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ORIGINAL PAPER

Efficacy of Sulforaphane in Treatment of Children with Autism Spectrum Disorder: A Randomized Double-Blind Placebo-Controlled Multi-center Trial

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Abstract

Sulforaphane has been reported to possibly improve core symptoms associated with autism spectrum disorders from mostly small size studies. Here we present results of a larger randomized clinical trial (N = 108) in China. There were no significant changes in caregiver rated scales between sulforaphane and placebo groups. However, clinician rated scales showed a significant improvement in the sulforaphane group, and one third of participants showed at least a 30% decrease in score by 12 weeks treatment. The effects of sulforaphane were seen across the full range of intelligence and greater in participants over 10 years. Sulforaphane was safe and well-tolerated even for young children. The inconsistent results between caregiver and clinician rated scales suggest more clinical trials are needed to confirm our findings.

Keywords Autism spectrum disorders (ASD) · Sulforaphane · Clinic trials · Cognitive impairment

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Background

Autism spectrum disorder (ASD) is a neurodevelopmental condition impacting about 2.3% of school age children (Maenner et al., 2021). The prevalence of ASD is reported to be 0.39% in China (Wang et al., 2018), but it is likely an underestimate as ASD is usually diagnosed in children only with severe symptoms in China (Jin et al., 2018). While educational and behavioral training programs show some effectiveness (Lord et al., 2018), core features typically persist at clinical levels in most cases. Intellectual disability commonly co-occurs in children with ASD, and has a negative effect on education and behavioral training programs (Walton & Ingersoll, 2013). No medications are approved for core features associated with ASD.

Sulforaphane (SF) is an isothiocyanate derived from its glucosinolate precursor, glucoraphanin, as found in broccoli, mostly in the sprouts, partially inactivated by cooking, and some studies suggest it may improve symptoms of ASD. Though mechanisms of action are not fully understood, SF may target several physiological mechanisms implicated ASD, such as redox metabolism/oxidative stress (Liu et al., 2020). In 2014, Zimmerman and colleagues first reported that SF significantly improved the clinical symptoms of autistic teens and young adults with ASD (Singh et al., 2014). Awareness, communication, stereotyped behavior and hyperactivity of individuals in SF group improved significantly during the 18 weeks treatment period, and symptoms returned during the follow-up period (4 week) off SF. Furthermore, a 3 year follow-up of this study using the subjective impressions of caregivers noted that many caregivers felt SF was beneficial and continued to use it (Lynch et al., 2017). However, subsequent published studies with small samples did partially but not fully replicate these findings (Bent et al., 2018; Momtazmanesh et al., 2020; Zimmerman et al., 2021). Results from an unpublished study by Laura Politte also showed no statistical difference between sulforaphane and placebo in any of the outcome measures (L. Politte personal communication) (ClinicalTrials.gov NCT02909959).

Accordingly, we examined the effect of sulforaphane in a broader age range of young children and adolescents with ASD through a randomized controlled trial in a new ethnic group, a Chinese Han population. To our knowledge, this is the largest sample size used to date in a study of effects on SF in ASD. Our more diverse sample allowed us to include evaluation of level of cognitive deficit and age as moderators of response to SF.

Methods

Study Design and Participants

The study was a 12 week, randomized, double-blind, placebo-controlled, multicenter trial. It was approved by the ethics committee of Second Xiangya Hospital and Guangzhou Huiai Hospital. This trial was registered at ClinicalTrials.gov (NCT02879110) and conducted between August 2016 and May 2019. All participants provided written informed consent prior to enrollment. The efficacy and safety measures were assessed at baseline and week 4, week 8 and week 12. Blood samples were collected at baseline and week 12.

Children with ASD were recruited if they met the following criteria: (1) age 3–15 years; (2) met Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) diagnostic criteria for ASD; (3) met instrument classification as ASD via validated Chinese versions of the Autism Diagnostic Interview-Revised (ADI-R) and Autism Diagnostic Observation Schedule (ADOS). Clinicians were trained and certified in ADI-R and ADOS assessment. Exclusions criteria were: (1) severe physical disease; (2) severe central nervous system disease, such as epilepsy; (3) known history of genetic syndromes co-occurring with ASD, (4) concomitant medications (see supplementary data for details).

Intelligence Assessment

The Chinese version of Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV) was used to assess intelligence quotient (IQ) of children at baseline if they were older than 6 years and able to comply with testing (Wechsler, 2003). The Peabody Picture Vocabulary Test, Fourth Edition (PPVT-4) was used to assess receptive vocabulary ability of children at baseline if they were younger than 6 years or unable to comply with WISC-IV testing (Dunn & Dunn, 2007). The PPVT has been used as proxy for verbal IQ of children with ASD (Krasileva et al., 2017).

Randomization and Masking and Procedures for Drug Administration

Participants were randomized 1:1 into SF or placebo (PBO) groups after initial screening and baseline assessments. Randomization and drug administration was conducted by a research assistant using a computer-generated random number table, who was not involved in the clinical assessment. SF was delivered as Avmacol® (Nutramax Laboratories Consumer Care, Inc., Edgewood, Maryland, USA). The matched placebo tablets were made mainly with starch

by a local drug company (Hunan dongting pharmaceutical co. LTD). Avmacol® tablets, contain both glucoraphanin and active myrosinase enzyme, are formulated to support sulforaphane production from ≥ 30 µmol of glucoraphanin per tablet. Participants took SF or PBO tablets daily once a day for 12 weeks, and dosing was weight-based: two tablets/day for 10–29 lb, three tablets/day for 30–49 lb, four tablets/day for 50–69 lb, six tablets/day for 70–89 lb, seven tablets/day for 90–109 lb, and eight tablets/day for 110–130 lb. Based on bioavailability study data from studies performed at John Hopkins University (Fahey et al., 2019) an estimated delivery of approximately 24, 36, 48, 72, 84 and 96 µmol of sulforaphane daily was expected in the respective SF dosage groups specified above.

Clinicians, evaluators, participants and parents were blinded to the randomization. Although the appearance of SF and PBO tablets were similar, parents of some children needed to grind the pills to facilitate swallowing; there might be a slightly different taste or smell in the ground SF vs PBO tablets. However, clinicians, evaluators, participants and parents were not told of any expected differences in taste or smell of placebo vs of sulforaphane, and since this was a new treatment not used in China previously, they had no basis of making a prior judgment. We also asked them not to discuss the nature of pills with each other during the trial.

We did not specify or monitor diet of our participants or controls for other potential sources of sulforaphane intake from cruciferous vegetables. However, sulforaphane itself has a high probability of being destroyed in Chinese meals because of China's high-temperature cooking methods for most foods.

