communication and sensorymotor function [58]. A typical trajectory of white matter development, observed in ASD with respect to age-matched neurotypical peers, is an initial hypermyelination at early postnatal stages, followed by marked hypomyelination, which lasts throughout lifespan.

In this regard, we observed a great variability of the g-ratio, a parameter widely used as a functional and structural index of the optimal axonal myelination [34]. Specifically, BTBR CD mice had a higher g-ratio compared with C57 CD and so, in the present study, a thinner myelin sheath layer was reported for the mouse model of idiopathic ASD. Such indications are fully in line with other studies in which myelination was significantly downregulated in BTBR mice compared with C57 mice [59]. Moreover, even the VPA-treated rats showed signs of neuronal degeneration, as indicated by the rarefaction and loss of neurofilaments in myelinated nerve fibers [18]. Recently, a significant increase in myelinated axon density, accompanied by thinner myelin sheaths, has been reported in the corpus callosum of Shank3B ASD mouse model compared with the wild type [60]. These ultrastructural alterations were widespread across the corpus callosum, suggesting a global disruption of myelinated axon integrity. In contrast, a significant reduction of the g-ratio value occurred in BTBR treated with KD, further supporting an important role of such a treatment in ASD pathophysiology. Indeed, some genetic modifications linked to this specific diet intervention were found in BTBR, which after exposure to KD showed temporal cortex and hippocampus improvements associated with myelin formation and white matter development [61]. However, recent evidence indicated that there is a significant increase in the expression of SOX2-dependent oligodendrocyte/myelination markers but only with a long-term KD intervention, at least in the aging mouse brain [62]. Hence, proinflammatory cytokines may be indicated as potent biomarkers of ASD [63] in which motor and emotional impairments may be considered signs of cerebellar neuroinflammation [64]. This brain area belongs to the main circuits that orchestrate not only motor activities but also cognitive processing and social behavior [65,66], to-

gether with anxiety responses [13,67], even in relation to ASD [68]. Interestingly in this study, dietary intervention with KD reduced the levels of the proinflammatory cytokines TNF-α, IL-6 and IL-1β in Cb of BTBR mice. Such an effect was similar to some functional foods that alleviate behavioral alterations, reversing the disorganization of the Cb and improving TLR-4/Myd88/NF-κB signaling cascade in VPA-induced autism [69]. The normalization of proinflammatory cytokine levels in this brain area is correlated to improvements of motor/exploratory performance, as previously reported for experimental autoimmune encephalomyelitis mouse model in which KD prevented motor deficiency, suppressed microglial activation and inhibit demyelination [70].

Emerging studies link neuroinflammation to altered white matter trajectories [71] like in our study. Our attention was focused on Cb expression patterns of the classical isoforms (from 14–21.5 kDa) of MBP. MBP is the major myelin protein in CNS that interacts not only with structural membrane-associated and cytoskeletal proteins, but also with proteins involved in protein expression, including those of the transcription-translation machinery, as well as with mitochondrial proteins, thus playing a regulatory role in the myelination process [72]. Interestingly, a downregulation of all MBP isoforms has been reported in BTBR CD mice and this is in good agreement with significant temporal and regional differences with lower levels in the CNS of Shank3Δ11(−/−) [73]. Noteworthy is the reduction of both 18.5-kDa isoform, which predominates in mature myelinating oligodendrocytes, and 17.5-kDa isoform that alone can fulfill the functional tasks of all isoforms in the nervous system [74]. Reduction of such isoforms could be involved in the hypomyelination that we observed in Cb of BTBR CD as well as in the activation of microglia, which may affect oligodendrocyte development through alteration of the proinflammatory cytokines that, in turn, may impair motor and anxiety-like behaviors. This possibility has already been proposed in Fmr1-knockout mice during postnatal development [75]. Additionally, this is in line with histopathological examination of BTBR brain tissue that displayed a downregulation of myelin-related proteins together with reduced levels of staining with ferric alum, indicating myelin disruption [76]. Recently, histochemical and immunofluorescence data suggested age-related demyelination in the central tract of the vermis, as indicated by reduced level of MBP in the arbor vitae [77]. Surprisingly, only the expression of 18.5/17-kDa isoforms resulted to be increased in BTBR treated with KD. Indeed, it has been previously shown that KD was able to restore oligodendrocyte integrity and increase CNS myelination, therefore ameliorating axonal defects and normalizing motor functions in Pelizaeus–Merzbacher disease [78]. In this case, MBP isoform could be responsible for compaction of the myelin sheath in Cb of BTBR KD by interacting with lipid membranes since the lipid composition of the myelin leaflet has a high impact on the interaction between the membrane and MBP [79]. It must be underlined that an overexpression of 17.2 kDa MBP isoform, even when the only 17.2-kDa MBP isoform is present, is able to elicit CNS myelination in transgenic shiverer mice [80]. Moreover, the unmodified C1 component of 18.5 kDa bovine MBP can assemble tubulin into microtubules in a dose-dependent manner via filamentous intermediates thereby simultaneously promoting the formation of microtubule bundles [81]. Such a condition is essential for a correct microtubules' stability and dynamics, including axonal transport, cell signaling, and overall neuronal integrity.

