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Safety and Efficacy of Rivastigmine in Children with Down Syndrome: A Double Blind Placebo Controlled Trial

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Individuals with Down syndrome (DS) have decreased cholinergic function and an uneven profile of cognitive abilities, with more pronounced deficits in learning, memory, and expressive language. Cholinesterase inhibitors may improve cognitive function in adults and adolescents with DS, but studies in children with DS have been limited. This study aimed to: (i) investigate the safety and efficacy of rivastigmine treatment; (ii) build upon our open-label studies in children with DS in a double-blind, placebo-controlled clinical trial; and (iii) investigate specific cognitive domains that may respond to rivastigmine treatment. We conducted a 20-week double-blind, placebo-controlled trial to investigate the safety and efficacy of rivastigmine in 22 children and adolescents with DS aged 10–17 years. Safety measures included reports of adverse events, laboratory parameters, and electrocardiograms. Efficacy measures included parental assessments of adaptive behavior and executive function, and direct measures of language and memory. No group differences were found on safety measures and 22 of 24 participants that passed study screening completed the study. The results did not demonstrate evidence for significant improvement in aspects of cognition, language, or overall function in the children receiving rivastigmine. Our results suggest that rivastigmine is safe and well-tolerated for children and adolescents with DS, but may not be effective for improving performance on the selected measures in this study. However, larger samples and/or alternate measures could possibly reveal improvements in cognitive function with rivastigmine treatment. Further research is needed to define a battery of cognitive measures that is sensitive to treatment effects in DS.

Key words: Down syndrome; trisomy 21; cholinesterase inhibitors; cognition; adaptive behavior

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INTRODUCTION

Individuals with Down syndrome (DS) show an uneven profile of cognitive abilities, with more pronounced deficits in learning, memory, and language [Kishnani et al., 2010]. There is currently an unmet need for strategies to improve independence and overall performance in these cognitive domains for individuals with DS. The cognitive deficits in DS may be associated with an innate cholinergic deficit or dysfunction, as abnormalities have been demonstrated in both

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peripheral and central cholinergic function [Sacks and Smith, 1989; Florez et al., 1990; Beccaria et al., 1998]. As the central cholinergic system is critical in cognition, memory, attention, and mood, enhancing cholinergic function may be an appropriate target for treatment in individuals with DS. DS has been associated with reductions in cholinergic neurons [Casanova et al., 1985], leading to the hypothesis that decreased neurogenesis or survival of cholinergic neurons could underlie altered cortical connectivity and delayed maturation of cortical neurons in children with DS [Becker et al., 1991; Berger-Sweeney, 2003]. This hypothesis implies that increasing cholinergic function early in development could potentially improve cognitive function by altering cortical connectivity [Berger-Sweeney, 2003]. Cholinesterase inhibitors (ChEIs), including rivastigmine and donepezil, have been investigated as potential therapeutic agents to improve cognitive performance.

Several clinical trials have been completed to test the safety and efficacy of ChEIs for improving cognitive and language function in adults with DS who do not have Alzheimer disease. The first clinical trial of ChEIs in individuals with DS demonstrated improved global functioning in a small sample of adults treated with donepezil [Kishnani et al., 1999]. A subsequent open-label study of donepezil indicated that donepezil was generally well-tolerated and was associated with improvements in expressive language in adults with DS [Heller et al., 2003]. Additionally, several double-blind, placebo-controlled studies of donepezil have shown gains in memory, language, and global function in individuals with DS [Johnson et al., 2003; Kishnani et al., 2009; Kondoh et al., 2011].

Studies investigating efficacy of ChEIs in children with DS have yielded mixed results. Using donepezil and rivastigmine tartrate, open-label trials with children (aged 8–13 years) [Heller et al., 2004; Spiridigliozzi et al., 2007] and adolescents (aged 10–17 years) [Heller et al., 2006b] with DS have shown similar increases in expressive language performance, memory, and attention. However, several recent clinical trials have failed to demonstrate significant effects of ChEIs on cognition in children with DS. A long-term follow-up study of rivastigmine in adolescents with DS indicated no between-group differences in cognitive or language performance change over time [Heller et al., 2010]. However, at least two participants in this study showed long-term gains in adaptive function, suggesting that there may be a subset of individuals that respond to the medication. A double-blind placebo-controlled study of donepezil in adolescents with DS also found no group differences in adaptive behavior, with both the experimental and placebo groups showing significant improvement over the course of the trial [Kishnani et al., 2010].

The previous studies of ChEIs in children with DS have been limited by open-label designs or by the specific cognitive assessments that were selected. The study by Kishnani et al. [2010] did not demonstrate improvement in cognition using a limited set of measures with administration of donepezil. We aimed in this study to investigate the safety and efficacy of rivastigmine treatment in a double-blind design and to expand the assessment battery to include other specific cognitive domains that may selectively respond to ChEIs. To our knowledge, there have been no double-blind randomized trials conducted using rivastigmine in children with DS.

MATERIALS AND METHODS

We conducted a 20-week double-blind, placebo-controlled trial for safety and efficacy of rivastigmine at Duke University Medical Center and at the Kennedy Krieger Institute at Johns Hopkins University (Clinical Trials Registry NCT01084135). The study was prospectively reviewed and approved by both the Duke University and Johns Hopkins University Institutional Review Boards.

Participants

Eligible participants for enrollment in the study included males and females ages 10 through 17 years of age with a diagnosis of DS (free trisomy 21 or Robertsonian translocation for trisomy 21 as determined by chromosome analysis). Participants were required to be outpatients with reliable caregivers. Participants were also required to have sufficient verbal skills for study participation as defined by the Expressive One-Word Picture Vocabulary Test (EOWPVT) [Brownell, 2000] and examiner assessment (described below). Exclusion criteria included any medical condition that was considered both significant and unstable, including an active medical history of gastric ulcer, glaucoma, poorly controlled asthma, and prolonged use (>2 weeks) of NSAIDs. Exclusion criteria also included any active or clinically significant conditions affecting absorption, distribution, or metabolism of the study drugs.

