Special Article

A systematic review and meta-analysis of the benefits of a gluten-free diet and/or casein-free diet for children with autism spectrum disorder

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Context: *It has been suggested that a gluten-free and casein-free (GFCF) diet may alleviate the symptoms of autism spectrum disorder (ASD) and facilitate neurodevelopment of children with ASD. Studies to date have been inconclusive.* ***Objective:*** *This study aimed to evaluate (through quantitative meta-analysis) the efficacy and safety of a GFCF diet for children with ASD. To our knowledge, this is the first time such an analysis has been carried out.* ***Data Sources:*** *Eight electronic databases were searched, from the establishment of each database up to March 27, 2020: PubMed, Web of Science, Embase (Ovid), PsycINFO (Ovid), Cochrane Library, CNKI, Wanfang, and VIP databases.* ***Data Extraction:*** *Two authors independently performed the data extraction and risk-of-bias assessment.* ***Data Analysis:*** *A quantitative meta-analysis was performed with standard procedures by using Stata SE 15 software. Within the total of 8 studies, with 297 participants, 5 studies reported significant reductions in stereotypical behaviors [standard mean difference (SMD) = –0.41, 95% confidence interval (CI): –0.68 to –0.15], and 3 studies reported improvements in cognition (SMD = –0.46, 95% CI: –0.91 to –0.01) following GFCF dietary intervention . No statistically significant changes were observed in other symptomatic categories (all $P > 0.05$).* ***Conclusion:*** *The current meta-analysis showed that a GFCF diet can reduce stereotypical behaviors and improve the cognition of children with ASD. Though most of the included studies were single-blind, the benefits of a GFCF diet that have been indicated are promising. Additional studies on a larger scale are warranted.*
Systematic Review Registration: *PROSPERO registration no. CRD42020177619.*

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Key words: autism spectrum disorder (ASD), dietary intervention, gluten-free diet and casein-free diet (GFCF), meta-analysis.

doi: 10.1093/nutrit/nuab073

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder, characterized by impaired social cognition and communication, and stereotypical behaviors. The prevalence of ASD ranges from 0.48% in South-East France to 3.13% in Iceland.[1] Child care can bring a great challenge to families with children with ASD.

Currently, the treatments for ASD are mainly focused on educational, psychosocial interventions, with an emphasis on early and intensive treatment,[2] and pharmacologic interventions. Yet, despite intensive intervention with these therapies in children with ASD during their early developmental stages, the beneficial effects on some of the symptoms are not significant.[3] Therefore, families are seeking alternative therapies, such as dietary interventions, and a gluten-free and casein-free (GFCF) diet displays promising effects. A gluten-free diet is a diet eliminating grains that contain gluten (such as wheat, rye, barley, and possibly oats). A casein-free diet requires elimination of mammalian milk and its products, such as butter and cheese.

The effectiveness and safety of a GFCF diet for ASD remain controversial. It has been suggested that a GFCF diet is helpful in ameliorating various symptoms, including issues associated with social, cognition, communication, stereotypical behaviors, attention, and emotion.[4–6] There are several theories about the mechanism by which a GFCF diet might improve the symptoms of autism, among which the opioid excess hypothesis is the most widespread. Peptides with opioid functions derived from gluten and casein are presumed to affect the central nervous system via a "leaky" gut, whereby opioids leak through an inflamed and thinned gut lining in children with ASD.[7] These peptides with opioid activity are then thought to play an important role in aggravating autistic symptoms in the central nervous system.[8] On the other hand, a number of studies have reported no significant change in the symptoms of ASD, throwing into question the beneficial effects of a GFCF diet for ASD.[9–12] Currently, most studies on the effects of a GFCF diet in ASD have been observational clinical trials or case reports, with mixed results. In addition, some safety issues for the GFCF diet, such as nutritional deficiency, are a concern, limiting its clinical application. Therefore, it is important to thoroughly evaluate the efficacy and safety of a GFCF diet in individuals with ASD. So far, the effects of a GFCF diet on individuals with ASD have not been systematically and quantitatively analyzed. Hence, this meta-analysis was conducted to evaluate the efficacy and safety of a GFCF diet, as compared with a normal diet, for individuals with ASD.

METHODS

The protocol of the study was registered on PROSPERO (CRD42020177619). This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (PRISMA) (see Table S1 in the Supporting Information online).[13] The PICOS (Participants, Intervention/exposure, Comparison, Outcomes, Study design) criteria used to structure the research question are shown in Table 1.