Overall, our findings imply that Cb is sensitive to KD therapeutic intervention for ASD motor and anxiety-related symptoms, also emphasizing its modulatory role on neuroinflammation, as previously reported for other brain regions [23]. In addition, we further underline the crucial role of the different MBP isoforms in the remyelination capacity of Cb in a validated mouse model of ASD during a crucial time window such as adolescence. Given the increasing evidence on individuals with ASD that exhibit impairments in white matter development and integrity [82,83], this study may represent a first preclinical step towards the understanding of the mechanisms by which metabolic interventions, such as KD, promote myelin plasticity and remyelination processes in ASD. This is in line with previous observations showing that, in another rat model of diffuse axonal injury, ketone bodies were able to protect myelin-forming oligodendrocytes and reduce axonal damage [84]. Moreover, the observed modulation of inflammatory markers supports the hypothesis that ketogenic metabolic states may exert beneficial effects through the attenuation of processes related to neuroinflammation very likely by remodeling gut–brain axis, as reported not only in animal models [23] but also in humans [22,85]. Indeed, it has been shown that KD provided a reduction in *Firmicutes, Bacteroidetes*, and *Proteobacteria* and a consistent increment in the alpha biodiversity thus indicating a new strategy to improve ASD-related symptoms. Nevertheless, there are still disagreements about the impact of gut microbiota-based interventions on patients with ASD, since clinical studies have reported heterogeneous outcomes in terms of behavioral improvement and gastrointestinal symptom relief [86]. We are still at the beginning and further studies will be necessary to clarify the contribution of KD in myelin plasticity and to evaluate whether the involvement of cerebro-cerebellar circuits may represent viable therapeutic targets for improving neurodevelopmental outcomes in individuals with ASD.

Declaration of competing interests

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. The authors hereby affirm that they have no conflicts of interest.

CRediT authorship contribution statement

Raffaella Alò: Writing – review & editing, Writing – original draft, Validation, Funding acquisition, Data curation, Conceptualization. **Ilaria Olivito:** Methodology, Investigation, Formal analysis, Data curation. **Damiana Minervini:** Methodology, Investigation, Formal analysis, Data curation. **Ennio Avolio:** Methodology, Investigation, Data curation. **Anna De Bartolo:** Methodology, Investigation, Formal analysis, Data curation. **Ida Perrotta:** Writing – review & editing, Methodology, Investigation. **Maria Carmela Cerra:** Writing – review & editing, Formal analysis, Data curation. **Tommaso Angelone:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Carmine Rocca:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Rosa Maria Facciolo:** Writing – review & editing, Writing – original draft, Validation, Funding acquisition, Data curation, Conceptualization.

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References

[1] Kodak T, Bergmann S. Autism spectrum disorder: characteristics, associated behaviors, and early intervention. Ped Clin North Am 2020;67(3):525–35. doi:10.1016/j.pcl.2020.02.007.