Clinical Outcome Measures

The change in Social Responsiveness Scale-First Version (SRS) (Constantino et al., 2003) was selected as a priori primary outcome measure. Secondary outcome measures included the change of Clinical Global Impression Scale (CGI-I for improvement and CGI-S for severity focused on overall symptomatology) (Choque Olsson & Bolte, 2014), and the Repetitive Behavior Scale—Revised (RBS-R) (Lam & Aman, 2007). The Autism Behavior Checklist (Volkmar et al., 1988) also been used and our analysis for sulforaphane effects concentrated on the social relating behavior subscore of this scale. The OSU Autism Rating Scale-DSM-IV (OARS-4) (OSU RUPP, 2005) was included as an exploratory secondary outcome. The SRS, RBS-R and Autism Behavior Checklist are caregiver rated scales and higher scores indicate more severe symptoms. The OARS-4 and CGI are clinician rated scales incorporating both input from caregivers and direct observation of the patient. Each item on the OARS-4 scale has a range of 0 to 3 with higher ratings indicating a higher presence of autistic features. Although the CGI-S scale has not been validated as an autism-specific scale, it is frequently used to measure outcomes in autism-focused clinical trials (Hollander et al., 2022; McCracken et al., 2002). The CGI-S scale has a range of 1 to 7 with higher ratings indicting higher levels of traits. The CGI-I scale has a range of 1 to 7 with lower ratings indicting better improvement. These clinicians rated scales were performed by child psychiatrists who were very familiar with ASD, trained in the scales, and certified by annual re-training for consistency. Additionally, Child Behavior Checklist scores (CBCL) (Achenbach & Rescorla, 2000, 2001) and Adaptive Behavior Assessment System scores, Second Edition (ABAS-II) (Harrison & Oakland, 2008) were only obtained from a subset of participants, so they could not be used as additional outcome measures.

Safety Measures

The primary safety and side effects measure was the Systematic Assessment for Treatment Emergent Effects-Specific Inquiry (SAFTEE-SI) (Levine & Schooler, 1992), which was reported by parents. Potential metabolic side effects were assessed by routine serum and urine laboratory chemistries (CBC, metabolic profile) drawn at baseline and week 12 of study. Heart rate, weight and height were measured at each visit.

Discontinuation

All participants were accompanied by their caregivers during each visit. Medication treatment was supervised by caregivers at home. The participant's adherence for each visit interval was defined as taking more than 80% of tablets prescribed for that interval. Those who were unable to demonstrate adequate adherence after multiple coaching was withdrawn from the study. In addition, participants could be withdrawn from the study for safety concerns under the discretion of investigators. (see Table S1 for reasons for discontinuation).

Statistical Analysis

The primary analysis was an intent to treat analysis. Statistical significance was set at $P < 0.05$, and trend level significance was $P < 0.10$. The analysis of symptom variables used mixed model analysis using SAS 9.4 mixed procedure to handle missing data, from drop-outs or other causes, in the analysis, using either unstructured covariance and autoregressive [AR (1)] that would best fit the models. Participants were included in the statistical analysis of the evaluation of each variable if they had at least one post-baseline value for that assessment. If variables deviated markedly from the normal distribution, transformations (log, square root) were attempted before analysis

to achieve a better approximation to normal distribution. The main analysis was a mixed model analysis of difference scores from baseline with baseline scores as covariate. Additional analyses used mixed model original values at the indicated time points without covariate, and completer analysis at each time point. Effect size for localized and overall mixed model treatment effect was computed for variables with statistically significant treatment effects or strong trends, using additionally developed SAS syntax based on the methods suggested by Selye and associates recently published in Frontiers of Psychology (Selya et al., 2012). Corrected significance levels across scales or subscales for a specific variable was assessed by Benjamini–Hochberg (BH) protected significance level (at $\alpha = 0.05$) (Benjamini & Hochberg, 1995; Hsueh et al., 2003). Effect size output used η^2 which we translated into Cohen's d (through Psychometrical, www.psychometrica.de/effect_size). Effect size at individual time points in the completer analyses was analyzed by computation in an excel program for treatment and control groups with Cohen's d and Hedges correction.

For the SAFTEE side-effect scale ratings we analyzed both mean number of occurrences of a side effect during each evaluation during study drug treatment (corrected for baseline occurrence), and the number of participants who had this side effect reported at least once during the period of study drug administration. Because of very low incidence of side effects and non-normal distributions of these scores, non-parametric analyses were used to compare the side effects between active and placebo participants.

For statistical analysis participants' surrogate IQ was divided into scores of < 60 or ≥ 60, based on their scores of the WISC-IV or PPVT-4. Twenty-two participants didn't have values for WISC or PPVT measures (12 in the SF group and 10 in the placebo group) because they could not cooperate with the assessment. These participants who could not complete or understand even the PPVT-4 were classified in the < 60 severe intellectual disability group. The 60 cut off point was chosen because it yielded approximately equal number of participants with and without more severe cognitive impairment. For some measures, analysis was repeated with the more standard cutoff of 70 used in clinical practice.

Routine clinical laboratory values were assessed at baseline and 12 weeks of treatment, and differences in changes in laboratory values in the two treatment groups were assessed by t-test or analysis of variance.

Results

Participant Characteristics

A total of 201 participants were accessed for eligibility, of whom 135 were randomized. However, before the distribution of the sulforaphane or placebo, some guardians of patients withdrew the informed consent without explanation or due to their children being unable to complete some additional tests, such as collecting enough blood samples for biological study. Only 60 participants in sulforaphane group and 48 participants in placebo group received allocated intervention (Fig. 1). There were no significant differences in any background characteristic between the SF and PBO participants, including age, sex, weight, or baseline scores on ADOS and OARS-4 rating scales and surrogate IQ assessment (Table 1). This was primarily a male sample (92% SF–98% PBO) with a high degree of autism traits based on ADOS scores (Table 1 and Table S2 for module details) and baseline total SRS scores (Table 2, means 101–104). Outside of exclusionary criteria, other concomitant co-occurring conditions were assessed by the CBCL based on *T*-scores for diagnostic syndrome classification (Table S3). About 20–30% of participants were classified as having either affective or anxiety co-occurring symptoms at clinical thresholds based on their symptom profile on the CBCL, and a smaller percentage on other types of *T*-scores defined diagnostic conditions. However, there were no statistically significant differences in the percent occurrence between placebo and sulforaphane participants for any type of CBCL subscale. For those participants for whom had baseline ABAS-II scores, there was also no difference between sulforaphane and placebo participants (Table S4). Intellectual ability on the basis of measured IQ ranged in the entire sample from 20 to 155 using either scores on WISC-IV (mean 70.2) or PPVT-4 (mean 63.6) scores. The validity of the classification of our two intellectual impairment sub-types (more cognitively impaired < 60, less cognitively impaired ≥ 60, as described in methods section) was supported by differences in their ABAS-II scores (Table S5). As expected, the more cognitively impaired group had significantly lower ABAS-II scores than the less cognitively impaired sub-group. Adaptive functions, as measured by ABAS-II, were similar between those who could not perform either WISC-IV or PPVT-4 and participants in the more cognitively impaired sub-group (measured IQ < 60). There were statistically significant correlations between intellectual impairment classification or WISC-IV or PPVT-4 scores and ABAS-II scores (see supplementary data page 8). All participants did not use other medications or herbal supplements, melatonin, and special diets during the trial, and most participants received applied behavior analysis school-based interventions.

