



Long-term follow-up of children with autism spectrum disorder and severe treatment-resistant behavioral symptoms treated with purified cannabidiol

Pablo Sebastián Fortini^{a,*}, Javier J. Toibaro^b, Roberto H. Caraballo^c

^a Equipo de Neurodesarrollo INIZIO, Santiago del Estero 321, Tucumán, Argentina

^b Hospital General de Agudos J.M. Ramos Mejía, Gral. Urquiza 609, 1221, Buenos Aires, Argentina

^c Department of Neurology, Hospital de Pediatría Prof. Dr. J.P. Garrahan, Combate de los Pozos 1881, 1245, Buenos Aires, Argentina

ARTICLE INFO

Keywords:

Autism spectrum disorder
Behavior disturbances
Cannabidiol
Long-term
Treatment

ABSTRACT

Background: Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental condition often associated with severe behavioral disturbances and limited pharmacological treatment options. Cannabidiol (CBD) has emerged as a potential therapeutic option; however, evidence on its long-term effectiveness and safety in children with ASD is scarce.

Objective: To evaluate the long-term effectiveness and safety of purified CBD as add-on therapy in children with severe ASD and treatment-resistant behavioral symptoms.

Material and methods: We conducted a prospective observational before-and-after study in children and adolescents (3–18 years) with ASD severity levels 2 or 3 and intellectual disability treated with add-on CBD. The primary outcome was change in caregiver-identified symptoms, while secondary outcomes included standardized behavioral scales (Repetitive Behavior Scale-Revised [RBS-R], Vineland Adaptive Behavior Scales-II maladaptive behavior domain, Aberrant Behavior Checklist [ABC], Pediatric Sleep Clinical Global Impressions–Severity, Autism Family Experience Questionnaire, and Parental Stress Scale). Safety and tolerability were assessed through caregiver-reported adverse events.

Results: Twenty children were enrolled, of whom 13 completed the long-term follow-up (mean 27.6 ± 1.3 months). Of the caregiver-identified symptoms, improvements observed during the initial short-term study were maintained or further improved during follow-up. Standardized scales showed modest but sustained improvements, particularly in irritability, social withdrawal, and hyperactivity. Mild, transient adverse events, mainly irritability or decreased appetite, did not recur during long-term followup, and concomitant medications were reduced in 40% of patients.

Conclusion: Long-term treatment with purified CBD in children with severe ASD was well tolerated and associated with sustained improvement in caregiver-reported outcomes and standardized scales.

1. Introduction

Autism spectrum disorder (ASD) is a group of heterogeneous neurodevelopmental conditions characterized by deficits in social communication and interaction associated with restricted and repetitive patterns of behavior, interests, or activities (American Psychiatric Association, DSM-5, 2013). In addition to these core features, children with ASD may present with a wide range of psychiatric and medical comorbidities, including intellectual disability (ID), epilepsy, irritability, aggression, anxiety, sleep disorders, and gastrointestinal disturbances (Bauman, 2010; Masi et al., 2017; Mostafavi and Gaitanis, 2020).

These associated symptoms can significantly worsen functional impairment, family stress, and quality of life.

Behavioral and educational interventions are an important part of the management (Anagnostou, 2018), but pharmacological options are limited. Currently, risperidone and aripiprazole are the only agents approved by the U.S. Food and Drug Administration for the treatment of irritability in ASD; however, both are associated with considerable adverse effects, including weight gain and metabolic syndrome (Holdman et al., 2022; Wink et al., 2014; Goel et al., 2018).

Nearly 40% of children with ASD and disruptive behaviors do not respond adequately to conventional behavioral or pharmacological

* Corresponding author at: Center Equipo de Neurodesarrollo INIZIO, Santiago del Estero 321, Tucumán, Argentina.

E-mail addresses: sebastianfortini@icloud.com (P.S. Fortini), jtoibaro@gmail.com (J.J. Toibaro), robertohcaraballo@gmail.com (R.H. Caraballo).

<https://doi.org/10.1016/j.pbb.2026.174220>

Received 31 March 2026; Received in revised form 27 May 2026; Accepted 2 June 2026

Available online 4 June 2026

0091-3057/© 2026 Elsevier Inc. All rights reserved, including those for text and data mining, AI training, and similar technologies.

interventions (Adler et al., 2015). Consistent with this unmet need, a recent network meta-analysis found that although risperidone and aripiprazole may reduce irritability in the short term, the overall certainty of the evidence is low (Meza et al., 2025).

Cannabidiol (CBD), a non-psychoactive cannabinoid, has gained increasing interest as a potential therapeutic option for both core symptoms of ASD and associated comorbidities, based on its anxiolytic, anti-inflammatory, and neuromodulatory properties, together with a generally favorable safety profile (Russo, 2011; Agarwal et al., 2019; Stolar et al., 2022). Alterations in the endocannabinoid system, a neuromodulatory network involved in the regulation of social behavior, anxiety, and sleep, have been described in individuals with ASD (Chakrabarti et al., 2015; Zamberletti et al., 2017; Karhson et al., 2018; Aran et al., 2019a; Zou et al., 2021). These findings provide a biological rationale for the clinical use of CBD as a therapeutic strategy in autism.

Several studies have evaluated the use of CBD in children with ASD (Aran et al., 2021; Barchel et al., 2019; Fleury-Teixeira et al., 2019; Bar-Lev Schleider et al., 2019; Silva et al., 2024; Bilge and Ekici, 2021; Hacoheh et al., 2022). However, most of these used CBD-enriched formulations with different ratios of CBD to tetrahydrocannabinol (THC), the psychoactive component of the cannabis plant.

In our previous study (Fortini et al., 2025), we reported the initial 3-month outcomes of this same cohort of children with ASD and severe treatment-resistant behavioral symptoms treated with purified CBD. We chose to use purified CBD to avoid exposure to THC and its potential psychoactive effects, which are of particular concern in pediatric populations. In that study, we observed favorable short-term outcomes with purified CBD in children with ASD and severe treatment-resistant behavioral symptoms, including ID and repetitive behaviors.