[2] Alò R, Olivito I, Fazzari G, Zizza M, Di Vito A, Avolio E, et al. Correlation of distinct behaviors to the modified expression of cerebral Shank1,3 and BDNF in two autistic animal models. Behav Brain Res 2021;404:113165. doi:10.1016/j.bbr.2021.113165.

[3] Casanova MF, Frye RE, Gillberg C, Casanova EL. Comorbidity and autism spectrum disorder. Front Psychiatry 2020;11:617395. doi:10.3389/fpsyt.2020.617395.

[4] Robinson-Agramonte MLA, Noris García E, Fraga Guerra J, Vega Hurtado Y, Antonucci N, Semprún-Hernández N, et al. Immune dysregulation in autism spectrum disorder: what do we know about it? Int J Mol Sci 2022;23(6):3033. doi:10.3390/ijms23063033.

[5] Palanivelu L, Chen YY, Chang CJ, Liang YW, Tseng HY, Li SJ, et al. Investigating brain-gut microbiota dynamics and inflammatory processes in an autistic-like rat model using MRI biomarkers during childhood and adolescence. Neuroimage 2024;302:120899. doi:10.1016/j.neuroimage.2024.120899.

[6] Bhat AN. Is motor impairment in autism spectrum disorder distinct from developmental coordination disorder? A report from the SPARK study. Phys Ther 2020;100(4):633–44. doi:10.1093/ptj/pzz190.

[7] Wang LAL, Petrulla V, Zampella CJ, Waller R, Schultz RT. Gross motor impairment and its relation to social skills in autism spectrum disorder: a systematic review and two meta-analyses. Psychol Bull 2022;148(3–4):273–300. doi:10.1037/bul0000358.

[8] Minissi ME, Gómez-Zaragozá L, Marín-Morales J, Mantovani F, Sirera M, Abad L, et al. The whole-body motor skills of children with autism spectrum disorder taking goal-directed actions in virtual reality. Front Psychol 2023;14:140731. doi:10.3389/fpsyg.2023.1140731.

[9] Hennessy A, Seguin D, Correa S, Wang J, Martinez-Trujillo JC, Nicolson R, et al. Anxiety in children and youth with autism spectrum disorder and the association with amygdala subnuclei structure. Autism 2023;27(4):1053–67. doi:10.1177/13623613221127512.

[10] Ohara R, Kanejima Y, Kitamura M, Izawa KP. Association between social skills and motor skills in individuals with autism spectrum disorder: a systematic review. Eur J Investig Health Psychol Educ 2019;10(1):276–96. doi:10.3390/ejihpe10010022.

[11] Briot K, Jean F, Jouni A, Geoffray MM, Ly-Le Moal M, Umbricht D, et al. Social anxiety in children and adolescents with autism spectrum disorders contribute to impairments in social communication and social motivation. Front Psychiatry 2020;11:710. doi:10.3389/fpsyt.2020.00710.

[12] Chin PW, Augustine GJ. The cerebellum and anxiety. Front Cell Neurosci 2023;7:1130505. doi:10.3389/fncel.2023.1130505.

[13] Chin PW, Augustine GJ. Serotonergic input into the cerebellar cortex modulates anxiety-like behavior. J Neurosci 2025;45(14):e1825242024. doi:10.1523/JNEUROSCI.1825-24.2024.

[14] Biswas MS, Roy SK, Hasan R, Pk MMU. The crucial role of the cerebellum in autism spectrum disorder: neuroimaging, neurobiological, and anatomical insights. Health Sci Rep 2024;7(7):e2233. doi:10.1002/hsr2.2233.

[15] van der Heijden ME, Gill JS, Sillitoe RV. Abnormal cerebellar development in autism spectrum disorders. Dev Neurosci 2021;43(3–4):181–90. doi:10.1159/000515189.

[16] Chao OY, Marron Fernandez de Velasco E, Pathak SS, Maitra S, Zhang H, Duvick L, et al. Targeting inhibitory cerebellar circuitry to alleviate behavioral deficits in a mouse model for studying idiopathic autism. Neuropsychopharmacology 2020;45(7):1159–70. doi:10.1038/s41386-020-0656-5.

