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CLINICAL TRIAL

Safety and preliminary efficacy on cognitive performance and adaptive functionality of epigallocatechin gallate (EGCG) in children with Down syndrome. A randomized phase Ib clinical trial (PERSEUS study)

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ABSTRACT

Purpose: Although some caregivers are using epigallocatechin gallate (EGCG) off label in hopes of improving cognition in young adults with Down syndrome (DS), nothing is known about its safety, tolerability, and efficacy in the DS pediatric population. We aimed to evaluate safety and tolerability of a dietary supplement containing EGCG and if EGCG improves cognitive and functional performance.

Methods: A total of 73 children with DS (aged 6-12 years) were randomized. Participants received 0.5% EGCG (10 mg/kg daily dose) or placebo for 6 months with 3 months follow up after treatment discontinuation.

Results: In total, 72 children were treated and 66 completed the study. A total of 38 participants were included in the EGCG group and 35 in the placebo group. Of 72 treated participants, 62 (86%) had 229 treatment-emergent adverse events (AEs). Of 37 participants in the EGCG group, 13 (35%) had 18 drug-related treatment-emergent AEs and 12 of 35 (34%) from the placebo group had 22 events. In the EGCG group, neither severe AEs nor increase in the incidence of AEs related to safety biomarkers were observed. Cognition and functionality were not improved compared with placebo. Secondary efficacy outcomes in girls point to a need for future work.

Conclusion: The use of EGCG is safe and well-tolerated in children with DS, but efficacy results do not support its use in this population.

Introduction

A clinical study in 16 to 34 years people with Down syndrome (DS) (TESDAD study) has shown that treatment with a flavanol from green tea, epigallocatechin gallate (EGCG), combined with cognitive training (delivered in the form of cognitive tasks provided via a telematic platform in which subjects perform them trice per week for about 30 minutes per session) is safe and provides health benefits improving cognition and adaptive functionality.[1] This is accompanied by increased functional connectivity detected in neuroimaging studies.[1] Despite this promising results, EGCG was

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not recommended for clinical use in patients. The modulation of the overexpressed *DYRK1A* gene was postulated as the most likely target of EGCG. *DYRK1A* overexpression has been linked to abnormal brain development in DS; however, the most appropriate time point for intervention to regulate *DYRK1A* expression has not been established.[2] Therefore, evaluation of safety and efficacy of EGCG in children, whose brain is characterized by high neural plasticity,[3] is a logical extension of studies conducted in people with DS aged 16 to 34 years.

In an animal model study overexpressing *DYRK1A*, the dietary supplement enriched with EGCG used in the present clinical trial showed no adverse events (AEs) on cardiac or hepatic function and was shown to pass through the blood-brain barrier.[4] Despite the available safety studies in adults with DS, there is a concern about the hepatotoxicity of EGCG[5] and the European Food Safety Authority has recommended limiting its intake of up to 800 mg/day.[5] There are no data related to its safety in the pediatric population.

The primary objective of the this report was to evaluate the safety and tolerability of a dietary supplement enriched with EGCG in children with DS aged 6 to 12 years. The secondary objective was to evaluate the effect of EGCG on cognitive performance and adaptive functionality.

Materials and Methods

This study was conducted between February 5, 2018 and March 24, 2020 in 5 centers: IMIM-Institut Hospital del Mar d'Investigacions Mèdiques, Barcelona; IHP-Instituto Hispalense de Pediatría, Sevilla; HIUNJ-Hospital Infantil Universitario Niño Jesús, Madrid; HUMV-Hospital Universitario Marqués de Valdecilla, Santander, and the IJL- Institut Jérôme Lejeune, Paris. Children with clinical diagnosis of DS were identified by their reference physicians in recruiting centers and through announcements in local DS associations. To reduce confounding factors in cognitive evaluation, only children with a mental age of at least 3 years and without severe disease were eligible for this study. Details on the inclusion and exclusion criteria are presented in Supplement 1.

Study design and treatment

This was a phase Ib randomized, multicenter, double-blind, placebo-controlled, parallel-groups study.

