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A Randomized, Double-Blind, Placebo-Controlled Trial of Low-Dose Sertraline in Young Children with Fragile X Syndrome

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Abstract

Objective—Observational studies and anecdotal reports suggest sertraline, a selective serotonin reuptake inhibitor (SSRI), may improve language development in young children with fragile X syndrome (FXS). We evaluated the efficacy of six months of treatment with low-dose sertraline in a randomized, double-blind, placebo-controlled trial in 52 children with FXS ages 2–6 years.

Results—Eighty-one subjects were screened for eligibility and 57 were randomized to sertraline (27) or placebo (30). Two subjects from the sertraline arm and three from the placebo arm discontinued. Intent-to-treat analysis showed no difference from placebo on the primary outcomes: the Mullen Scales of Early Learning (MSEL) expressive language age equivalent and Clinical Global Impression-Improvement (CGI-I). However, analyses of secondary measures showed significant improvements, particularly in motor and visual perceptual abilities and social

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participation. Sertraline was well tolerated, with no difference in side effects between sertraline and placebo groups. No serious adverse events occurred.

Conclusion—This randomized controlled trial of six-months of sertraline treatment showed no primary benefit with respect to early expressive language development and global clinical improvement. However, in secondary, exploratory analyses there were significant improvements seen on motor and visual perceptual subtests, the Cognitive T score sum on the MSEL, and on one measure of Social Participation on the Sensory Processing Measure–Preschool. Further, post hoc analysis found significant improvement in early expressive language development as measured by the MSEL among children with ASD on sertraline. Treatment appears safe for this 6-month period in young children with FXS, but we do not know the long-term side effects of this treatment. These results warrant further studies of sertraline in young children with FXS using refined outcome measures, as well as longer term follow-up studies to address long-term side effects of low-dose sertraline in early childhood.

INTRODUCTION

Fragile X syndrome (FXS) is a monogenic neurodevelopmental disorder caused by a CGG repeat expansion in the fragile X mental retardation (*FMR1*) gene on the X chromosome. The full mutation – an expansion of greater than 200 CGG repeats – leads to methylation of the gene and a subsequent lack of the protein it encodes, the fragile X mental retardation protein (FMRP)[1–3]. FXS is the most common genetic form of intellectual disability (ID) and single-gene cause of autism spectrum disorder (ASD). The full mutation allele is present in approximately 1 in 5,000 males and 1 in 8,000 females in the total population[4]. FXS is characterized by significant behavioral, cognitive, and emotional dysregulation including symptoms of anxiety, ASD, attention deficit hyperactivity disorder (ADHD), self-injurious behavior, irritability, aggression, impulsiveness, sensory processing vulnerabilities, and language deficits[5–11]. In addition, there are motor coordination deficits[11,12] and visual perceptual deficits; the latter of which have been documented even in infancy with eye tracking studies[13,14]. FXS is also highly associated with ASD; approximately 60% of patients with FXS also have ASD[7,15,16]. In addition, Iossifov *et al.* found that 30–50% of de novo gene mutations resulting in ASD are regulated by or associated with FMRP[17].

Underlying these behaviors is the deficit in FMRP throughout development[18]. FMRP is a selective mRNA-binding protein that negatively regulates the translation of mRNA into proteins at the synapse[1,19]. The proteins regulated by FMRP are important for synaptic plasticity and include cytoskeleton proteins, metabotropic glutamate receptors (mGluRs), and γ-aminobutyric acid (GABA) receptor subunits[20]. The lack of FMRP leads to upregulation of protein synthesis and altered synaptic function[1]. Targeted treatments for FXS would ideally reverse these neurophysiological changes.

To date, the mGluR5 antagonists, proposed as targeted treatments, have not demonstrated efficacy in FXS in trials[21], although they have not been studied in children under 5 years old[22]. There is emerging evidence that treatment in young children with neurodevelopmental problems may have the best effects because the brain is still rapidly developing and thus most susceptible to intervention[23–27]. For example, there was no effect in adolescents and

adults with FXS treated with arbaclofen, but children ages 5 to 11 years demonstrated benefit in several behavioral measures[28]. This is also the case for behavioral interventions in ASD utilizing the Early Start Denver Model (ESDM); young children under 5 years of age demonstrate improvements not only in behavioral and developmental symptoms, but also improvements in EEG parameters compared to community intervention[29].

Young children with autism show lower levels of serotonin production on PET scanning compared to young neurotypically developing children[30,31]. Serotonin levels are abnormally low during early development (under 5 years) when synapse formation is most rapid, suggesting a developmental window in the first several years of life in which a selective serotonin reuptake inhibitor (SSRI) could be beneficial[24]. Evidence also shows that SSRIs stimulate brain-derived neurotrophic factor (BDNF) when given early in development in mouse models of Down syndrome[32]. Given the lack of maturation of synapses in FXS along with BDNF's role in synaptic maturation, plasticity, and neurogenesis[33,34], SSRIs are of particular interest for the treatment of FXS[24].

