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Horizons of autism spectrum disorder and attention deficit hyperactivity disorder in clinical practice

Edited by
Tingyu Li, Fei Li and Yiping Shen

Published in
Frontiers in Psychiatry
Frontiers in Pediatrics

ISSN 1664-8714
ISBN 978-2-8325-2203-5
DOI 10.3389/978-2-8325-2203-5

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Horizons of autism spectrum disorder and attention deficit hyperactivity disorder in clinical practice

Topic editors

Tingyu Li — Chongqing Medical University, China

Fei Li — Shanghai Jiao Tong University, China

Yiping Shen — Harvard Medical School, United States

Citation

Li, T., Li, F., Shen, Y., eds. (2023). *Horizons of autism spectrum disorder and attention deficit hyperactivity disorder in clinical practice*.

Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-2203-5

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frontiers | Frontiers in Psychiatry

TYPE Editorial
PUBLISHED 04 April 2023
DOI 10.3389/fpsyt.2023.1168785

OPEN ACCESS

EDITED AND REVIEWED BY
Cesar A. Soutullo,
University of Texas Health Science Center at Houston, United States

*CORRESPONDENCE
Tingyu Li
✉ tyli@vip.sina.com

SPECIALTY SECTION
This article was submitted to ADHD, a section of the journal Frontiers in Psychiatry

RECEIVED 18 February 2023
ACCEPTED 21 March 2023
PUBLISHED 04 April 2023

CITATION
Li F, Shen Y and Li T (2023) Editorial: Horizons of autism spectrum disorder and attention deficit hyperactivity disorder in clinical practice. *Front. Psychiatry* 14:1168785. doi: 10.3389/fpsyt.2023.1168785

Editorial: Horizons of autism spectrum disorder and attention deficit hyperactivity disorder in clinical practice

Fei Li[1], Yiping Shen[2,3] and Tingyu Li[4]*

[1]Developmental and Behavioral Pediatric Department and Child Primary Care Department, Brain and Behavioral Research Unit of Shanghai Institute for Pediatric Research, MOE- Shanghai Key Laboratory for Children's Environmental Health, Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China, [2]Division of Genetics and Genomics, Boston Children's Hospital, Boston, MA, United States, [3]Department of Neurology, Harvard Medical School, Boston, MA, United States, [4]Ministry of Education Key Laboratory of Child Development and Disorders, Chongqing Key Laboratory of Childhood Nutrition and Health, National Clinical Research Center for Child Health and Disorders, Children Nutrition Research Center, Children's Hospital of Chongqing Medical University, Chongqing, China

KEYWORDS

autism spectrum disorder, attention deficit hyperactivity disorder, risk factor, management, clinical practice

Editorial on the Research Topic
Horizons of autism spectrum disorder and attention deficit hyperactivity disorder in clinical practice

Introduction

Autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD) are two types of developmental disorders that have gained significant attention in the field of developmental and behavioral pediatrics. However, in clinical practice, the etiology of these disorders is under investigation and their management is still challenging. The current topic, therefore, aims to focus on the horizons of the two developmental disorders in clinical practice (1, 2). So far, nine papers have been published on this topic, of which seven are mostly about ASD studies.

Early identification and screening for ASD

Early identification and screening for ASD are crucial for improving the prognosis of autistic children through evidence-based early intervention (3). The early clinical symptoms of ASD can be typically manifested as dysfunctions in eye contact, pointing by forefinger, response to name calling, communication, and inappropriate object use or abnormal sensory or perceptual in toddlers. However, most children (≥80%) who are diagnosed with ASD after a comprehensive evaluation at <3 years have retained their diagnosis (4). According to Wang T. et al., children with ASD are introduced to complementary foods later in their early developmental stage, which is associated with later feeding problems. Compared to typically developing controls, children with ASD also experience more feeding problems that can be

linked to core symptoms of the disorder. Pediatricians should therefore advise parents to watch for difficulties in adjusting to the introduction of complementary foods, as this may indicate a higher risk of feeding problems later in life and atypical social interaction and communication development. Chen et al. have also found that the communication warning behavior sub-scale of the children neuropsychological and behavioral scale-revision 2016, a commonly used developmental assessment tool for children aged 0–6 years in China, can be used to screen for ASD. A communication warning behavior score that is 12 points or more above the norm requires further attention and comprehensive diagnostic evaluation to achieve early detection and diagnosis of ASD in children.

Possible biomarker for ASD

ASD diagnosis has been made according to ASD criteria of DSM-5, which are mainly describing developmental milestones in areas of social interaction, communication, restricted interest, and stereotyped behaviors. Biomarkers for ASD diagnosis are desirable and are the topic for extensively research. Many ASD-related genes are found in subjects with ASD, especially for syndromic autism (5). In their research, Zhang et al. have observed a significant increase in sialidase NEU1 mRNA levels in children with autism, and have also found a correlation between increased NEU1 expression and social dysfunction. These findings suggest the need for further investigation into the relationship between NEU1 and ASD. Maternal low vitamin D status is regarded as a possible risk factor for ASD in offspring. Serum hypo vitamin D is common and has been recommended as a possible biomarker for ASD diagnosis (6). Several studies have reported that vitamin D supplementation is useful in improving the autistic symptoms. Shan et al. have discovered that the vitamin D levels in children with ASD are linked to electronic screen time, age, and duration of exposure to sunlight. These findings suggest that regulating screen time could be an alternative approach to managing the vitamin D nutritional status of children with ASD.

Effect of the Early Start Denver Model (ESDM) on children with ASD is related to different traditional Chinese medicine type

Early intervention of ASD has a significant effect on the rehabilitation of ASD. Although the effectiveness of the Early Start Denver Model (ESDM) for treating ASD has been established, there remains significant variability in treatment response among individuals. As per a prior study, a collection of social cognitive skills such as receptive and expressive language, intention to communicate, and attention to faces, have consistently been associated with response to ESDM (7). It is rare to explore the application of Chinese traditional medicine in the treatment of Children with ASD. In their research, Wang L. et al. examined the relationship between three different types of children with ASD, as classified by traditional Chinese medicine (kidney jing deficiency, liver qi stagnation, and deficiency of both the heart and spleen), and the effectiveness of the Early Start Denver Model (ESDM) treatment. The authors discovered that ESDM was effective in treating all three types of children with ASD, with the group experiencing liver qi stagnation exhibiting the most notable improvements. A multi-center prospective investigation of outcome of behavioral intervention on the different traditional Chinese medicine types of ASD may be useful to give strong evidence to predict the effect of the behavioral intervention.

Screen time in early life is a possible environmental risk factor for ADHD

The etiology of ADHD is still uncertain now, however, genetic conditions and many environmental factors are involved in the pathogenic process. Excessive screen exposure time is adverse to children's health (8). According to the research conducted by Wu et al., there could be a link between early exposure to screens and hyperactive behaviors in children. The study revealed that more than 90 minutes of screen time per day in children under the age of 3 was associated with hyperactive behaviors. These findings suggest that restricting screen time in toddlers may prove advantageous in preventing ADHD.

Nonpharmacological intervention for ADHD management

Mainstreams of ADHD management include medication and non-medication therapy. Medications including stimulants (methylphenidate, amphetamine, etc.) and non-stimulants (atomoxetine, clonidine, etc.) are usually used in ADHD children over 6 years (9). However, in clinic many ADHD children over 6 years managed under medication or not, and most of ADHD children under 6 years old still need non-medication management.

In their study, Chu et al. investigated the impact of combining group executive functioning and online parent training on school-aged children (between 6 to 8 years old) diagnosed with ADHD. Their findings suggest that this combination approach holds potential as a non-pharmaceutical therapeutic option for younger students with ADHD. Wang Y-c et al. have designed the scheme that effects of high-definition transcranial direct current stimulation (HD-tDCS) on the right orbital frontal cortex in the treatment of ADHD. This study draws cautious conclusions that HD-tDCS leads to significant improvements in cognitive measures of attention maintaining.

Conclusions

The current work explores the basic practice in clinics for ASD and ADHD. These results support that early behavioral study for recognition, diagnosis and early intervention for ASD are crucial to improve the clinical symptoms, limiting screening time in toddlers may be beneficial for ADHD prevention in toddlers and nutritional vitamin D status in children with ASD, and non-medication therapy is still important in clinical practice. More studies based on

clinical practice would be valuable in guiding treatment decisions and improving the prognosis of ASD and ADHD.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Funding

This work was supported by the National Natural Science Foundation of China (Nos. 81771223 and 81770526).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers in Pediatrics

ORIGINAL RESEARCH
published: 13 January 2022
doi: 10.3389/fped.2021.806981

Screen Time, Age and Sunshine Duration Rather Than Outdoor Activity Time Are Related to Nutritional Vitamin D Status in Children With ASD

*Ling Shan†, Hanyu Dong†, Tiantian Wang, Junyan Feng and Feiyong Jia**

Department of Developmental and Behavioral Pediatrics, The First Hospital of Jilin University, Changchun, China

Objective: This study aimed to investigate the possible association among vitamin D, screen time and other factors that might affect the concentration of vitamin D in children with autism spectrum disorder (ASD).