Data sources and search strategy

A search was conducted in the 8 databases (PubMed, Web of Science, Embase (Ovid), PsycINFO (Ovid), Cochrane Library, CNKI, Wanfang, and VIP databases) with the following literature strategy: ("gluten free diet" OR "diet, gluten free" OR "gluten restriction diet" OR "diet, gluten restriction" OR "casein free diet" OR "diet, casein free" OR "casein restriction diet" OR "diet, casein restriction") AND ("spectrum disorders, autism" OR "autism spectrum disorders" OR "disorder, autistic" OR "autistic disorder" OR "kanner syndrome" OR "autism, infantile" OR "infantile autism" OR "autism" OR "autism, early infantile" OR "early infantile autism" OR "infantile autism, early" OR "syndrome, Asperger" OR "Asperger syndrome" OR "Asperger's disease" OR "disease, Asperger's" OR "Asperger disorder" OR "disorder, Asperger" OR "pervasive developmental disorder not otherwise specified" OR "child development disorders, pervasive"). There was no any restriction on the language. Studies published from the establishment of each database to March 27, 2020 were searched.

Inclusion and exclusion criteria

Inclusion criteria for eligible studies were as follows: (1) studies that investigated (or evaluated data on) a gluten-free diet and/or casein-free diet for autistic children; (2) randomized controlled trials with a parallel or crossover design.

Exclusion criteria were as follows: (1) abstracts only, case reports, reviews, and non-clinical studies; (2) studies with insufficient data for estimating the standard mean difference (SMD) and 95% confidence interval (CI); (3) studies reporting duplicated data or repeated analysis.

Study selection

Two autism spectrum disorder specialists independently screened titles and abstracts to identify potential articles on the basis of the inclusion criteria. The full texts of the potential articles were then read and assessed by 2 researchers to determine the studies to be

Table 1 **PICOS criteria for inclusion of studies**

Parameter	Criterion
Participants	Children with autism spectrum disorder
Intervention/exposure	Gluten-free diet and/or casein-free diet
Comparison	Normal diet or regular diet
Outcomes	11 symptomatic groups
Study design	Randomized controlled trials with a parallel or crossover design

included in the analysis. During the process, disagreements were resolved and consensus was reached by discussion. For example, the study of Hyman et al[9] had a single-subject design (ie, it was an N=1 randomized trial), but it was excluded, during discussion, because it was not a randomized controlled trial with a parallel or crossover design.

Data extraction

Data was independently extracted by 2 authors. Again, disagreements were resolved and consensus was reached through discussion. Detailed data were acquired by contacting the corresponding author, when necessary. GetData Graph Digitizer software (Version2.26.0.20) was utilized for measurements when detailed data were not available. It is a program for getting raw data out of visual graphs by setting the minimum and maximum values of x and y axes, followed by marking the points whose raw data are unknown. For studies with a crossover design, only the data from the first phase were extracted, so as to avoid a carryover effect.[14] The following items were extracted from each study: first author, year of publication, country, sample size, age, gender, diagnostic criteria utilized, interventions, follow-up, outcomes, mean and standard deviation, and side effects.

Quality assessment

The Cochrane Collaboration's risk-of-bias tool[15] was adopted for bias assessment and quality evaluation of RCTs in the following aspects: selection bias, performance bias, detection bias, attrition bias, reporting bias, and other types of bias.

Classification of outcome variables

Approximately 20 different instruments were used to assess various outcome variables in the 8 included studies. In order to perform a straightforward analysis of multiple studies with just one end point, we focused on the 3 core groups symptoms and cognition, with reference to the definition of ASD in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR).[16] The three core symptomatic groups include communication difficulties, social disorders, and stereotypical behaviors. In addition to the above symptoms quantified in the studies, gastrointestinal (GI) symptoms have been narratively but not quantifiably described.

Quantitative data synthesis

A quantitative meta-analysis was performed with standard procedures using Stata SE 15 software. The treatment effect and standard deviation were precalculated for analytical purposes. The change between pre-treatment and after-treatment was measured based on the recommended formula:

$$\text{mean (change)} = \text{mean (post)} - \text{mean (pre)}.$$

Mean (post) and mean (pre) represent the mean scores on the scales before treatment and after treatment, respectively. Standard deviation of the change [SD (change)] was estimated from the following formula:

$$\text{SD (change)} = \sqrt{\text{SD(post)}^2 + \text{SD(pre)}^2 - (2*\text{corr}*\text{SD(post)}*\text{SD(pre)})}.$$

SD (pre) and SD (post) represent the standard deviations of scores on the scales before and after treatment, respectively. The corr is the coefficient and is estimated to be 0.5, taking a conservative approach.[17] The tests or scales were transformed into a form in which lower scores indicated better performance. In the original data, in which higher scores of a scale corresponded to better outcomes, multiplication by –1 was conducted to accomplish the transformation, with the SD remaining unchanged. SMDs, using Hedges' adjusted g as generated by Stata SE 15 software, were used as estimates of the effect size of the dietary intervention. The magnitude of Hedges' g can be interpreted as small (<0.5), moderate (0.5–0.8), or large (>0.8).[18] The false discovery rate (FDR) correction by Benjamini–Hochberg was implemented for comparisons of the 3 core symptoms.