EGCG was administered combined with a dietary supplement (see FontUp, Grand Fontaine Laboratories in Supplemental Table 1, Supplement 2). The powdered formulation was chocolate flavored to mask the bitterness of EGCG. Patients received either active FontUp (containing 0.5% EGCG—daily dose of 10 mg/kg EGCG—5 mg/kg twice/day) or placebo (FontUp without EGCG twice a day for 6 months). EGCG dosage was established by body weight, up to a maximum daily dose of 400 mg EGCG because of safety concerns. The powder was dosed by families with a measuring spoon and administered orally, dissolved in 100 mL of water. The first dose was given in the morning and the second midafternoon. Caregivers had to provide boxes of the dietary supplement that were weighted to verify treatment compliance.

The clinical trial is registered in ClinicalTrials.gov (NCT03624556).

Procedures

The duration of individual's participation was 10 months: up to 15 days between screening and baseline, 6 months of treatment, and 3 months of follow up (Figure 1). Patients were contacted for the first visit (v1) and informed consent was signed. Patients were screened for eligibility, which included clinical examination, electroencephalogram (EEG), electrocardiography (ECG), echocardiography (EcCG), blood and urine analysis, and mental age assessment. Patients were enrolled and centrally randomized 1:1, 15 days before baseline visit (v2, day 1), using a conditional random sequence provided by the Hospital del Mar Pharmacy Department, which also provided the dietary supplement to each participant after treatment allocation. At v2, clinical and neuropsychological data were collected. At month 3 (v3), clinical assessment, EEG, blood, and urine analysis (chemistry, safety, and biomarkers) were performed and AEs were recorded. At 6 months (v4), an ECG, EcCG, and neuropsychological assessment were performed. Liver function was monitored on day 5 and liver and thyroid function on months 1.5, 3, 4.5, and 6. At the follow-up visit, 3 months after the end of treatment, clinical and neuropsychological assessments were performed and AEs were recorded.

Because a preclinical study showed signs of goiter and elevated thyroid-stimulating hormone (TSH) after 2 to 8 weeks of oral administration of diet containing green tea extract catechins to male rats,[6] we paid special attention to thyroid function. Alterations on thyroid function, depending on the degree of TSH elevation, were defined as mild or severe, according to whether TSH serum levels ranged between 4.5 to 10 mIU/L or were >10 mIU/L.[6] TSH concentrations of >6.3 mIU/L were initially considered a criterion for discontinuation. However, given thyroid function instability in individuals with DS,[7] the protocol was amended and the upper limit was raised to 10 mIU/L.

Drug accountability and participant's diary were used to measure compliance.

Outcome measures

Primary end point

Safety outcome

Safety and tolerability of treatments were evaluated by means of incidence, nature, severity, and causality of AEs and serious AEs (SAEs) as defined by the clinician through biochemical analysis (liver, renal, and thyroid function) and

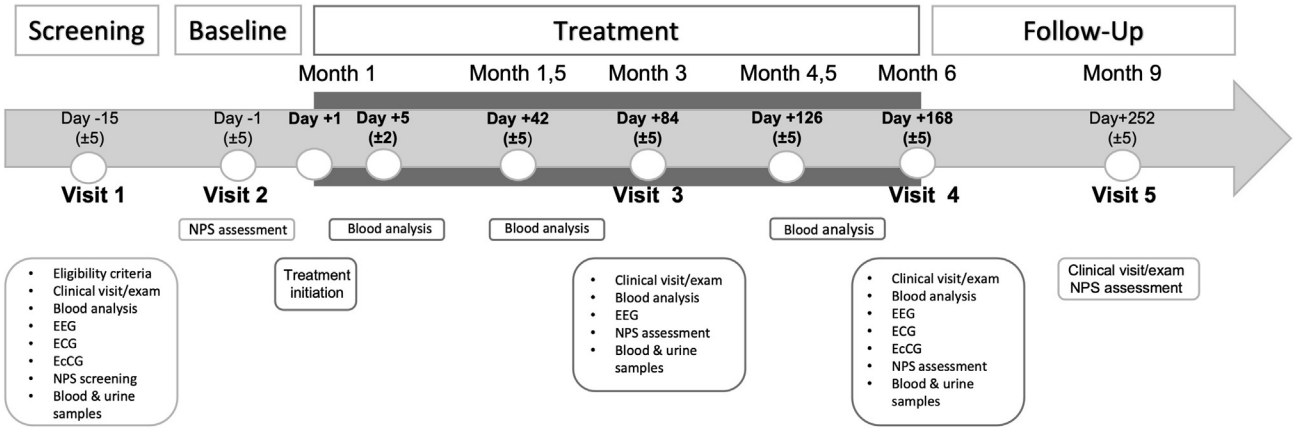

Figure 1 PERSEUS diagram of explorations. EcCG, echocardiography; ECG, electrocardiography; EEG, electroencephalogram; NPS, neuropsychological.

assessment of neurophysiological activity (EEG) and cardiac function by means of ECG and EcCG. Safety criteria can be found in Supplement 3.