Methods: In total, 306 children with ASD were recruited, and data, including their age, sex, height, weight, screen time, time of outdoor activity, ASD symptoms [including Autism Behavior Checklist (ABC), Childhood Autism Rating Scale (CARS) and Autism Diagnostic Observation Schedule–Second Edition (ADOS-2)] and vitamin D concentrations, were collected. A multiple linear regression model was used to analyze the factors related to the vitamin D concentration.

Results: A multiple linear regression analysis showed that screen time ($\beta = -0.122$, $P = 0.032$), age ($\beta = -0.233$, $P < 0.001$), and blood collection month (reflecting sunshine duration) ($\beta = 0.177$, $P = 0.004$) were statistically significant. The vitamin D concentration in the children with ASD was negatively correlated with screen time and age and positively correlated with sunshine duration.

Conclusion: The vitamin D levels in children with ASD are related to electronic screen time, age and sunshine duration. Since age and season are uncontrollable, identifying the length of screen time in children with ASD could provide a basis for the clinical management of their vitamin D nutritional status.

Keywords: autism spectrum disorder, sedentary behavior, multiple linear regression, environmental factor, 25(OH)D

OPEN ACCESS

Edited by:
Tingyu Li,
Chongqing Medical University, China

Reviewed by:
Khaled Saad,
Assiut University Hospital, Egypt
Jorge Pérez-Gómez,
University of Extremadura, Spain

****Correspondence:***
Feiyong Jia
jiafy@jlu.edu.cn

†These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Child and Adolescent Psychiatry, a section of the journal Frontiers in Pediatrics

Received: *01 November 2021*
Accepted: *21 December 2021*
Published: *13 January 2022*

Citation:
Shan L, Dong H, Wang T, Feng J and Jia F (2022) Screen Time, Age and Sunshine Duration Rather Than Outdoor Activity Time Are Related to Nutritional Vitamin D Status in Children With ASD. Front. Pediatr. 9:806981. doi: 10.3389/fped.2021.806981

INTRODUCTION

Autism spectrum disorder (ASD) is characterized in the Diagnostic and Statistical Manual of Mental Disorders-fifth edition (DSM-5) by persistent deficits in social interaction and communication and stereotyped or repetitive patterns of behavior, interests or activities (1). The latest ASD prevalence (2) reported by the U.S. Centers for Disease Control and Prevention (CDC) in 2020 was one in 54 children at the age of 8 years. Currently, ASD is a relatively common neurodevelopmental disorder in children that has a serious impact on children's social adaptability. Unfortunately, the etiology of ASD is unclear. Recent research (3, 4) has shown that ASD is the

result of a combination of genetic and environmental factors. Environmental factors could include nutritional factors, heavy metal exposure, air pollution, socioeconomic factors (including lifestyles), etc. Vitamin D might be an environmental factor involved in ASD (5, 6), and screen time has been proven to influence childhood development and social behaviors (7, 8).

Previous studies (9–11) have shown that the vitamin D levels in children with ASD are lower than those in typically developing children. Furthermore, there are negative correlations between the vitamin D levels and core symptoms of ASD (12). Moreover, vitamin D supplementation might improve the core symptoms of ASD (5, 11, 13). Skin under sun irradiation is a major source of vitamin D *in vivo*. Other factors (14) also affect the vitamin D concentrations, including genetic polymorphisms, age, geographical location and latitude, lifestyle (exposure behavior and culture), UVB dose, clothing and body surface area (BSA) exposure.

A sedentary lifestyle (15, 16) is an important cause of an insufficient vitamin D status. Zittermann (15) reported that adult male subjects with low levels of physical activity have lower blood vitamin D concentrations. Solis-Urra's study (16) showed that greater sedentary time is associated with vitamin D deficiency in adult and older women. Some studies have distinguished among various types of sedentary behavior (17, 18). Social activities, such as talking or hanging around, reading and playing musical instruments, are regarded as nonscreen-based sedentary behavior, whereas watching television (TV) and videos and playing traditional video games are regarded as screen-based sedentary behavior. Therefore, the use of electronic devices is an important aspect of sedentary behavior. Children with ASD could have longer screen times (19, 20). The latest World Health Organization (WHO) Guidelines (21) on Physical Activity and Sedentary Behavior released in 2020 suggested that children and adolescents should limit the amount of time spent being sedentary, particularly the amount of recreational screen time.

There have been limited studies concerning vitamin D levels and children's screen time. Soden and coworkers (22) showed that ∼54% of ASD children had insufficient serum 25-hydroxyvitamin D levels, and the mean electronic media use was 251 min/day; however, these authors did not consider the association between these two factors. Absoud's study (23) showed that vitamin D deficiency occurs in children (not ASD children) who exercised less outdoors, watched more TV, and were overweight. To date, no studies considered the relationship between the vitamin D levels and electronic screen time of ASD children. Based on the above studies, we hypothesize that the excessive screen time of children with ASD could be related to insufficient vitamin D concentrations due to decreased sun exposure because of less outdoor activity.

Our team conducted several preliminary studies investigating the relationship among ASD, vitamin D (5, 9, 12) and the screen activities of children with ASD (7, 8). Based on previous research, we conducted this study to explore the associations among vitamin D, screen time and other factors that can affect the concentration of vitamin D in children with ASD, such as age, sunshine duration, Body Mass Index (BMI), and outdoor activity. This study aimed to further reveal the environmental factors of ASD and provide evidence for the clinical management of vitamin D levels in children with ASD.

METHODS

Participants

In total, 306 children diagnosed with ASD for the first time in the Department of Developmental and Behavioral Pediatrics of the First Hospital of Jilin University were recruited for this study. Recruitment started in March 2021 and was completed in August 2021. Inclusion criteria are as following. All children were from northeastern China (38°N−53°N) with an age under 7 years-old. The DSM-5 and Autism Diagnostic Observation Schedule–Second Edition (ADOS-2) were utilized for the diagnosis of ASD. The participants were diagnosed for the first time and without systematic intervention. The Autism Behavior Checklist (ABC) and Childhood Autism Rating Scale (CARS) were also used to evaluate the symptoms of ASD to assist in the diagnosis of ASD. Exclusion criteria are: children with severe physical disabilities, uncontrolled epilepsy, vitamin D supplementation for the past 3 months, and clear metabolic diseases or genetic diseases. The study was approved by the ethics committee of our hospital, and informed consent was provided by the parents or caregivers of the children.

Procedures

We investigated the children's characteristics (age, sex, height, and weight), ASD symptoms, outdoor activity time, screen time and serum concentration of vitamin D. Height and weight were measured by physicians in the clinic. The parents provided the children's other basic characteristics and mean outdoor activity time per day when visiting the evaluator. The children's ASD symptoms were examined using the ABC and CARS. The ABC is a 57-item screening checklist for autistic symptoms containing five subscales (body behavior, sensory, self-care, language and social interaction). This scale is designed for parent interviews. The CARS consists of 15 subscales, each of which is scored on a continuum from normal to severely abnormal. The CARS requires observation of the behavior of ASD children in a consulting room. The CARS was evaluated by experienced evaluators from our department. The evaluator also collected the screen time per day on weekdays and weekends and calculated and recorded the average daily screen time as follows: average daily screen time (hours)= [screen time per day on weekdays (min)*5+screen time per day on weekends (min)*2]/7/60. The ADOS-2 was utilized in this study as a diagnostic tool for ASD. The ADOS-2 (24) is a semistructured, standardized assessment tool for individuals with suspected ASD and measures autism symptoms in the domains of social relatedness, communication, play, and repetitive behaviors; the ADOS-2 is considered the gold standard for ASD diagnostic evaluation. We also tested the serum vitamin D concentration of the children with ASD. 25-Hydroxyvitamin D (25(OH)D) is the main circulating form of vitamin D. Therefore, we measured the concentration of 25(OH)D to reflect the nutritional status of vitamin D in the children with ASD. All samples were tested by Guangzhou KingMed Diagnostics Group Co., Ltd. (KingMed Diagnostics,

TABLE 1 | Patient characteristics.

	***N* = 306**
Age (M ± SD) (years)	3.39 ± 1.07
Vitamin D (M ± SD) (ng/ml)	25.26 ± 9.29
Screen time (M ± SD) (hours)	2.12 ± 2.14
ABC[a] score (M ± SD)	53.31 ± 16.31
CARS[b] score (M ± SD)	33.92 ± 4.36

[a]*ABC, Autism Behavior Checklist.*
[b]*CARS, Childhood Autism Rating Scale.*

SSE 603882) using the liquid chromatography tandem mass spectrometry method.