Currently, there are no data on the prolonged use of purified CBD in children with ASD. Long-term outcomes are of important to evaluate the ongoing efficacy and the potential for tolerance.

The aim of the present study was therefore to assess the long-term effectiveness and safety of purified CBD as an add-on treatment in the same cohort of children with ASD and severe treatment-resistant behavioral disturbances. By extending the follow-up, we aimed to evaluate the persistence of clinical benefits, the durability of treatment response, and the long-term tolerability of CBD.

2. Material and methods

This study represents the long-term follow-up of a previously published prospective observational before-and-after study evaluating purified CBD in children with ASD and severe treatment-resistant behavioral symptoms (Fortini et al., 2025). The present analysis was conducted to assess the long-term effectiveness and safety of purified CBD in the same cohort.

Children and adolescents (3 to 18 years of age) with a diagnosis of ASD according to DSM-5 criteria were included. Participants were eligible if they had ASD severity levels 2 or 3, requiring substantial or very substantial support and showing marked impairments in social communication and restricted, repetitive patterns of behavior; the presence of ID, severe behavioral disturbances with daily episodes of self- and hetero-aggression requiring physical restraint by caregivers and antipsychotic treatment that had proved ineffective as monotherapy or in combination; daily, frequent, and sustained (>10 min) stereotyped or repetitive behaviors; and hyperactivity significantly interfering with daily functioning and adherence to medical or behavioral interventions.

Patients were excluded if they had epilepsy, given its known responsiveness to CBD and the potential for confounding effects. Additional exclusion criteria included ASD severity level 1 without ID; a history of metabolic, immunologic, cardiac, genetic, infectious, or bleeding disorders; known allergy to any component of the CBD oil; pregnancy; cannabis use within seven days prior to study initiation; and a high likelihood of non-compliance with the study protocol.

2.1. Intervention

All patients received treatment with highly purified CBD oral solution (Convupidiol®), a product approved by the Argentine National Administration of Drugs, Foods, and Medical Devices (ANMAT).

Our dosing strategy was based on gradual and individualized titration according to tolerability and clinical response. For children weighing ≤ 45 kg, the starting dose was 2 mg/kg/day, and for those > 45 kg, 5 mg/kg/day, given in two divided doses. Drug-sensitive patients began at 1 mg/kg/day. Doses were then increased by the same amount as the initial dose (2 mg/kg/day or 1 mg/kg/day), according to tolerance and clinical response, up to a maximum of 25 mg/kg/day or 700 mg. The initial dose was maintained for two weeks, and from week three onward, titration was adjusted based on the child's clinical evolution. Similar individualized titration protocols and broad dosing ranges have been reported in previous ASD studies using CBD, with starting doses ranging from 1 to 5 mg/kg/day and target doses commonly around 7.5–10 mg/kg/day (Aran et al., 2021; Bilge and Ekici, 2021; Hacoheh et al., 2022).

CBD was administered as an add-on therapy to ongoing stable treatments, including antipsychotics, stimulants, or behavioral therapies.

Clinical evolution was assessed through structured caregiver reports obtained during scheduled follow-up contacts conducted either by telephone or during in-person visits, depending on the study phase. Follow-up interviews were performed at regular intervals of approximately every 7, 10, or 15 days. Information obtained during the interviews was recorded using standardized data collection forms and incorporated into the longitudinal outcome analysis.

2.2. Assessments

Baseline demographic and clinical characteristics were systematically collected at study entry, including age, sex, body weight, and comorbidities.

2.2.1. Primary outcome

The primary outcome was defined as longitudinal changes in individualized target symptoms identified by caregivers as having the greatest impact on the child's daily functioning and family quality of life across home, recreational, and therapeutic settings. At baseline, prior to CBD initiation, caregivers identified five symptoms for each patient. These same target symptoms were reassessed at approximately three-month intervals during treatment and at the extended follow-up evaluation.

Whenever feasible, both parents jointly identified the target symptoms and contributed to the follow-up ratings. When this was not possible, the primary caregiver—most often the mother—completed the evaluations. Follow-up assessments were conducted through scheduled in-person visits or structured telephone interviews. The principal investigator reviewed caregiver reports, confirmed symptom classification, and ensured consistency in longitudinal data collection throughout the study period.

The reported symptoms/behaviors were grouped into nine pre-defined categories: aggressive behavior, irritability, hyperactivity, repetitive behaviors, sleep disturbances, impulsivity, difficulty remaining dressed, exaggerated responses to sensory stimuli, and feeding difficulties.

Treatment response was assessed by evaluating changes in the frequency of these behaviors using a six-point ordinal scale: 1 = much improved ($> 75\%$ reduction in frequency); 2 = improved (50–74% reduction); 3 = slightly improved (25–49% reduction); 4 = no change (0–24% reduction); 5 = slightly worse; and 6 = much worse. A positive treatment response was defined as a score of 1, 2, or 3.

2.2.2. Secondary outcomes

Secondary outcomes were evaluated using standardized instruments administered at baseline and at approximately three-month intervals throughout CBD treatment. The assessments included the Repetitive Behavior Scale-Revised (RBS-R) (Bodfish et al., 2000; Martínez-González et al., 2021), the Maladaptive Behavior domain of the Vineland Adaptive Behavior Scales, Second Edition (VABS-II) (Sparrow et al., 2005), the Pediatric Sleep Clinical Global Impressions-Severity scale (Sleep CGI-S) (Malow et al., 2016), selected subscales of the Aberrant Behavior Checklist (ABC: irritability, social withdrawal, and hyperactivity) (Aman et al., 1985), the Autism Family Experience Questionnaire (AFEQ) (Leadbitter et al., 2018), and the Parental Stress Scale (PSS) (Berry and Jones, 1995), as explained in our previous study (Fortini et al., 2025).