Secondary endpoints

Primary efficacy outcome

Cognitive and functional performances were assessed with an ad hoc created battery (battery for children with Intellectual or Developmental disabilities, IDD-CHILD Battery). The cognitive evaluation included executive functions, attention, language, and memory domains. The functional assessment involved adaptive behavior (see details in Supplement 4 and Supplemental Table 2 in Supplement 2).

Secondary efficacy outcome

Secondary efficacy outcomes included proxy measures reported by parents on executive skills, memory, and quality of life (QoL) (see details in Supplement 4 and Supplement Table 2 in Supplement 2) and the modulation of *DYRK1A* kinase activity by EGCG measured as total plasma homocysteine and *DYRK1A* concentrations in plasma as surrogate efficacy biomarkers.[4]

Sample size considerations

Sample size computation was based on the primary efficacy outcome. The differences observed in the composite variable of Vineland Adaptive Behavior Scales (VABS)-II Supplemental Norm Score reported in a previous study in young adults with DS[8] were considered for sample size calculation. With a 2-sided alpha risk of 0.05 and a beta risk of 0.2, 35 patients were needed per group to detect a difference greater than 2.9 units with a common SD of 4. A dropout rate of 10% was anticipated.

Statistical analysis

Baseline characteristics of all study patients were described with either mean, SD, median, and range for quantitative variables and frequencies for qualitative variables. AEs and SAEs were recorded individually. AEs were reported from start of the treatment until 3 weeks after the last dose using Medical Dictionary for Regulatory Activities (MedRA version 24.0) and frequencies were presented per study group. The absolute differences between the risks of AEs between both treatment arms were estimated in percentage points together with 95% CIs, and tests for equality of risk differences were carried out.

Follow-up data of neuropsychological testing including cognitive and functional scores at 6 months of treatment and after a wash-out period of 3 months, respectively, were analyzed using linear mixed models (LMMs) that included treatment and baseline scores as fixed effects and site as random effect. The models' effect size measure is the baseline-adjusted mean difference after 6 and 9 months. For the follow-up data of efficacy biomarkers and safety markers, the LMMs used to analyze the data at 3 and 6 months included time, treatment, and time-treatment interaction as fixed effects and nested random effects per individual within each study site. However, because none of the time-treatment interactions were statistically significant, these were then removed from the models. Post hoc analyses were carried out to explore the possible effects of sex and mental age on treatment effect. Both sex-treatment and mental age-treatment interactions were included in the LMMs for the cognitive and functional scores at 6 and 9 months, respectively. If interaction was statistically significant, the treatment effect was estimated separately among girls and boys or according to mental age. The principal treatment effect differences found between girls and boys are shown with radar plots that represent the standardized mean difference (Cohen's d) under both treatments after 6 and 9 months, respectively. The computation of Cohen's d does not adjust for baseline differences, but enables the comparison of differences on different scales. For this purpose, differences on reverse scales were multiplied by –1.

All statistical analyses were carried out using the software package R, version 4.0.4. Statistical significance was

set at 0.05. In particular, the R package fmsb was used to compute the risk differences. A detailed analyses plan is provided in Supplement 4.

Results

Enrollment and study completion

Of the 93 participant candidates at screening, 20 were not included: 19 did not achieve a minimal cognitive development (mental age ≥ 3 years using the Brunet-Lézine scale C version, picture naming, and receptive vocabulary of the Wechsler Preschool and Primary Scale of Intelligence-IV [WPPSI-IV]) and 1 declined to participate. During the study, 7 subjects dropped out (2 consent withdrawn, 1 before treatment initiation but after randomization and another by weight gain, 3 cases of elevated levels of TSH, 1 case by EEG alterations, and 1 case by cow protein intolerance). A total of 73 patients were randomized, 72 patients took at least 1 treatment dose (safety population) and 66 patients completed the study (completed population) (Figure 2).