According to medication usage surveys, approximately 50% of patients over 5 years old with FXS are prescribed an SSRI[6,35]. Sertraline (trade name Zoloft) is an SSRI that is approved by the Food and Drug Administration (FDA) for the treatment of Obsessive Compulsive Disorder in children 6–17 years old, as well as Major Depressive Disorder, Social Anxiety Disorder, Panic Disorder, Posttraumatic Stress Disorder, and Premenstrual Dysmorphic Disorder in adults. Sertraline has been used in clinical practice to treat anxiety, irritability, and socialization deficits in individuals with FXS[36]. Sertraline was initially used clinically to lower the symptom of anxiety in children with FXS[36]. In addition, a retrospective study of low dose sertraline (2.5 to 5 mg per day) in 45 children with FXS ages 12 to 50 months demonstrated significant improvements in the trajectory of receptive and expressive language in those on sertraline compared to those not treated with sertraline[37]. This study emphasized the need for a controlled trial of low dose sertraline in young children with FXS. Sertraline may be an optimal SSRI for FXS because it is less activating than fluoxetine, has minimal interaction with the metabolism of other medications compared to other SSRIs, and prevents re-uptake of dopamine, particularly in the striatum[24,38–40]. Dopamine is dysregulated in FXS because of impaired dopamine-receptor modulation in cells lacking FMRP [41]. Such evidence has supported the need for further study of low-dose sertraline in young children with FXS as the one reported here.

MATERIALS AND METHODS

Participants and Design

This was an exploratory, first trial of sertraline in children with fragile X syndrome ages 2 to 6 years using a randomized, double-blind, placebo-controlled parallel two-arm design between February 2012 and August 2015. Inclusion criteria included molecular documentation of FXS, age between 2 and 6 years, English speaking, and willingness to travel and participate in this controlled trial. Exclusion criteria included CNS disease other than FXS or other disease state. Patients with FXS both with and without ASD were included in this study. The ASD diagnosis was made when children scored in the ASD range on the Autism Diagnostic Observation Scale, Second Edition (ADOS-2)[42] and by

Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)[43] criteria. Randomization to sertraline or placebo was carried out independently by XXXXXXXXXX Investigational Drug Services. The study drug was administered in liquid form (20 mg per mL). Subjects ages 2 to 3 years received sertraline liquid or placebo liquid in a dose of 2.5 mg per day (0.125 mL). Subjects ages 4 years to 5 years, 8 months received a dose of 5.0 mg per day (0.25 mL). The doses were based on those used in the retrospective study that originally suggested sertraline may help improve the trajectory of language development[37]. Assessments were conducted in clinic at baseline and at a six-month follow-up visit, except the Clinical Global Improvement (CGI-I) scale, which was given only at the six-month visit. Weekly telephone calls in the first month monitored side effects by reviewing a side effects checklist, with subsequent calls occurring monthly. A follow-up phone call at 3 months also included a review of behavior and side effects.

Assessments

All assessments (except the CGI-I) were completed at both baseline and approximately six-month follow up visits. Global assessments included the CGI-I[44], and the MSEL[45] Early Learning Composite (ELC) and four of its subscales: fine motor, visual reception, expressive language, and receptive language skills. The Preschool Language Scale, Fifth Edition (PLS-5)[46] was also used to evaluate Auditory Comprehension (AC) and Expressive Communication (EC). More specific assessments included a Visual Analog Scale (VAS) of the three most problematic behaviors reported by parents, the Sensory Processing Measure – Preschool Home Form (SPM-P)[47,48] to assess sensory processing, social participation and praxis, and the Autism Diagnostic Observation Scale, Second Edition (ADOS-2)[49] to diagnose comorbid autism in subjects. Medication compliance was also tracked with a daily dosing diary. Safety assessments involved a physical exam, vital signs, and standard blood labs including a Comprehensive Metabolic Panel (CMP) and Complete Blood Count with Differential (CBC).

Statistical Analysis

The study design included three primary outcomes at six months: MSEL expressive language raw score, MSEL expressive language standard score, and CGI-I. The pre-specified efficacy analysis for the expressive language score was analysis of covariance (ANCOVA) with baseline scores and the t-test for CGI-I. Thus, tests for primary efficacy analysis were adjusted at the significance level of 0.016 for n = 52 subjects. For three subjects who exceeded 68 months of age at the 6-month visit, standard and age-equivalent scores were approximated using the 60-month age conversion chart. All other measures and associated analyses are secondary/exploratory, and the analysis was also based on the ANCOVA model for outcomes with baseline measures. Post hoc analyses of primary outcomes by subgroup (full mutations, mosaics), ASD, and males were conducted. To assess the robustness of the main analyses to missing data, we have conducted a post hoc principled sensitivity analysis for primary outcomes based on pattern mixture model estimated by a mean score approaching using the *rctmiss* package in Stata®. Adverse events were summarized by severity, relation to drug, and AE resolution status (ongoing/not ongoing). Student's t-test and Fisher's exact test were applied to continuous and categorical variables.