Statistical Analysis

We used Statistical Product and Service Solutions (SPSS) software version 23.0 (SPSS for Windows, SPSS Inc., Chicago, IL, USA) to analyze all data. The continuous variables with normal distributions are represented as the means ± standard deviations (SDs), and the categorical variables are represented as frequencies (percentages). The continuous variables with normal distributions were compared by Student's *t*-test or ANOVA. The correlations among the serum concentration of vitamin D, age, and screen time were detected by a Pearson's correlation test. A multiple linear regression model was used to analyze the factors related to the vitamin D concentrations. The results were considered significant at $P < 0.05$.

RESULTS

The clinical and sociodemographic characteristics are presented in **Table 1**. There were 233 boys and 73 girls among the children with ASD (76.14 vs. 23.86%). Their age ranged from 1.7 to 7 years old (3.39 ± 1.07 y). Their mean screen time was 2.12 ± 2.14 h per day. The mean concentration of serum 25(OH)D was 25.26 ± 9.29 ng/ml. We grouped the ASD children according to sex, BMI, time of outdoor activities and blood collection month (reflecting the sunshine duration) and compared the vitamin D concentrations among the groups (**Table 2**). The vitamin D concentration was not statistically significant in the comparison between the male and female groups ($t = -0.537$, $P = 0.591$). We calculated the BMI of all enrolled children (kg/m^2). According to their BMI (25), the children with ASD were divided into normal or underweight, overweight and obese groups. The comparison of the vitamin D levels among the groups was not statistically significant ($F = 1.441$, $P = 0.239$). However, as the BMI increased, the vitamin D levels tended to decrease (**Table 2**). According to the time of outdoor activities per day, we divided the children into four groups (<30 min, ≥30 and <60 min, ≥60 and <90 min, ≥90 min). The comparison of the vitamin D levels among the groups was not statistically significant ($F = 1.193$, $P = 0.313$). However, as the outdoor activity time increased, the vitamin D levels tended to increase (**Table 2**). According to the blood collection month, we divided the children into six groups (March, April, May, June, July, and August).

TABLE 2 | Comparison of vitamin D in each group (grouped by gender, BMI, time of outdoor activities and blood collection month).

	***N* (%)**	**Vitamin D (M ± SD) (ng/ml)**	**t/F**	***P***
Gender			−0.537	0.591
Male	233 (76.1)	25.10 ± 9.05		
Female	73 (23.9)	25.77 ± 10.04		
BMI[a]			1.441	0.239
Normal or underweight	177 (61.0)	25.87 ± 9.48		
Overweight	61 (21.0)	25.30 ± 10.12		
Obese	52 (18.0)	23.37 ± 7.53		
Time of outdoor activities[b]			1.193	0.313
<30 min	89 (29.6)	24.20 ± 10.14		
≥30 and <60 min,	94 (31.2)	25.00 ± 8.76		
≥60 and <90 min	53 (17.6)	25.81 ± 8.15		
≥90 min	65 (21.6)	26.96 ± 9.77		
Blood collection month			2.728	0.020*
March	100 (32.7)	22.86 ± 8.98		
April	80 (26.1)	24.98 ± 11.38		
May	36 (11.8)	26.89 ± 9.61		
June	46 (15.0)	27.24 ± 7.67		
July	26 (8.5)	27.95 ± 4.87		
August	18 (5.9)	27.64 ± 5.36		

[a]*Body Mass Index (BMI) data were not available for 16 children.*
[b]*Time of outdoor activities data were not available for five children.*
*$P < 0.05$.

TABLE 3 | Correlations among vitamin D, age, and screen time.

	R	***P***
Age	−0.115	0.045*
Screen time	−0.272	<0.001*

*$P < 0.05$.

The comparison of the vitamin D levels among the groups was statistically significant ($F = 2.728$, $P = 0.020$). It seems that the longer the sunshine duration, the higher the vitamin D concentration (**Table 2**). The correlation analysis showed that the vitamin D concentration of the children with ASD was negatively correlated with age ($r = -0.115$, $P = 0.045$) and screen time ($r = -0.272$, $P < 0.001$) (**Table 3**; **Figures 1**, **2**). The older the age and the longer the screen time, the lower the vitamin D concentration.

We incorporated age, screen time, BMI, time of outdoor activity, and blood collection month into a multiple linear regression model (**Table 4**) with vitamin D as the dependent variable. We analyzed whether these factors were related to the vitamin D concentrations in the children with ASD. The results of the multiple linear regression showed that age ($\beta = -0.233$, $P < 0.001$), screen time ($\beta = -0.122$, $P = 0.032$) and blood collection month ($\beta = 0.177$, $P = 0.004$) were related to the vitamin D concentrations in the children with ASD.

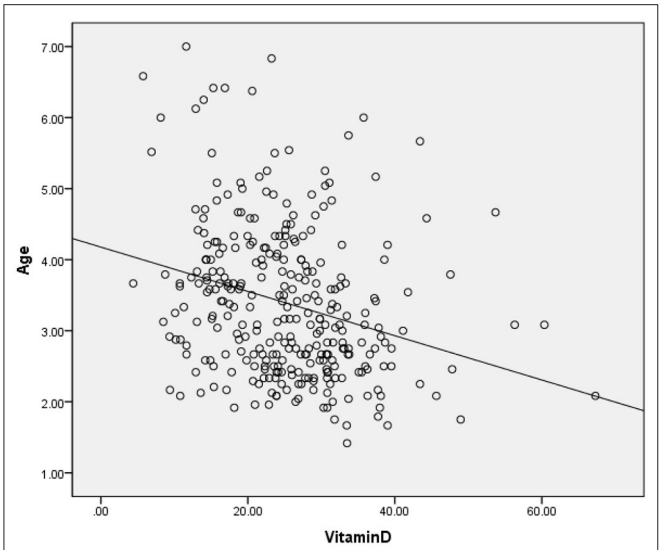

FIGURE 1 | Correlation of vitamin D (ng/ml) and age (y).

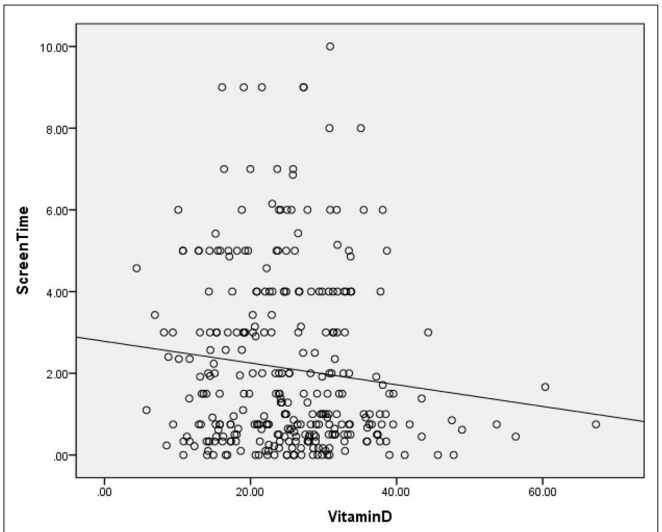

FIGURE 2 | Correlation of vitamin D (ng/ml) and screen time (h).

TABLE 4 | Multiple linear regression model of vitamin D.

	β	T	95% CI	P
Age	−0.233	−4.076	−0.260, −0.091	<0.001*
Screen time	−0.122	−2.157	−0.017, −0.001	0.032*
Body Mass Index (BMI)	−0.059	−1.038	−2.071, 0.641	0.300
Time of outdoor activities	0.014	0.235	−0.887, 1.127	0.814
Blood collection month	0.177	2.928	0.346, 1.767	0.004*

*$P < 0.05$.

DISCUSSION

Our results suggest that the vitamin D concentrations in children with ASD are negatively correlated with screen time, and other factors that might be related to the vitamin D concentration include age and sunshine duration.

Screen Time and Vitamin D

Our results suggest that there is an association between screen time and the vitamin D concentrations in children with ASD. A study (26) based on adults suggested that screen time could be related to a lack of time for physical activity. A Brazilian study (27) involving 12- to 17-year-old adolescents also showed that they spend a significant amount of time each day in front of electronic screens, while ∼50% of teenagers do not engage in any physical activity in their spare time. Dong (28) conducted a study involving 559 adolescents aged 14 to 18 years in the southern USA and identified physical activity to be associated with the plasma 25(OH)D concentrations. Lenders (29) also reached a similar conclusion that physical activity was positively associated with the 25(OH)D levels, although the sample size was small. As mentioned earlier, we speculate that a very long screen time (as one of the most important sedentary behaviors of children) might affect children's outdoor activity time and further affect their vitamin D levels.

However, our results do not seem to fully support this speculation. Our results suggest that screen time is related to the vitamin D concentrations, but the outdoor activity time is not related to the vitamin D concentrations. Although our data show that vitamin D has a tendency to increase with increasing outdoor activity time, it is not statistically significant. According to a 2014 meta-analysis (30), sedentary behavior and physical activity were negatively correlated in young people, but the effect size was small, indicating that longer sedentary behavior cannot be completely equal to shorter activity times. Thus, sedentary behavior and less physical activity are different behaviors (31, 32), and their effects on the vitamin D concentrations cannot be substituted for each other. Inactivity and screen time might have distinct pathophysiological mechanisms and implications for illness (33). Our results suggest that screen time (but not outdoor time) is associated with the vitamin D levels, which is not ambivalent.