Of the 73 randomized patients, 38.4% were females. Mean (SD) chronological age and mental age were 9.5 (2) and 5.0 (0.7) years, respectively (Table 1). As a result of the random treatment allocation, characteristics of the patients in both treatment groups were very similar.

Primary end point

Safety outcome

Of 72 patients who took at least 1 treatment dose, 62 (86.1%) had a total of 229 treatment-emergent AEs. The distribution of AEs per group is presented in Table 2.

Of 37 patients, 13 (35.1%) had 18 drug-related treatment-emergent AEs in the EGCG group and 12 of 35 (34.3%) had 22 events in the placebo group (test for equality of proportions: $P = .94$). No severe AE was observed in the EGCG group.

Among the 229 AEs, 214 were mild, 12 moderate, and 3 severe. The 3 severe AEs were observed in the placebo group, including an SAE (EEG alteration). A total of 38 AEs were possibly related and 2 were definitely related (1 in the EGCG group—stye in the eye and 1 in the placebo group—TSH alteration). A list of all AEs and SAEs is available in Supplemental Table 3 in Supplement 2. Globally, the number of AEs was similar in both treatment arms; none of the differences among the proportions of patients with drug-related AEs in either treatment arms were statistically significant.

Regarding safety biomarkers, no alterations in liver enzymes nor in renal function were observed (Supplemental Table 4, Supplemental Figures 1 and 2, in Supplement 2). Regarding thyroid hormones homeostasis, 5 events of TSH alterations were observed in the EGCG group and 6 in the placebo group according to the ranges allowed by the protocol (Supplemental Table 3 in Supplement 2). Neither cardiotoxicity (evaluation of ECG and EcCG) nor neurophysiological alterations in EEG were observed.

Secondary endpoints

For both primary and secondary efficacy outcomes, results are available for all measures in Supplemental Table 5 in Supplement 2. No statistically significant and consistent differences were observed at 6 and 9 months for cognitive and functional assessments in favor of EGCG. Children receiving EGCG had a better VABS-written score at 9 months than those receiving placebo (baseline-adjusted treatment effect 1.84, 95% CI = 0.01-3.67; $P = .05$), but not at 6 months. Statistically significant improvements were observed for the placebo group compared with EGCG for comprehension, blocks design total, and VABS interpersonal relationship. Regarding QoL, a statistically significant improvement was observed in the EGCG group at 9 months (Pediatric Quality of Life Inventory [PedsQL]-school functioning, 9.67; 95% CI = 1.56-17.78; $P = .02$). For parent-reported outcomes assessed using the Behavior Rating Inventory of Executive Function-Preschool Version (BRIEF-P) executive function questionnaire, improvements in favor of EGCG were reported for working memory, plan and organize, and emergent metacognition index ($P = .02$ at 9 months, .02 at 6 months and .03 at 9 months, respectively).

No significant changes were found for *DYRK1A* plasma levels in neither EGCG nor active placebo groups (data not shown). Plasma homocysteine concentrations did not change over time in the EGCG group but those of the placebo group were lower at 6 months (4.6 vs 5.3 μmol/L; $P = .01$) when compared with baseline.

Interaction with sex and age

Results from post hoc analyses of interactions with mental age and sex are shown in Table 3 and Supplemental Figures 3 and 4 in Supplement 2.

For girls treated with EGCG, improvements in the composite of executive functions reported by caregivers (emergent metacognition [BRIEF-P] and in its 2 components, working memory and plan/organize), after 6 months, were observed. At 9 months, the improvements in plan/organize were still present. Improvements were also observed in girls treated with EGCG in PedsQL-total core module and in one of its components, PedsQL-social functioning. No effect was observed for boys.

A statistically significant interaction with mental age was found for PedsQL-emotional at 6 months but not at 9 months. Statistically significant improvements were also shown for VABS-expressive, picture naming, and picture memory at 9 months.