Safety and tolerability were monitored through daily caregiver-completed adverse event forms. Adverse events were graded by severity, and those leading to treatment discontinuation or emergency department evaluation were classified as severe. Treatment adherence and clinical status were reviewed during scheduled in-person visits and structured telephone follow-up interviews conducted every 7, 10, or 15 days, depending on the study phase.

The study was approved by the Bioethics in Research Committee (accreditation nr. MSGCBA 00012) before participant enrollment. Written informed consent was obtained from parents or legal guardians of all participants prior to inclusion. All procedures were performed in accordance with institutional guidelines, national regulations, and the ethical standards of the Declaration of Helsinki and its later amendments.

3. Results

3.1. General features

Between January and December 2023, 20 children were enrolled in the study, of whom 13 completed prospective follow-up through June 2025, allowing evaluation of the sustained effects and safety profile of CBD treatment over a mean follow-up period of 27.6 ± 1.3 months. Demographic and clinical characteristics of the long-term follow-up cohort are summarized in Table 1.

According to DSM-5 criteria, five patients (38%) were classified as ASD severity Level 2 and eight (62%) as Level 3. All patients had intellectual disability, classified as mild in two (16%), moderate in six (46%), and severe in five (38%). Language impairment was present in 11 patients (85%), including eight (73%) who were nonverbal. The remaining two patients had fluent speech but showed deficits in morphosyntactic, semantic, and pragmatic domains consistent with their cognitive level.

Patients were receiving multiple concomitant medications at baseline, most commonly risperidone, aripiprazole, levomepromazine, and melatonin. During follow-up, six patients (46%) were able to reduce the dose or partially discontinue at least one concomitant medication, most

Table 1

Demographic and clinical characteristics of the 13 patients with moderate-to-severe ASD included in the long-term extension study of purified cannabidiol ($n = 13$).

Variable	Value
Male sex, n (%)	10 (76%)
Age at inclusion, years, mean $\pm$ SD	9.9 $\pm$ 4.51
Weight at baseline, kg, mean $\pm$ SD	53.6 $\pm$ 35.8
Weight at follow-up, kg, mean $\pm$ SD	60.6 $\pm$ 33.8
Final CBD dose, mg, mean $\pm$ SD	373.8 $\pm$ 182.4
Number of concomitant drugs, mean $\pm$ SD (median, range)	2.84 $\pm$ 1.46 (2, 1–5)
Drugs with dose reduction, mean $\pm$ SD (median, range)	0.61 $\pm$ 0.96 (0, 0–3)
Discontinued drugs, mean $\pm$ SD (median, range)	0.53 $\pm$ 1.12 (0, 0–3)
Treatment duration, days, mean $\pm$ SD (median)	853.3 $\pm$ 42.2 (872)

frequently aripiprazole or levomepromazine. No clinically relevant drug–CBD interactions were observed.

The median initial CBD dose was 100 mg (mean 135.7 mg; range 35–400), and the mean final dose was 373.8 ± 182.4 mg. Overall, patients remained within the same age-adjusted weight percentiles throughout follow-up.

The demographic and clinical characteristics of the cohort are summarized in Table 1.

3.2. Efficacy

3.2.1. Primary outcome

At baseline, caregivers identified five severe target symptoms per patient (91 total), most commonly aggressive behaviors, irritability, impulsivity, hyperactivity, repetitive behaviors, sensory hypersensitivity, and difficulties related to sleep, eating, or remaining dressed. After three months of CBD treatment, improvement in at least one target symptom was observed in 18 of 20 patients (90%), with 83.5% of all reported symptoms showing improvement. Among the 13 children included in the long-term follow-up, caregivers initially identified a total of 62 severe symptoms. Improvement of all target symptoms remained steady or slightly improved further over the course of treatment. At the 26-month follow-up, 65% (41 symptoms) were rated as much improved ($>75\%$ reduction), 25% (15) as improved (50–74% reduction), and 10% (6) as slightly improved (25–49% reduction).

Table 2 summarizes the median weekly frequency of the nine caregiver-identified target symptom/behavior categories at baseline and at three and 26 months after CBD initiation.

3.2.2. Secondary outcomes

For secondary outcomes, the children were evaluated using standardized instruments. The severity of restricted and repetitive behaviors was assessed using the RBS-R at baseline, after three months of CBD treatment, and at long-term follow-up. At baseline, severity was classified as mild in one patient (5.5%), moderate in 11 (61.1%), and severe in six (33.3%). After three months of CBD treatment, severity was mild in three patients (16.6%), moderate in 13 (72.2%), and severe in two (11.1%). At that time point, compared with baseline, severity improved in nine patients (50%), remained unchanged in seven (38.8%), and worsened in two (11.1%).

Among the 13 patients who completed the long-term follow-up, five (38.5%) demonstrated additional improvement beyond that observed at

Table 2

Weekly frequency of caregiver-identified symptom/behavior categories at baseline, 3 months, and long-term follow-up in the 13 patients included in the long-term extension study.

Symptom/behavior (n) ^a	Baseline (n = 13) Median (IQR)	3 months (n = 13) Median (IQR)	26 months (n = 13) Median (IQR)
Aggressive behavior (n = 12) ^a	72 (21–140)	19 (5–30)	9 (3–28) (n = 12)
Irritability (n = 10) ^a	39 (21–70)	10 (3–14)	8 (2–14) (n = 10)
Hyperactivity (n = 4) ^a	36 (30–42)	17 (9–30)	6 (4–21) (n = 4)
Repetitive behavior (n = 5) ^a	75 (28–210)	37 (18–100)	20 (12–50) (n = 5)
Sleep difficulties (n = 1) ^a	7 (7)	7 (7)	7 (7) (n = 1)
Impulsivity (n = 2) ^a	38 (28–49)	11 (8–15)	8 (4–12) (n = 2)
Difficulties staying dressed (n = 2) ^a	24 (13–35)	4 (3–5)	3 (1–5) (n = 2)
Exaggerated response to stimuli (n = 2) ^a	25 (14–35)	2 (0–4)	2 (0–4) (n = 2)
Eating difficulties (n = 2) ^a	28 (28–28) ^b	7 (4–10)	7 (4–10) (n = 2)

^a Number of patients with the symptom at long-term follow-up.