Another study showed a different result. A 2019 Brazilian study showed that moderate-to-vigorous physical activity could play an important role in increasing serum 25(OH)D concentrations in adolescence, especially in boys, regardless of the screen time. The sample size of the study was large (n = 1,152), but the subjects were typically developing adolescents aged 12–17 years, who were much older than our participants. Since vitamin D in children with ASD might have different metabolic statuses (6) and related metabolic gene polymorphisms (34, 35) compared with typically developing children, the associations among screen time, sedentary behavior, outdoor activity time and vitamin D deserve further discussion.

Age and Vitamin D

Our results suggest that the age of children with ASD is negatively correlated with the vitamin D concentrations. Older children with ASD have lower vitamin D concentrations. Andiran's

research (36) conducted correlation analyses in Turkey and revealed that the 25(OH)D levels were negatively correlated with age (0–5 age group, 34.2 ± 16.2 ng/ml; 5–10 age group, 20.5 ± 8.7 ng/ml; 10–16 age group, 18.7 ± 11.5 ng/ml). This finding is likely related to the preventive measures in primary health care implemented by the Ministry of Health in Turkey (36). Since 2005, vitamin D supplements have been distributed to all newborns throughout their infancy at no financial cost (37). A US survey (38) involving 4,558 children and adolescents aged 1–11 years also showed that the vitamin D concentration of the children aged 1–5 was higher than that of the children aged 6–1 (70 vs. 66 nmol/L). However, the United States is a multiracial country, and non-Hispanic black and Hispanic children have the lowest levels of 25(OH)D, which might have had an impact on the results.

The situation is different in China. Vitamin D is recommended for routine supplementation of 400–800 units from birth to early childhood (rather than school age), and it is not a free-cost drug in China. Universal primary health care for children must be further strengthened. Similarly, in the UK, a population-based study conducted in 2011 (23) also showed that the plasma vitamin D levels decreased progressively with age. Although there are no recommendations for vitamin D supplementation in older children, in younger children, the recommended supplement uptake is low. The participants in this study were all children who had not taken vitamin D regularly in the previous 3 months; thus, vitamin D supplements can be ignored. The reason for this phenomenon is not yet clear.

Sunshine Duration and Vitamin D

Our research suggests that the vitamin D levels in the summer (June-August) are higher than those in the spring (March-May). Most humans depend on sunlight exposure to satisfy their requirements for vitamin D (39). Solar ultraviolet B photons are absorbed by the skin, leading to the transformation of 7-dehydrocholesterol into vitamin D3 (cholecalciferol) (40). Vitamin D levels are related to ultraviolet light, which is easy to understand. Similarly, some studies (41–43) have shown that season is an important factor affecting the vitamin D status. The vitamin D levels in the human body are the highest in the summer (41).

Interestingly, regarding sunshine duration, June is the longest month of sunshine in the year, but the level of vitamin D in July and August is slightly higher than that in June, which seems to be a delayed phenomenon. There was indeed a Brazilian study that supports our speculation. In Brazil, the results of the São Paulo vitamin D evaluation study (44) showed that the lowest UV radiation levels were recorded in the winter, while the lowest 25(OH)D concentrations occurred in the spring, corresponding to a delay of a season. A strong correlation was observed between the current mean 25(OH)D concentration and the mean UVR value from the previous season ($r = 0.98$) (45).

Possible Intervention Strategies

We recommend limiting the screen time of children with ASD. Children younger than 2 years who have a deviation in social and language development but have not yet been confirmed with a diagnosis of ASD according to the AAP recommendation (46) should avoid electronic screen devices. The limited screen time requires high-quality content, high-quality company and interaction with parents. In addition, it is still recommended that children with ASD take vitamin D while monitoring their vitamin D levels, especially those who are older and have longer screen times, during short sunshine duration seasons and in areas with high latitudes (low UV), emphasizing the management of multilevel related environmental factors, in addition to behavioral interventions and education for children with ASD. In the future, a cohort study will be performed to verify the effectiveness of our management strategy.

Limitations and Further Directions

We ignored the vitamin D intake in the diet. Although vitamin D produced through the skin is the most important source, under the condition of insufficient sunlight (especially in the spring at high latitudes in northeastern China), food can supply ∼10 to 20% of vitamin D (39). Future research should consider dietary factors.

This study is only a cross-sectional study and cannot provide causal conclusions. Further prospective cohort studies are needed.

We only investigated the vitamin D levels of children with ASD in the spring and summer, while the sunshine duration is shorter in the autumn and winter. Therefore, we must conduct a whole year of research in the future to verify our conclusions.

CONCLUSION

The vitamin D levels in children with ASD are related to their electronic screen time, age and sunshine duration. While age and season are uncontrollable, identifying the length of screen time in children with ASD could provide a basis for the clinical management of their vitamin D levels.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the First Hospital of Jilin University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

LS: methodology, investigation, and writing the initial manuscript. HD: methodology, investigation, formal analysis, and some writing. TW: data curation and formal analysis and editing the manuscript. JF: investigation, formal analysis, and editing the manuscript. FJ: conceptualization, funding acquisition, supervision, and oversight and resources. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the National Natural Science Foundation of China (Grant Number: 81973054), Key Scientific and Technological Projects of Guangdong Province (Grant Number: 2018B030335001), Joint Fund Bethune Medical Special Project of Jilin Province (Grant Number: 20200201507JC), and the Project of Jilin Provincial Department of Finance (Grant Number: 2018SCZWSZX-60).

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frontiers in Pediatrics

CLINICAL TRIAL
published: 11 February 2022
doi: 10.3389/fped.2021.813305

Effects of Combing Group Executive Functioning and Online Parent Training on School-Aged Children With ADHD: A Randomized Controlled Trial

Liting Chu[1,2], *Peiying Zhu*[1], *Chenhuan Ma*[1], *Lizhu Pan*[1], *Li Shen*[3], *Danmai Wu*[1,2], *Yu Wang*[1]* *and Guangjun Yu*[1,2]*

[1] *Department of Child Health Care, Shanghai Children's Hospital, Shanghai Jiao Tong University, Shanghai, China,* [2] *School of Public Health, Shanghai Jiao Tong University, Shanghai, China,* [3] *Clinical Research Center, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China*

Objective: The acceptance of drug treatment for younger children with attention-deficit/hyperactivity disorder (ADHD) in China remains low. Here, we explored the clinical benefits of a non-pharmaceutical intervention method combining a group and executive function training and an online parent training program, termed group executive functioning and online parent training (GEF-OPT), for school-aged students with ADHD through a randomized controlled trial.

Method: A total of 145 children (aged 6–8 years) were formally registered and randomized to the intervention group ($n = 73$) and waitlist group ($n = 72$). The enrolled children received eight sessions of GEF-OPT treatment, which consists of a hospital-based children executive function (EF) training program and an online parent training program. Treatment outcome was assessed by a parent/teacher report questionnaire and neurophysiological experiment.

Results: After eight sessions of intervention, children in the intervention group showed a significant improvement in inattentive symptom compared to the waitlist group (14.70 ± 4.35 vs. 16.03 ± 2.93; $p = 0.024$), but an insignificant difference in hyperactive-impulsivity (9.85 ± 5.30 vs. 10.69 ± 5.10; $p = 0.913$). Comorbid oppositional defiant disorder was significantly reduced in the intervention group (7.03 ± 4.39 vs. 8.53 ± 4.41; $p = 0.035$). Children in the intervention group had greater reduction in the scores of behavioral regulation index (inhibition, emotional control) and metacognition index (working memory, planning/organization, monitoring) in executive function than those in the waitlist group ($p < 0.05$). Significant effects were also found in learning problem of Weiss Functional Impairment Scale–Parent form and parental distress between two groups at post-treatment ($p < 0.05$). In line with this, the result of go/no-go task showed significant improvements in accuracy change (4.45 ± 5.50% vs. 1.76 ± 3.35%; $p = 0.001$) and reaction time change (47.45 ± 62.25 s vs. 16.19 ± 72.22 s; $p = 0.007$) in the intervention group compared with the waitlist group.