Participants were recruited from DS clinics and the community. A total of 24 participants with DS were enrolled into the study, including 12 participants enrolled at Johns Hopkins University and 10 participants at Duke University. Two participants at the Johns Hopkins site declined to continue with the study following the screening visit. A total of 12 participants received rivastigmine and 10 received placebo. The treatment group included four males and eight females, and the placebo group included four males and six females. Participant ages are presented in Table I by treatment group and gender. No significant differences were found between treatment groups for age ($P = 0.24$) or gender ($P = 1.0$). No participants had a history of epilepsy or seizures. A history of cardiac surgery for structural heart defects such as atrioventricular

TABLE I. Demographic Characteristics

	Rivastigmine			Placebo		
	Male = 4	Female = 8	Total (n = 12)	Male = 4	Female = 6	Total (n = 10)
Mean age (SD)	13.02 (1.79)	13.89 (1.98)	13.6 (1.88)	12.79 (1.51)	12.54 (2.08)	12.64 (1.78)
Age range	10–14	10–17	10–17	10–14	10–16	10–16

canal defects and valvular defects was noted for five individuals in the treatment group and four individuals in the placebo group. Four individuals in the treatment group had comorbid neurodevelopmental disabilities including ADHD (three participants) and sensory integration disorder (one participant). In the placebo group, one individual had ADHD, two had oppositional defiant disorder, and one had pervasive developmental disorder. No significant differences were found between treatment groups in the number of individuals with comorbid neurodevelopmental disabilities ($P = 1.0$) or history of cardiac surgery ($P = 1.0$).

Overview of Study Visits

The study consisted of a screening visit (−4 weeks), a baseline visit (week 0), a safety visit at week 10, and a final visit at week 20 (Fig. 1). Efficacy measures of overall function, language, and cognition (described below) were administered at the screening, baseline, and final (week 20) visits. Vital signs, physical examinations, and pregnancy testing for females of childbearing potential were conducted at all visits. An electrocardiogram (EKG) was performed at the screening and week 20 visits only. Participants receiving placebo maintained the same schedule as those receiving the medication.

Screening visit. At the screening visit, participants' caregivers provided informed consent and the child provided assent prior to all study activities. Blood was drawn to check thyroid function, liver function, and complete blood count. Participants were required to have normal bloodwork to continue participation in the study. A 12-lead EKG was also performed for all participants.

Participants were administered the examples and items one to nine of the EOWPVT to determine eligibility to proceed with the study. The examiner also spoke informally with the child regarding a subject of interest to the child for approximately 5 min. In order to continue participation in the study, participants were required to provide correct verbal responses for seven of nine of the EOWPVT items. The child was required to be able to put at least two to three words together in conversational speech. The child's speech was required to be understandable to the examiner for the majority of the time and the child could not use sign language or an augmentative communication tool as the primary means of communication. The parent and child were also interviewed for about 30 min by a clinician about the child's medical history and to assess the parent's impression of the child's functioning.

Baseline visit. At the baseline visit, participants were randomized by the Investigational Pharmacies at Duke University and Johns Hopkins University to receive liquid rivastigmine or liquid placebo orally. Blinded data was maintained by each site's Investigational Pharmacy. Rivastigmine was titrated, based on tolerability, on the following schedule: At the baseline visit (week 0), participants began rivastigmine treatment at a dose of 1.5 mg/day (0.75 mg bid). This dose was continued for 2 weeks. Parents of participants received a phone call from the study coordinator after 2 weeks to determine the child's dose tolerance. The information about the child's dose tolerance was shared with the study PI, who determined whether or not to increase the child's dose to 3.0 mg/day (1.5 mg bid) for an additional 6 weeks.

Caregivers were provided with study diaries to record drug administration compliance and changes in medical history. Compliance diaries were reviewed at each visit and adverse events (AEs) and serious adverse events (SAEs) were recorded. AEs were classified by the study investigators as being potentially related or as likely unrelated to rivastigmine. A SAE was defined as: (i) results in death; (ii) is life-threatening; (iii) requires hospitalization; (iv) results in persistent or significant functional disability; or (v) other medical event that may jeopardize the participant or require intervention to prevent one of the outcomes listed above.

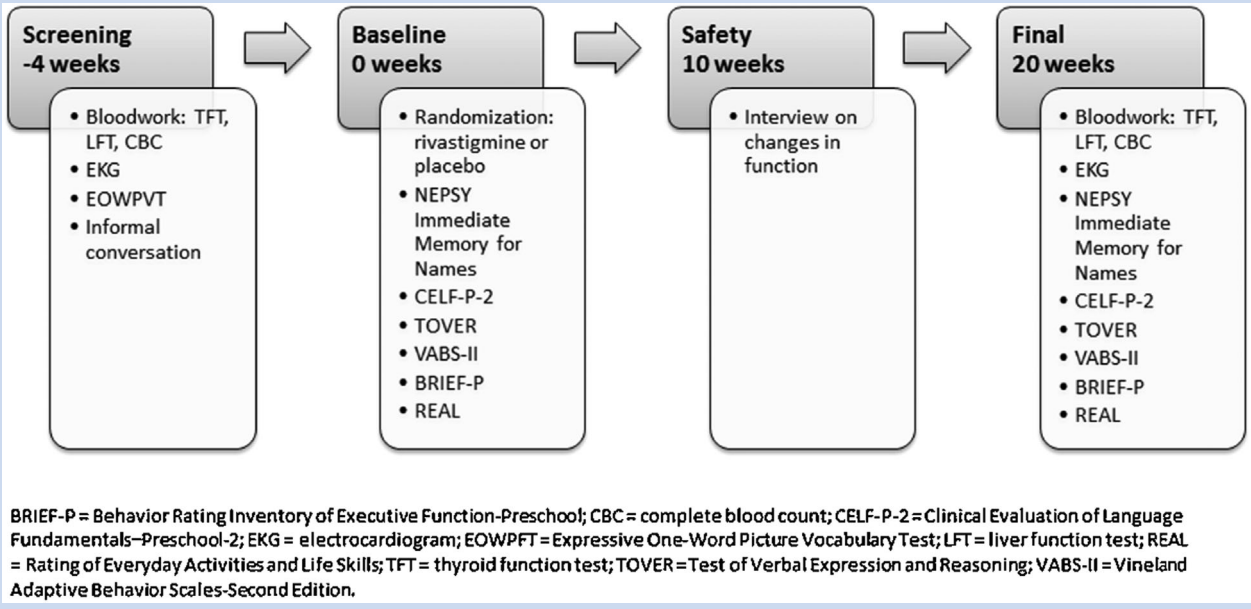

FIG. 1. Timeline of study visits.