Outcomes on Efficacy Measures

There were no significant effects of SF vs PBO on any measures of the SRS scale (Table 2 and Table S6). However, SF participants showed significantly more reductions

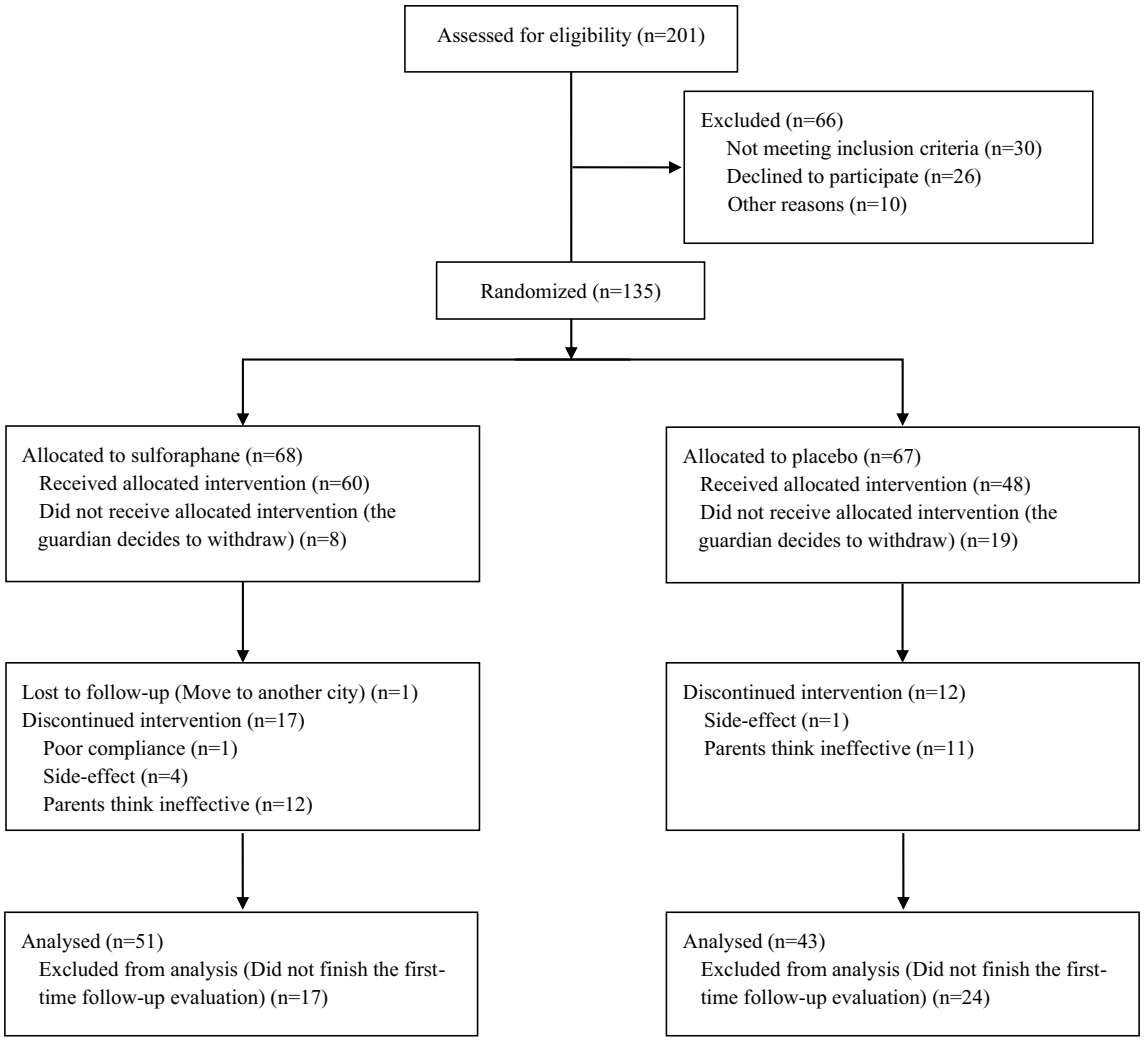

Fig. 1 Flow of participants through the trial

or improvements of autism features on the CGI-I scale ($P < 0.001$) and the OARS-4 scale ($P < 0.01$ to $P < 0.002$) (Table 2, Fig. S1). Differences from baseline in OARS-4 total average scores, impaired social interaction scores and communication barriers scores were greatest at the 12-week treatment time point and all these measures showed statistically significant decreases. The difference score analysis also showed significant effects at week 8 of treatment. The mean decreases in the OARS-4 scales at 12 weeks represent a percent decrease ranging from 19.4 to 29.5% from baseline. The overall analyses (F-treatment effects) for these OARS-4 variables and CGI-I continued to show statistically significant effects for SF vs PBO on these measures at BH corrected significance levels for the protected comparisons (at $\alpha = 0.05$) using all of the nine analyses included in Table 2. Completer analysis (Table S7, Fig. S2) showed similar effects to the mixed model analysis. The impaired social interaction decreases of the OARS-4 appeared to show the greatest effect among the OARS-4 sub-scores, and 38.5% of SF participants showed at least a 30% decrease in these scores by week 12 of treatment (Figs. S1 and S2). Ninety percent of SF treated participants showed at least mild improvement or better (score 3 or less) on the CGI-I by week 12 compared to 41% of PBO participants (Fig. S2), and 18% of SF participants showed moderate improvement or better (score 2 or less) vs 5% of PBO participants. The effect sizes for several items from of the clinician rated scales (OARS-4 and CGI-I) was large for the overall summary of the three time points in the mixed model analysis (Table 2, 0.96–1.15) with the greatest effect size seen at the 12 week time point. The completer analysis showed similar but slightly smaller moderate to large effect sizes at individual time points (Table S7). Since the effects of SF increased with length of treatment, the effect size at 12 weeks may be the most relevant. Total scores on motor components of autism features did not change; there was no significant effect of SF on

Table 1 Characteristics of the subjects who were randomized and received initial study medication