All tests, except for primary efficacy, were at level of 0.05 and analyses were implemented in SAS ® software Version 9.4[50].

The study was designed with 80% power to detect a standardized effect size of about 0.82 in a two-arm parallel design with endpoint at six month at level 0.016 for three primary measures. The required sample size is 60 (30 per group), but resource limitations limited the number of patients randomized to 57.

RESULTS

Subject Characteristics

Eighty-one subjects were assessed for eligibility and 57 participants met the inclusion criteria stated above. There was no significant difference in MSEL mean Early Learning Composite (ELC) at baseline for sertraline 56.6 (+13.55) versus placebo 54.8 (+8.53). Nor was there any difference in the non-pharmacological therapies, such as speech and language therapy and occupational therapy, that were utilized for >93% of each treatment group. There was no significant difference in the level of education of the mothers in either treatment group (Table 1). 56% of families included a parent with a college degree or higher in the sertraline arm compared to 67% in the placebo arm. These 57 participants were randomized: 27 to sertraline and 30 to placebo (Figure 1). Two subjects from the sertraline arm and three subjects from the placebo arm discontinued. The demographic characteristics are shown in Table 1. There were no significant demographic differences between the two treatment arms. The majority of participants were males (78% in sertraline and 90% in placebo) and Caucasian (70% in sertraline and 50% in placebo) with an average age of 3.9 (SD 1.1) years in the sertraline group and 3.9 (SD 1.1) years in the placebo group. Fifty-two participants completed six months of treatment.

Pre-specified primary outcome analysis

Pre-specified intent-to-treat (“as randomized”) analyses were conducted for three designated primary outcome measures: Mullen Scales of Early Learning (MSEL) expressive language raw score, MSEL expressive language standard score, and Clinical Global Impression Scale–Improvement (CGI-I). Observed changes in scores were on average greater for the sertraline group, but there was not a significant difference compared to placebo for the three pre-specified primary outcomes: MSEL expressive language raw score (mean for sertraline vs. placebo: 25.04 [10.78] vs. 21.3 [9.59], $P = 0.586$), expressive language standard score (25.76 [10.87] vs. 22.59 [6.47], $P = 0.607$), and CGI-I (2.28 [1.06] vs. 2.59 [0.84], $P = 0.244$); see Table 2. Descriptively, 8 (32%) on sertraline compared to 2 (7.4%) on placebo reported “very much improved”; 5 (20%) on sertraline compared to 10 (40.7%) on placebo reported “much improved”; and the percent in each arm reporting “minimally improved” or “no change” were essentially the same.

Secondary/exploratory outcome measures

Secondary measures included (A) MSEL subscales: fine motor, visual reception and receptive language score; (B) social affect, restricted and repetitive behavior total of the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2); (C) Visual Analog

Scale (VAS); (D) Sensory Processing Measure-Preschool (SPM-P; Home Form); and (E) Preschool Language Scale, Fifth Edition (PLS-5). The results show significant improvement on sertraline in fine motor age equivalent (28.44 [10.91] vs. 25.04 [6.91], $P = 0.005$), the fine motor raw score (27.32 [8.06] vs. 25.19 [4.96], $P = 0.008$), the age equivalents for visual perception (33.68 [15.06] vs. 30.59 [9.6], $P = 0.031$) and the Cognitive T score sum (105.36 [40.27] vs. 93.0 [20.33], $P = 0.047$). Post hoc analysis combining all MSEL age-equivalent scores (expressive, visual, receptive, and fine motor) indicates significant improvements on sertraline compared to placebo (30.09 [12.64] vs. 23.60 [10.76], $P = 0.007$) (Figure 2). Observed average scores on other secondary measures were typically improved in the sertraline group, although not significantly improved compared to placebo (Table 3). The SPM-P social participation subtest score, however, demonstrated significantly lower dysfunction on sertraline compared to placebo at follow up (raw score: 18.01 [6.2] vs. 19.65 [6.4], $P = 0.013$). Details are presented in Table 3.

We noted that standard scores (T scores) were typically not significant, which may be due to the fact that the many participant scores were floored at the lowest score of 20. Thus, a post hoc analysis of the age-equivalent MSEL combined score (combining the expressive, visual, receptive and fine motor subtest scores) was conducted. Based on this combined MSEL measure, there was significant improvement in follow-up scores, adjusted for baseline score (mean: 30.09 months [12.64]on the vs. 23.60 months [10.76], P = 0.007) (Table 3 and Figure 4).