OPEN ACCESS

Edited by:
Fei Li,
Shanghai Jiaotong University, China

Reviewed by:
Antonella Gagliano,
University of Cagliari, Italy
Sharon Zlotnik,
University of Haifa, Israel

***Correspondence:**
Yu Wang
wy_rain@126.com
Guangjun Yu
gjyu@shchildren.com.cn

Specialty section:
This article was submitted to Child and Adolescent Psychiatry, a section of the journal Frontiers in Pediatrics

Received: *11 November 2021*
Accepted: *22 December 2021*
Published: *11 February 2022*

Citation:
Chu L, Zhu P, Ma C, Pan L, Shen L, Wu D, Wang Y and Yu G (2022) Effects of Combing Group Executive Functioning and Online Parent Training on School-Aged Children With ADHD: A Randomized Controlled Trial. Front. Pediatr. 9:813305. doi: 10.3389/fped.2021.813305

Conclusion: We conclude that participants in the GEF-OPT program improved outcomes for inattentive symptom, executive function, learning problems, and parental distress. GEF-OPT is a promising non-pharmaceutical therapeutic option for younger children.

Trial Registration: ChiCTR2100052803.

Keywords: ADHD, non-pharmacological treatment, executive function, online intervention, parent training

INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is a common neurodevelopmental disorder in childhood, characterized by hyperactivity, impulsivity, and inattention that are not commensurate with the developmental level. ADHD not only impedes the development of children's learning and social abilities but also brings a heavy burden on their families and society (1). A meta-analysis indicates that the prevalence of ADHD among children and adolescents in China is 6.26%, generally consistent with the worldwide prevalence (2). Medical treatment (methylphenidate, atomoxetine, etc.) can relieve the core symptoms of ADHD (3–6); however, a considerable proportion of patients fail to tolerate or respond to the stimulant treatment (7). Further, the evidence that drug therapy can prevent a series of comorbidities in later childhood or adulthood is lacking (5, 8). Recently, many treatment guidelines emphasize the importance of multimodal treatment for ADHD, which consists of combining drug treatment and non-drug treatment (i.e., parent training and social skills training) (9–11).

Executive function (EF) deficits are major contributors to poorer outcomes in ADHD patients (12, 13), which have been directly related to impairments in academic, interpersonal, and social functioning (14, 15). EF is the high-level cognitive function of the central nervous system that promotes new behaviors (16). ADHD patients with deficiencies in EF show functional impairments, including inhibition, planning, work memory, plan organization, and cognitive flexibility (17). These impairments associated with ADHD highlight the importance of the early and appropriate interventions in improving the developmental trajectories (18). Group-based EF training is currently recommended to help children with ADHD symptoms. Lan et al. (19) compared the effects of group EF training with social skills training in children with ADHD and found that EF training produced more effective and lasting changes on peer relationship difficulties. Qian et al. (20) found 33 school-aged students who benefitted from ecological executive skills training, and these children exhibited less core symptoms 1 year later, compared with the control group. Therefore, it is necessary to give EF training for school-aged children with ADHD.

Parent management training (PMT) is a psychosocial intervention program that allows the parents of ADHD children to apply the behavior management methods to effectively manage children's challenging behaviors (21). These methods are favored by parents who are resistant to medication (22). These parent training programs include Incredible Years (23), the New Forest Parenting Program (24), and Positive Parenting Program (25), some of which have achieved positive therapeutic effects (26). Most efficacious studies are traditional on-site interaction (23, 27), which refers to parents receiving training lessons from doctors or therapists face to face, then conducting behavioral training for children at home. However, this type of training is often hindered by time and traffic restraints. Retention in Barkley's study is poor, with only 25% of parents attending more than 4 of 14 sessions (28). Moreover, the benefit of parent training intervention in long-term follow-ups has generally not been demonstrated. In a notable exception, Shelton's research proposed that the effects of parent management training did not persist at a 2-year follow-up (29). Coincidentally, some studies also pointed out that parent training and pharmacological treatment are not so effective for children with ADHD and that parental compliance is very important (30, 31). Currently, the rapid development of digital health has made it possible for the Internet-based parental training. Studies have confirmed that digital health intervention provides patients with high accessibility, scalability, and cost-effectiveness while still improving patient outcomes (32). For example, Franke and colleagues demonstrated that an online parenting program is an effective intervention for preschool children (33). The efficacy of a web-assisted self-help parenting program was also verified by a large sample size (34). Thus, it can be considered that web-based parenting training is a feasible measure in ADHD intervention.

Given that parents are more willing to accept non-pharmacological interventions for school-aged children with ADHD, we explored the clinical benefits of non-pharmacological interventions combining the group executive functioning and online parent training (GEF-OPT) for ADHD children aged 6–8 years old. To do so, two hypotheses were examined. The first is whether the intervention group (parents and teachers) reports lower levels of child core ADHD symptoms after intervention compared with parents/teachers in the waitlist group. The second is whether the non-pharmacological interventions show some key improvements over the waitlist group, including: (a) improvements in executive functioning; (b) improvements in peer relationship, learning, and social function; and (c) lower levels of parental pressure and anxiety. Our research was performed within a hospital-based group training center plus online platforms in order to facilitate child intervention and parent training. A randomized controlled trial (RCT) was conducted to investigate the training effects of GEF-OPT after intervention.

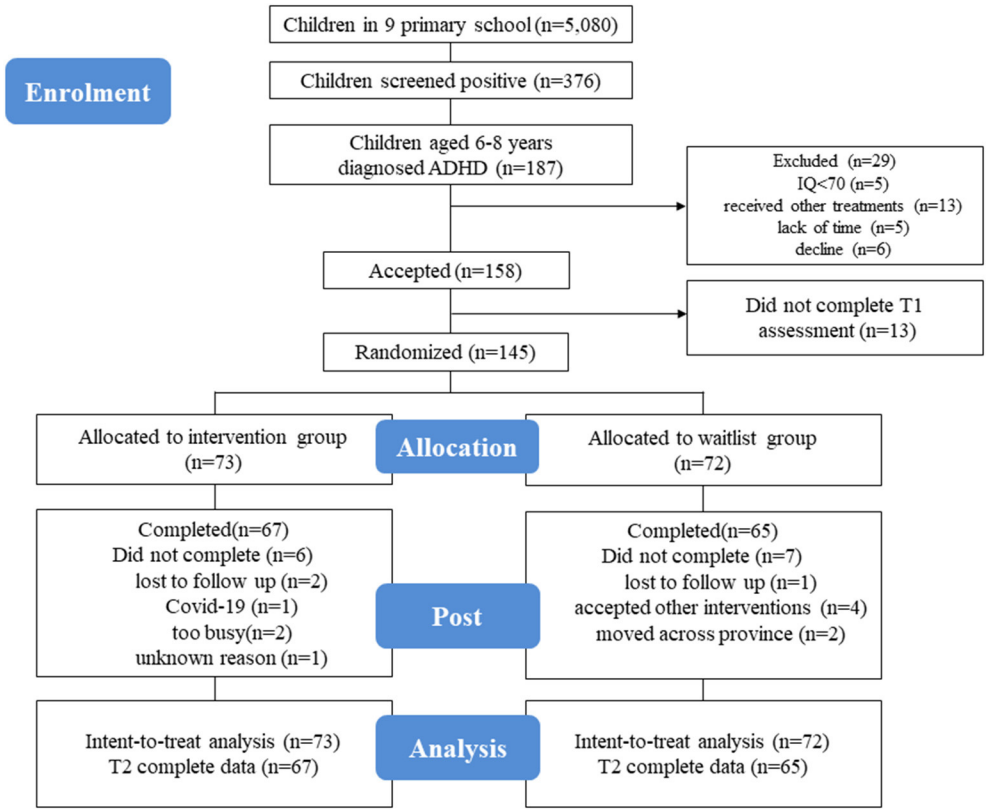

FIGURE 1 | This figure shows the consort diagram of the RCT. All outcomes were measured before and after the intervention for both groups. The waitlist group received the same intervention after the second assessment. Six participants in the intervention group (two lost to follow-up, one was unwilling to go to the hospital due to COVID-19, two were too busy, and one of an unknown reason) and seven participants in the waitlist group (one lost to follow-up, four accepted other interventions, and two moved across a province) dropped out of the study.

METHODS

Study Design and Population

All participants were from primary schools in Putuo District, Shanghai, China. Similar to our previous study (35), an invitation and information letter was sent to in-house healthcare professionals and the headmasters of involved schools, informing them about the study. The hospital pediatricians then conducted an online meeting about the purpose of this project and the type of intervention for both teachers and parents. Parents who wanted to participate in this project would contact the research assistant, and then be registered in a WeChat group, where all related matters and screening system would be informed.

Participants, recruited between January 2021 and June 2021, are screened for ADHD *via* a mobile app Swanson Nolan and Pelham, Version IV (SNAP-IV) Scale. Parents would directly receive the positive or negative results after completing the electronic scale, and then, they could decide on their own whether to take their children to Shanghai Children's Hospital for diagnosis. After 1 month of parents self-filling the electronic scale, pediatricians identified 187 6–8-year-old children diagnosed with ADHD according to the DSM-5 criteria by detailed medical history collection and behavioral observation (36). Research assistants sent the project invitation and informed consent form to the parents of these children. Eventually, there were 145 ADHD children who participated in this study. Scale evaluation was done by those who were familiar with children's daily life at home and school, mainly parents and class teachers. Assessment took place at two time points: at pre-intervention (T1) and post-intervention (T2; 8 weeks after T1). After T1 assessment, families were randomly allocated to the intervention or waitlist group. The waitlist group received the intervention after T2 assessment. The consort diagram of each stage of RCT and drop-out reasons is shown in **Figure 1**.