Characteristics	Sulforaphane (N = 60)	Placebo (N = 48)	F	P
Age—yrs. (age range)	9.0 ± 3.6 (3–15)	9.1 ± 3.8 (3–15)	0.020	0.888
Male sex—no. (%)[a]	55 (92)	47 (98)		0.223
Height—cm	137.1 ± 24.7	135.1 ± 25.3	0.179	0.673
Weight—kg	36.5 ± 16.1	35.2 ± 16.4	0.175	0.677
Body–mass index	18.3 ± 3.0	18.3 ± 3.8	0.001	0.977
Daily dose—tablet	5.4 ± 2.0	5.1 ± 2.0	0.559	0.456
Autism diagnostic interview-revised score				
Qualitative abnormalities in reciprocal social interaction	23.0 ± 4.6	23.7 ± 4.6	0.736	0.393
Qualitative abnormalities in communication	17.4 ± 4.5	16.5 ± 4.4	0.913	0.342
Restricted, repetitive, and stereotyped patterns of behavior	6.0 ± 2.8	6.0 ± 2.8	< 0.001	0.994
Abnormality of development evident at or before 36 months	3.6 ± 1.4	4.0 ± 1.1	3.910	0.051
Autism diagnostic observation schedule scoreb				
Communication	7.2 ± 2.1	7.6 ± 2.1	1.004	0.319
Reciprocal social interaction	10.2 ± 2.7	10.8 ± 3.0	1.009	0.317
Communication + social interaction	17.8 ± 3.7	18.6 ± 4.3	1.161	0.284
Play/imagination and creativity	2.2 ± 1.1	2.4 ± 1.1	0.554	0.458
Stereotyped behavior and restricted interests	2.6 ± 2.0	2.8 ± 2.0	0.335	0.564
OARS-4 score at baseline				
Total score	1.7 ± 0.5	1.7 ± 0.4	0.056	0.814
Impaired social interaction	2.2 ± 0.6	2.2 ± 0.5	0.226	0.635
Communication barrier	1.8 ± 0.6	1.7 ± 0.6	0.549	0.460
Stereotypes model	1.2 ± 0.6	1.1 ± 0.6	0.064	0.800
Intelligence assessment[c]				
WISC-4 total score (no, SFN 13 vs PLA 10)	69.6 ± 18.7	68.5 ± 20.2	0.019	0.892
PPVT-4 T score (no, SFN 35 vs PLA 28)	67.5 ± 25.3	58.8 ± 26.6	1.780	0.187

Each number presents mean ± SD except for sex distribution where number of pale sex and percent is given. Test of differences between groups was by analysis of variance, except for sex distribution

[a]Fisher's exact test

[b]The detailed information of ADOS modules is presented in the supplementary Table S2

[c]A total of 22 children did not complete the intelligence assessment due to lack of cooperation or inability to perform tests

RBS scale score or the stereotyped behavior component of the OARS-4 scale (Table 2) and the perseverative behavior subscale of the RBS-R showed an opposite trend favoring PBO (Table S8).

SF had fairly similar effects on decreasing impairment scores in participants with higher vs lower degrees of intellectual-cognitive deficits. There was no significant interaction effects of IQ class and clinician rated symptomatic improvement measures, although some analyses showed a non-significant trend for a slightly greater effect in the more severely impaired group (surrogate IQ < 60) (Table 3). Division of participants by surrogate IQ at 70 as a more commonly accepted IQ cutoff in clinical diagnosis of intellectual disability yielded similar results (Table S9). We had 76 participants with IQ scores and outcome measures for whom we could do continuous rather than group cut point analysis. For this sub-sample correlation analysis of surrogate IQ score vs clinical improvement on OARS-4 variables at 12 weeks, showed no significant correlation between these variables in either the placebo or sulforaphane group, suggesting a lack of effect of this surrogate IQ measure on clinical response. When surrogate IQ was added as a covariate to the mixed model analysis of the OARS-4 variables of total average score and impaired social interaction, the main drug effect of SF vs PBO remained significant and the covariate effect was statistically significant. Additional statistical analysis showed an influence of the covariate, suggesting IQ influencing the main drug effect for OARS-4 total average score difference and communication impairment score difference (see supplementary data page 13 for statistics of coefficient of change percent).

Correlation analysis suggested that older age of participants was associated with a small but statistically significant greater improvement in the SF participants in some OARS-4

Table 2 Effects of sulforaphane on autism rating scales scores in all subjects

Scale	Measure or sub-scale	Treatment	Baseline score (mean ± s.e.m.)	Adjusted estimated difference from baseline at specified time point (mean ± s.e.m.) (stars indicate significance between SF vs PBO at specific time point-see legend for details)			Overall analysis F_{TR} = treatment
				4 weeks	8 weeks	12 weeks	
OSU Autism rating scale-DSM-IV (OARS-4)	Total average score	Sulforaphane (n = 51)	1.70 ± 0.06	− 0.12 ± 0.05	− 0.23 ± 0.05** (d = 1.08)	− 0.33 ± 0.05** (d = 1.39)	F_{TR} = 10.25, DF = 1,91, **P = 0.002**^{BH}, d = 0.96
		Placebo (n = 43)	1.66 ± 0.07	− 0.01 ± 0.05	− 0.03 ± 0.05	− 0.09 ± 0.06	
	Impaired social interaction	Sulforaphane (n = 51)	2.17 ± 0.08	− 0.25 ± 0.06	− 0.43 ± 0.06** (d = 1.35)	− 0.64 ± 0.07*** (d = 1.44)	F_{TR} = 13.21, DF = 1,91, **P < 0.001**^{BH}, d = 1.15
		Placebo (n = 43)	2.21 ± 0.08	− 0.12 ± -0.06	− 0.16 ± 0.07	− 0.24 ± 0.07	
	Communication barriers	Sulforaphane (n = 51)	1.79 ± 0.08	− 0.06 ± 0.06	− 0.21 ± 0.08* (d = 0.99)	− 0.37 ± 0.07** (d = 1.29)	F_{TR} = 9.73, DF = 1,91, **P = 0.002**^{BH}, d = 0.97
		Placebo (n = 43)	1.67 ± 0.08	0.08 ± 0.07	− 0.01 ± 0.07	− 0.05 ± 0.07	
	Stereotyped behaviors	Sulforaphane	1.15 ± 0.08	− 0.14 ± 0.16	0.07 ± 0.16	− 0.04 ± 0.22	F_{TR} = 1.08, DF = 1,91, P = 0.300
		Placebo (n = 43)	1.11 ± 0.09	− 0.06 ± 0.06	0.19 ± 0.16	0.37 ± 0.23	
Social responsiveness scale (SRS)	Total average score	Sulforaphane (n = 51)	101.14 ± 3.41	− 2.98 ± 2.80	− 1.92 ± 3.06	− 1.83 ± 3.14	F_{TR} = 0.02, DF = 1,91, P = 0.885
		Placebo (n = 41)	104.53 ± 3.80	− 0.79 ± 3.11	− 0.40 ± 3.42	− 3.88 ± 3.54	
Repetitive behavior scale—revised (RBS-R)	Total average score	Sulforaphane (n = 48)	28.96 ± 2.39	− 3.23 ± 1.57	− 4.65 ± 1.93	− 3.54 ± 2.06	F_{TR} = 1.91, DF = 1,86, P = 0.171
		Placebo (n = 41)	30.51 ± 2.59	− 6.22 ± 1.68	− 4.29 ± 2.12	− 9.82 ± 2.22	
Autism behavior checklist (AUBC)	Social relating behavior	Sulforaphane (n = 51)	12.14 ± 0.72	− 1.01 ± 0.64	− 0.57 ± 0.80	− 0.87 ± 0.84	F_{TR} = 0.14, DF = 1,89, P = 0.706
		Placebo (n = 40)	12.03 ± 0.82	− 1.10 ± 0.72	− 1.15 ± 0.89	− 1.30 ± 0.93	
CGI-severity		Sulforaphane (n = 51)	4.88 ± 0.15	0.13 ± 0.10	0.10 ± 0.11	− 0.17 ± 0.11	F_{TR} = 0.27, DF = 1,91, P = 0.604
		Placebo (n = 43)	5.16 ± 0.17	0.17 ± 0.11	0.09 ± 0.11	0.01 ± 0.12	
CGI-improvement (estimated score at specified time point)		Sulforaphane (n = 49)	NR	3.61 ± 0.08	3.26 ± 0.08*** (d = 0.87)	2.89 ± 0.09*** (d = 1.10)	F_{TR} = 22.80, DF = 1,89, **P < 0.001**^{BH}, d = 1.07
		Placebo (n = 42)	NR	3.79 ± 0.08	3.71 ± 0.08	3.51 ± 0.09	