The χ^2-based Q statistic and the I^2 statistic were performed to assess the interstudy heterogeneity. A fixed-effects model was used if there was no significant heterogeneity ($P > 0.05$ for the Q test and $I^2 < 50\%$). Otherwise, a random effects model was used. A sensitivity analysis was performed to evaluate the effect of each study on the pooled results by excluding a single study sequentially. Consistent

results were assumed to indicate reliable results and vice versa. Finally, the use of the funnel plot was limited due to the small number of studies evaluated.

Meta-regression

In addition to the method previously planned, a meta-regression was performed to test the effects of potential moderators (length of intervention, sample size, mean age of the intervention group, and percentage of males in the intervention group) on individual effect sizes.

RESULTS

Study selection

Initially, 812 studies were retrieved through the search strategy, and then 58 studies were selected by screening the titles and abstracts. Of these 58 candidate trials, 34 were removed due to not being RCTs, 7 explored other interventions with or without a GFCF diet, 5 were duplicate trials, 2 were not related to ASD or included ASD comorbid with other diseases, 1 was unable to be extracted, and 1 crossover study didn't provide baseline data. Finally, a total of 8 studies qualified for inclusion in the meta-analysis. The flow chart of the screening of the studies can be seen in Fig. 1.[4,11,12,19–23]

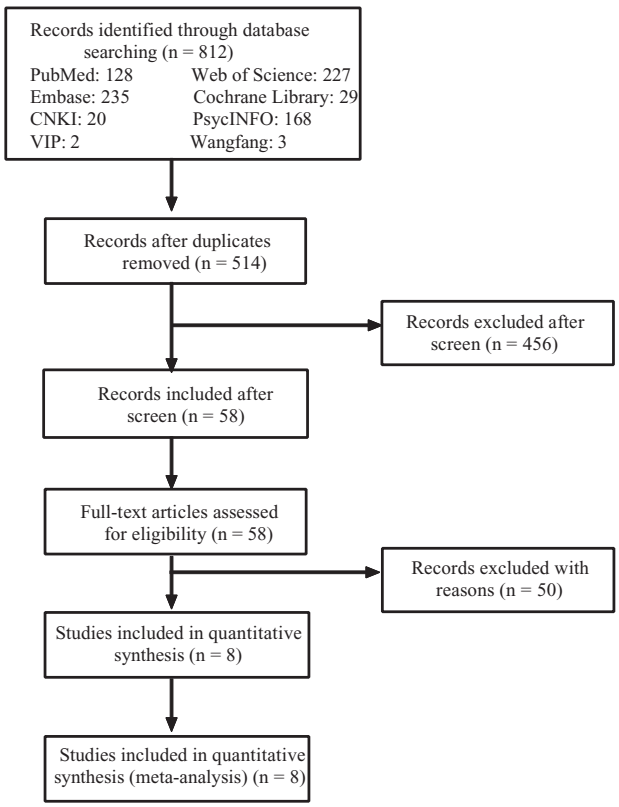

Figure 1 **The flowchart for study selection in the meta-analysis.**

Characteristics of included studies

This meta-analysis included 8 RCT studies, comprising 297 participants with ASD (whose diagnosis conformed with the International Statistical Classification of Diseases and Related Health Problems 10th Revision or DSM-IV-TR), of which 144 were in the intervention groups and 153 were in the control group. There was male predominance in these studies, which is consistent with the gender disparity in ASD incidence. Five studies investigated the effectiveness of a GFCF diet in individuals with ASD, while the other 3 studies assessed the efficacy of a gluten-free diet (GFD) for children with ASD. These trials were conducted in Iran, Norway, Poland, Spain, the USA, and Denmark, and were published between 2002 and 2019. The sample sizes of these trials ranged from 12 to 76, with durations varying from 1.5 months to 12 months. Further characteristics of the included studies are shown in Table 2.[4,11,12,19–23]