[17] Elsherif R, Mm Abdel-Hafez A, Hussein OA, Sabry D, Abdelzaher LA, Bayoumy AA. The potential ameliorative effect of mesenchymal stem cells-derived exosomes on cerebellar histopathology and their modifying role on PI3k-mTOR signaling in rat model of autism spectrum disorder. J Mol Histol 2025;56(1):65. doi:10.1007/s10735-024-10335-w.

[18] Mokhtar Tawfeek ES, Aly Abou Elez Gawish S, Hamed WS, Asker SA. Construction of an animal model of autism based on interaction between cerebellar histological, immunohistochemical, and biochemical changes in adult male albino rat. Ultrastruct Pathol 2025;49(1):39–57. doi:10.1080/01913123.2024.2438382.

[19] Heine VM, Dooves S. Neuroglia in autism spectrum disorders. Handb Clin Neurol 2025;210:303–11. doi:10.1016/b978-0-443-19102-2.00006-5.

[20] Hasan-Olive MM, Lauritzen KH, Ali M, Rasmussen LJ, Storm-Mathisen J, Bergersen LH. A ketogenic diet improves mitochondrial biogenesis and bioenergetics via the PGC1α-SIRT3-UCP2 axis. Neurochem Res 2019;44:22–37. doi:10.1007/s11064-018-2588-6.

[21] Jiang Z, Yin X, Wang M, Chen T, Wang Y, Gao Z, et al. Effects of ketogenic diet on neuroinflammation in neurodegenerative diseases. Aging Dis 2022;13(4):1146–65. doi:10.14336/ad.2021.1217.

[22] Allan NP, Yamamoto BY, Kunihiro BP, Nunokawa CKL, Rubas NC, Wells RK, et al. Ketogenic diet induced shifts in the gut microbiome associate with changes to inflammatory cytokines and brain-related miRNAs in children with autism spectrum disorder. Nutrients 2024;16(10):1401. doi:10.3390/nu16101401.

[23] Olivito I, Avolio E, Minervini D, Soda T, Rocca C, Angelone T, et al. Ketogenic diet ameliorates autism spectrum disorders-like behaviors via reduced inflammatory factors and microbiota remodeling in BTBR T+ Itpr3tf/J mice. Exp Neurol 2023;366:14432. doi:10.1016/j.expneurol.2023.114432.

[24] Schrickel A, Groeneweg J, Dekeyster E. Exploring the potential of the ketogenic diet in autism spectrum disorder: metabolic, genetic, and therapeutic insights. Metab Brain Dis 2025;40(1):94. doi:10.1007/s11011-024-01518-1.

[25] Arakawa H. Implication of the social function of excessive self-grooming behavior in BTBR T+ltpr3tf/J mice as an idiopathic model of autism. Physiol Behav 2021;237:113432. doi:10.1016/j.physbeh.2021.113432.

[26] Bagheri H, Friedman H, Hadwen A, Jarweh C, Cooper E, Oprea L, et al. Myelin basic protein mRNA levels affect myelin sheath dimensions, architecture, plasticity, and density of resident glial cells. Glia 2024;72(10):1893–914. doi:10.1002/glia.24589.

[27] Alò R, Zizza M, Fazzari G, Facciolo RM, Canonaco M. Genistein modifies hamster behavior and expression of inflammatory factors following subchronic unpredictable mild stress. Neuroendocrinology 2019;108(2):98–108. doi:10.1159/000495209.

[28] Urakawa S, Takamoto K, Hori E, Sakai N, Ono T, Nishijo H. Rearing in enriched environment increases parvalbumin-positive small neurons in the amygdala and decreases anxiety-like behavior of male rats. BMC Neurosci 2013;14:13. doi:10.1186/1471-2202-14-13.

[29] Sweis BM, Bachour SP, Brekke JA, Gewirtz JC, Sadeghi-Bazargani H, Hevesi M, et al. A modified beam-walking apparatus for assessment of anxiety in a rodent model of blast traumatic brain injury. Behav Brain Res 2016;296:149–56. doi:10.1016/j.bbr.2015.09.015.