All participants were administered the NEPSY Immediate Memory for Names subtest [Korkman et al., 1998], subtests from the Clinical Evaluation of Language Fundamentals-Preschool-2 (CELF-P-2) [Semel et al., 2004], and the Test of Verbal Expression and Reasoning (TOVER) [Heller et al., 2000]. Parents/caregivers were administered the Vineland Adaptive Behavior Scales, Second Edition, Survey Interview Form (VABS-II) [Sparrow et al., 2005], the Behavior Rating Inventory of Executive Function-Preschool Version (BRIEF-P) [Gioia et al., 2002], and the Rating of Every Day Activities and Life Skills (REAL) [Spiridigliozzi et al., 2010].

Safety visit. At the week 10 safety visit, the child's dose tolerance was reviewed again. Parents and the child were interviewed for about 30 min about changes in his/her functioning. Based on the participant's response to his/her dose, the dose was increased to 4.5 mg/day (3.0 mg and 1.5 mg). All participants were able to tolerate the 4.5 mg/day dose.[1]

Final visit. Assessments at study termination (week 20) included a full physical exam and medical history intake, vital signs, routine blood work, and a thyroid profile. Participants were also given an EKG and liver function tests. Efficacy measures administered at the baseline visit with the participants and their parents/caregivers were repeated at this final visit.

Efficacy Measures

Efficacy measures were obtained at the baseline and final visits (Fig. 1) and included both parental report measures and direct assessments of the child's performance. Parental report measures included the VABS-II Survey Interview Form, REAL, and BRIEF-P. The VABS-II is a measure of adaptive behavior in children, adolescents, and adults. It yields an overall standard score (Adaptive Behavior Composite, ABC) and age standard scores in three domains (Communication, Daily Living Skills, and Socialization). These scores have a mean of 100 and a standard deviation of 15 (range = 20–160). Higher scores suggest a higher level of adaptive functioning. In this study, the change between each participant's ABC at baseline and the final visit was computed. A rise in standard scores from baseline to the final visit indicates improvement.

The REAL is a 29-item parent report measure of an individual's behavior, speech and language ability, mood, social interactions, and executive functioning/attention during the past month. It was developed in an attempt to briefly characterize each child's functioning in those areas that may be sensitive to a treatment effect. Higher scores suggest a higher level of overall functioning. In this study, we analyzed subscale scores for behavior and executive function/attention and the Total Scores only.

The BRIEF-P is a parent report measure of executive function behaviors in children in their home setting. It yields an overall score (Global Executive Composite, GEC), that is, based on its five clinical scales. Raw scores range from 63 to 189. Higher scores suggest that an individual's executive function skills are more problematic. In this study, the change between each subject's raw score at baseline and the final visit was computed for the Global Executive Composite. A decline in raw scores from baseline to the final visit indicates improvement.

Direct assessments of the child's performance included the NEPSY Immediate Memory for Names subtest, the CELF-P-2 Recalling Sentences and Word Classes subtests, and the TOVER.

The NEPSY Memory for Names subtest measures the child's ability to learn and recall the names of eight pictured children over three learning trials. The total number of names recalled immediately after each learning trial was analyzed. These scores range from 0 to 24.

[1]Following the initial 10-week period of low dose study drug, two participants could not have their dose increased to 4.5 mg due to an insufficient supply of the study drug. The study time was extended for these two participants in order for them to have a dosage of 4.5 mg for 10 weeks.

TABLE II. Treatment-Emergent Adverse Events by Preferred Term, All Causalities

	Treatment group	
Any adverse event	Rivastigmine (n = 12), n (%)	Placebo (n = 10), n (%)
Stomachache	5 (41.7)	3 (30.0)
Diarrhea	3 (25.0)	2 (20.0)
Nausea	3 (25.0)	3 (30.0)
Vomiting	3 (25.0)	1 (10.0)
Cold	3 (25.0)	2 (20.0)
Menstrual cramps	2 (16.7)	0
Stomach flu	2 (16.7)	0
Sleepy	2 (16.7)	0
Dizziness	1 (8.3)	0
Headache	1 (8.3)	1 (10.0)
Trouble sleeping	1 (8.3)	1 (10.0)
Decreased appetite	1 (8.3)	1 (10.0)
Weakness	1 (8.3)	0
Lumps in neck	1 (8.3)	0
Nasal congestion	1 (8.3)	0
Boil	1 (8.3)	0
Cut	1 (8.3)	0
Restless legs	1 (8.3)	0
Pale coloring	1 (8.3)	0
Foot twitching	1 (8.3)	0
Itchy	1 (8.3)	0
Eye twitch	1 (8.3)	0
Rash	1 (8.3)	0
Indigestion	1 (8.3)	0
Constipation	1 (8.3)	0
Worsening acne	1 (8.3)	0
Irritable	1 (8.3)	0
Leg cramps	1 (8.3)	0
Incontinence	1 (8.3)	1 (10.0)
"Assertive, stubborn, more emotional"	1 (8.3)	0
Fainted	1 (8.3)	0
Fatigue	1 (8.3)	0
Shakiness	0	1 (10.0)
UTI, yeast infection	0	1 (10.0)
Increased bilirubin	0	1 (10.0)
Fever	0	2 (20.0)
Worsening alopecia	0	1 (10.0)
Frequent urination	0	1 (10.0)
Weight gain	0	1 (10.0)

TABLE III. Electrocardiogram (EKG) Findings for Participants Receiving Rivastigmine or Placebo

	Rivastigmine			Placebo			Group difference in change from screening-final
EKG measure	Screening average value (SD)	Final average value (SD)	*P*-value*	Screening average value (SD)	Final average value (SD)	*P*-value*	*P*-value
Ventricular rate (BPM)	76.38 (14.5)	73.6 (14.5)	0.25	73.14 (19.1)	72.14 (16.25)	0.75	0.64
P–R interval (ms)	137.0 (21.1)	133.6 (21.1)	0.12	138.46 (24.6)	135.00 (20.41)	0.53	0.62
QRS duration (ms)	88.0 (25.4)	89.2 (28.3)	0.41	85.57 (12.1)	85.07 (13.95)	0.74	0.71
QT interval (ms)	368.38 (73.8)	391.4 (38.4)	0.25	384.42 (39.1)	384.57 (35.77)	0.98	0.45
QTc interval (ms)	431.56 (27.6)	427.6 (19.4)	0.46	415.71 (24.1)	416.00 (31.09)	0.97	0.37

Individual EKG findings present at both screening and final visits

Participant	Rivastigmine group[a]
1	AV dual-paced rhythm (Pacemaker)
2	Sinus rhythm; ST elevation anterior leads; possible right ventricular hypertrophy; possible left ventricular hypertrophy

Participant	Placebo group[b]
1	Sinus rhythm; first degree AV block; left atrial abnormality; left ventricular hypertrophy
2	Normal sinus rhythm; left axis deviation; borderline prolonged QT interval or TU fusion
3	Sinus bradycardia; left axis deviation; right bundle branch block

No significant differences were found on EKG measures between screening and final visits for either group and no significant differences were found between treatment groups.
[a]Left axis deviation was found in two additional participants. Isolated sinus bradycardia was found in one participant.
[b]Isolated mild sinus bradycardia was found in three additional participants.
*Two sample *t*-test assuming equal variance.