^b Episodes were the same (28/week) in both patients.

the three-month evaluation. Specifically, one patient shifted from severe to moderate, two from moderate to mild, and two from mild to no problem. The remaining eight patients showed no further change and remained within the moderate severity category.

In the Maladaptive Behavior domain of the VABS-II, among the 13 patients who completed the extended evaluation, the median baseline score was 22, corresponding to the clinically significant range. Following CBD treatment, the median score decreased to 20.5, as reported in the initial study, shifting to the elevated range. At long-term follow-up, the median score further declined to 20.1, remaining within the elevated range.

In the initial study, scores on the ABC decreased following CBD initiation, with mean improvements of 33% in irritability, 40% in social withdrawal, and 30% in hyperactivity. At long-term follow-up, among the 13 patients who completed the extended evaluation, these improvements were sustained and in some cases further reduced. Median scores decreased to 22.1 (range, 5–38) for irritability, 4.8 (range, 0–14) for social withdrawal, and 17.3 (range, 6–34) for hyperactivity. Compared with baseline values, these corresponded to mean reductions of 30%, 46%, and 38%, respectively.

In the initial study, the Pediatric Sleep CGI-S showed that sleep disturbances were present in all 18 patients who remained on treatment. Although no significant overall changes were observed after three months of CBD therapy, some improvements were noted in specific sleep parameters. Difficulties in falling asleep and remaining asleep independently from parents were reported in 13 patients (72.2%) at baseline and persisted in all, although symptoms were generally less severe. Bedtime resistance decreased from 16 patients (88.8%) at baseline to nine (50%) after CBD initiation, delayed sleep onset from 16 (88.8%) to seven (38.8%), and nocturnal awakenings from 14 (77.7%) to seven (38.8%).

At long-term follow-up, all 13 patients who completed the extended evaluation continued to present some degree of sleep disturbance. Difficulties in falling asleep and remaining asleep independently from parents were reported in seven patients (54%), bedtime resistance in seven (54%), delayed sleep onset in five (38%), and nocturnal awakenings in six (46%), similar to baseline and the 3-month evaluation.

Among the 13 patients who completed the long-term follow-up, the median score on the AFEQ was 155.7 at baseline and decreased to 136.6 after CBD initiation, with a further reduction to 134.5. The median score on the PSS was 54.4 at baseline and 49 after CBD initiation, remaining stable at 49 throughout the long-term follow-up.

The results of the behavioral assessments conducted at baseline and at three and 26 months after treatment initiation in our cohort of patients with ASD are summarized in [Table 3](#).

3.3. Safety

During short-term follow-up, adverse events were observed in 10 of the 13 patients included in the long-term follow-up cohort (76.9%), most commonly increased irritability and decreased appetite ([Table 4](#)). All events were mild to moderate and transient, and were managed by slowing dose escalation or reducing the dose. During long-term follow-up, these adverse events did not recur, and no new or unexpected adverse events were observed.

3.4. Follow-up

Our initial report presented the outcomes of 18 of the 20 patients originally enrolled, as two discontinued CBD treatment—one due to increased irritability and the other because of poor adherence. In the present report, we describe the long-term outcomes of 13 of these 18 patients. Five patients were not included in the extended follow-up: three discontinued treatment due to adherence difficulties related to family dynamics, and two discontinued for maternal health-related reasons. In one family within the long-term follow-up cohort, CBD

Table 3

Behavioral assessments at baseline, 3 months, and 26 months in the 13 patients included in the long-term extension study.

Measure	Baseline (n = 13)	3 months (n = 13)	26 months (n = 13)
RBS-R	Mild 1, moderate 8, severe 4	Mild 3, moderate 9, severe 1	None 2, mild 3, moderate 8
Maladaptive Behavior Domain VABS-II – score median (range)	22 (19–26)	20.5 (19–24)	20.1 (19–22)
ABC, median (range)			
Irritability	31.2 (10–39)	19.8 (9–36)	22.1 (5–38)
Social withdrawal	9 (2–29)	6 (0–14)	4.8 (0–14)
Hyperactivity	28 (9–36)	20 (9–36)	17.3 (6–34)
AFEQ, median (range)	155.7 (128–178)	136.6 (111–168)	134.5 (116–157)
PSS, median (range)	54.4 (38–70)	49 (29–68)	49 (36–65)
Pediatric Sleep CGI-S – n (%)			
Difficulty falling asleep and sleeping independently	7 patients (54%)	7 patients (54%)	7 patients (54%)
Bedtime resistance	7 patients (54%)	7 patients (54%)	7 patients (54%)
Delayed sleep onset	5 patients (38%)	5 patients (38%)	5 patients (38%)
Nocturnal awakenings	6 patients (46%)	6 patients (46%)	6 patients (46%)

ABC, Aberrant Behavior Checklist; AFEQ, Autism Family Experience Questionnaire; CGI-S, Clinical Global Impressions–Severity; PSS, Parental Stress Scale; RBS-R, Repetitive Behavior Scale–Revised; VABS-II, Vineland Adaptive Behavior Scales-II.

Table 4

Adverse effects observed in the 13 patients included in the long-term extension study.

Adverse effect	Number of patients
Increased irritability	3
Decreased appetite	2
Hand tremor	1
Worsening behavior	1
Fever	1
Sleep disturbances	1
Nervousness	1

treatment was independently discontinued without medical indication. Three weeks later, the parents reported worsening behavioral symptoms, including increased aggression and irritability. CBD was subsequently reintroduced, resulting in a clear improvement in symptoms.