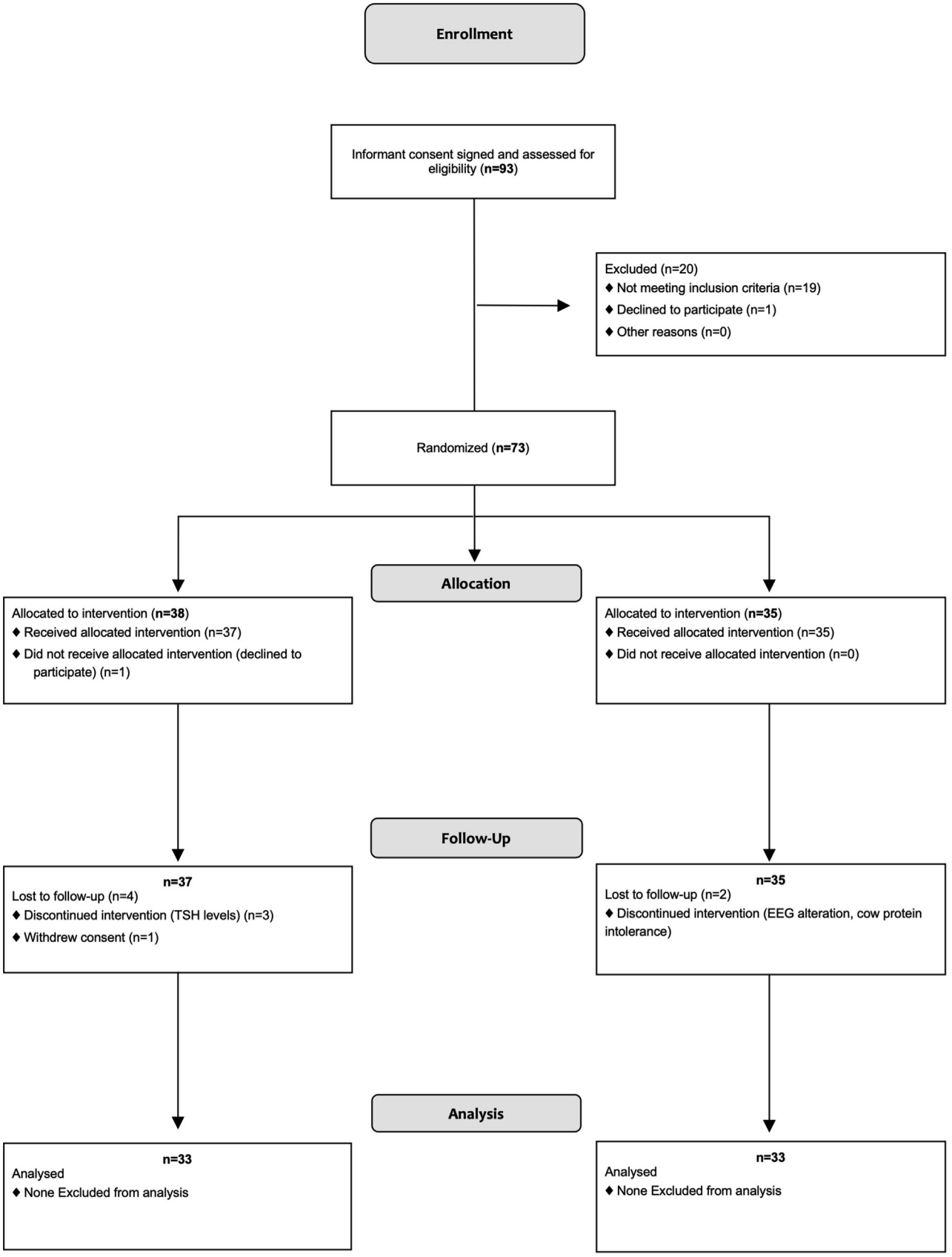

Figure 2 PERSEUS consort diagram. EEG, electroencephalogram; TSH, thyroid stimulating hormone.