TABLE IV. Baseline Performance on Cognitive Measures

	Baseline average score (SD)		
Outcome measure	Rivastigmine	Placebo	*P*-value
VABS-II (survey interview form): Standard scores			
Communication	67.55 (7.98)	67.20 (4.37)	0.84
Daily living skills	71.80 (17.88)	67.10 (9.39)	0.47
Socialization	74.50 (14.42)	72.00 (9.57)	0.65
Adaptive behavior composite	69.90 (13.60)	67.00 (6.75)	0.55
BRIEF-P: Raw scores			
Global executive composite	107.55 (21.03)	100.10 (23.44)	0.45
REAL: Raw scores			
Behavior	18.30 (3.56)	17.78 (4.29)	0.78
Executive function/attention	10.27 (3.58)	10.00 (3.97)	0.78
Total	61.10 (12.99)	63.89 (14.78)	0.67
CELF-P-II: Raw scores			
Recalling sentences	11.83 (10.54)	6.10 (6.84)	0.16
Word classes expressive	7.75 (6.24)	6.40 (5.56)	0.6
Word classes receptive	13.67 (6.11)	11.0 (5.58)	0.3
Word casses total	21.42 (11.34)	17.4 (10.9)	0.41
NEPSY: Immediate memory for names	10.83 (6.18)	10.20 (5.09)	0.8
TOVER	22.5 (15.92)	12.8 (7.48)	0.09

BRIEF-P, behavior rating inventory of executive function-preschool; CELF-P-II, clinical evaluation of language fundamentals-preschool; REAL, rating of everyday activities and life skills; TOVER, test of verbal expression and reasoning; VABS-II, Vineland adaptive behavior scales—second edition.

TABLE V. Between-Group Differences on Cognitive Measures

	Number of participants analyzed		Mean difference final-baseline (SD)		
Outcome Measure	**Rivastigmine**	**Placebo**	**Rivastigmine**	**Placebo**	***P*-value**
VABS-II (survey interview form): Standard scores					
Communication	11	10	0.9 (3.3)	2.0 (3.6)	0.45
Daily living skills	10	10	−2.3 (4.6)	2.8 (5.2)	0.03*
Socialization	10	10	−3.2 (5.0)	2.0 (6.1)	0.05
Adaptive behavior composite	10	10	−1.7 (3.2)	2.0 (3.6)	0.03*
BRIEF-P: Raw scores					
Global executive composite	11	10	−3.6 (7.7)	−6.1 (12.0)	0.58
REAL: Raw scores					
Behavior	10	9	−0.2 (2.5)	0.9 (1.2)	0.25
Executive function/attention	11	9	−0.2 (1.9)	1.1 (3.1)	0.24
Total	10	9	0.8 (7.1)	3.4 (8.3)	0.46
CELF-P-II: Raw scores					
Recalling sentences	12	10	−0.5 (4.1)	2.0 (1.9)	0.09
Word classes expressive	12	10	3.75 (4.25)	1.3 (3.23)	0.15
Word classes receptive	12	10	1.17 (3.66)	1.0 (3.89)	0.92
Word classes total	12	10	4.92 (7.06)	2.3 (6.31)	0.38
NEPSY: Immediate memory for names	12	10	2.25 (3.67)	1.9 (5.38)	0.86
TOVER	11	10	0.09 (6.07)	1.80 (3.39)	0.44

BRIEF-P, behavior rating inventory of executive function-preschool; CELF-P-II, clinical evaluation of language fundamentals-preschool; REAL, rating of everyday activities and life skills; TOVER, test of verbal expression and reasoning; VABS-II, Vineland adaptive behavior scales—second edition.
*Significant group difference at $P < 0.05$.

The CELF-P-2 Recalling Sentences subtest is a measure of expressive language where the child is asked to repeat sentences of increasing length and complexity immediately after hearing them. The Word Classes subtest requires the child to choose two words that are related best and then verbalize how they go together. The Total, Receptive, and Expressive raw scores for this subtest were analyzed.

The TOVER is an expressive language measure designed to assess an individual's ability to find solutions to questions from pictured stimuli and to think about answers using visual information. It was developed to assess change in response to treatment in individuals with DS. The TOVER consists of 23 pictures and 64 related questions. The total score (range 0–64) was analyzed.

Analysis

Descriptive statistics were computed for all study variables of interest (overall and stratified by treatment group). EKG measurements of ventricular rate, QRS complex duration, QT interval, PR interval, and QTc interval were also compared between the treatment and placebo groups. Short-term efficacy was determined by comparing differences in language and cognitive performance between baseline and week 20 visits across treatment and placebo groups. Repeated measures ANOVA models were used to examine pairwise changes between baseline and week 20 visits within and between groups. Age and gender were included as covariates in the analyses of changes between baseline and final visits. Given the exploratory nature of the study, we did not correct for multiple comparisons.

RESULTS

Safety Measures

Adverse events. No SAEs were reported for any individuals in the treatment or placebo groups and no participants needed to terminate study participation after starting the study medication. There were 11 AEs related or possibly related to the study medication. A total of six AEs occurred in the first 8 weeks of the treatment (one at the 1.5 mg and five at the 3 mg levels) and five occurred in the second 8 weeks of treatment at the 4.5 mg level. None of the AEs was unexpected and all were categorized as mild. Of the 12 participants taking rivastigmine, 1 participant reported no AEs and 8 participants reported between one and five mild, transient AEs considered to be potentially related to rivastigmine effects, including diarrhea, nausea, vomiting, stomachache, incontinence, decreased appetite, dizziness, weakness, headache, irritability, and "assertive, stubborn, and more emotional" behavior (Table II). The remaining three participants taking rivastigmine reported AEs that were considered to be unrelated to the study medication, including sleepiness, and diarrhea related to viral illness.