4. Discussion

The present long-term follow-up study builds on our initial report evaluating purified CBD in children with severe ASD. In that study, we included 20 children aged 3–17 years with moderate or severe ASD Level 2 or 3, all presenting with ID and language impairment. In this study, we followed 13 of the original children for a period of 26 months, and we found that purified CBD continued to have a good safety profile and was well tolerated.

As ASD is highly heterogeneous, with a wide range of core and associated symptoms as well as multiple comorbidities, standardized questionnaires may not fully capture the complexity of symptoms in individual patients, particularly in those with more severe presentations ([Aran et al., 2021](#)). Therefore, for the primary outcome measure, parents and caregivers were asked at baseline to identify the five behaviors they considered most disruptive in their child's daily life. Treatment effectiveness was then evaluated based on changes in these individualized target symptoms after CBD initiation. Among the 13 patients who

completed the long-term follow-up, the initial improvement observed in these target symptoms was maintained or slightly increased over the 26-month follow-up.

For the secondary outcomes, several psychometric instruments were used to assess treatment effects. Restricted and repetitive behaviors measured with the RBS-R improved in half of the evaluated patients and remained stable in most others; in the long-term follow-up, five of the 13 patients showed further improvement and eight remained stable, while none of the children worsened. In the Maladaptive Behavior domain of the VABS-II, the mean score decreased from the clinically significant range at baseline to the elevated range after three months of CBD treatment, and this improvement was maintained among the 13 patients who completed the extended follow-up. On the ABC, irritability, social withdrawal, and hyperactivity improved by at least 30% at three months in the initial study. During the long-term follow-up, five children showed further improvement and eight remained stable, with no worsening. Finally, sleep improvements were modest in our cohort. At long-term follow-up, sleep disturbances persisted in all 13 patients, although some parameters showed partial improvement.

Previous studies have reported somnolence, decreased appetite, irritability, and weight loss as the most common adverse effects of CBD, generally mild or moderate and rarely leading to treatment discontinuation (Barchel et al., 2019; Aran et al., 2019a; Aran et al., 2019b, 2021; Fleury-Teixeira et al., 2019; Bar-Lev Schleider et al., 2019; Silva et al., 2024). In our cohort, adverse events were similar, although somnolence was not observed. The adverse effects were managed by slowing dose titration or reducing the dose. During long-term follow-up, the adverse effects did not recur and no new adverse events were reported.

In children with severe ASD, blood sampling may be particularly challenging due to behavioral and sensory difficulties, which limited systematic laboratory monitoring in our study, including routine transaminase measurements. Nevertheless, no clinical evidence suggestive of hepatotoxicity was observed during long-term follow-up. Concerns regarding CBD-associated hepatotoxicity have mainly emerged from epilepsy studies using higher CBD doses (10–29 mg/kg/day), particularly in combination with valproic acid (Stohs and Ray, 2020). In children with ASD, lower doses are generally used, and available studies have reported acceptable biochemical safety profiles, although systematic laboratory monitoring has been inconsistent (Stolar et al., 2022; Aran et al., 2021).

An additional advantage of CBD is the possibility of reducing the number or dosage of concomitant medications, as reported in previous studies (Fusar-Poli et al., 2020; Aran et al., 2019a; Fleury-Teixeira et al., 2019; Bar-Lev Schleider et al., 2019). In our cohort, doses of concomitant medications could be reduced or partially discontinued in 40% of the patients, although complete discontinuation was not achieved. However, Ismail et al. (2025) described biological mechanisms that may support combination therapy in ASD. The authors analyzed shared proteomic targets between CBD, risperidone, and fluoxetine, suggesting that these drugs may act on common serotonin-related pathways and neuroinflammatory mechanisms, potentially producing synergistic effects that stabilize neurotransmitter imbalances. In our cohort, the most common combination was CBD with risperidone, which may have contributed to the clinical improvements observed.

Unlike most previous studies that used CBD-enriched cannabis products (Aran et al., 2021; Barchel et al., 2019; Fleury-Teixeira et al., 2019; Bar-Lev Schleider et al., 2019; Silva et al., 2024; Bilge and Ekici, 2021; Hacoen et al., 2022), we used purified CBD to ensure formulation consistency and avoid exposure to THC in pediatric patients. In contrast, Parrella et al. (2026) recently evaluated a broad-spectrum CBD oil combined with terpenes and negligible amounts of THC in a randomized, double-blind, placebo-controlled crossover trial. In earlier studies, CBD:THC ratios varied widely, ranging from 9:1 to 75:1 (Silva et al., 2024; Fleury-Teixeira et al., 2019), making comparisons difficult. The so-called entourage effect, referring to the potential synergistic action of other cannabis plant compounds, may also

influence outcomes, especially regarding social functioning (Hacoen et al., 2022; Parrella et al., 2026; Pereira et al., 2025); however, Aran et al. (2021) found no clear advantage of whole-plant extracts over purified cannabinoids.

Trauner et al. (2025) also used purified CBD for the treatment of severe ASD. In their double-blind, placebo-controlled crossover study using the RBS-R, Child Behavior Checklist (CBCL), and Autism Diagnostic Observation Schedule, Second Edition (ADOS-2). Both the CBD and placebo groups showed improvement, with no statistically significant differences between groups, highlighting a notable placebo effect. However, improvements in repetitive behaviors measured by the RBS-R were nearly twice as large in the CBD group, although not statistically significant. In addition, blinded clinicians correctly identified the majority of children in the phase they were taking the active drug and observed clinical benefits, including reduced aggression and hyperactivity, improved communication, fewer self-injurious behaviors, and lower anxiety.