Post hoc analysis: Mosaicism, ASD, Gender, Missing data

We considered post hoc analyses with respect to a) mosaicism, b) ASD, c) male gender, and d) missing data sensitivity. No differences in treatment effect were found among individuals with mosaicism and among full mutation subgroups (results not shown). Among the cohort with ASD, overall improvement as measured by CGI-I was not different between sertraline and placebo. However, MSEL expressive language raw score improvement was significantly higher in the children with ASD in the sertraline group compared to placebo group (23.5 [10.5] vs. 17.6 [6.8], P = 0.029). The MSEL expressive language T score was not significant due to the flooring effect. For the children without ASD, the difference in expressive language raw score was not significantly different in the sertraline compared to placebo (31.0 [7.7] vs. 26.6 [10.8], P = 0.785), although the results should be interpreted with caution since the sample size is very small. In a post hoc analysis based on only males (excluding 7.7% who were females), the results/conclusions were the same as the primary analysis describe above.

Post hoc sensitivity analyses were conducted to examine whether the main study analyses were robust to missing data. The sensitivity analysis for expressive language (EL) score assumes that the average score may change up to 10 units (mean score for main analysis range from 21 to 26) in both arms, in the sertraline arm only, and in control arm only. For CGI-I (score range 1 to 7), the sensitivity analysis assumes that the average score could change up to three units. These ranges for difference in outcome measures for sensitivity analysis are extremely wide in order to accommodate any reasonable change in the outcome variable (representing small to very large clinical departure) from the primary analysis

(ANCOVA models and t-test for CGI-I). Not surprisingly, due to the small number of missing values, the conclusions remain unchanged (95% confidence interval contains zero, i.e. no difference between sertraline and placebo for each sensitivity parameter value).

Safety

There were 253 adverse events (AEs) reported and they were similar between sertraline and placebo groups. The top three types of AEs were upper respiratory infection, diarrhea, and gastrointestinal issues. Details of the types of AEs are provided in Table 4. Table 5 summarizes characteristics of AEs by severity, relation to drug, AE status (whether ongoing) and serious AEs. No serious AEs were reported. No significant differences in characteristics of AEs were found between subjects on sertraline and placebo, respectively, for severity (any moderate/severe AE: 54% vs. 48%, P = 0.789), relationship to drug (any drug related AE: 81% vs. 79%, P = 1.0), and whether the AE status was ongoing (any ongoing AE: 3% vs. 10%, P = 0.455).

DISCUSSION

The developmental window of rapid synapse formation and network connectivity in the first few years of life may perhaps be the best time to deliver a beneficial treatment[23,25,29]. The first 5 years of life is also a time of low serotonin production in the frontal cortex in children with ASD[51,52]. A metabolomics study of a variety of forms of ASD have also demonstrated a deficit of the enzymes critical for the transformation of tryptophan into serotonin in lymphoblastoid lines[53]. These data suggest that serotonin levels may be low in the first few years of life in children with ASD and perhaps children with FXS.

In a retrospective study[37], the use of an SSRI (sertraline) in a low dose that does not lead to activation or hyperarousal was associated with improvement in receptive and expressive language trajectories in young children with FXS. This finding led to the current controlled trial of sertraline in FXS reported here. Expressive language was chosen as a primary outcome measure, primarily based on clinical observations and the published retrospective study, but the current study was considered exploratory and represented the first controlled trial of sertraline in young children with FXS with limited knowledge and guidance on outcome measures. Although children treated with sertraline did not significantly improve in the pre-specified primary outcome measures compared to placebo, they demonstrated nominal significant improvements in the age-equivalent combined subtest scores of the MSEL, the Cognitive T score sum of the MSEL, and both the age equivalent and raw scores of the visual reception and fine motor coordination subscales of the MSEL in secondary/exploratory analyses. Similarly, a nominal significant improvement in those treated with sertraline compared to placebo was also demonstrated in the social participation subscale raw score from the SPM-P Home Form, wherein families indicated positive changes in social aspects of daily routines including play with others, meals, family outings such as holidays or birthday parties, and community participation such as going to the grocery store. Therefore, sertraline not only demonstrated a nominal significant effect on certain aspects of development and cognition, but also limited evidence of social improvements. Further, post hoc analysis found significant improvement in the primary outcome variable, early

expressive language development, among children with ASD on sertraline compared to placebo. These are important outcome measurement domains that warrant consideration in future studies because potentially useful outcome measures for randomized controlled studies in children with FXS are quite limited. All families that completed the study wanted to continue their children on sertraline clinically at the end of the study.