Inclusion and Exclusion Criteria

Children were newly diagnosed with ADHD, following the criteria of *Diagnostic and Statistical Manual of Mental Disorders, fifth edition* (DSM-5) (36), ranging from 6 to 8 years old. IQ should be 70 or above established with the Wechsler Intelligence Scale for children–fifth edition (WISC-V) (37). Moreover, parents or primary caregivers did not want to receive drug therapy, could read and write the Chinese language, were legally able to sign informed consent, and signed the informed consent.

Children with autism spectrum disorder, schizophrenia, epilepsy, head injury, or verified neurological disorder, intellectual disability (IQ <70, based on WISC-V) (38), and sensory impairment (hearing/vision problems) and those

TABLE 1 | Contents of GEF-OPT.

Week	Targeted executive function	Part of group executive function training	Online parent training
1	Sustained attention	Commitment: Each child was asked to tell a class rule and then wrote it down or express with pictures in the notebook. Visual tracking: The therapist took out three playing cards and put them face up in a row, and asked children to choose one (for example, spades A). Then the therapist put them back to its original position, asked children to focus on the card, and moved the card quickly from side to side. After several moves, children were asked to point out the position of spades A from the three playing cards. The number and type of cards would be changed.	Knowledge about ADHD and methods of family attention training
2	Planning and time management	Schedule: The therapist taught children planning and time management skills and gave each child a timetable as well as asked them to formulate the time they spend on necessary events and other activities for the following week (homework, tutoring class, extracurricular activities, etc.). Children were required to complete the weekly schedule.	Help children manage time and supervise them to complete each task according to the schedule
3	Organization skills	Room and desk organization: Children should be first asked to distinguish clean and cluttered room and desk. The therapist used teaching aids to classify and organize possessions in the room and study with children. Homework was to tidy up the room and desk, and complete a task list for hosting a birthday party.	Learn to mobilize children's enthusiasm and praise them in time
4	Inhibition	Simon says: One child acted as Simon and gave instructions to other children (nodding, stomping, touching nose, etc.). When he started with "Simon says," the rest of children needed to follow instructions, otherwise they should keep still.	Learn behavioral strategies such as positive reinforcement and punishment to manage conduct problems
5	Working memory	Sherlock: The therapist gave out 8 cards with arrows of different clues (daily necessities, fruits, animals, clothing, etc.). Children needed to remember the evidence on the cards. Then the therapist turned the card face down and picked up the doll. The doll moved according to the arrow and the number of steps on the card. If the child answered correctly and the card the doll stayed on was turned over, the child would get this card.	Strategies for effective learning skills and communication with teachers
6	Spatial intelligence	Matchmaker: The therapist gave a card surrounded by 10 blocks (from easy to difficult). Children needed to flip 5 long blocks in the shortest time to match the corresponding pattern.	Guidelines for giving effective instructions
7	Cognitive flexibility	My first journey: The therapist taught children to understand the map of China. Four city tickets were randomly selected on the table. Each child had another four city tickets, then took turns rolling the dice, and chose the route according to the color of the dice and city tickets. When the arrival city was the same as the four tickets on the table, the child could get the ticket of the stated characteristics of the city.	Games of improving parent-child relationship and methods for stress management
8	Consolidate and summarize	Consolidate and reinforce the poorly-performed projects completed before. Children shared their positive changes and received rewards.	Questions and answers Review and identified obstacles resolution

receiving other ADHD treatments were excluded. Neither the intervention nor waitlist group were treated with medication.

Randomization and Blinding

The participants who met all eligibility criteria and provided written informed consent were randomly assigned (1:1) to receive intervention or wait to do intervention using a computer-generated randomization sequence. Randomization was done by research staff using statistics software (SAS 9.4, SAS Institute, Cary, NC, USA). Given the nature of this study, participants could not be blind to their assigned group, so the participants and pediatrician were aware of group allocation. Other research staff were blind to the group. Analyses were done by a statistician masked to group allocation.

Online Parent Training and Group Executive Function Training

We provided a multimodal treatment for children and parents in the intervention group. The GEF-OPT in this study was based on Training Executive, Attention, and Motor Skills (TEAMS) (39), which was modified to be more suitable for Chinese elementary school families.

The training program consisted of eight 90-min sessions, composed of separate child and parent groups (four-to-six families per group). Before treatment, parents were told to help children prepare a notebook, pencil, and eraser. The children took part in group EF training in a clinical setting, and parents received OPT *via* Voov Meeting (computer, tablet, or mobile phone). Parents had a 30-min lesson to learn about ADHD and behavioral management skills and conduct behavioral management while assisting children in completing homework after EF training class. Child groups were led by a team of three staff: typically one senior psychologist and two graduate students. Parent groups were run by three professional pediatricians specializing in child healthcare. The outline for each session is presented in **Table 1**.

Sample Size Calculation

The primary endpoint was the total scores of parents reported SNAP-IV scale after intervention. It was estimated that a total sample of 140 (1:1) would be sufficient to demonstrate a

statistically significant difference between the intervention and waitlist group with 90% power and an alpha of 0.05 and expected dropout of 10%.

Statistical Analysis

Data analyses were performed with SAS v9.4 (SAS Institute). The difference between two groups was assessed by an independent sample *t*-test for continuous data and chi-square test for categorical data. Analysis of covariance (ANCOVA) was used to compare the intervention effects between two groups with pre-intervention data as covariates. The magnitude of effect sizes was expressed in Cohen's *d*, which is computed by comparing the change scores between intervention and waitlist groups and dividing them by the pooled standard deviation (SD) of change scores. Data were shown as mean ± SD and frequency (percentage). Missing data were imputed by last observation carried forward (LOCF) and followed by intention-to-treat (ITT) analysis guidelines. All statistical analyses were two-tailed, and $P < 0.05$ was considered statistically significant.

Questionnaires and Experiments

Swanson Nolan and Pelham, Version IV Rating Scale

The SNAP- IV is composed of 26 items using a four-point scale ranging from 0 to 3, including three subscales: inattention, hyperactivity, and Oppositional-defiant disorder (ODD). A higher score indicates greater levels of symptoms. This scale was reported to have good reliability and validity (40). The SNAP- IV was completed by parents and teachers via a mobile app, with ∼15 min to complete. The primary outcome in this study was the total scores of the parent-rated SNAP-IV scale between the intervention and waitlist group at T2.

Behavior Rating Inventory of Executive Function-Parent Form (BRIEF)

The Behavior Rating Inventory of Executive Function-Parent Form (BRIEF) is a questionnaire for parents of school-aged children that enables professionals to assess EF behaviors at home. It contains 86 items within eight theoretically and empirically derived clinical scales that measure the different aspects of EF: Inhibition, Shift, Emotional Control, Initiate, Working Memory, Planning/Organization, Organization of Materials, and Monitor (41). The Chinese version of this scale has good reliability and validity and is suitable for those with a Chinese cultural background (42).

Go/No-Go Task

Go/No-Go task is frequently used to investigate response inhibition (43). In this study, the test was performed according to Monden's research (44), which includes six block sets, namely, alternating Go, No-Go, and Go/No-Go blocks. In the Go block, a child was asked to recognize a picture of elephants and tigers (100%) and then quickly pressed the space bar. In the Go/No-Go block, a child was provided with lion pictures (50%) that require a button press and giraffe pictures that do not require a button press (50%). Each block lasted for 24 s. Before each block, there were 3 s of instruction in Chinese telling children to press the space bar when they saw elephants and tigers, pressed the space bar when they saw lions, and not press any button when they saw giraffes. The total block setting time was 54 s, and the overall session time was about 6 min.

The accuracy (RC) and reaction time (RT) of each child were recorded for the behavior analysis. The Go/No-Go task of this experiment were presented on a 24-in. computer screen by E-Prime 2.0 software. The distance between child's eyes and the computer screen is approximately 50 cm. Before collecting data, all participants must receive guidance and actually perform several experimental tasks, and the examiner observed the completion of participants to ensure that the participants correctly understand the experimental tasks.

Weiss Functional Impairment Scale–Parent Form

The Weiss Functional Impairment Scale–Parent form (WFIRS-P) is a social function assessment tool compiled based on the characteristics of ADHD. It is used by parents based on children's emotional and behavioral aspects in the recent month. The scale has a total of 50 items, including six subscales of family, learning and school, life skills, children's self-concept, social activities, and risky activities. Previous research showed that the WFIRS-P of Chinese version has good reliability and validity, with an internal consistency of 0.70–0.92, and a test–retest reliability of 0.61–0.87 (42).