Parenting a child with autism spectrum disorder is associated with higher levels of parental stress. In our cohort, five of the 18 children initially included did not continue long-term treatment, three due to adherence difficulties related to family dynamics and two due to maternal health-related reasons, suggesting that family burden may influence treatment continuity. Improvements in parental well-being have been associated with better outcomes in autistic children, emphasizing the importance of evaluating both child- and caregiver-related outcomes in clinical studies (Parrella et al., 2026).

As in our initial study, standardized measures of parental stress (Berry and Jones, 1995) and family experience (Leadbitter et al., 2018) did not show statistically significant changes during long-term follow-up. However, all 13 families reported improvements in their child's behavior, sleep, and social interaction. Caregivers described a positive impact of CBD on daily management, family dynamics, perceived symptom severity, stress levels, and overall quality of life. These perceived benefits also motivated families to continue treatment, as they expressed reluctance to return to the behavioral difficulties observed before to CBD initiation.

Assessing behavioral change in children with ASD remains methodologically challenging, particularly in those with severe ID and language impairment. Selecting appropriate outcome measures is difficult, as many commonly used instruments were originally developed for diagnostic classification rather than for monitoring treatment response. For example, diagnostic tools such as the ADOS-2 provide severity metrics useful for case definition and phenotyping, but were not designed to detect short-term therapeutic effects. Longitudinal studies have shown that ADOS-2 scores tend to remain relatively stable over time and are only modestly associated with functional change, with limited sensitivity to pharmacological interventions administered over weeks or months (Aragão et al., 2026). Similarly, the difficulty of identifying outcome measures capable of adequately capturing behavioral change in this diverse population has been reported, showing that clinical improvement observed by clinicians or parents is not always paralleled by measurable changes on standardized scales (Hacoen et al., 2022; Trauner et al., 2025).

Because many important healthcare outcomes, such as symptom reduction, decreased disability, and improved quality of life, are best evaluated by patients and families (Black, 2013), caregivers in our study identified the five symptoms most disruptive to their child's daily life and monitored their evolution during CBD treatment. This approach was particularly relevant because all patients had ID and language impairment, limiting participation in structured assessments. Consistent with the concept of Patient-Centered Outcome Measures (PCOMs) (Scheffer et al., 2025), this strategy may better capture clinically meaningful changes, even though it relies on subjective caregiver reports.

Parental expectations may influence subjective reports of treatment outcomes in children with ASD and contribute to placebo responses in clinical trials (Barchel et al., 2019). However, caregivers remain a key

source of information for assessing behavioral changes and adverse events, particularly in patients with limited communication abilities. While parental optimism may increase placebo responses, caregiver stress may reduce them (Masi et al., 2017). Importantly, a recent study found that parental expectations regarding cannabinoid treatment were not associated with placebo response (Aran et al., 2023). This long-term follow-up study has several limitations. Most importantly, the long-term results are based only on patients who completed follow-up and remained on treatment, which may have introduced selection bias, as these individuals were more likely to have experienced clinical benefit. In addition, the small sample size limits the generalizability of our findings. The absence of a placebo or comparison group makes it difficult to determine whether the observed changes were solely due to CBD treatment. In addition, most outcomes were based on caregiver reports, which are inherently subjective. However, although standardized scales were used, they may not fully reflect the individual changes that families considered most meaningful. For these reasons, the results should be interpreted with caution.

Nevertheless, our findings support the long-term tolerability and safety of CBD at the administered doses, including its use in combination with other medications, with no clinically relevant drug interactions observed in the majority of patients.

In addition, even from a conservative perspective, the potential value of CBD in this population should be considered in the context of previous treatment failures. All patients included in this study had persistent severe behavioral symptoms despite multiple pharmacological interventions. Although seven of the 20 patients did not continue in the protocol, the majority of families chose to maintain treatment, and 13 patients were followed long term.

Our findings emphasize the importance of caregiver-perceived outcomes and suggest that conventional questionnaires may not fully capture clinically meaningful changes in children with severe ASD. Larger controlled studies using validated, patient-centered outcome measures are needed to better define the long-term efficacy and safety of CBD in this population.

5. Conclusions

In this long-term follow-up of children with severe ASD treated with purified CBD, no significant differences were observed between the three-month and the 26-month evaluations, suggesting that the initial improvements were maintained over time. Among patients who completed the extended follow-up, purified CBD was well tolerated and associated with sustained improvement in several symptoms, particularly those identified by families as most disruptive in daily life.

These findings suggest a potential therapeutic role for CBD in children with severe ASD while also highlighting the limitations of conventional assessment scales in capturing meaningful change in this complex population. However, given the observational design and small sample size, the results should be interpreted with caution.

CRediT authorship contribution statement

Pablo Sebastián Fortini: Writing – original draft, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Javier J. Toibaro:** Validation, Supervision, Methodology. **Roberto H. Caraballo:** Writing – review & editing, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Patient consent statement

Parents of the participants provided written informed consent and written assent was obtained from participants when appropriate.

Ethics approval statement

The study was approved by the Bioethics in Research Committee (accreditation nr. MSGCBA 00012) before participant enrollment.

Permission to reproduce material from other sources

Permission to use the Parental Stress Scale was obtained from Judy O. Berry, EdD, Professor Emerita of Psychology, the University of Tulsa.

Funding statement

Laboratory Alef, Argentina, provided the CBD for the families as well as support for the methodology and statistical analysis.

Declaration of competing interest

The authors have no conflict of interests to declare.

Acknowledgements

We would like to thank the parents of the children with ASD who participated in this study and generously shared their observations, Mariana Fazzina for her assistance in the methodology.

Data availability

Data available on request from the authors.