Significant developmental change in both groups was expected in this study because early childhood is when significant language abilities normally arise, particularly over the prolonged course of a six-month treatment period. Therefore the “placebo” effect seen most easily in the CGI-I gain also represents a developmental gain expected at this age. We would expect that the developmental gain would also be additive to the sertraline effect. Although sertraline did not produce significant language gains over placebo, it is interesting and encouraging for the families that the visual perceptual and fine motor skills of children with FXS were significantly improved by sertraline because these are common deficits seen in FXS. Visual perceptual problems, specifically reduced contrast sensitivity to second order movement, can be detected in the first year of life in eye-tracking studies of babies with FXS[13,14]. Subsequent visual perceptual problems including visual memory, visual spatial perception, and visual motor coordination are problematic in both males and females with FXS[54–57]. Both fine motor and gross motor coordination problems occur in FXS and can sometimes lead to delayed motor milestones, especially if hypotonia is present. Referral to an occupational and physical therapist is recommended to give early intervention for these problems[11,12]. It is possible that the effects of sertraline on the dopamine system, specifically up-regulation of dopamine in the nucleus accumbens and striatum[58], may have supported improvements in motor and perceptual skills.

Animal studies have also demonstrated a procognitive and neuroprotective effect of sertraline in wild type mice[59]. The procognitive effect in young mice is thought to relate to up-regulation of BDNF, which in turn up-regulates serotonin, dopamine and GABAergic systems[60,61]. Lauterborn and colleagues rescued the synaptic plasticity deficits in the KO mouse with BDNF treatment[62]. Perhaps BDNF up-regulation is the key mechanism for the procognitive benefits of sertraline seen in the young patients with FXS treated here[62].

Another possibility is that sertraline did not directly affect motor or perceptual systems per se, but that the positive effects on the fine motor and visual perception subscales of the MSEL were due to a general increase of focused attention in the treatment group. Thus, by allowing subjects to be in a calmer and more focused attentional state during test administration, sertraline might have yielded more positive scores on these subscales because their completion requires focused attention and physical compliance. These improvements in behavior may also have been linked to reduced anxiety during testing, therefore contributing to better performance in the participants. It should be noted, however, that one subjective parent measure of anxiety, the VAS, did not demonstrate significant improvements in behavior including anxiety or mood compared to placebo.

There are a number of limitations in this study and the results should be considered preliminary. There were several secondary analyses, which increases the likelihood of finding a significant result that is spurious. Subjects and caregivers were unblinded at the

time they completed the study with their child as opposed to the conclusion of the overall study, introducing potential bias. Another limitation of the study is the limited number of girls in the study (nine total); thus, this does not allow for an assessment of differential effects between girls and boys since it is likely that girls likely will show greater developmental progress during a 6-month time span.

Importantly, the side effects of sertraline were not significantly different from placebo and there were no serious adverse events. This medication appears to be safe in young children with FXS when used for 6 months, but follow-up is essential, particularly if young patients continue with longer-term treatment. Indeed, all of the caregivers elected to continue sertraline for their children at the end of the trial. Their follow-up is necessary to better understand if any long-term problems are associated with low-dose sertraline use in young children.

CONCLUSION

This preliminary controlled trial demonstrated that expressive language is not significantly improved by 6 months of treatment with sertraline in young children with FXS. However, sertraline did produce modest but nominal significant gains in visual perception, fine motor skills, social participation, and overall development in exploratory/secondary analyses, and improvement in expressive language among children with ASD in post hoc analysis. This study suggests that further trials to replicate these preliminary results and studies coupled with enhanced language/educational interventions are warranted in FXS and could provide guidance on effect sizes and refined outcome measures to be used in future trials.