[30] Kraeuter AK, Guest PC, Sarnyai Z. The open field test for measuring locomotor activity and anxiety-like behavior. Methods Mol Biol 2019;1916:99–103. doi:10.1007/978-1-4939-8994-2_9.

[31] Walker H, Frost N. Protocol for the generation of single-nuclei RNA-seq libraries and quantification of heterogeneous cell types activate during social interaction. STAR Protoc 2024;5(4):103395. doi:10.1016/j.xpro.2024.103395.

[32] Godbout JP, Chen J, Abraham J, Richwine AF, Berg BM, Kelley KW, et al. Exaggerated neuroinflammation and sickness behavior in aged mice after activation of the peripheral innate immune system. FASEB J 2005;19:1329–31. doi:10.1096/fj.05-3776fje.

[33] Wang Y, Sun B, Shibata B, Guo F. Transmission electron microscopic analysis of myelination in the murine central nervous system. STAR Protoc 2022;3(2):101304. doi:10.1016/j.xpro.2022.101304.

[34] De Bartolo A, Pasqua T, Romeo N, Rago V, Perrotta I, Giordano F, et al. The redox-active defensive selenoprotein T as a novel stress sensor protein playing a key role in the pathophysiology of heart failure. J Transl Med 2024;22(1):375. doi:10.1186/s12967-024-05192-w.

[35] Rocca C, Femminò S, Aquila G, Granieri MC, De Francesco EM, Pasqua T, et al. Notch1 mediates preconditioning protection induced by GPER in normotensive and hypertensive female rat hearts. Front Physiol 2018;9:521. doi:10.3389/fphys.2018.00521.

[36] De Bartolo A, Romeo N, Marrone A, Rago V, Granieri MC, Vommaro ML, et al. A recombinant fragment antigen-binding (Fab) of trastuzumab displays low cytotoxic profile in adult human cardiomyocytes: first evidence and the key implication of fcγriia receptor. Acta Pharmacol Sin 2025;46(3):618–31. doi:10.1038/s41401-024-01397-3.

[37] Rocca C, Grande F, Granieri MC, Colombo B, De Bartolo A, Giordano F, et al. The chromogranin A1-373 fragment reveals how a single change in the protein sequence exerts strong cardioregulatory effects by engaging neuropilin-1. Acta Physiol (Oxf) 2021;231(4):e13570. doi:10.1111/apha.13570.

[38] Öztürk E, Aslan Çin NN, Cansu A, Akyol A. Ketogenic diet as a therapeutic approach in autism spectrum disorder: a narrative review. Metab Brain Dis 2024;40(1):67. doi:10.1007/s11011-024-01506-5.

[39] Ijomone OK, Oria RS, Ijomone OM, Aschner M, Bornhorst J. Dopaminergic perturbation in the aetiology of neurodevelopmental disorders. Mol Neurobiol 2025;62(2):2420–34. doi:10.1007/s12035-024-04418-8.

[40] Adès N, Bouslama-Oueghlani L. Myelin dysfunction in autism spectrum disorder: insights into core symptoms and mechanisms of brain development. Mol Psychiatry 2026. doi:10.1038/s41380-026-03490-x.

[41] Block AR, Skaletski EC, Sheedy CM, Vanderpool EA, Travers BG. Motor performance in autistic youth from childhood through adolescence: evidence for both sustained and widening group differences. Autism Res 2026:e70211. doi:10.1002/aur.70211.

[42] Xiao R, Zhong H, Li X, Ma Y, Zhang R, Wang L, et al. Abnormal cerebellar development is involved in dystonia-like behaviors and motor dysfunction of autistic BTBR mice. Front Cell Dev Biol 2020;8:231. doi:10.3389/fcell.2020.00231.

[43] Faraji J, Karimi M, Lawrence C, Mohajerani MH, Metz GAS. Non-diagnostic symptoms in a mouse model of autism in relation to neuroanatomy: the BTBR strain reinvestigated. Transl Psychiatry 2018;8:234. doi:10.1038/s41398-018-0280-x.

[44] O'Connor R, van De Wouw M, Moloney GM, Ventura-Silva AP, O'Riordan K, Golubeva AK, et al. Strain differences in behaviour and immunity in aged mice: relevance to autism. Behav Brain Res 2021;399:113020. doi:10.1016/j.bbr.2020.113020.