Electrocardiogram data. EKG findings are presented in Table III. No group differences were found between the treatment and placebo groups on EKG measures, including ventricular rate, QRS complex duration, QT interval, PR interval, and QTc interval. Within groups, no significant changes between baseline and final visits were found on EKG measures. Three participants in the placebo group and two participants in the treatment group had abnormal EKG findings at baseline and the same findings at the final visit.

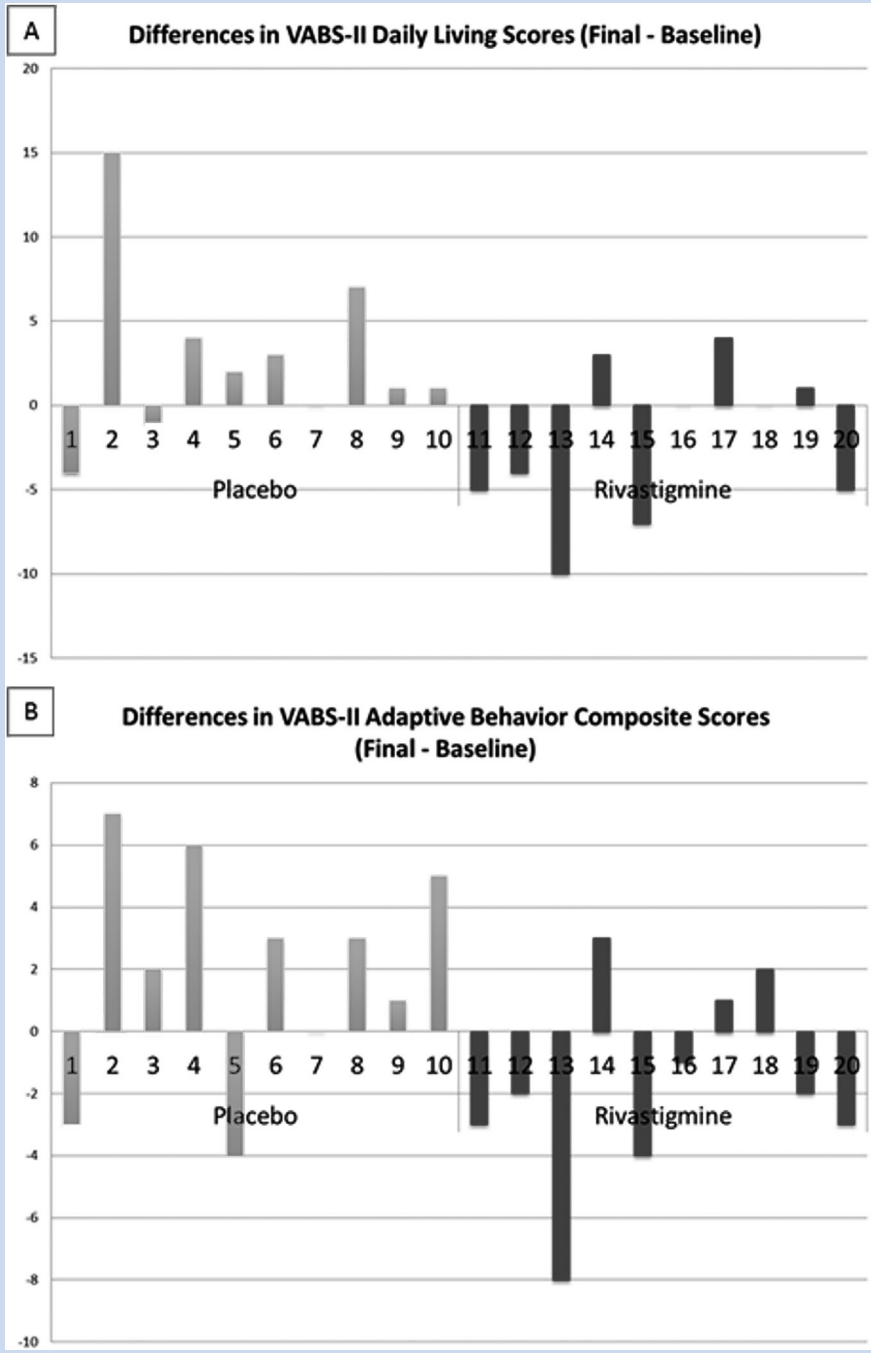

FIG. 2. Differences in VABS-II scores between final and baseline visits for individual study participants (numbered 1–20) for (A) Daily Living scores and (B) Adaptive Behavior Composite scores. A rise in standard scores from baseline to the final visit indicates improvement. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

Efficacy Measures

Performance on baseline efficacy measures did not significantly differ between groups, although there was a trend toward higher performance on the TOVER at baseline for the rivastigmine group (Table IV). Group differences on performance change between baseline and final visits are presented in Table V. Three participants receiving rivastigmine were missing data for some measures. Two of these participants did not complete all final visit assessments and the other participant's data was excluded due to discrepant reports

TABLE VI. Within-Group Differences Between Baseline and Final Visits on Cognitive Measures