FTR is overall treatment effect, sulforaphane vs placebo for all time points considered. Bold values indicate the treatment effects which are statistically significant

Each value of adjusted mean difference, is estimated mean difference from all subjects having at least one post-value value, derived from SAS mixed model analysis with baseline value as covariate. For CGI-improvement there is no baseline score and no baseline covariate; N's-OARS-4 sulforaphane (n = 51) placebo (n = 43); SRS sulforaphane (n = 51) placebo (n = 41); RBS-R sulforaphane (n = 48) placebo (n = 41); AUBC sulforaphane (n = 52) placebo (n = 40); Statistical significance of difference in estimated mean of SF vs PBO groups at specific time point, by t-test comparison from mixed model results: *P < 0.05, **P < 0.01, ***P < 0.001; d = effect size Cohen's d. ^{BH}=Statistically significant with BH correction for nine analyses in this table (at α = 0.05). For the OARS-4 scale total average difference and sub-scale differences there was a significant time effect, P < 0.01, with most scores in both sulforaphane and placebo decrease over the course of the study (except for the stereotyped behavior sub-scale)

scores (age vs total average difference 8 week r = − 0.38. P = 0.01 n = 44; age vs impaired social interaction difference 8 week r = − 0.25, P = 0.02, n = 44; age vs impaired communication barriers difference 12 week r = − 0.33, P = 0.04, n = 39), but not with overall global improvement on CGI-I. Participants aged 10 or higher showed a

Table 3 Effects of level of surrogate IQ measure on sulforaphane's effect on OARS-4 difference scores and CGI-I scores

Subject group	Treatment	Adjusted estimated difference from baseline at specified time point (mean ± s.e.m.) (stars indicate significant difference between SF vs PBO at specific time point)			Overall analysis (difference scores) F_{TR} = overall treatment effect F_{ITR} = treatment* IQ class interaction effect
		4 weeks	8 weeks	12 weeks	
OARS-4 total average score					
IQ 60 or above	Sulforaphane (n = 26)	− 0.06 ± 0.07	− 0.26 ± 0.07†	− 0.33 ± 0.07	F_{TR} = 9.32 DF = 1,89, **P = 0.003**
	Placebo (n = 19)	− 0.04 ± 0.08	− 0.05 ± 0.09	− 0.22 ± 0.09	F_{ITR} = 1.31, DF = 1,89, P = 0.237
Less than IQ 60	Sulforaphane (n = 25)	− 0.18 ± 0.07*	− 0.21 ± 0.08†	− 0.33 ± 0.08**	
	Placebo (n = 24)	0.02 ± 0.07	− 0.01 ± 0.08	− 0.00 ± 0.07	
OARS-4 subscale scores					
Impaired social interaction					
IQ 60 or above	Sulforaphane (n = 26)	− 0.13 ± 0.08	− 0.47 ± 0.09	− 0.69 ± 0.08*	F_{TR} = 12.13, DF = 1,89, **P = 0.001**
	Placebo (n = 19)	− 0.27 ± 0.10	− 0.26 ± 0.10	− 0.40 ± 0.10	F_{ITR} = 1.62, DF = 1,89, P = 0.175
Less than IQ 60	Sulforaphane (n = 25)	− 0.34 ± 0.09**	− 0.40 ± 0.09*	− 0.59 ± 0.09***	
	Placebo (n = 24)	0.01 ± 0.08	− 0.08 ± 0.08	− 0.13 ± 0.08	
Communication barriers					
IQ 60 or above	Sulforaphane (n = 26)	− 0.04 ± 0.08	− 021 ± 0.09	− 0.43 ± 0.09	F_{TR} = 7.92, DF = 1,89, **P = 0.006**
	Placebo (n = 19)	0.03 ± 0.10	− 0.11 ± 0.11	− 0.25 ± 0.11	F_{ITR} = 0.90, DF = 1,89, P = 0.469
Less than IQ 60	Sulforaphane (n = 25)	− 0.08 ± 0.09	− 0.21 ± 0.10*	− 0.31 ± 0.09**	
	Placebo (n = 24)	0.11 ± 0.09	0.08 ± 0.09	0.08 ± 0.09	
Stereotyped behaviors					
IQ 60 or above	Sulforaphane (n = 26)	− 0.02 ± 0.10	− 0.12 ± 0.11	− 0.30 ± 0.10	F_{TR} = 2.71, DF = 1,89, P = 0.116
	Placebo (n = 19)	0.10 ± 0.11	0.19 ± 0.12	− 0.04 ± 0.12	F_{ITR} = 1.65, DF = 1,89, P = 0.169
Less than IQ 60	Sulforaphane (n = 25)	− 0.07 ± 0.10	0.03 ± 0.11	− 0.06 ± 0.10	
	Placebo (n = 24)	− 0.02 ± 0.10	0.01 ± 0.10	0.06 ± 0.10	
CGI-improvement (estimated score at specified time point)					
IQ 60 or above	Sulforaphane (n = 25)	3.76 ± 0.10	3.27 ± 0.11	2.79 ± 0.12†	F_{TR} = 19.10, DF = 1,87, **P < 0.001**
	Placebo (n = 18)	3.75 ± 0.12	3.54 ± 0.13	3.15 ± 0.15	F_{ITR} = 3.75, DF = 1,87, P = 0.056
Less than IQ 60	Sulforaphane (n = 24)	3.46 ± 0.10*	3.24 ± 0.11***	3.02 ± 0.12***	
	Placebo (n = 24)	3.80 ± 0.10	3.80 ± 0.10	3.71 ± 0.11	