References

- Adler, B.A., Wink, L.K., Early, M., et al., 2015. Drug-refractory aggression, self-injurious behavior, and severe tantrums in autism spectrum disorders: a chart review study. *Autism* 19 (1), 102–106. <https://doi.org/10.1177/1362361314524641>.
- Agarwal, R., Burke, S.L., Maddux, M., 2019. Current state of evidence of cannabis utilization for treatment of autism spectrum disorders. *BMC Psychiatry* 19 (1), 328. <https://doi.org/10.1186/s12888-019-2259-4>, 29.
- Aman, M.G., Singh, N.N., Stewart, A.W., et al., 1985. The aberrant behavior checklist: a behavior rating scale for the assessment of treatment effects. *Am. J. Ment. Defic.* 89 (5), 485–491.
- American Psychiatric Association, 2013. *Diagnostic and Statistical Manual of Mental Disorders*, 5th ed. American Psychiatric Association, Arlington, VA.
- Anagnostou, E., 2018. Clinical trials in autism spectrum disorder: evidence, challenges and future directions. *Curr. Opin. Neurol.* 31, 119–125. <https://doi.org/10.1097/WCO.0000000000000542>.
- Aragão, G.F., Brandão, C.B., Júnior, J.E.L., 2026. Cannabidiol in autism: clinical pharmacology priorities for trial design. *Br. J. Clin. Pharmacol.* 92 (2), 357–359. <https://doi.org/10.1002/bcp.70400>.
- Aran, A., Eylon, M., Harel, M., et al., 2019a. Lower circulating endocannabinoid levels in children with autism spectrum disorder. *Mol. Autism* 10, 2. <https://doi.org/10.1186/s13229-019-0256-6>.
- Aran, A., Cassuto, H., Lubotzky, A., et al., 2019b. Brief report: cannabidiol-rich cannabis in children with autism spectrum disorder and severe behavioral problems—a retrospective feasibility study. *J. Autism Dev. Disord.* 49 (3), 1284–1288. <https://doi.org/10.1007/s10803-018-3808-2>.
- Aran, A., Harel, M., Cassuto, H., et al., 2021. Cannabinoid treatment for autism: a proof-of-concept randomized trial. *Mol. Autism* 12 (1), 6. <https://doi.org/10.1186/s13229-021-00420-2>.
- Aran, A., Harel, M., Ovadia, A., et al., 2023. Mediators of placebo response to cannabinoid treatment in children with autism spectrum disorder. *J. Clin. Med.* 12 (9), 3098. <https://doi.org/10.3390/jcm12093098>.
- Barchel, D., Stolar, O., De-Haan, T., et al., 2019. Oral cannabidiol use in children with autism spectrum disorder to treat related symptoms and co-morbidities. *Front. Pharmacol.* 9, 1521. <https://doi.org/10.3389/fphar.2018.01521>.
- Bar-Lev Schleider, L., Mechoulam, R., Saban, N., et al., 2019. Real life experience of medical cannabis treatment in autism: analysis of safety and efficacy. *Sci. Rep.* 9 (1), 200. <https://doi.org/10.1038/s41598-018-37570-y>.
- Bauman, M.L., 2010. Medical comorbidities in autism: challenges to diagnosis and treatment. *Neurother. J. Am. Soc. Exp. Neurother.* 7 (3), 320–327. <https://doi.org/10.1016/j.nurt.2010.06.001>.
- Berry, J.O., Jones, W.H., 1995. The parental stress scale: initial psychometric evidence. *J. Soc. Pers. Relat.* 12, 463–472.
- Bilge, S., Ekici, B., 2021. CBD-enriched cannabis for autism spectrum disorder: an experience of a single center in Turkey and reviews of the literature. *J. Cannabis Res.* 3 (1), 53. <https://doi.org/10.1186/s42238-021-00108-7>, 16.
- Black, N., 2013. Patient reported outcome measures could help transform healthcare. *BMJ (Clin. Res. Ed.)* 346, f167. <https://doi.org/10.1136/bmj.f167>.