	Rivastigmine			Placebo		
Outcome measure	Baseline average score (SD)	Final average score (SD)	*P*-value	Baseline average score (SD)	Final average score (SD)	*P*-value
VABS-II (survey interview form): Standard scores						
Communication	67.55 (7.98)	68.45 (8.49)	0.38	67.20 (4.37)	69.20 (4.10)	0.11
Daily living skills	71.80 (17.88)	69.50 (15.17)	0.15	67.10 (9.39)	69.90 (11.0)	0.12
Socialization	74.50 (14.42)	71.3 (12.30)	0.07	72.00 (9.57)	74.00 (6.04)	0.32
Adaptive behavior composite	69.90 (13.60)	68.20 (11.71)	0.13	67.00 (6.75)	69.00 (5.85)	0.11
BRIEF-P: Raw scores						
Global executive composite	107.55 (21.03)	103.91 (17.72)	0.15	100.10 (23.44)	94.00 (20.87)	0.14
REAL: Raw scores						
Behavior	18.30 (3.56)	18.10 (2.77)	0.80	17.78 (4.29)	18.70 (3.57)	0.05
Executive function/attention	10.27 (3.58)	10.09 (2.39)	0.76	10.00 (3.97)	11.10 (4.31)	0.31
Total	61.10 (12.99)	61.90 (10.50)	0.73	63.89 (14.78)	67.30 (12.85)	0.25
CELF-P-II: Raw scores						
Recalling sentences	11.83 (10.54)	11.33 (9.33)	0.68	6.10 (6.84)	8.10 (6.54)	0.01*
Word classes expressive	7.75 (6.24)	11.5 (6.52)	0.01*	6.40 (5.56)	7.7 (4.32)	0.24
Word classes receptive	13.67 (6.11)	14.83 (7.13)	0.29	11.0 (5.58)	12.0 (5.01)	0.44
Word classes total	21.42 (11.34)	26.3 (13.3)	0.03*	17.4 (10.9)	19.7 (9.12)	0.28
NEPSY: Immediate memory for names	10.83 (6.18)	13.08 (6.96)	0.06	10.20 (5.09)	12.10 (4.48)	0.29
TOVER	22.5 (15.92)	24.45 (15.12)	0.96	12.8 (7.48)	14.6 (7.83)	0.13

BRIEF-P, behavior rating inventory of executive function-preschool; CELF-P-II, clinical evaluation of language fundamentals-preschool; REAL, rating of everyday activities and life skills; TOVER, test of verbal expression and reasoning; VABS-II, Vineland adaptive behavior scales—second edition.
*Significant effect of visit at $P < 0.05$.

between parental ratings at the baseline and final visits. Additionally, one participant receiving placebo did not complete the REAL at the baseline visit.

Significant differences between the treatment and placebo groups were found on the overall score for the Vineland Adaptive Behavior Scale-II and one domain. Significant group differences were found on the standard score changes between baseline and final visits for the Adaptive Behavior Composite score ($P = 0.03$) and the Daily Living Skills domain ($P = 0.03$). Group differences on the standard score changes for the Socialization domain were of borderline significance ($P = 0.05$). However, the patterns of these group differences on all three VABS-II domain scores indicated relative improvements in performance over time in the placebo group, while the treatment group showed relative decreases in performance over time. Figure 2 illustrates the differences in VABS-II scores between final and baseline visits across individual participants. No significant group differences were found on the VABS-II Communication domain standard scores or on the other parent report measures (REAL and BRIEF-P). Additionally, no significant group differences were found on the direct assessments of the child's performance, including the NEPSY Immediate Memory for Names subtest score, the CELF-P-2 subtest scores, or the TOVER total score.

Within-group analyses of the effects of time (baseline versus final visit) are presented in Table VI. Within-group analyses of time effects indicated that the treatment group showed significant improvement between baseline and final visits on the CELF-P-2 Word Classes Expressive Scores ($P = 0.01$) and Total Scores ($P = 0.03$). Figure 3 illustrates the differences from baseline to final visits in CELF-P-2 Word Classes Expressive and Total scores across individual participants. Differences between baseline and final visits also approached statistical significance for the treatment group on the NEPSY Memory for Names ($P = 0.06$). In contrast, only the placebo group showed improvement between the baseline and final visits on the CELF-P-2 Recalling Sentences subtest ($P = 0.01$). No significant differences were found between baseline and final visits for either the treatment or placebo groups on any of the VABS-II, BRIEF-P, REAL, or TOVER scores. Finally, no significant effects of age or gender were found for either group on any outcome measures.

DISCUSSION

This double-blind, placebo-controlled clinical trial of rivastigmine treatment in children and adolescents with DS did not show significant improvement in aspects of cognition, language, or overall function. The results suggest that rivastigmine is not associated with significantly increased adverse events or increased risk for cardiac complications compared to placebo.

The current study's findings build upon previous investigations of the efficacy of ChEIs in DS. A previous open-label trial of