Table 1 Summary description by groups of treatment

Sociodemographic Variables	All Randomized	All Completed	EGCG	Placebo
Study Site	*N* = 73 (%)	*N* = 66 (%)	n = 38 (%)	n = 35 (%)
Site 1	10 (13.7)	9 (13.6)	5 (13.2)	5 (14.3)
Site 2	19 (26.0)	18 (27.3)	10 (26.3)	9 (25.7)
Site 3	16 (21.9)	12 (18.2)	8 (21.1)	8 (22.9)
Site 4	19 (26.0)	18 (27.3)	10 (26.3)	9 (25.7)
Site 5	9 (12.3)	9 (13.6)	5 (13.2)	4 (11.4)
Sex				
Female	28 (38.4)	24 (36.4)	13 (34.2)	15 (42.9)
Male	45 (61.6)	42 (63.6)	25 (65.8)	20 (57.1)
Age, y, mean (SD)	9.53 (2.00)	9.42 (2.00)	9.61 (1.90)	9.46 (2.13)
Median (range)				
Median (range)	10 (5-12)	10 (5-12)	10 (5-12)	10 (6-12)
BMI (kg/m^2), mean (SD)	18.0 (2.86)	17.8 (2.65)	18.0 (2.36)	17.9 (3.35)
Median (range)	17.7 (13.7-28.7)	17.7 (13.7-28.7)	17.8 (15.0-26.6)	17.1 (13.7-28.7)
Mental age (mo), mean (SD)	60.5 (8.76)	60.2 (8.75)	60.9 (9.37)	60.1 (8.16)
Median (range)	63 (40.5-78)	63 (40.5-75)	63 (42-78)	63 (40.5-72)
Mental age (y), mean (SD)	5.04 (0.73)	5.01 (0.73)	5.07 (0.78)	5.01 (0.69)
Median (range)	5.2 (3.4-6.5)	5.2 (3.4-6.2)	5.2 (3.5-6.5)	5.2 (3.4-6)
Developmental quotient, mean (SD)	51.7 (9.55)	51.9 (9.18)	51.1 (8.41)	52.3 (10.7)
Median (range)	51.2 (33.5-79.3)	51.6 (33.5-79.3)	49.9 (36.8-79.3)	53.8 (33.5-78.1)
Manual dominance				
Left	4 (5.48)	4 (6.06)	2 (5.26)	2 (5.71)
Right	69 (94.5)	62 (93.9)	36 (94.7)	33 (94.3)
First language				
Catalan	6 (8.22)	4 (6.06)	3 (7.89)	3 (8.57)
French	19 (26.0)	18 (27.3)	10 (26.3)	9 (25.7)
Spanish	48 (65.8)	44 (66.7)	25 (65.8)	23 (65.7)
School years, mean (SD)	6.61 (2.89)	6.37 (2.82)	6.97 (2.67)	6.21 (3.10)
Median (range)	6 (1-12)	6 (1-11)	7 (2-12)	6 (1-11)

EGCG, epigallocatechin gallate.

Table 2 Description of adverse effects in patients who took at least 1 treatment dose

	EGCG (n = 37)			Placebo (n = 35)			Risk Difference
Adverse Events	Number of Events	Number of Patients	Percentage of Patients (%)	Number of Events	Number of Patients	Percentage of Patients (%)	Estimated (95% CI)
Treatment-emergent adverse events	103	30	81.1	126	32	91.4	–0.1 (–0.26 to 0.05); *P* = .2
Treatment-emergent drug-related adverse events	17	13	35.1	22	12	34.3	0.01 (–0.21 to 0.23); *P* = .94
Treatment-emergent moderate adverse events	5	5	13.5	7	5	14.3	–0.01 (–0.17 to 0.15); *P* = .92
Treatment-emergent moderate drug-related adverse events	2	2	5.4	1	1	2.9	0.03 (–0.07 to 0.12); *P* = .58
Treatment-emergent severe adverse events	0	0	0.0	3	3	8.6	–0.09 (–0.18 to 0.01); *P* = .07
Treatment-emergent severe drug-related adverse events	0	0	0.0	1	1	2.9	–0.03 (–0.08 to 0.03); *P* = .31

The denominator for each percentage is the number of patients in the safety set within the column. The risk difference is the absolute difference of the proportions of patients with adverse effects. Mild side effects are not shown. Severe is defined as grade 3 or above. Drug-related is defined as definitely, probably, or possibly related. A missing severity is considered grade 3. A missing relationship is considered definitely related, if the event is treatment-emergent. Treatment-emergent adverse events leading to withdrawal from the study are defined as those treatment-emergent adverse events in which the field "Withdrawn from Study?" is marked as Yes.

EGCG, epigallocatechin gallate.