Quality assessment

In relation to selection bias, 4 studies reported the detailed methods of the random sequence generation and allocation, while the remainder simply declared that they performed random studies, lacking sufficient details to validate this. In relation to performance bias, individuals knew the interventions they were receiving in 4 studies, but there was insufficient data to assess whether subjects knew the intervention in 3 studies. Only 1 study described the blinding of child participants with respect to the intervention. None of the 8 studies showed evidence of reporting bias or attribution bias. The results of the quality assessments are shown in Supplementary Fig. 1.[4,11,12,19–23]

Symptomatic groups

Regarding the symptomatic groups, social and communication issues, and stereotypical behaviors were investigated in 5 trials and cognition in 3. The meta-analysis suggested statistically significant improvement in stereotypical behaviors (SMD = –0.41, 95% CI: –0.68 to –0.15, adjusted $P = 0.006$) and cognition (SMD = –0.46, 95% CI: –0.91 to –0.01, $P = 0.045$) following a GFCF dietary intervention (Figs. 2[4,11,19,21,23] and 3[4,11,12]).

No statistically significant changes were observed in communication or social issues (all $P > 0.05$) (Figs. 4[4,11,19,21,23] and 5[4,11,19,21,23]). The details of this data can be seen in Table S2 in the Supporting Information online.[4,11,12,19,21,23]

Regarding GI symptoms, Ghalichi et al[19] reported significant improvements in the GFD group, whereas there was no significant difference in the control

Table 2 **The characteristics of the included studies in the meta-analysis**

Study (year)	Knivsberg et al (2002)[4]	Piwowarczyk et al (2019)[11]	Navarro et al (2015)[12]	Ghalichi et al (2016)[19]	Gonzalez Domenech et al (2019)[a20]	Johnson et al (2011)[21]	Gonzalez Domenech et al (2019)[a22]	Whiteley et al (2010)*[23]
Country	Norway	Poland	USA	Iran	Spain	USA	Spain	Denmark
Diagnosis	ASD	ASD	ASD	ASD	ASD	ASD	ASD	ASD
Intervention	GFCF	GFD	GFD	GFD	GFCF	GFCF	GFCF	GFCF
Sample size, intervention group	10	28	6	38	13	8	15	26
Sample size, control group	10	30	6	38	12	14	14	29
Duration, months	12	6	1	1.5	3	3	6	8–12
Mean age (years), intervention group	7.6	3.8	5.5	7.8	8	3.3	8.8	8.22
Mean age (years), control group	7.2	3.8	6	8	8.3	3.3	9.1	8.13
Male, %, intervention group	NA	94	NA	74	75	88	65	NA
Male, %, control group	NA	76	NA	74	92	79	94	NA

[a]Crossover study. *Abbreviations*: GFCF, gluten-free and casein-free diet; GFD, gluten-free diet; ASD, autism spectrum disorder; NA, not available.

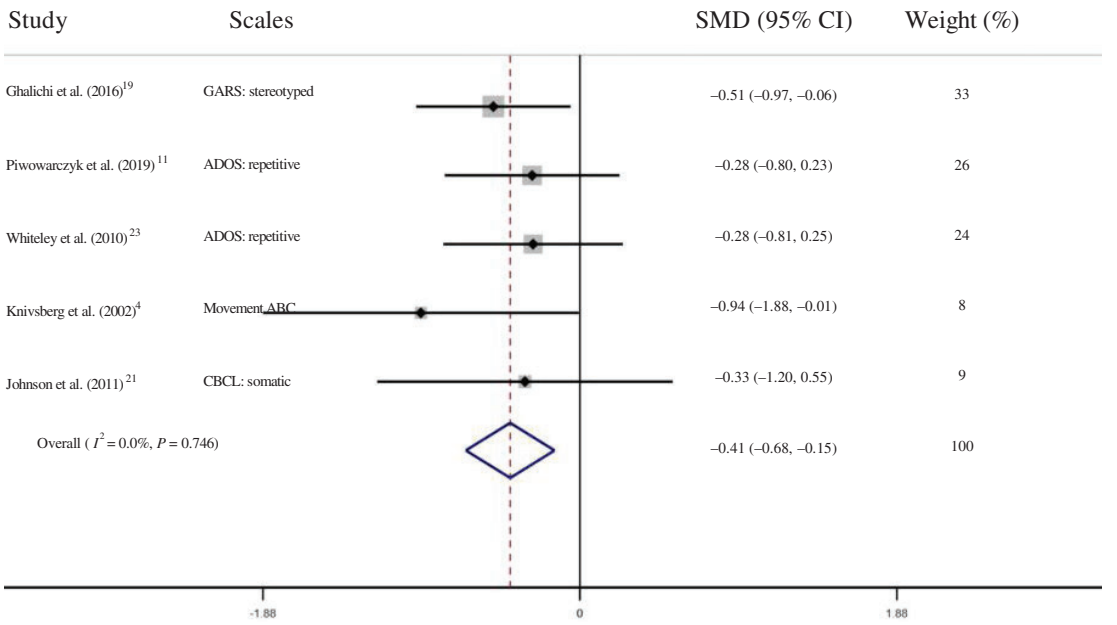

Figure 2 **Meta-analysis results and scales for stereotypical behaviors.**

Abbreviations: ADOS, Autism Diagnostic Observation Scale; CBCL, Child Behavior Checklist; CI, confidence interval; GARS, Gilliam Autism Rating Scale; Movement ABC, Movement Assessment Battery for Children; SMD, standard mean difference.