Parenting Stress Index

Parenting Stress Index (PSI) refers to the difficulties, anxiety, tension, and other pressures that parents have in the process of fulfilling their parental roles and parent–child interactions. There are 36 items in total, including three subscales: parenting distress, dysfunctional interaction, and child difficulty. High scores show great levels of parenting stress. The PSI has shown adequate reliability and high validity in Chinese children (45).

RESULTS

Descriptive Analyses

A total of 187 (3.7%) students aged 6–8 years were diagnosed with ADHD. After exclusion, 145 children were enrolled and randomized to the intervention group ($n = 73$) and waitlist group ($n = 72$). Attrition included six children in the intervention group (two were lost to follow-up, one withdrew due to COVID-19, two were too busy, and one with an unknown reason) and seven waitlist group children (one was lost to follow-up, four accepted other interventions, and the other two moved out of a province) (**Figure 1**). Eventually, there were 132 families (91.0%) that completed the study at T2 (**Figure 1**).

Analyzing the basic demographic information, including the age, IQ, gender, ADHD subtypes, comorbidities, and family status, of the intervention and waitlist groups, did not reveal a significant difference between these two treatment conditions on any of the demographics or baseline variables (**Table 2**, $P > 0.05$).

Effects of GEF-OPT by SNAP-IV Scales

For assessing the changes in ADHD symptoms, we applied a Chinese version of SNAP-IV, which has good reliability and validity (46). As shown in **Table 3**, the primary outcome was

TABLE 2 | Demographic characteristics of the intervention group and the waitlist group.

Variable	Intervention ($n = 73$)	Waitlist ($n = 72$)	t/χ^2	P
Age (years), mean ± SD	7.10 ± 0.47	7.04 ± 0.61	0.666	0.506
IQ, mean ± SD	97.01 ± 17.31	96.36 ± 12.23	0.262	0.794
Gender, *n* (%)			0.667	0.414
Boy	57 (78.1)	52 (72.2)		
Girl	16 (21.9)	20 (27.8)		
ADHD subtype, *n* (%)			1.002	0.606
Inattentive	45 (61.6)	42 (58.3)		
HI	8 (11.0)	12 (16.7)		
Combined	20 (27.4)	18 (25.0)		
Comorbidity, *n* (%)				
ODD	15 (20.5)	13 (18.1)	0.145	0.704
Anxiety and depression	2 (2.7)	4 (5.6)	0.725	0.395
Family structure, *n* (%)			1.242	0.265
Core family	40 (54.8)	46 (63.9)		
Non-core family	33 (45.2)	26 (36.1)		
Family annual income, yuan *n* (%)			2.687	0.261
∼100,000	9 (12.3)	10 (13.9)		
100,000–200,000	19 (26.0)	27 (37.5)		
200,000∼	45 (61.6)	35 (48.6)		
Parental relationship, *n* (%)			0.090	0.764
Harmony	49 (67.1)	50 (69.4)		
General	24 (32.9)	22 (30.6)		
Father's education, *n* (%)			1.602	0.449
College∼	12 (16.4)	16 (22.2)		
High school-college	48 (65.8)	40 (55.6)		
∼Junior high school	13 (17.8)	16 (22.2)		
Mother's education, *n* (%)			0.510	0.775
College∼	9 (12.3)	9 (12.5)		
High school-College	53 (72.6)	49 (68.1)		
∼Junior high school	11 (15.1)	14 (19.4)		
Parent-child communication time, *n* (%)			0.222	0.638
<3 d/w	2 (2.7)	3 (4.2)		
≥3 d/w	71 (97.3)	69 (95.8)		
Parent-child outdoor activities, *n* (%)			2.846	0.092
<3 d/w	43 (58.9)	52 (72.2)		
≥3 d/w	30 (41.1)	20 (27.8)		
Children's exposure to electronic screens time, *n* (%)			5.239	0.073
1 h/d∼	38 (52.1)	27 (37.5)		
0.5–1 h/d	19 (26.0)	17 (23.6)		
∼0.5 h/d	16 (21.9)	28 (38.9)		

ADHD, Attention deficit hyperactivity disorder; IQ, Intelligence quotient; HI, Hyperactive-impulsivity; ODD, Oppositional-defiant disorder; SD, Standard deviation.

presented as SNAP-IV scales of the core items. After adjusting the baseline scale data of pre-intervention, the significant difference could be observed in parent-rated inattentive [$F_{(1, 143)} = 5.17$, $P = 0.024$, $d = 0.27$] and ODD [$F_{(1, 143)} = 4.55$, $P = 0.035$, $d = 0.27$] as well as teacher-rated inattentive [$F_{(1, 143)} = 13.23$, $P < 0.001$, $d = 0.53$], ODD [$F_{(1, 143)} = 13.05$, $P < 0.001$, $d = 0.53$], and total score [$F_{(1, 143)} = 14.76$, $P < 0.001$, $d = 0.43$]. Both Hyperactive-impulsivity (HI) and the total score in parent-rated SNAP-IV scales did not show significant treatment effects, while only HI in teacher-rated SNAP-IV scales was not statistically different between two groups.

Effects of GEF-OPT by BRIEF Scales

To assess the EF behaviors of patients at home, the BRIEF scales were then analyzed. There were significant effects in inhibition [$F_{(1, 143)} = 21.85$, $P < 0.001$, $d = 0.69$], emotional control [$F_{(1, 143)} = 7.24$, $P = 0.008$, $d = 0.33$], working memory [$F_{(1, 143)} = 6.81$, $P = 0.010$, $d = 0.27$], planning/organization [$F_{(1, 143)} = 5.10$, $P = 0.025$, $d = 0.32$], monitor [$F_{(1, 143)} = 7.45$, $P = 0.007$, $d = 0.34$], behavioral regulation index [$F_{(1, 143)} = 14.77$, $P < 0.001$, $d = 0.42$], metacognition index [$F_{(1, 143)} = 7.39$, $P = 0.007$, $d = 0.30$], and total score [$F_{(1, 143)} = 12.67$, $P = 0.001$, $d = 0.32$]. Although the subscale scores of waitlist group also decreased at T2, the effects of intervention group were improved more significantly than that of the waitlist group (**Table 4**).

Effects of GEF-OPT by WFIRS-P and PSI Scores

To further confirm the beneficial effects of GEF-OPT, we assessed the WFIRS-P and PSI scores. In line with the BRIEF scales, significant differences were also observed in the Learning and School subscale, [$F_{(1, 143)} = 8.52$, $P = 0.004$, $d = 0.60$], and the total score of WFIRS-P [$F_{(1, 143)} = 6.99$, $P = 0.009$, $d = 0.30$] between GEF-OPT and waitlist groups (**Table 5**). At T2, parents in the GEF-OPT group showed a significantly greater decrease in parenting distress [$F_{(1, 143)} = 28.45$, $P < 0.001$, $d = 0.73$], dysfunctional interaction [$F_{(1, 143)} = 37.72$, $P < 0.001$, $d = 0.98$], child difficulty [$F_{(1, 143)} = 14.39$, $P < 0.001$, $d = 0.91$], and the total score of PSI [$F_{(1, 143)} = 48.75$, $P < 0.001$, $d = 1.20$] than their counterparts in the waitlist group (**Table 5**).

Effects of GEF-OPT by Go/No-Go Task Analysis

The Go/No-Go task is frequently used to investigate response inhibition (43). We then set out to assess the effect of GEF-OPT intervention on enrolled children using the Go/No-Go task. There was no significant difference in RC between the intervention group (85.67 ± 6.75) and waitlist group (86.12 ± 8.08) at T1, while a significant difference was found in the increase of RC in two time points, with 4.45 ± 5.50 in the intervention group and 1.76 ± 3.35 in the waitlist group ($t = 3.561$, $P = 0.001$) (**Figure 2A**). The RT of the intervention group was 478.33 ± 56.46 at T1 and 430.87 ± 54.21 at T2, while the waitlist group was 483.95 ± 43.70 and 467.75 ± 53.90. The reduction of RT between the two groups was also statistically different ($t = 2.736$, $P = 0.007$) (**Figure 2B**).

DISCUSSION

Clinical guidelines suggest that drug treatment is the preferred treatment for school-aged children with ADHD (10), and the side effects of most drugs are mild and gradually tolerated. However,

TABLE 3 | Effects of GEF-OPT by SNAP-IV scales.