Table 3 Estimated treatment effects and 95% CI after 6 and 9 months among girls and boys

		Sex-Treatment Interaction	Baseline and Mental Age-adjusted Treatment Effects[a]	
			Boys	Girls
Functional Assessment	Time Period	*P*-value	Adjusted mean difference (95% CI) *P*-value	Adjusted mean difference (95% CI) *P*-value
BRIEF				
Inhibit[b]	6 months	$P = .521$		
	9 months	$P = .209$		
Working memory[b]	6 months	$P = .026$	0.07; (−3.12, 3.26); $P = .999$	−5.07; (−9.33, −0.80); $P = .016$ (better performance under EGCG)
	9 months	$P = .258$		
Plan/organize[b]	6 months	$P = .036$	−0.42; (−1.49, 2.33); $P = .857$	−3.88; (−6.72, −1.03); $P = .005$ (better performance under EGCG)
	9 months	$P = .046$	0.53; (−2.64, 1.58); $P = .821$	−2.67; (−5.24, −0.09); $P = .041$ (better performance under EGCG)
Emergent metacognition index[b]	6 months	$P = .023$	−0.34; (−4.89, 4.16); $P = .979$	−7.82; (−13.91, −1.74); $P = .008$ (better performance under EGCG)
	9 months	$P = .130$		
PedsQL	9 months			
Physical functioning	6 months	$P = .062$		
	9 months	$P = .099$		
Emotional functioning	6 months	$P = .912$		
	9 months	$P = .587$		
Social functioning	6 months	$P = .158$	−3.83 (−13.91, 6.25); $P = .634$	16.09 (2.5, 29.68); $P = .016$ (better performance under EGCG)
	9 months	$P = .016$		
School functioning	6 months	$P = .285$		
	9 months	$P = .067$		
Total core module	6 months	$P = .077$	−0.04; (−7.59, 7.51); $P = 1$	13.14; (3.02, 23.26); $P = .007$ (better performance under EGCG)
	9 months	$P = .028$		
Cognitive score	6 months	$P = .841$		
	9 months	$P = .085$		

Positive values indicate higher scores of EGCG with respect to placebo.

BRIEF, Behavior Rating Inventory of Executive Function; *EGCG*, epigallocatechin gallate; *PedsQL*, Pediatric Quality of Life Inventory.

[a]Obtained from linear mixed models with treatment, sex, mental age, sex-treatment, and mental age-treatment interactions, and baseline scores as fixed effects and a random intercept per study center. CIs and *P* values are adjusted for multiple comparisons.

[b]Negative scores indicate improvement on this cognitive test.

Discussion

The primary objective of this study was to evaluate the safety and tolerability of EGCG in children with DS. The treatment was well-tolerated by patients, with a similar rate of AEs in both treatment groups. Neither cardio, renal, and hepatotoxicity nor neurophysiological alterations were observed.

Special attention was paid to thyroid homeostasis, because preclinical results have highlighted a potential risk of goiter and elevated TSH levels.[6] TSH alterations and/or hypothyroidism were observed in both treatment groups (5 patients per group), suggesting no increased thyroid-related toxicity due to EGCG administration. An acceptable safety profile of EGCG and omega-3 supplements was also reported in a recent study in children with DS aged 1 to 8 years.[9] In that study, EGCG was administered at a dose of 10 mg/kg per day for 6 months and the only safety finding was a decline in plasma folates in 3 patients, which was likely related to inhibition of folic acid uptake by EGCG.

Regarding secondary objectives on efficacy, no improvements in cognitive and functional outcomes were observed in the EGCG group compared with placebo. Statistically significant differences were identified both in the EGCG and placebo group for isolated scores, but these improvements were not consistent over time.