group, based on the ROME III questionnaire, which is a valuable tool for assessment of GI symptoms . Piwowarczyk et al[11] revealed no significant change in abdominal pain or constipation during follow-up. In addition to the statistical comparison, there was a narrative description. The GI symptoms before and after the intervention in the 2 groups were only narratively reported by Navarro et al,[12] without statistical analysis.

Safety issues

Only 4 studies analysed safety issues into consideration during the intervention, and it was consistently reported that the intervention group did not differ from the control group in terms of side effects.[11,21–23] Johnson et al[21] reported that, in the GFCF diet group, 2 subjects complained of irregular bowel movements, 1 had stomach aches, 1 reported nausea/vomiting, 1 had night waking, and 1 reported decreased appetite. However, similar complaints were reported in the control group. No significant difference ($\chi^2 = 2.064$; $P = 0.151$) was observed between the 2 groups. Piwowarczyk et al[11] reported 1 case of cerebral ataxia in the GFD group, and 1 of celiac disease and 1 of epilepsy in the control group. No significant difference was found between the 2 groups. González-Domenech et al[22] suggested that changes in autoimmunity; the concentrations of calcium, vitamin D, ferritin, folic acid, and hematocrit; weight and height; the history of

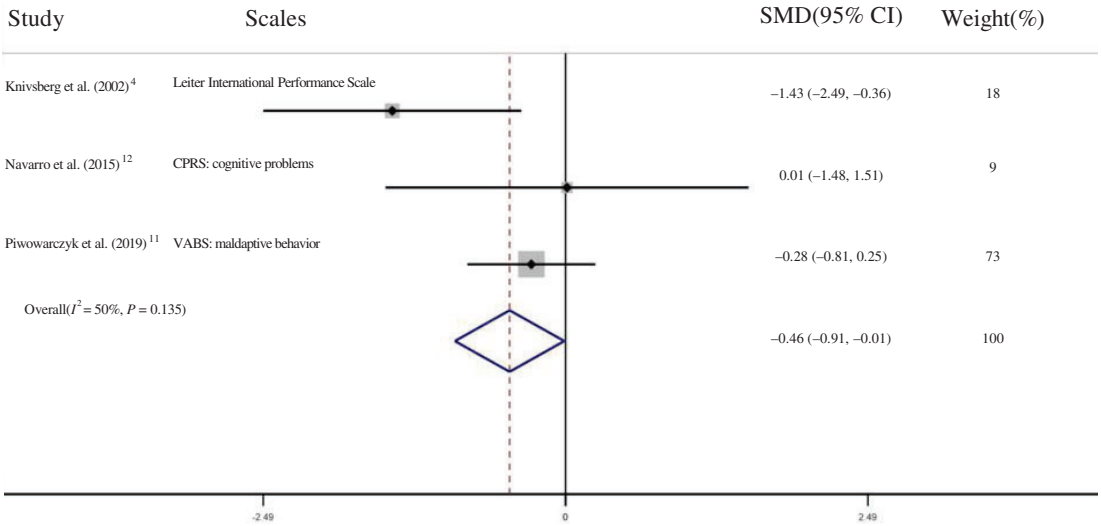

Figure 3 **Meta-analysis results and scales for cognition.**

Abbreviations: CI, confidence interval; CPRS, Children's Psychiatric Rating Scale; SMD, standard mean difference; VABS, Vineland Adaptive Behavior Scale.