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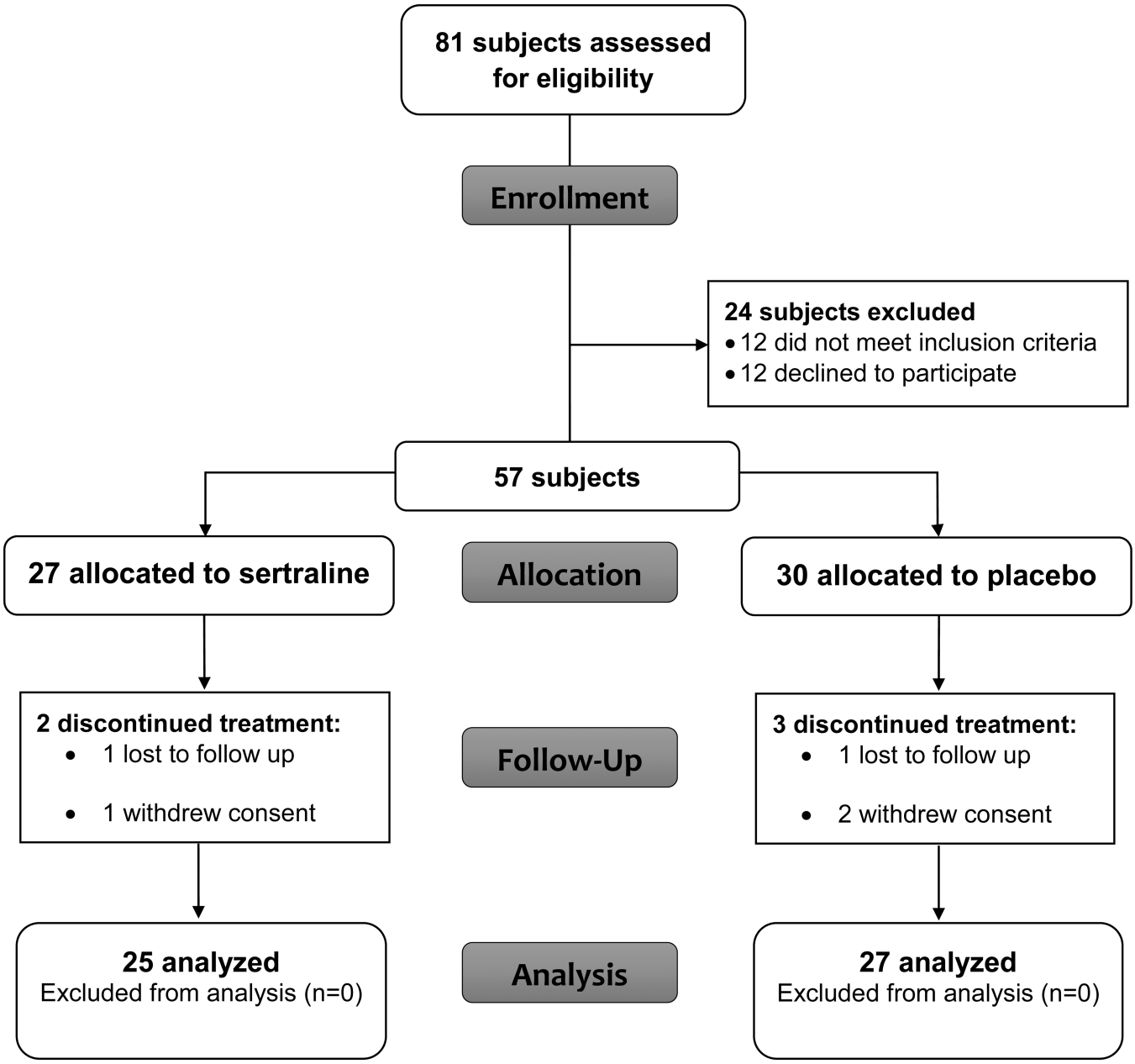

Figure 1.
Consolidated Standards of Reporting Trials (CONSORT) flow diagram of subject disposition.