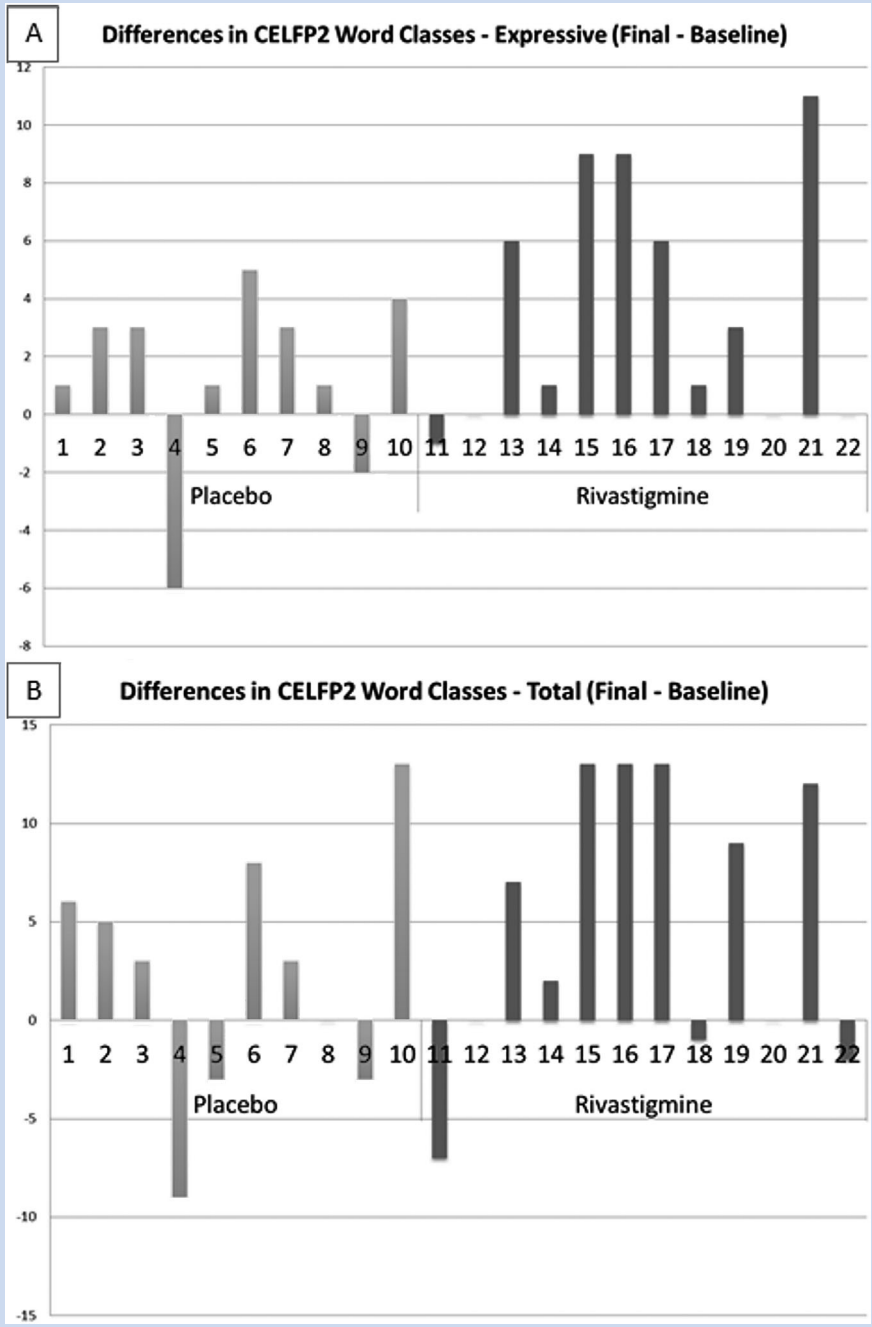

FIG. 3. Differences in CELF-P-2 scores between final and baseline visits for individual study participants (numbered 1–22) for (A) Word Classes Expressive scores and (B) Word Classes Total scores. A rise in raw scores from baseline to the final visit indicates improvement. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

ChEIs in children with DS [Heller et al., 2004] indicated that donepezil was associated with significant improvements in expressive and receptive language. Similarly, an investigation of rivastigmine efficacy in adolescents with DS indicated improvements in overall adaptive function, attention, memory, and language [Heller et al., 2006b]. However, these studies were limited by sample size (with totals of 7 children and 11 adolescent participants, respectively) and by the lack of a placebo-controlled comparison group. In the current study, there was a great deal of variability in the performance of participants who were treated with rivastigmine. Although the treatment group showed significant improvement on the measures emphasizing expressive language and short-term visual memory, this change was not statistically significantly different from the placebo group. Still,

these measures targeting the participants' expressive language skills continue to hold promise as a way to detect change in larger medication trials, as it is possible that only a subset of individuals with DS show improvements in expressive language with rivastigmine treatment.

The results of the current study were consistent with a long-term follow-up study of rivastigmine efficacy in adolescents with DS [Heller et al., 2010] and a double-blind placebo-controlled study of donepezil in adolescents with DS [Kishnani et al., 2010]. Neither study demonstrated significant changes in cognitive or language performance related to the study medication. While the study by Heller et al. [2010] suggested the potential for a small subset of individual responders to the medication for adaptive behavior, the data from the current study did not provide evidence for a larger subset of individual responders in the treatment group compared to the placebo group. Furthermore, performance in the treatment group appeared to decline on several VABS-II domains from the baseline to final visits, raising the question of whether a subset of individuals may have had a detrimental response to rivastigmine. However, these performance differences between visits did not reach statistical significance and no individual score changes on the VABS-II were within a clinically meaningful range. Larger studies may be needed to address whether a subset of individuals could show clinically relevant changes on cognitive measures related to the participant's overall intelligence quotient (IQ) or other variables, such as genetic or epigenetic factors, that may modify the response to ChEIs.

While cholinergic deficits have been established to be present in adults with DS, the timing of the onset of these deficits is less clear. Data from animal models have suggested that cholinergic function related to projections from the basal forebrain is critical for cortical development and establishment of neural circuits that underlie complex cognitive functions in adulthood [Berger-Sweeneyand Hohmann, 1997; Berger-Sweeney, 1998]. It is unclear, however, whether the influence of the cholinergic basal forebrain system is similar during development and adulthood [Berger-Sweeney, 2003]. It will be important for future research to aim to gain an understanding of how the developing cholinergic system is affected in DS. Additionally, it will be critical to characterize the roles of other modulatory systems such as glutamate, which has been implicated in learning and memory difficulties in DS [Costa, 2014]. In order to develop cognitive interventions that target these systems, further research is necessary to characterize the pathogenic roles of these neurotransmitter systems across different stages of brain development in DS.

There are multiple challenges inherent in conducting clinical trials in children with DS. One important challenge is determining appropriate cognitive testing measures that will be sensitive to medication effects. DS is associated with changes in a broad range of cognitive functions, including adaptive function, expressive and receptive language, attention, memory, and executive function. When selecting study measures to investigate efficacy of treatment, there is often a trade-off between investigating multiple cognitive domains and obtaining sufficient statistical power to demonstrate significant effects [Heller et al., 2006a]. The current study highlights the current unmet need to define a set of the most appropriate cognitive measures that are reliable, valid, and sensitive to treatment effects. It is also critical to minimize the variability within each participant's performance at each time point.

A limitation of the current study is that it may not be adequately powered to detect small changes in cognitive performance related to rivastigmine effects. It may be that many hundreds of participants are required to detect more subtle changes in cognitive functioning in children with DS related to rivastigmine. Larger studies would be necessary to investigate factors such as dose and titration tolerance, especially when wide variability in these factors may be observed [Heller et al., 2006a]. Additionally, the current study did not investigate the potential role of factors such as educational or other therapeutic interventions that could influence change in cognitive functioning. Future studies may benefit from including these types of factors as covariates to distinguish potential medication effects.

This study also highlights the challenges of conducting clinical trials using liquid rivastigmine. The liquid form of rivastigmine is helpful for children who may have difficulty swallowing pills and allows for flexibility in dosing and titration [Heller et al., 2006a]. However, we experienced challenges related to a shortage of the study medication mid-way through the study that required two participants to follow a modified protocol and extended study time. Partnering with the pharmaceutical industry is necessary to ensure adequate supply of study drug, as well for attaining the necessary study funding and sample sizes that are required for conducting clinical trials for enhancement of cognitive function in DS [Heller et al., 2006a].

CONCLUSIONS

The current study did not demonstrate evidence for efficacy of rivastigmine in improving cognitive function in children with DS. However, our results suggested that rivastigmine does appear to be safe and well-tolerated for children and adolescents with DS. Future investigations with larger samples of participants may be needed to define a profile of specific cognitive measures that are reliable and sensitive to treatment effects for use in clinical trials for children with DS.