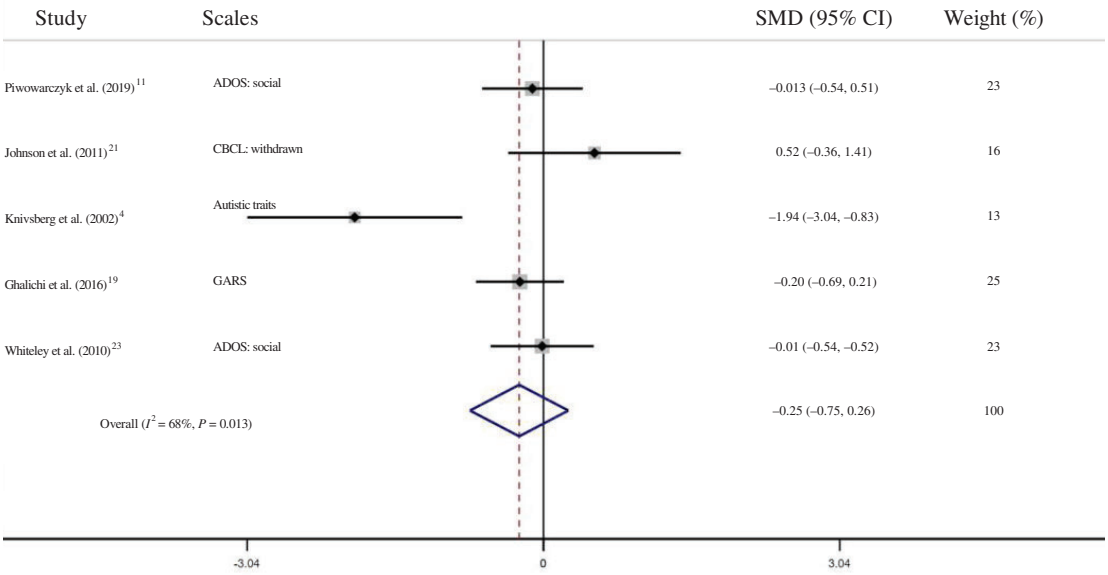

Figure 4 **Meta-analysis results and scales for social behaviors .**

Abbreviations: ADOS, Autism Diagnostic Observation Scale; CBCL, Child Behavior Checklist; CI, confidence interval; GARS, Gilliam Autism Rating Scale; SMD, standard mean difference.

GI; and eating disorders were comparable following interventions between the GFCF group and the control group. There were no adverse effects observed in the Whiteley et al[23] study.

Sensitivity analysis

A sensitivity analysis was performed to assess the results of the meta-analysis for cognition, social and communication issues, and stereotypical behaviors. The effect of each study on the pooled results was evaluated by excluding a single study sequentially. The results of the sensitivity analysis showed stability of the results for stereotypical behaviors, which validated the rationality and reliability of the analysis. Notably, a single study performed by Knivsberg et al[4] may have influenced the cognition results of the meta-analysis. For further details of the outcomes of the sensitivity analysis, see Supplementary Fig. 2.[4,11,12,19,21,23]

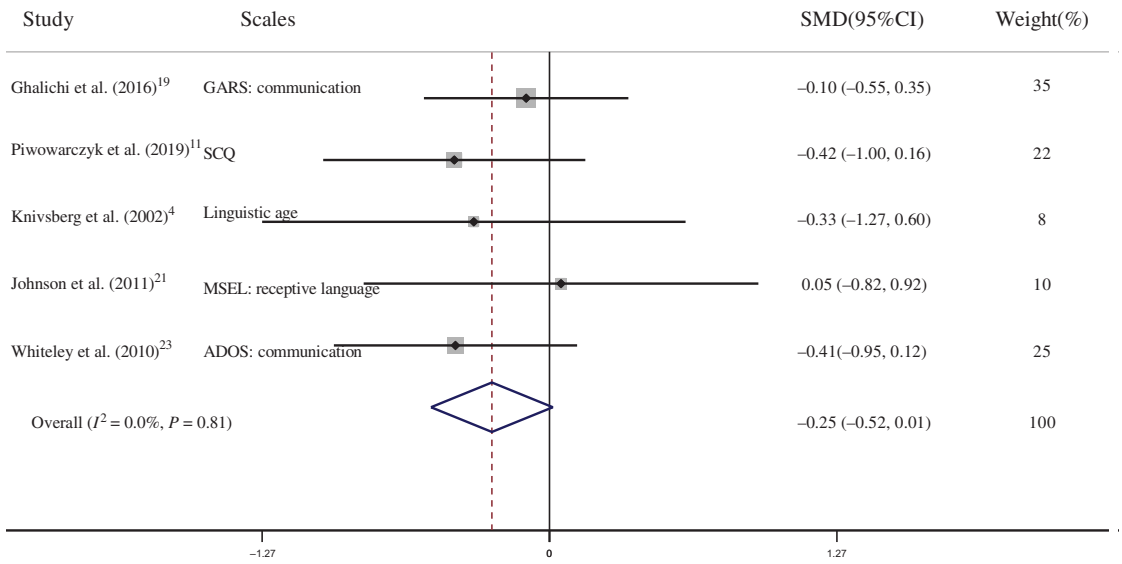

Figure 5 **Meta-analysis results and scales for communication.**

Abbreviations: ADOS, Autism Diagnostic Observation Scale; CI, confidence interval; GARS, Gilliam Autism Rating Scale; MSEL, Mullen Scales of Early Learning; SCQ, Social Communication Questionnaire; SMD, standard mean difference.