FTR is overall treatment effect, sulforaphane vs placebo for all time points considered. Bold values indicate the treatment effects which are statistically significant

Data is from mixed-model analysis of difference scores with drug treatment and IQ as factors. Difference between SF vs PBO at specific time point, by t-test from mixed-model analysis: †P < 0.10, *P < 0.05, **P < 0.01, ***P < 0.001

greater decrease by SF on OARS-4 total average scores and social responsiveness scores than participants below age 10 (Table 4).

In a more complex analyses, when both age and IQ class were added as additional factors or covariates to the overall mixed model analysis of OARS-4 scores, the overall positive effects of SF vs PBO on these clinicians rated outcome scales remained strong (Table S10).

There were no significant SF versus placebo difference on the AUBC, SRS and RBS-R total average difference score when subdivided by age (under 10 vs above) or IQ (over 60 vs below) (Tables S11 and S12).

Safety and Adverse Events

Sulforaphane treatment was safe and well-tolerated. There were no serious adverse events reported during this trial, and there were no statistically significant differences between changes in routine laboratory values, weight, or heart rate in the SF vs PBO group during the trial (Tables S13–S16). There were also no differences (P < 0.05) in reported side effects on the SAFTEE scale between SF and PBO groups (Table 5). The two most common reported adverse events were difficulty concentrating and dysphasia and the percentage of SF vs PBO participants who had at least one occurrence during study period were not significantly different. The caregivers in the SF group reported 28% participants feeling nervous or excited at least one-time versus 11.6%

Table 4 Age differences on effects of sulforaphane on OARS-4 and CGI-I scores

Subject group	Treatment	Adjusted estimated difference from baseline at specified time point (mean ± s.e.m.) (stars indicate significant difference between SF vs PBO at specific time point)			Overall analysis (difference scores) F_{TR} = overall treatment effect F_{ATR} = treatment* age class interaction effect
		4 weeks	8 weeks	12 weeks	
OARS-4 total average score					
Age 10 or less	Sulforaphane (n = 30)	− 0.13 ± 0.06	− 0.15 ± 0.07	− 0.29 ± 0.07	F_{TR} = 12.47, DF = 1,88, **P = 0.001**
	Placebo (n = 26)	− 0.08 ± 0.07	− 0.07 ± 0.07	− 0.12 ± 0.07	F_{ATR} = 3.74, DF = 1,88, P = 0.056
Age over 10	Sulforaphane (n = 20)	− 0.13 ± 0.07*	− 0.34 ± 0.08**	− 0.39 ± 0.09*	
	Placebo (n = 17)	0.10 ± 0.08	0.04 ± 0.08	− 0.04 ± 0.09	
OARS-4 subscale scores					
Impaired social interaction					
Age 10 or less	Sulforaphane (n = 30)	− 0.24 ± 0.08	− 0.38 ± 0.08	− 0.67 ± 0.08*	F_{TR} = 20.00, DF = 1,88, **P < 0.001**
	Placebo (n = 26)	− 0.31 ± 0.08	− 0.32 ± 0.08	− 0.36 ± 0.08	F_{ATR} = 9.78, DF = 1,88, **P = 0.002**
Age over 10	Sulforaphane (n = 20)	− 0.31 ± 0.09**	− 0.53 ± 0.10***	− 0.61 ± 0.10***	
	Placebo (n = 17)	0.17 ± 0.10	0.08 ± 0.10	− 0.07 ± 0.10	
Communication barriers					
Age 10 or less	Sulforaphane (n = 30)	− 0.07 ± 0.08	− 0.11 ± 0.09	− 0.39 ± 0.08**	F_{TR} = 9.01, DF = 1,88, **P = 0.004**
	Placebo (n = 26)	0.02 ± 0.09	− 0.04 ± 0.09	− 0.02 ± 0.09	F_{ATR} = 0.40, DF = 1,88, P = 0.527
Age over 10	Sulforaphane (n = 20)	− 0.05 ± 0.10	− 0.32 ± 0.11*	− 0.34 ± 0.12	
	Placebo (n = 17)	0.16 ± 0.10	0.05 ± 0.11	− 0.10 ± 0.11	
Stereotyped behaviors					
Age 10 or less	Sulforaphane (n = 30)	− 0.09 ± 0.09	0.03 ± 0.10	− 0.14 ± 0.09	F_{TR} = 2.51, DF = 1,88, P = 0.116
	Placebo (n = 26)	0.05 ± 0.10	0.13 ± 0.10	− 0.02 ± 0.10	F_{ATR} = 0.05, DF = 1,88, P = 0.827
Age over 10	Sulforaphane (n = 20)	− 0.01 ± 0.11	− 0.17 ± 0.12	− 0.22 ± 0.13	
	Placebo (n = 17)	− 0.01 ± 0.12	0.01 ± 0.11	0.07 ± 0.12	
CGI-improvement (estimated score at specified time point)					
Age 10 or less	Sulforaphane (n = 29)	3.59 ± 0.10	3.20 ± 0.10**	2.74 ± 0.11***	F_{TR} = 21.86, DF = 1,86, **P < 0.001**
	Placebo (n = 25)	3.72 ± 0.10	3.67 ± 0.11	3.38 ± 0.12	F_{ATR} = 0.01, DF = 1,86, P = 0.929
Age over 10	Sulforaphane (n = 19)	3.63 ± 0.12	3.25 ± 0.12**	3.18 ± 0.15*	
	Placebo (n = 17)	3.88 ± 0.13	3.76 ± 0.13	3.67 ± 0.15	

FTR is overall treatment effect, sulforaphane vs placebo for all time points considered. FATR is interaction effect between treatment and age class. Bold values indicate the treatment effects which are statistically significant

Data is from mixed-model analysis of difference scores with drug treatment and age as factors. For OARS-4 total average score and impaired social interaction sub-score, and CGI-I scores the was an overall significant effect of time, P < 0.01, with differences scores becoming more negative and CGI-I scores lower (indicating more improvement), over time. Difference between SF vs PBO at specific time point, by t-test from mixed-model analysis: *P < 0.05, **P < 0.01, ***P < 0.001

in the placebo (P = 0.54). Other common adverse events that occurred in at least 10% were similar between the SF and the PBO groups. Similar results were seen when side effects were analyzed by means number of occurrences per rating occasion during study period (Table 5). Six participants (12%) in the SF group, whose parents administered the study drug in crushed tablets, reported "taste abnormality" for the SF tablets but did not drop out of the study due to this reason. Even for these participants the parents reported that their children could tolerate the taste of the crushed tablets and did not appear to affect the blinding. There were no metabolic side-effects (Tables S13–S16).