[45] Zhang L, Xu X, Ma L, Wang X, Jin M, Li L, et al. Zinc water prevents autism-like behaviors in the BTBR mice. Biol Trace Elem Res 2023;201:4779–92. doi:10.1007/s12011-022-03548-1.

[46] Martin L, Sample H, Gregg M, Wood C. Validation of operant social motivation paradigms using BTBR T+tf/J and C57BL/6J inbred mouse strains. Brain Behav 2014;4:54–764. doi:10.1002/brb3.273.

[47] Reshetnikov VV, Ayriyants KA, Ryabushkina YA, Sozonov NG, Bondar NP. Sex-specific behavioral and structural alterations caused by early-life stress in C57BL/6 and BTBR mice. Behav Brain Res 2021;414:113489. doi:10.1016/j.bbr.2021.113489.

[48] Ryabushkina YA, Ayriyants KA, Sapronova AA, Mutovina AS, Kolesnikova MM, Mezhlumyan EV, et al. Effects of different types of induced neonatal inflammation on development and behavior of C57BL/6 and BTBR mice. Physiol Behav 2024;280:114550. doi:10.1016/j.physbeh.2024.114550.

[49] Eissa N, Jayaprakash P, Aljneibi S, Alsaadi A, Alzaabi S, Łazewska D, et al. Mitigating effects of H3 receptor antagonism on cerebellar autophagic pathways and behavioral phenotypes in BTBR T+ tf/J mouse model of autism spectrum disorder. Eur J Pharmacol 2025;997:177481. doi:10.1016/j.ejphar.2025.177481.
[50] Pobbe RL, Defensor EB, Pearson BL, Bolivar VJ, Blanchard DC, Blanchard RJ. General and social anxiety in the BTBR T+ tf/J mouse strain. Behav Brain Res 2011;216(1):446–51. doi:10.1016/j.bbr.2010.08.039.
[51] Shelp J, Chaves C, Terpstra A, Koning E, Gomes FA, Fabe J, et al. Perspectives on the ketogenic diet as a non-pharmacological intervention for major depressive disorder. Trends Psych Psychother 2025. doi:10.47626/2237-6089-2024-0932.
[52] Olivito I, Simona F, Tarsitano A, Pagliuso M, Tarantino C, De Lorenzo A, et al. Mediterranean ketogenic diet accounts for reduced pain frequency and intensity in patients with chronic migraine: a pilot study. Clin Nutr 2024;43(8):1781–7. doi:10.1016/j.clnu.2024.06.015.
[53] Smith J, Rho JM, Teskey GC. Ketogenic diet restores aberrant cortical motor maps and excitation-to-inhibition imbalance in the BTBR mouse model of autism spectrum disorder. Behav Brain Res 2016;304:67–70. doi:10.1016/j.bbr.2016.02.015.
[54] Mahajan VR, Nadel JA, King MT, Pawlosky RJ, Davis MI, Veech RL, et al. Ketone ester-enriched diet ameliorates motor and dopamine release deficits in MitoPark mice. Eur J Neurosci 2024;60(11):6875–90. doi:10.1111/ejn.16601.
[55] Avolio E, Olivito I, Leo A, De Matteo C, Guarnieri L, Bosco F, et al. Vasostatin-1 restores autistic disorders in an idiopathic autism model (BTBR T+ Itpr3tf/J mice) by decreasing hippocampal neuroinflammation. Prog Neuropsychopharmacol Biol Psychiatry 2024;135:111131. doi:10.1016/j.pnpbp.2024.111131.
[56] Noori AS, Rajabi P, Sargolzaei J, Alaghmand A. Correlation of biochemical markers and inflammatory cytokines in autism spectrum disorder (ASD). BMC Pediatr 2024;24(1):696. doi:10.1186/s12887-024-05182-3.
[57] Usui N, Kobayashi H, Shimada S. Neuroinflammation and oxidative stress in the pathogenesis of autism spectrum disorder. Int J Mol Sci 2023;24(6):5487. doi:10.3390/ijms24065487.
[58] Canada K, Evans TM, Pelphrey KA. Microglial regulation of white matter development and its disruption in autism spectrum disorder. Cereb Cortex 2025;35(4):bhaf109. doi:10.1093/cercor/bhaf109.