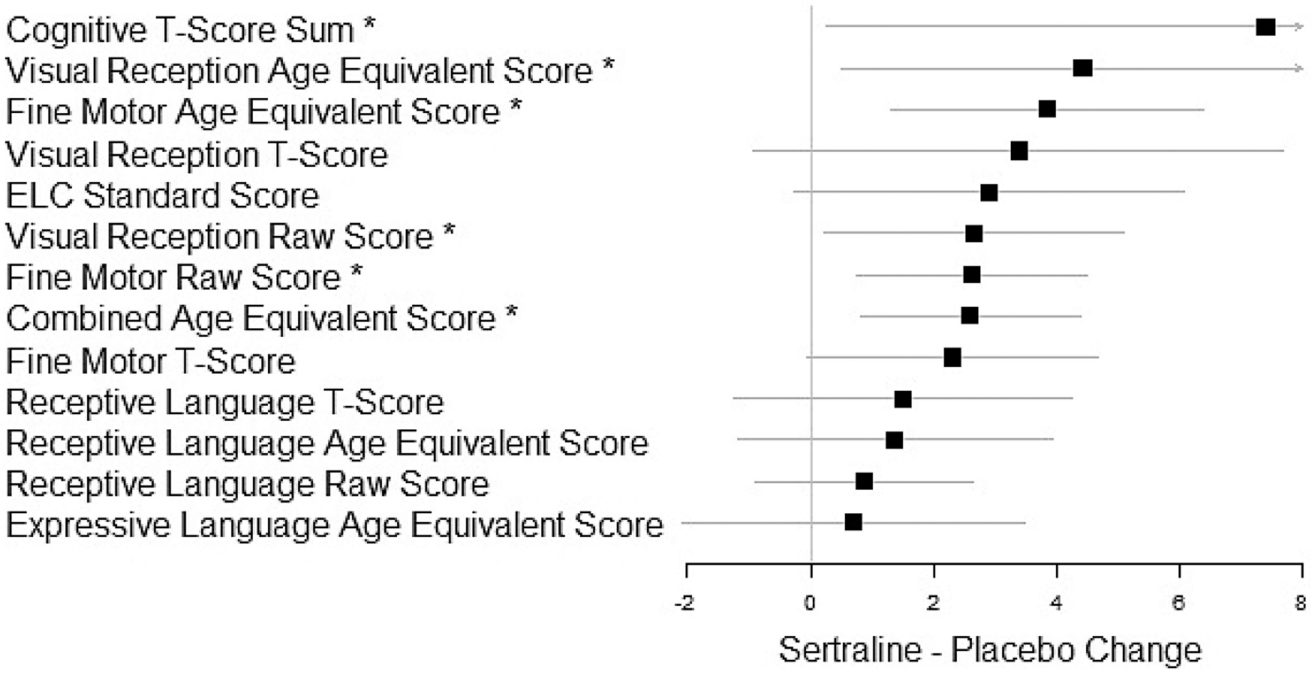

Figure 2.

Effect sizes and 95% confidence intervals (CIs) for exploratory secondary measures for the Mullen Early Scales of Learning (MSEL). Given effect size are post treatment score difference estimates (sertraline minus placebo) adjusted for baseline measure, along with 95% CIs (arrows indicate CI length truncated for display). Asterisks indicate $P < 0.05$.

Table 1

Baseline characteristics study subjects. Included are demographic and clinical characteristics of 57 participants, randomly assigned to sertraline or placebo, used for analysis of primary and secondary variables.

Variable	Sertraline			Placebo			
	N	Mean	SD	N	Mean	SD	P-value
Age at visit 1	27	3.89	1.09	30	3.92	1.11	0.9218
		%			%		
Gender							
Female	6	22.22		3	10		0.283
Male	21	77.78		27	90		
Race							
White	19	70.37		15	50		0.1767 *
Asian	2	7.41		5	16.67		
Black	1	3.7		5	16.67		
American Indian/Alaska Native	1	3.7		0	0		
Unknown/not reported	4	14.81		5	16.67		
Ethnicity							
Hispanic	6	22.22		6	20		0.2755
Not Hispanic or Latino	11	40.74		7	23.33		
Unknown/not reported	10	37.04		17	56.67		
Autism Diagnostic Observation Schedule (ADOS-2)							
No ASD	9	33.33		13	43.33		0.354
ASD	15	55.6		17	56.7		
Missing	3	11.11		0	0		
Molecular category							
Mosaic	11	40.74		10	33.33		0.5938
Full mutation	16	59.26		20	66.67		
Concomitant medications							
Alpha2 agonists	2	7.41		2	6.67		--
Anticonvulsant	2	7.41		2	6.67		--

Variable	Sertraline			Placebo		
	N	Mean	SD	N	Mean	SD	P-value
Minocycline	1	3.7		1	3.33		--
Non-pharmacological intervention							
Yes	26	96.3		28	93.33		--
Education level							
Missing	3	11.11		2	6.67		0.5616**
Elementary School	0	0		1	3.33		.
High school graduate	5	18.52		1	3.33		
Partial college	4	14.81		6	20		
College Degree	6	22.22		7	23.33		.
Graduate degree/professional training	9	33.33		13	43.33		

* White vs. Others

** College degree/graduate degree vs. lower education level

Table 2

Primary efficacy results. Given are results for three pre-specified outcome measures.

Variables	Sertraline						Placebo						
	Baseline			Follow Up			Baseline			Follow Up			
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	P-value[a]
Mullen Scales of Early Leaning													
Expressive Language Raw Score	26	21.3	10.32	25	25.0	10.78	30	19.3	9.68	27	21.3	9.59	0.586
Expressive Language T Score	27	25.8	11.78	25	25.8	10.87	30	23.3	5.80	27	22.6	6.47	0.607
Clinical Global Impression-Improvement (CGI-I)	27	--	--	25	2.3	1.06	30	--	--	27	2.6	0.84	0.244

[a]Adjusted significance level 0.016; All available data analyzed.

Table 3

Results of secondary measures. Exploratory analysis for Mullen Scales of Early Learning, Autism Diagnostic Observation Scale, Second Edition, Visual Analog Scale, Sensory Processing Measure-Preschool, and Preschool Language Scale, Fifth Edition.