Discussion

This is the largest study of the effects of sulforaphane in autism and the first study in the Chinese Han population. Inclusion of children as young as 3 years allows probing of treatment effects in earlier developmental cohorts. Although there were no effects of SF vs PBO on primary outcome measure, SRS, or on other caregiver rated scales, we found statistically significant effects of SF improving ASD features on clinician rated CGI-I and OARS-4 scales, especially in the core features related to social interaction and communication deficits, with moderate to large effect sizes by week 12 of treatment. In completer analysis at week 12,

Table 5 Sulforaphane and placebo subject reported side effects during study drug administration period

Side effect	Sulforaphane (N = 50)		Placebo (N = 43)		Statistical test (sulforaphane vs placebo)	
	Percent of subjects having at least one occurrence during study	Mean number of occurrences per rating occasion during study	Percent of subjects having at least one occurrence during study	Mean number of occurrences per rating occasion during study	At least one occurrence during study drug treatment (ChiSq or FET) (P values)	Mean number of occurrences per rating session (Mann–Whitney U) (P values)
Difficulty concentrating	42.0	0.287 (0.393)	32.6	0.264 (0.421)	ChiSq = 0.349	P = 0.547
Dysphasia	30.0	0.200 (0.337)	32.6	0.279 (0.430)	ChiSq = 0.791	P = 0.501
Feeling nervous or excited	28.0	0.153 (0.295)	11.6	0.062 (0.196)	ChiSq = 0.051	P = 0.054
Difficult sitting	26.0	0.160 (0.303)	27.9	0.186 (0.336)	ChiSq = 0.836	P = 0.778
Irritable	24.0	0.173 (0.345)	20.9	0.101 (0.212)	ChiSq = 0.724	P = 0.548
Akathisia	24.0	0.147 (0.295)	25.6	0.186 (0.351)	ChiSq = 0.860	P = 0.744
Poor memory	18.0	0.140 (0.324)	11.6	0.077 (0.239)	ChiSq = 0.392	P = 0.366
Flattening of affect	18.0	0.093 (0.224)	16.3	0.132 (0.326)	ChiSq = 0.826	P = 0.977
Speak with a lisp	16.0	0.087 (0.231)	14.0	0.116 (0.308)	ChiSq = 0.783	P = 0.901
Difficulty falling asleep	14.0	0.067 (0.190)	14.0	0.077 (0.216)	ChiSq = 0.995	P = 0.964
Poor coordination	14.0	0.087 (0.241)	16.3	0.116 (0.299)	ChiSq = 0.759	P = 0.724
Hidrosis	12.0	0.060 (0.174)	4.7	0.023 (0.112)	FET = 0.279	P = 0.211
Nasal congestion	10.0	0.047 (0.151)	7.0	0.039 (0.166)	ChiSq = 0.604	P = 0.612
Constipation	10.0	0.080 (0.257)	4.7	0.039 (0.181)	FET = 0.445	P = 0.333

Mean (SD) is mean number of occurrences per rating occasion during study drug treatment. The maximum value of mean occurrence is + 1.00 which would indicate that the side effects was present in all subjects on every rating occasion. Side effects listed in table were chosen from the side-effects showing the highest occurrence during active sulforaphane treatment. There were no significant differences ($P < 0.05$) for either mean number of occurrences of side effects listed above, or the number of subjects who had at least one occurrence of a side effect. Other side effects were rare and had very low occurrence, and all statistical comparisons showed no significant difference between sulforaphane and placebo

ChiSq Chi-square test, *FET* Fisher's exact test

[a]One patient missed the assessment of adverse events

90% of SF participants showed mild improvement or better compared to 41% assigned to PBO, and 39% of participants on SF showed at least 30% improvement on impaired social interaction score on the OARS-4 compared to 11% on PBO. Nevertheless, the substantive clinical meaning of the degree of improvement on clinician rated scales needs further clarification.

Our study replicates and extends some of the positive findings of SF effects in several earlier smaller studies conducted in the United States. The original US double-blind study by Zimmerman's group (Singh et al., 2014) found significant improvement following SF treatment on the autism behaviors in teens and young adults measured on the SRS scale, Aberrant Behavior Checklist (ABC), and CGI-I improvement scales. An open label study by Hendren and associates found significant improvement on selected components of the SRS and ABC scale (Bent et al., 2018). In these two studies the percent decrease in scores in the SF treated participants on the SRS scale (8.9 to 16.2%) was similar or smaller than the percent decrease in the OARS-4 scales of SF treated participants in the current study (19.4 to 29.5%). Results from a subsequent double-blind sulforaphane study from Zimmerman's group (Zimmerman et al., 2021) showed no effect of SF vs PBO on a clinician rated scale, Ohio Autism Clinical Impression Scale (OACIS), or the caregiver rated SRS scale during the double blind portion of the study, although there was a highly significant effect of SF on improving scores with the caregiver rated Aberrant Behavior Checklist at the end of the 15 week double-bind study point, but the data they presented was analyzed only on a sub-sample of the enrolled participants. There were also some significant improvements by SF on SRS scale when data was combined from the open label extension phase.

The length and dose of studies may influence the degree of positive effects from sulforaphane. The original Zimmerman groups study found significant effects at 18 weeks and only a trend at 10 weeks. Their group's more recent

study (Zimmerman et al., 2021) found differences on some scales that only became significant at later time points after the end of the 15 week double-blind period study. The current study found the greatest effect at 12 weeks. On the basis of data from one review (Yagishita et al., 2019), it has been suggested that the maximal biological effect may be obtained with a tolerated dose of approximately 200 µmol sulforaphane/day (for 70 kg person) or 1.3 µmol/lb/day, which is somewhat higher than our dose (0.9 µmol/lb/day), or doses used in some other studies (Momtazmanesh et al., 2020; Singh et al., 2014; Zimmerman et al., 2021) of about 1 µmol/lb/day. Relatively few dose–response studies have been conducted in human subjects, so it is not possible to determine whether a dose–response plot for sulforaphane for autism will take the form a linear "S" or ∩ shape. No maximally effective dose has been established for autism. The formulation of administration of sulforaphane could also have affected results in different studies. The original Zimmerman study (Singh et al., 2014) used cryopreserved sulforaphane rich broccoli sprout extracts capsules whereas most of the other studies used including the present study used commercial Avmacol tablets supplied by Nutramax; however, we do not have sufficient data to assess the effects of differences in the form of the preparations.