[59] Gong H, Lu Y, Deng SL, Lv KY, Luo J, Luo Y, et al. Targeting S100A9 attenuates social dysfunction by modulating neuroinflammation and myelination in a mouse model of autism. Pharmacol Res 2025;211:107568. doi:10.1016/j.phrs.2024.107568.
[60] Zhao G, Cheng A, Shi J, Shi P, Guo J, Yin C, et al. Large-scale EM data reveals myelinated axonal changes and altered connectivity in the corpus callosum of an autism mouse model. Front Neuroinform 2025;19:1563799. doi:10.3389/fninf.2025.1563799.
[61] Mychasiuk R, Rho JM. Genetic modifications associated with ketogenic diet treatment in the BTBRT+Tf/J mouse model of autism spectrum disorder. Autism Res 2017;10(3):456–71. doi:10.1002/aur.1682.
[62] Stratton MS, López-Domínguez JA, Canella A, Ramsey JJ, Cortopassi GA. Differential effects of short-term and long-term ketogenic diet on gene expression in the aging mouse brain. J Nutr Health Aging 2025;29(2):100427. doi:10.1016/j.jnha.2024.100427.
[63] Arteaga-Henríquez G, Gisbert L, Ramos-Quiroga JA. Immunoregulatory and/or anti-inflammatory agents for the management of core and associated symptoms in individuals with autism spectrum disorder: a narrative review of randomized, placebo-controlled trials. CNS Drugs 2023;37(3):215–29. doi:10.1007/s40263-023-00993-x.
[64] Eissa N, Sadeq A, Sasse A, Sadek B. Role of neuroinflammation in autism spectrum disorder and the emergence of brain histaminergic system. Lessons also for BPSD? Front Pharmacolol 2020;11:886. doi:10.3389/fphar.2020.00886.
[65] Kakei S, Tanaka H, Mitoma H, Manto M. The cerebellum as a predictor: recent insights into cognitive control and extension of Kalman filter theory to cognition. Discov Med 2025;37(196):791–800. doi:10.24976/discov.med.202537196.69.
[66] D'Angelo E. The cerebellum gets social. Science 2019;363:229. doi:10.1126/science.aaw2571.
[67] Alò R, Fazzari G, Zizza M, Avolio E, Di Vito A, Olvito I, et al. Emotional and spontaneous locomotor behaviors related to cerebellar daidzein-dependent TrkB expression changes in obese hamsters. Cerebellum 2023;22(4):698–707. doi:10.1007/s12311-022-01432-1.
[68] Rodrigues BA, Silva JDN, Kawamoto EM. Cerebellar alterations in autism spectrum disorder: a mini-review. Cerebellum 2025;24(2):52. doi:10.1007/s12311-025-01806-1.
[69] Molinari F, Fusco R, Siracusa R, D'Amico R, Impellizzeri D, Abdelhameed AS, et al. Functional foods alleviate behavioral alterations and improve GABAergic system regulating TLR-4/NF-κb axis in valproic-induced autism. Brain Behav 2025;15(6):e70591. doi:10.1002/brb3.70591.
[70] Sun W, Wang Q, Zhang R, Zhang N. Ketogenic diet attenuates neuroinflammation and induces conversion of M1 microglia to M2 in an EAE model of multiple sclerosis by regulating the NF-κb/NLRP3 pathway and inhibiting HDAC3 and P2X7R activation. Food Funct 2023;14(15):7247–69. doi:10.1039/d3fo00122a.
[71] Bozzi Y. Unraveling white matter alterations in autism: the role of oligodendrocytes, microglia, and neuroinflammation. Cereb Cortex 2025;35(4):bhaf094. doi:10.1093/cercor/bhaf094.
[72] Smirnova EV, Rakitina TV, Ziganshin RH, Arapidi GP, Saratov GA, Kudriaeva AA, et al. Comprehensive atlas of the myelin basic protein interaction landscape. Biomolecules 2021;11(11):1628. doi:10.3390/biom11111628.