Meta-regression analyses

Meta-regression analyses revealed that none of the putative moderators (length of intervention, sample size, mean age of the intervention group, or percentage of males in the intervention group) had a significant effect on the individual effect size.

DISCUSSION

Eight RCT studies with 297 subjects were included in the meta-analysis. The results of the meta-analysis indicated benefits of the GFCF dietary intervention in terms of stereotypical behaviors and cognition. All studies reporting safety issues agreed that the GFCF diet did not increase the risk of safety concerns compared with the regular diet.

The nature of the pathology in ASD remains controversial. One possible mechanism for the effect of dietary interventions in ASD is the opioid excess theory. In this theory, milk proteins and gluten/gliadin epitopes reach the mucosa and stimulate the underlying immune system, leading to the release of cytokines and inflammatory mediators, which further enhance the degradation of the epithelial barrier.[24] On the other hand, they also cause a shift in the composition of the gut microbiota, resulting in an increased abundance of Bacteroidetes, *Clostridium*, *Caloramator*, *Sarcina*, *Desulfovibrio*, and *Lactobacillus*, and a decreased abundance of *Haemophilus parainfluenzae* and *Bifidobacterium*.[25,26] Changed gut microbiota influence the integrity of the intestinal barrier by regulating the expression and distribution of tight-junction proteins.[27] As a consequence, milk proteins and gluten/gliadin enter the blood stream via the permeable gut, then cross over the blood–brain barrier and finally reach the central nervous system.[28] The hydrolytic digestion of casein and gliadin releases peptides with opioid activity, which are associated with changes in the intracellular antioxidant glutathione and the methyl donor S-adenosylmethionine in neurons, thus aggravating autistic symptoms.[8] In addition, gluten and casein are common allergens, and can lead to inflammatory reactions and the production of specific IgA and IgG antibodies to gluten and casein.[29] Another theory is that exposure to the soluble folate receptor in milk, which has structural homology with the human folate receptor, elicits a cross-reactive immune response and facilitates the production of folate receptor autoantibodies. These autoantibodies can block folate transport across the blood–brain barrier, resulting in a decreased concentration of 5-methyltetrahydro-folate and folate in the cerebrospinal fluid, which may increase the risk of autism.[30–32] In addition, ileal transcripts encoding disaccharidases and hexose transporters (which are important factors necessary for carbohydrate digestion and transport in enterocytes) were found to be deficient in children with autism, resulting in problems in digesting lactose in milk.[33]

The results of the meta-analysis support previous studies, exploring the schizophrenia and other psychiatric disorders, with sufficient evidence-based power. Studies of patients with schizophrenia and other psychiatric disorders on a GFCF diet similarly suggested that

a GFCF diet had a favorable impact on symptoms.[34] Previous studies have observed remarkable statistical improvements in various behaviors in ASD children,[35,36] whereas some other studies have suggested that there was no significant difference between the diet group and control group or between pre-diet and after-diet in individuals with ASD.[9,10] The result of the current meta-analysis also revealed a narrative description of reduction of GI symptoms. Pennesi et al[37] showed that a GFCF diet was associated with a significant improvement in ASD behaviors, in physiological symptoms in the subgroup with GI symptoms (especially constipation and diarrhea), and in the subgroup with allergy symptoms, and that the benefits were more distinct in those who stuck to a strict GFCF diet for more than 6 months. Coincidentally, Mulloy et al[38] also recommended GFCF diets should be used when behavioral changes appear to be associated with dietary changes and/or in the presence of a confirmed allergy to gluten and/or casein. Nonetheless, most current systematic reviews claim that there is insufficient evidence to support a GFCF diet as a treatment for ASD and that further studies with large samples and/or a double-blind design are needed.[9,38–43]

The quality and risk of bias of the included studies varied, and performance bias was the primary source of bias. Only 1 of the 8 included studies stated that the child participants were blinded with respect to the dietary intervention, 3 were unclear about the blinding, and 4 did not have blinding (because of the particularity of the dietary intervention, which was a long-term intervention and unlikely to be implemented in hospitals). In most studies, family members of the children received dietary intervention training and guidance by telephone or follow-up and implemented it at home. The children actually knew little about the dietary intervention they received due to the impaired cognition. Therefore, the high risk of performance bias would have had little influence on the quality of the included studies.