- Bodfish, J.W., Symons, F.J., Parker, D.E., et al., 2000. Varieties of repetitive behavior in autism: comparisons to mental retardation. *J. Autism Dev. Disord.* 30 (3), 237–243. <https://doi.org/10.1023/a:1005596502855>.
- Chakrabarti, B., Persico, A., Battista, N., et al., 2015. Endocannabinoid signaling in autism. *Neurother. J. Am. Soc. Exp. Neurother.* 12 (4), 837–847. <https://doi.org/10.1007/s13311-015-0371-9>.
- Fleury-Teixeira, P., Caixeta, F.V., Ramires da Silva, L.C., et al., 2019. Effects of CBD-enriched *Cannabis sativa* extract on autism spectrum disorder symptoms: an observational study of 18 participants undergoing compassionate use. *Front. Neurol.* 10, 1145. <https://doi.org/10.3389/fneur.2019.01145>.
- Fortini, P.S., Toibaro, J.J., Caraballo, R.H., 2025. Purified cannabidiol leads to improvement of severe treatment-resistant behavioral symptoms in children with autism spectrum disorder. *Pharmacol. Biochem. Behav.* 249, 173971. <https://doi.org/10.1016/j.pbb.2025.173971>.
- Fusar-Poli, L., Cavone, V., Tinacci, S., et al., 2020. Cannabinoids for people with ASD: a systematic review of published and ongoing studies. *Brain Sci.* 10 (9), 572. <https://doi.org/10.3390/brainsci10090572>.
- Goel, R., Hong, J.S., Findling, R.L., Ji, N.Y., 2018. An update on pharmacotherapy of autism spectrum disorder in children and adolescents. *Int. Rev. Psychiatry (Abingdon, England)* 30 (1), 78–95. <https://doi.org/10.1080/09540261.2018.1458706>.
- Hacohen, M., Stolar, O.E., Berkovitch, M., et al., 2022. Children and adolescents with ASD treated with CBD-rich cannabis exhibit significant improvements particularly in social symptoms: an open label study. *Transl. Psychiatry* 12 (1), 375. <https://doi.org/10.1038/s41398-022-02104-8>.
- Holdman, R., Vigil, D., Robinson, K., et al., 2022. Safety and efficacy of medical cannabis in autism spectrum disorder compared with commonly used medications. *Cannabis Cannabinoid Res.* 7 (4), 451–463. <https://doi.org/10.1089/can.2020.0154>.
- Ismail, M., Aananou, S., Fogue, C., et al., 2025. A systematic computational analysis of pharmacological options in neuroinflammatory-induced autism spectrum disorder in children: a potential for drug repositioning. *J. Biol. Methods* 12 (4), e99010079. <https://doi.org/10.14440/jbm.0219>.
- Karhson, D.S., Krasinska, K.M., Dallaire, J.A., et al., 2018. Plasma anandamide concentrations are lower in children with autism spectrum disorder. *Mol. Autism.* 9, 18. <https://doi.org/10.1186/s13229-018-0203-y>.
- Leadbitter, K., Aldred, C., McConachie, H., PACT Consortium, et al., 2018. The Autism Family Experience Questionnaire (AFEQ): an ecologically-valid, parent-nominated measure of family experience, quality of life and prioritised outcomes for early intervention. *J. Autism Dev. Disord.* 48 (4), 1052–1062. <https://doi.org/10.1007/s10803-017-3350-7>.
- Malow, B.A., Connolly, H.V., Weiss, S.K., et al., 2016. The pediatric sleep clinical global impressions scale-a new tool to measure pediatric insomnia in autism spectrum disorders. *J. Develop. Behav. Pediatr. JDBP* 37 (5), 370–376. <https://doi.org/10.1097/DBP.0000000000000307>.
- Martínez-González, A.E., Piqueras, J.A., Bodfish, J.W., 2021. Adaptación española de la Escala de Conductas Repetitivas Revisada (Repetitive Behavior Scale-Revised, RBS-R). *ABA España.* <https://doi.org/10.26741/978-84-09-28002-5>.
- Masi, A., DeMayo, M.M., Glozier, N., et al., 2017. An overview of autism spectrum disorder, heterogeneity and treatment options. *Neurosci. Bull.* 33 (2), 183–193. <https://doi.org/10.1007/s12264-017-0100-y>.
- Meza, N., Franco, J.V., Squassero, Y., et al., 2025. Atypical antipsychotics for autism spectrum disorder: a network meta-analysis. *Cochrane Database Syst. Rev.* 5 (5), CD014965. <https://doi.org/10.1002/14651858.CD014965.pub2>.
- Mostafavi, M., Gaitanis, J., 2020. Autism spectrum disorder and medical cannabis: review and clinical experience. *Semin. Pediatr. Neurol.* 35, 100833. <https://doi.org/10.1016/j.spen.2020.100833>.
- Parrella, N.F., Hill, A.T., Enticott, P.G., et al., 2026. Effects of cannabidiol on social relating, anxiety, and parental stress in autistic children: a randomized controlled crossover trial. *Autism Res. Off. J. Int. Soc. Autism Res.* 19 (2), e70159. <https://doi.org/10.1002/aur.70159>.
- Pereira, D.A., Cheidde, L., Megiolaro, M.D.R., et al., 2025. Efficacy and safety of cannabinoids for autism spectrum disorder: an updated systematic review. *Cureus* 17 (3), e80725. <https://doi.org/10.7759/cureus.80725>.
- Russo, E.B., 2011. Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. *Br. J. Pharmacol.* 163 (7), 1344–1364. <https://doi.org/10.1111/j.1476-5381.2011.01238.x>.
- Scheffer, I.E., French, J., Valente, K.D., et al., 2025. Operational definition of developmental and epileptic encephalopathies to underpin the design of therapeutic trials. *Epilepsia* 66 (4), 1014–1023. <https://doi.org/10.1111/epi.18265>.
- Silva, E.A.D., Junior, Medeiros, W.M.B., Santos, et al., 2024. Evaluation of the efficacy and safety of cannabidiol-rich cannabis extract in children with autism spectrum disorder: randomized, double-blind, and placebo-controlled clinical trial. *Trends Psychiatry Psychother.* 46, e20210396. <https://doi.org/10.47626/2237-6089-2021-0396>.
- Sparrow, S.S., Cicchetti, D., Balla, D.A., 2005. Vineland Adaptive Behavior Scales, Second Edition (Vineland-II) [Database record]. *APA PsycTests.* <https://doi.org/10.1037/t15164-000>.
- Stohs, S.J., Ray, S.D., 2020. Is cannabidiol hepatotoxic or hepatoprotective: a review. *Toxicol. Res. Appl.* 4, 1–13. <https://doi.org/10.1177/2397847320922944>.
- Stolar, O., Hazan, A., Vissoker, R.E., et al., 2022. Medical cannabis for the treatment of comorbid symptoms in children with autism spectrum disorder: An interim analysis of biochemical safety. *Front. Pharmacol.* 13, 977484. <https://doi.org/10.3389/fphar.2022.977484>.
- Trauner, D., Umlauf, A., Grelotti, D.J., et al., 2025. Cannabidiol (CBD) treatment for severe problem behaviors in autistic boys: a randomized clinical trial. *J. Autism Dev. Disord.* <https://doi.org/10.1007/s10803-025-06884-y>. Advance online publication.
- Wink, L.K., Early, M., Schaefer, T., et al., 2014. Body mass index change in autism spectrum disorders: comparison of treatment with risperidone and aripiprazole. *J. Child Adolesc. Psychopharmacol.* 24 (2), 78–82. <https://doi.org/10.1089/cap.2013.0099>.
- Zamberletti, E., Gabaglio, M., Parolaro, D., 2017. The endocannabinoid system and autism spectrum disorders: insights from animal models. *Int. J. Mol. Sci.* 18 (9), 1916. <https://doi.org/10.3390/ijms18091916>.
- Zou, M., Liu, Y., Xie, S., et al., 2021. Alterations of the endocannabinoid system and its therapeutic potential in autism spectrum disorder. *Open Biol.* 11 (2), 200306. <https://doi.org/10.1098/rsob.200306>.