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REFERENCES

Beccaria L, Marziani E, Manzoni P, Arvat E, Valetto MR, Gianotti L, Ghigo E, Chiumello G. 1998. Further evidence of cholinergic impairment of the neuroendocrine control of the GH secretion in Down's syndrome. Dement Geriatr Cogn Disord 9:78–81.

Becker L, Mito T, Takashima S, Onodera K. 1991. Growth and development of the brain in Down syndrome. Prog Clin Biol Res 373:133–152.

Berger-Sweeney J. 1998. The effects of neonatal basal forebrain lesions on cognition: Towards understanding the developmental role of the cholinergic basal forebrain. Int J Dev Neurosci 16:603–612.

Berger-Sweeney J. 2003. The cholinergic basal forebrain system during development and its influence on cognitive processes: Important questions and potential answers. Neurosci Biobehav Rev 27:401–411.

Berger-Sweeney J, Hohmann CF. 1997. Behavioral consequences of abnormal cortical development: Insights into developmental disabilities. Behav Brain Res 86:121–142.

Brownell R. 2000. Expressive one-word picture vocabulary test (EOWPVT). Ann Arbor, MI: Academic Therapy Publications.

Casanova MF, Walker LC, Whitehouse PJ, Price DL. 1985. Abnormalities of the nucleus basalis in Down's syndrome. Ann Neurol 18:310–313.

Costa AC. 2014. The glutamatergic hypothesis for Down syndrome: The potential use of N-methyl-D-aspartate receptor antagonists to enhance cognition and decelerate neurodegeneration. CNS Neurol Disord Drug Targets 13:16–25.

Florez J, del Arco C, Gonzalez A, Pascual J, Pazos A. 1990. Autoradiographic studies of neurotransmitter receptors in the brain of newborn infants with Down syndrome. Am J Med Genet Suppl 7:301–305.

Gioia GA, Espy KA, Isquith PK. 2002. Behavior rating inventory of executive function, preschool version (BRIEF-P). Odessa, FL: Psychological Assessment Resources.

Heller JH, Spiridigliozzi GA, Crissman BG, McKillop JA, Yamamoto H, Kishnani PS. 2010. Safety and efficacy of rivastigmine in adolescents with Down syndrome: Long-term follow-up. J Child Adolesc Psychopharmacol 20:517–520.

Heller JH, Spiridigliozzi GA, Crissman BG, Sullivan-Saarela JA, Li JS, Kishnani PS. 2006a. Clinical trials in children with Down syndrome: Issues from a cognitive research perspective. Am J Med Genet Part C Semin Med Genet 142C:187–195.

Heller JH, Spiridigliozzi GA, Crissman BG, Sullivan JA, Eells RL, Li JS, Doraiswamy PM, Krishnan KR, Kishnani PS. 2006b. Safety and efficacy of rivastigmine in adolescents with Down syndrome: A preliminary 20-week, open-label study. J Child Adolesc Psychopharmacol 16: 755–765.

Heller JH, Spiridigliozzi GA, Doraiswamy PM, Sullivan JA, Crissman BG, Kishnani PS. 2004. Donepezil effects on language in children with Down syndrome: Results of the first 22-week pilot clinical trial. Am J Med Genet Part A 130A:325–326.

Heller JH, Spiridigliozzi GA, Kishnani PS. 2000. TOVER: Test of verbal expression and reasoning. Durham, NC: Duke University Medical Center.

Heller JH, Spiridigliozzi GA, Sullivan JA, Doraiswamy PM, Krishnan RR, Kishnani PS. 2003. Donepezil for the treatment of language deficits in adults with Down syndrome: A preliminary 24-week open trial. Am J Med Genet Part A 116A:111–116.

Johnson N, Fahey C, Chicoine B, Chong G, Gitelman D. 2003. Effects of donepezil on cognitive functioning in Down syndrome. Am J Ment Retard 108:367–372.

Kishnani PS, Heller JH, Spiridigliozzi GA, Lott I, Escobar L, Richardson S, Zhang R, McRae T. 2010. Donepezil for treatment of cognitive dysfunction in children with Down syndrome aged 10–17. Am J Med Genet Part A 152A:3028–3035.

Kishnani PS, Sommer BR, Handen BL, Seltzer B, Capone GT, Spiridigliozzi GA, Heller JH, Richardson S, McRae T. 2009. The efficacy, safety, and tolerability of donepezil for the treatment of young adults with Down syndrome. Am J Med Genet Part A 149A:1641–1654.

Kishnani PS, Sullivan JA, Walter BK, Spiridigliozzi GA, Doraiswamy PM, Krishnan KR. 1999. Cholinergic therapy for Down's syndrome. Lancet 353:1064–1065.

Kondoh T, Kanno A, Itoh H, Nakashima M, Honda R, Kojima M, Noguchi M, Nakane H, Nozaki H, Sasaki H, Nagai T, Kosaki R, Kakee N, Okuyama T, Fukuda M, Ikeda M, Shibata Y, Moriuchi H. 2011. Donepezil significantly improves abilities in daily lives of female Down syndrome patients with severe cognitive impairment: A 24-week randomized, double-blind, placebo-controlled trial. Int J Psychiatry Med 41:71–89.

Korkman M, Kirk U, Kemp S. 1998. NEPSY: A developmental neuropsychological assessment, manual. San Antonio, TX: The Psychological Corporation.

Sacks B, Smith S. 1989. People with Down's syndrome can be distinguished on the basis of cholinergic dysfunction. J Neurol Neurosurg Psychiatry 52:1294–1295.

Semel E, Wiig EH, Secord WA. 2004. Clinical evaluation of language fundamentals-preschool-2. San Antonio, TX: Harcourt Assessment.

Sparrow SS, Cicchetti DV, Balla DA. 2005. Vineland adaptive behavior scales. Survey forms manual, 2nd edition. Minneapolis, MN: NCS Pearson, Inc.

Spiridigliozzi GA, Heller JH, Crissman BG, Sullivan-Saarela JA, Eells R, Dawson D, Li J, Kishnani PS. 2007. Preliminary study of the safety and efficacy of donepezil hydrochloride in children with Down syndrome: A clinical report series. Am J Med Genet Part A 143A:1408–1413.

Spiridigliozzi GA, Heller JH, Kishnani PS. 2010. Rating of everyday activities and life skills. Durham, NC: Duke University Medical Center.