In some parents-reported outcomes, positive treatment effects were observed. Participants receiving EGCG improved in the BRIEF-P emergent metacognition index and in its components, working memory and planning skills. This index represents the child's difficulties in initiating, planning, organizing, implementing, and maintaining future-oriented problem solving. They also improved in its functioning at school (PedsQL) according to the parents. These results are in line with those previously found in the TESDAD study,[1] in which improvements in executive function task's and academic skills were observed. Post hoc analysis indicated a potential interaction between mental age for emotional functioning (PedsQL), in naming and memory tasks, and in adaptive behavior with a larger EGCG treatment effect observed with increasing mental age. There was also an apparent sex interaction; girls receiving EGCG showed improvements globally in executive functioning, working memory, and planning skills, and QoL, which was

not observed in boys (data reported by parents). These improvements are again in accordance with the results of a previous study in young adults with DS treated with EGCG already mentioned. Although our results should be interpreted with caution given the small number of patients in subgroups, in this previous study, some sex differences in the treatment effects on lipid profile and body composition were shown,[10] which should be considered among other factors that may explain the observed sex interaction. EGCG has poor bioavailability and erratic pharmacokinetics, leading to large intersubject variability in catechin pharmacokinetics that might contribute to variations in the beneficial effects of EGCG.[11,12]

Homocysteine and *DYRK1A* plasma concentrations were used as surrogate biomarkers for EGCG activity.[13] No significant change in *DYRK1A* concentration levels were observed, whereas a decrease in homocysteine levels was noted after 6 months in the placebo group. In contrast to our results, previous findings in adults with DS showed a rise in homocysteine concentrations after EGCG administration.[1] This discrepancy might be due to the EGCG dietary supplement used in this study that also contained omega-3 and vitamin B to facilitate EGCG dosing in children. Omega-3 fatty acids and vitamin B were previously shown to act synergistically leading to a decrease in homocysteine levels.[14] Conversely, EGCG can inhibit folic acid uptake, which would lead to an increase in homocysteine levels.[10] Taken together, EGCG has the opposite effect on homocysteine levels compared with omega 3 and vitamin B. The lack of measurable effect of EGCG on *DYRK1A* plasma concentrations precludes arguing *DYRK1A* inhibition. Future studies are needed to assess EGCG disposition in children with DS and the effect of sex, dose, and formulation on EGCG plasma concentrations.[15]

The strength of this study is the inclusion of a large, homogeneous sample of children with DS. All patients went to school and received similar levels of cognitive stimuli. Lack of treatment effect is unlikely because of selection bias of the study population. However, there are several potential reasons for the absence of detected treatment effect: (1) the administered EGCG dose was insufficient to reach pharmacologically active concentrations, (2) EGCG lacks efficacy in this population, (3) EGCG concentration dropped below pharmacologically active levels during night time, when consolidation of new information takes place, (4) other constituents of the nutritional supplement, ie, omega-3 and vitamin B could have exerted a positive treatment effect (active placebo), thereby masking a potential EGCG treatment effect, and (5) the neuropsychological tests were not adequate to detect an EGCG mediated effect. This last point is supported by the fact that parent-reported outcomes were not fully aligned with those obtained by neuropsychologists using instruments for patients with intellectual disabilities.

The largest limitation of this study is the fact that EGCG plasma concentrations were not measured for pharmacokinetics, precluding analysis of a correlation between EGCG exposure and *DYRK1A* inhibition. The dose was selected on the basis of safety considerations and previous studies with EGCG in young adults with DS[1,10] and mice models. Further research is needed to describe the effect of EGCG on *DYRK1A* concentrations in children with DS and to identify biomarkers for spatiotemporal expression patterns of genes involved in the development of children with DS.

Conclusion

The intake of the dietetic supplement enriched with EGCG up to a dose of 400 mg/day is safe and well-tolerated in children with DS of both sexes aged 6 to 12 years. Although there was no improvement in cognitive and functional outcomes over the treatment period, potential benefits in the participants treated with EGCG, particularly in girls, was found. However, until further research is done, the administration of EGCG in children with DS is not clinically justified.

Data Availability

All data supporting the engagement methods described and outcomes gleaned are available from the authors on request.

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Ethics Declaration

The study protocol was approved by the local Institutional Review Board (CEIM-PSMAR at IMIM, 2017/7194-PERSEUS/1 v7 and further endorsed by the other institutional review boards of Spanish participating centers) in Spain and by the Comité de Protection des Personnes and authorized by the French competent authorities (ANSM)-(IDRCB 2017-A01984-49) in France; it adheres to the standards of the Declaration of Helsinki, European Commission standards for conducting clinical trials, and Good Clinical Practices and complies with the General Data Protection Regulation (GDPR UE 2016/976). The participants provided their written informed consent to participate in this study.

Conflict of Interest

The authors declare no conflicts of interest.

Additional Information

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