Since the present study recruited both young children and adolescent participants and had larger sample size, we were able to assess the effect of age and intelligence (as measured by standard instruments) on the efficacy of sulforaphane. Overall SF showed a fairly similar effect across age groups, but for some measures, OARS-4 social responsiveness scores, there was a statistical difference between SF and PBO in the above ten age group, in preadolescent or adolescent participants with autism.

Our study found that the sub-group of participants with lower cognitive ability appeared to respond in a similar manner to SF treatment as more cognitively abled participants. Typically autistic children who have co-occurring cognitive impairment are less responsive to education and behavioral training programs, requiring substantial support (Walton & Ingersoll, 2013). Therefore, this may provide an intervention for individuals with ASD and cognitive impairment who often have more limited treatment options. At least one type of X-linked intellectual disability has been associated with impaired oxidative stress response (Bosshard et al., 2017), a deficit potentially correctable by SF since pharmacological research suggests that SF may act in part though decreasing oxidative stress and increasing acetylation of some histones (Fahey et al., 2019; Guerrero-Beltran et al., 2012; Tortorella et al., 2015).

Although in vitro, preclinical, and some clinical studies (Liu et al., 2020; Myzak et al., 2007; Sedlak et al., 2017) document that SF may act partially though its effects on improving response to oxidative stress, anti-inflammatory effects and/or and acetylation of histones, we have no direct evidence that these mechanisms were responsible for SF effects in our autism participants in the current study since we were unable to measure biomarkers related to these mechanisms. However, studies conducted by Zimmerman's colleagues on SF in autism (Liu et al., 2020; Zimmerman et al., 2021), which used doses of SF in the same range as our study, did show that sulforaphane decreased oxidative stress by reducing GSH/GSSG ratio (ratio of reduced to oxidized glutathione) compared to placebo which showed no change and deceased some inflammatory markers [IL-6 (Interleukin 6), IL-1β (Interleukin 1 beta), TNF-α (Tumor Necrosis Factor alpha)] although the relationship of these changes to the degree of clinical response was not specified.

Limitations

The primary limitation of our study is that the significantly positive results for sulforaphane were found only on our secondary and exploratory outcomes measures, OARS-4 and overall CGI-I, and not on the hypothesized a priori primary outcome on the SRS scale, which showed significant effects in the Zimmerman groups original study. The CGI is not a validated autism specific measure. Second, though the PPVT-4 is a measure of receptive vocabulary it has not been validated as a cognitive assessment or a proxy for verbal IQ (which may affect the validity of our conclusions about the degree to which surrogate IQ affects clinical response). Third, our sample were in an intensive educational training environment with a professional staff of teachers who provided input to for the clinician administered ratings. Fourth, the parents did not receive very extensive training in the SRS and related scales, so they might not have been sensitive to subtle differences. Because parents in our study did not have access to the type of observations that professional staff could make in the supportive education environment, they may not have incorporated these types of changes in their self-report scale ratings. Furthermore, recent evidence (published after the design of this study was finalized) has supported the idea that the SRS may not be an optimal assessment for tracking of changes in social and communication related ASD features as an outcome in clinical trials (Anagnostou et al., 2015). Finally, cultural differences in China, with a preference for more quiet orderliness in families, may have led some families to interpret the greater expression of communication and social relationship activation in their SF treated children as disruptive. The fact that parents rated the SAFTEE side effect of nervous or excited as present slightly more frequently in the sulforaphane

than placebo participants may be consistent with this interpretation.

There are some other potential limitations linked to study design. The exclusion of children with epilepsy and the exclusion of participants on concomitant medication may limit the generalizability of our findings. The fact that some participants withdrew before start of drug administration is not ideal and could potentially introduce bias due to different dropout rates in the SF and PBO group. However, our analysis showed there was no difference in characteristics of participants who later received SF or PBO tablets. Withdrawing participants who demonstrate poor adherence to the medication may not be optimal and is another limitation.

Conclusions

Overall, the treatment of ASD participants with sulforaphane in our study showed significant effects in improving some ASD symptoms in clinician rated scales across a range of age and cognitive status. However, the lack of improvement in parent rated symptom scales, and an a priori primary outcome measure SRS, makes the interpretation of the clinical meaning of our results ambiguous. Our results are consistent with results of several other studies which have shown positive effects of sulforaphane in ASD on a few scales but no difference on other scales. Since autism is such a devastating condition, even limited positive findings offer a glimmer of hope and justify further studies. More studies with consistent results on multiple scales are needed to verify positive effects of SF in ASD. However, we believe the state of the evidence at this time does not support a recommendation for general clinical use of SF for treatment of ASD. Complementary and alternative medicine treatments have been recommended in the past and later proven without efficacy, and some can produce harm (Levy et al., 2003; Perrin et al., 2012). Sulforaphane was safe and well-tolerated in our participants. The finding justifies further RCTs to determine whether longer treatment or higher doses of SF will show greater effects on ASD symptoms. The potential effects of sulforaphane as a preventive strategy, at an age before full ASD symptoms are manifest, should also be explored.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10803-022-05784-9.

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Author Contributions JO in conjunction with the help of RCS, HJ, and JMD designed the study. RCS and JA performed the statistical analysis. RCS, JO and RHT wrote the first draft of the manuscript with input from HJ and JMD, and JMD helped revise the final manuscript. RW helped coordinate parts of the research and its analysis JMD, JEF, BC, and HJ contributed to important revisions. All authors reviewed and asppproved the final manuscript. JZ provided overall supervision for the entire study.

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Declarations

Conflict of interest Brian Cornblatt is an employe of Nutramax Laboratories, Inc. He reviewed the manuscript as coauthor but did not have a role in interpretation of the results. After the conclusion of this trial, Dr. Fahey retired from the full-time faculty at Johns Hopkins, and now serves as a scientific advisor to Brassica Protection Products LLC (Baltimore, MD, USA), which produces TrueBroc® glucoraphanin-rich broccoli seed extract. Dr. Tobe has received research support with Roche and Janssen and served on ad boards for Roche, but these are not related to the current study. The other authors have declared that there are no conflicts of interest relevant to the study.

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