The question of whether the GFCF diet brings a risk of nutritional deficiencies to children with ASD has been raised, especially with respect to calcium deficiency related to the exclusion of dairy products, which then may lead to a reduction in bone cortical thickness.[44,45] A few researchers have carried out a series of studies on this issue. Some have reported there is no difference in adverse effects and nutritional deficiency between the GFCF group and the control group,[9] which is consistent with the results of the current meta-analysis.

Regarding the sensitivity analysis for cognition, a single study performed by Knivsberg et al[4] may influence the current meta-analysis results for cognition. Navarro et al[12] and Piwowarczyk et al[11] focused on the effectiveness of the GFD in children with ASD, whereas Knivsberg et al[4] explored the effect of the GFCF diet in individuals with ASD. The sensitivity analysis showed that the GFD was not significantly associated with improvement in cognition, which is consistent with the study of Mageshwari et al[46]

The effect sizes were relatively small, but were comparable with those for other treatments, such as using behavioral interventions to improve language, adaption (the ability of changing to fit some purpose or situation), and IQ in ASD.[47] The current psychological intervention for attention deficit hyperactivity disorder, as recommended in the guideline, also has little effect on the symptoms, with the SMD < 0.5.[48,49] Our results suggest that the GFCF diet exerts a specific effect in ASD, which though small is beneficial. This is consistent with the reported clinical efficacy of dietary supplementation in ASD, with similar effect size.[17] Thus, for ASD, which has no unified therapy, GFCF dietary therapy could be a beneficial supplementary treatment for some people with ASD.

Strength and limitations

The strength of this study is that it is the first study to quantitatively and systematically analyze the effectiveness of a GFCF diet for individuals with ASD. As commonly occurs in a meta-analysis, there are limitations. First, although the results of meta-regression showed that none of the putative moderators had a significant effect on the individual effect size, there were great disparities in the study designs, participants, trial durations, measurement techniques, etc., resulting in large heterogeneity among the included studies. Second, to unify the efficacy evaluation, the variables were classified into symptomatic groups, which caused the loss of some data. Third, the sample sizes of the included RCTs were relatively small, increasing the possibility of a type 2 error. Fourth, although the included studies were all RCTs, which have the strongest evidence power, there were several variables that may have affected the results, such as the adherence to the dietary intervention, and other treatments in addition to the dietary intervention (eg, behavioral interventions, drug treatments, education, etc.).

In future, more sensitive measurement tools should be developed to detect the subtle changes that may be observed during interventions in children with ASD. Double-blind, randomized controlled studies equipped with compliance testing to ensure adherence to the dietary intervention, and controlled combined therapies are needed. Most of the current studies lasted less than 4 months, so clinical trials with a longer duration

should be launched for evaluating longer-term effects. Finally, as shown in some trials, the GFCF diet seemed to play a greater role in children with ASD with GI symptoms than in those without GI symptoms. Therefore, it is important that larger sample sizes are studied in order to identify whether specific subgroups of individuals with ASD could benefit more from the GFCF diet.

CONCLUSION

The current meta-analysis showed that a GFCF diet can reduce the stereotypical behaviors and improve the cognition of children with ASD. Although there is no unified agreement regarding a mechanism to date, the current data concerning the benefits of a GFCF diet are promising. Studies with larger sample sizes, multicenter involvement, a double-blind design, and more sensitive measurements should be carried out in the future to validate the above conclusions.

Acknowledgments

Author contributions. X.Y., R.L.H., D.O., L.Z., and L.Q. contributed to the study design, provided the methodology for the study, and edited and revised the manuscript. L.Q. and X.X. assessed and analyzed the records, and contributed to drafting of the manuscript. H.H. and Y.C. searched the databases, extracted the data and contributed to drafting of the manuscript.

Funding. This work was supported by the Autism Special Fund of the Peking Union Medical Foundation, the CAMS Innovation Fund for Medical Science (CIFMS) (2017I2M–3–017), and the Non–profit Central Research Institute Fund of the Chinese Academy of Medical Sciences (2019XK320030). The funders had no role in study design, data collection, or preparation of the manuscript.

Declaration of interest. The authors have no relevant interests to declare.

Supporting Information

The following Supporting Information is available through the online version of this article at the publisher's website.

Table S1 **PRISMA 2009 checklist**

Table S2 **The meta-analysis of the included studies in social, communication, stereotypical behaviors, and cognition**

Figure S1 **Risk of bias in the included RCT studies in the meta-analysis**

Figure S2 **The results of the sensitivity analysis in the meta-analysis**

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