	Sertraline						Placebo						
	Baseline			Follow Up			Baseline			Follow Up			
Variables	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**P-value**
Mullen Scales of Early Leaning													
Visual Reception Raw Score	26	27.0	9.17	25	32.4	10.46	30	28.9	7.94	27	30.9	6.73	0.038
Visual Reception Age Equivalent Score	27	25.3	11.59	25	33.7	15.06	30	28.2	10.53	27	30.6	9.60	0.031
Fine Motor Raw Score	26	23.6	6.76	25	27.3	8.06	30	24.8	5.26	27	25.2	4.96	0.008
Fine Motor T Score	27	22.1	5.40	25	23.7	7.42	30	21.8	4.24	27	21.2	3.14	0.062
Fine Motor Age Equivalent Score	27	22.7	8.66	25	28.4	10.91	30	24.5	6.97	27	25.0	6.91	0.005
Cognitive T score sum	27	99.7	33.46	25	105.4	40.27	30	96.0	21.97	27	93.0	20.33	0.047
ELC Standard Score	27	56.6	13.55	25	59.0	17.20	30	54.8	8.53	27	53.6	7.90	0.077
Mullen summary Age Equivalent Score	27	26.8	8.89	25	30.1	12.64	30	24.8	9.33	27	23.6	10.76	0.007
Sensory Processing Measure-Preschool (SPM-P)													
Social Participation: Raw Score	26	20.5	5.49	22	18.0	6.23	30	20.6	6.85	23	19.7	6.41	0.013
Social Participation: T-Score	26	65.3	10.44	22	61.5	12.15	30	65.2	11.92	23	63.5	11.33	0.053

Other secondary measures measured analyzed with P ≥ 0.1 – Mullen Scales of Early Leaning: Expressive Language Age Equivalent Score, Visual Reception T Score, Receptive Language Raw Score, Receptive Language T Score, Receptive Language Age Equivalent Score; Autism Diagnostic Observation Scale (ADOS): ADOS2 Social Affect & Restricted & Repetitive, Behavior Total Score; Visual Analog Scale (VAS): Severity of Target Behavior 1, 2 and 3 (in cm); Sensory Processing Measure-Preschool (SPM-P): Vision: Raw Score, Hearing: Raw Score, Touch: Raw Score, Items43to46, Body Awareness: Raw Score, Balance & Motion: Raw Score, Planning & Ideas: Raw Score, Total: Raw Score, Vision: T- Score, Hearing: T- Score, Touch: T- Score, Body Awareness: T- Score, Balance & Motion: T-Score, Planning & Ideas: T- Score, Total: T- Score; Preschool Language Scale-Fifth Edition: Auditory Comprehension (AC) Raw Score, Expressive Communication (EC) Raw Score, AC+EC Raw Score Total, AC Standard Score, EC Standard Score, Total Language - AC + EC Standard Score, AC Age Equivalent (in months), EC Age Equivalent (in months), AC+EC Age Equivalent (in months)

Table 4

Types of adverse events.

Adverse Event	Sertraline		Placebo	
	N	%	N	%
Upper respiratory infection	30	29.13	36	24
Diarrhea	20	19.42	31	20.67
Gastrointestinal issues	11	10.68	11	7.33
Vomiting	7	6.8	16	10.67
Rash	5	4.85	5	3.33
Drowsiness	3	2.91	7	4.67
Hyperactivity	3	2.91	7	4.67
Ear infection	4	3.88	5	3.33
Loss of appetite	4	3.88	3	2
Bruxism	3	2.91	0	0
Anxiety	0	0	3	2
Nausea	1	0.97	3	2
Sweating	0	0	3	2
Decreased appetite	2	1.94	2	1.33
Nervousness	2	1.94	1	0.67
Headache	0	0	2	1.33
Sleep disturbance	0	0	2	1.33
Aggression	1	0.97	1	0.67
Biting clothing	1	0.97	0	0
Dilated pupils	1	0.97	0	0
Drooling	1	0.97	1	0.67
Dry skin	1	0.97	0	0
Eye infection	1	0.97	1	0.67
Falling	1	0.97	0	0
Irritability	1	0.97	0	0
Abnormal EEG	0	0	1	0.67
Bruising	0	0	1	0.67
Deciduous teeth eruption	0	0	1	0.67
Decreased verbalization	0	0	1	0.67
Genital infection	0	0	1	0.67
Hand flapping	0	0	1	0.67
Seizures	0	0	1	0.67
Self-injurious behavior	0	0	1	0.67
Tantrums	0	0	1	0.67
Tremor	0	0	1	0.67

Table 5

Characteristics of reported adverse events.

	Sertraline		Placebo	
Variable	**No. of Adverse Events**	**%**	**No. of Adverse Events**	**%**
Severity				
Mild	80	77.67	121	80.67
Moderate	22	21.36	25	16.67
Severe	1	0.97	4	2.67
Drug Related				
Not related	31	30.1	54	36
Possibly not related	4	3.88	8	5.33
Probably not related	1	0.97	8	5.33
Possibly related	55	53.4	78	52
Probably related	12	11.65	2	1.33
Serious Adverse Event				
No	103	100	150	100
Adverse Event Status				
Ongoing	8	7.77	4	2.67
Resolved	94	91.26	146	97.33
Withdraw	1	0.97	0	0