[73] Malara M, Lutz AK, Incearap B, Bauer HF, Cursano S, Volbracht K, et al. SHANK3 deficiency leads to myelin defects in the central and peripheral nervous system. Cell Mol Life Sci 2022;79(7):371. doi:10.1007/s00018-022-04400-4.
[74] Harauz G, Boggs JM. Myelin management by the 18.5-kDa and 21.5-kDa classic myelin basic protein isoforms. J Neurochem 2013;125(3):334–61. doi:10.1111/jnc.12195.
[75] Pacey LK, Xuan IC, Guan S, Sussman D, Henkelman RM, Chen Y, et al. Delayed myelination in a mouse model of fragile X syndrome. Hum Mol Genet 2013;22(19):3920–30. doi:10.1093/hmg/ddt246.
[76] Wei H, Ma Y, Liu J, Ding C, Hu F, Yu L. Proteomic analysis of cortical brain tissue from the BTBR mouse model of autism: evidence for changes in STOP and myelin-related proteins. Neuroscience 2016;312:26–34. doi:10.1016/j.neuroscience.2015.11.003.
[77] Kútna V, O'Leary VB, Hoschl C, Ovsepian SV. Cerebellar demyelination and neurodegeneration associated with mTORC1 hyperactivity may contribute to the developmental onset of autism-like neurobehavioral phenotype in a rat model. Autism Res 2022;15(5):791–805. doi:10.1002/aur.2688.
[78] Stumpf SK, Berghoff SA, Trevisiol A, Spieth L, Düking T, Schneider LV, et al. Ketogenic diet ameliorates axonal defects and promotes myelination in Pelizaeus-Merzbacher disease. Acta Neuropathol 2019;138(1):673–4. doi:10.1007/s00401-019-01985-2.
[79] Träger J, Widder K, Kerth A, Harauz G, Hinderberger D. Effect of cholesterol and myelin basic protein (MBP) content on lipid monolayers mimicking the cytoplasmic membrane of myelin. Cells 2020;9:529. doi:10.3390/cells9030529.
[80] Kimura M, Sato M, Akatsuka A, Saito S, Ando K, Yokoyama M, et al. Overexpression of a minor component of myelin basic protein isoform (17.2 kDa) can restore myelinogenesis in transgenic shiverer mice. Brain Res 1998;785(2):245–52. doi:10.1016/s0006-8993(97)01383-8.
[81] Hill CM, Libich DS, Harauz G. Assembly of tubulin by classic myelin basic protein isoforms and regulation by post-translational modification. Biochemistry 2005;44(50):16672–83. doi:10.1021/bi050646+.
[82] Adès N, Bouslama-Oueghlani L. Myelin dysfunction in autism spectrum disorder: insights into core symptoms and mechanisms of brain development. Mol Psychiatry 2026. doi:10.1038/s41380-026-03490-x.
[83] Luo D, Zhao C, Nie L, Yin Y, Liu X, Yu H, et al. Alterations in white matter integrity and asymmetry in children with autism spectrum disorder: an automated fiber quantification tractography study. J Psychiatr Res 2026;194:312–20. doi:10.1016/j.jpsychires.2026.01.018.
[84] Mu J, Wang T, Li M, Guan T, Guo Y, Zhang X, et al. Ketogenic diet protects myelin and axons in diffuse axonal injury. Nutr Neurosci 2022;25(7):1534–47. doi:10.1080/1028415x.2021.1875300.
[85] Wang Y, Guo Y, Zhu M, Zhang L, Wang L, Liu Z, et al. A case report: effects of a ketogenic diet on PTEN mutation-associated autism spectrum disorder. Front Nutr 2026;13:1721018. doi:10.3389/fnut.2026.1721018.
[86] Gao X, Fu N, Ben Q, Bu X. A meta-analysis of the effects of gut microbiota-based interventions on gastrointestinal and behavioral symptoms in children with Autism spectrum disorder. Nutr Rev 2026;84(3):500–13. doi:10.1093/nutrit/nuaf050.