Scales	Intervention group (n = 73)		Waitlist group (n = 72)		*F*	*P*	*d* [95% CI]
	Pre	Post	Pre	Post			
SNAP-IV, parent rated							
Inattentive	15.66 ± 3.99	14.70 ± 4.35	15.86 ± 4.03	16.03 ± 2.93	5.17	0.024	0.27 [−0.06, 0.60]
HI	11.47 ± 5.19	9.85 ± 5.30	12.58 ± 5.52	10.69 ± 5.10	0.01	0.913	−0.41 [−0.69, −0.14]
ODD	8.53 ± 4.78	7.03 ± 4.39	8.93 ± 3.94	8.53 ± 4.41	4.55	0.035	0.27 [−0.03, 0.57]
Total score	35.66 ± 9.79	31.58 ± 11.32	37.38 ± 10.74	35.25 ± 10.44	3.34	0.070	0.06 [−0.21, 0.33]
SNAP-IV, teacher rated							
Inattentive	16.19 ± 2.99	14.56 ± 3.96	15.90 ± 4.05	16.06 ± 2.74	13.23	<0.001	0.53 [0.24, 0.82]
HI	12.74 ± 4.10	10.64 ± 4.79	12.46 ± 4.53	11.28 ± 4.16	2.59	0.110	−0.09 [−0.36, 0.18]
ODD	9.60 ± 3.89	7.86 ± 3.93	8.92 ± 3.79	8.90 ± 3.62	13.05	<0.001	0.53 [0.28,0.78]
Total score	38.53 ± 7.76	33.07 ± 10.06	37.28 ± 10.54	36.24 ± 9.48	14.76	<0.001	0.43 [0.17, 0.69]

All data are shown as mean ± SD.
SNAP-IV, Swanson Nolan and Pelham, Version IV Rating Scale; HI, Hyperactive-impulsivity; ODD, oppositional-defiant disorder; SD, Standard deviation.

TABLE 4 | Effects of GEF-OPT by BRIEF scales.

Scales	Intervention group (*n* = 73)		Waitlist group (*n* = 72)		*F*	*P*	*d* [95% CI]
	Pre	Post	Pre	Post			
BRIEF							
Inhibition	19.88 ± 5.44	17.21 ± 4.37	19.85 ± 4.12	19.44 ± 4.58	21.85	<0.001	0.69 [0.43,0.95]
Shift	13.00 ± 2.76	13.07 ± 2.69	13.85 ± 2.53	13.60 ± 2.70	0.00	0.982	0.00 [−0.33,0.33]
Emotional control	17.36 ± 4.80	15.82 ± 4.27	18.36 ± 4.47	17.78 ± 4.59	7.24	0.008	0.33 [0.11,0.55]
Initiate	15.33 ± 2.98	14.79 ± 2.80	15.29 ± 2.84	14.99 ± 3.16	0.34	0.562	0.06 [−0.21,0.33]
Working memory	22.47 ± 3.60	21.22 ± 4.12	23.54 ± 3.46	23.32 ± 3.80	6.81	0.010	0.27 [0.01,0.54]
Planning/organization	25.71 ± 4.67	24.42 ± 4.62	25.63 ± 4.84	25.50 ± 4.50	5.10	0.025	0.32 [0.09,0.56]
Organization of materials	12.08 ± 2.31	11.52 ± 2.51	12.00 ± 2.44	11.93 ± 2.17	2.89	0.091	0.28 [0.03,0.53]
Monitor	19.53 ± 3.14	17.92 ± 3.09	19.64 ± 2.95	19.01 ± 2.86	7.45	0.007	0.34 [0.05,0.63]
BRI	50.23 ± 10.41	46.10 ± 8.68	52.06 ± 9.17	50.82 ± 10.41	14.77	<0.001	0.42 [0.21,0.63]
MI	95.12 ± 13.38	89.88 ± 14.15	96.10 ± 15.03	94.75 ± 14.93	7.39	0.007	0.30 [0.07,0.53]
Total score	145.36 ± 20.71	135.97 ± 19.83	148.15 ± 23.23	145.57 ± 24.33	12.67	0.001	0.32 [0.09,0.54]

All data are shown as mean ± SD.
BRIEF, Behavior Rating Inventory of Executive Function-Parent Form; BRI, Behavioral Regulation Index; MI, Metacognition Index; SD, Standard deviation.

parents, especially the parents of the younger age group, are still worried about the potential side effects, causing a low acceptance of and adherence to pharmacological intervention (47). Further, given that some parents might not be able to take part in field parent training due to varieties of reasons while children participated in GEF training, we launched the OPT course. We hypothesize that a program combing the traditional field intervention and online interventions could improve the effects of intervention. To our knowledge, this study demonstrates for the first time that traditional field intervention in conjunction with digital health technology has been successfully applied in both the screening and treatment of ADHD. In this RCT, all participants of online training courses are children's parents. We investigated the children's core ADHD symptoms, EF, behavioral function, and parental pressure through parent report questionnaire data and neurophysiological experiment (Go/No-Go task) at pre-treatment (T1) and post-treatment (T2, after 8 weeks). The benefits of GEF–OPT intervention can be clearly observed in the parents' and teachers' reported reduction of children's core ADHD symptoms and learning problem as well as improvements in EF with a lower level of parental distress in the intervention group at T2. This investigation indicates that the GEF-OPT training program could be a convinced choice of non-pharmacological intervention for younger school-aged ADHD children.

Our GEF-OPT programs combine two training programs that cover a range of symptoms in ADHD. Inattentive symptoms in individuals with ADHD occur due to the lack of sustained effort over time, whereas hyperactivity and impulsiveness originate from the delay aversion and the lack of future sight that is a consequence of altered time perception (48). The differences in long and short time duration perception could be followed with neural correlation. Beyond its core symptoms, ADHD comprises a range of higher-level executive dysfunctions,

TABLE 5 | Effects of GEF-OPT by WFIRS-P and PSI scores.

Scales	Intervention group ($n = 73$)		Waitlist group ($n = 72$)		*F*	*P*	*d* [95% CI]
	Pre	Post	Pre	Post			
WFIRS-P							
Family	8.03 ± 4.14	6.84 ± 3.61	8.50 ± 3.64	7.69 ± 3.81	1.43	0.233	0.11 [−0.25, 0.48]
Learning and school	6.25 ± 3.50	5.23 ± 2.94	5.26 ± 3.12	6.14 ± 3.39	8.52	0.004	0.60 [0.27, 0.94]
Life skills	9.55 ± 3.86	9.18 ± 3.32	9.40 ± 4.03	9.64 ± 4.85	0.82	0.365	0.17 [−0.15, 0.48]
Self-concept	2.26 ± 1.91	2.07 ± 1.78	2.10 ± 1.46	2.07 ± 1.35	0.03	0.855	0.11 [−0.31, 0.53]
Social activities	5.81 ± 3.69	4.85 ± 2.99	5.94 ± 2.87	5.63 ± 3.42	2.05	0.155	0.20 [−0.21, 0.61]
Risky activities	2.91 ± 2.09	2.64 ± 2.25	3.18 ± 1.82	2.97 ± 2.05	0.35	0.553	0.05 [−0.30, 0.39]
Total score	34.80 ± 12.79	30.81 ± 11.47	34.39 ± 12.50	34.14 ± 10.49	6.99	0.009	0.30 [0.03, 0.56]
PSI							
Parenting distress	27.78 ± 4.87	25.16 ± 4.17	28.79 ± 4.38	28.51 ± 4.03	28.45	<0.001	0.73 [0.43, 1.03]
Dysfunctional interaction	28.78 ± 5.98	24.99 ± 4.77	28.72 ± 5.93	28.29 ± 4.41	37.72	<0.001	0.98 [0.67, 1.29]
Difficult child	27.74 ± 6.14	25.52 ± 4.96	27.21 ± 5.54	27.08 ± 5.38	14.39	<0.001	0.91 [0.65, 1.16]
Total score	84.30 ± 13.11	75.67 ± 10.23	84.72 ± 13.71	83.89 ± 11.27	48.75	<0.001	1.20 [0.89, 1.50]

All data are shown as mean ± SD.
WFIRS-P, WEISS Functional Impairment Scale-Parent form; PSI, Parent Stress Index; SD, Standard deviation.

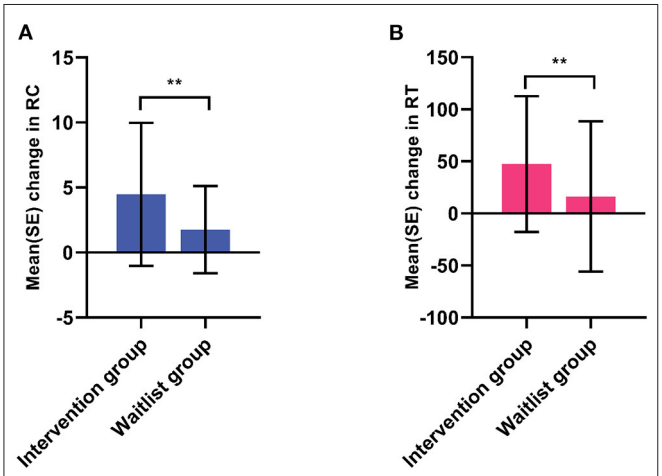

FIGURE 2 | Analysis of Go/No-Go task. The mean change of accuracy **(A)** and reaction time **(B)** of enrolled students in GEF-OPT intervention group ($n = 73$) and waitlist group ($n = 72$) were analyzed using Student *t*-test. Significant differences were found in difference of accuracy ($P = 0.001$) and reaction time mean change ($P = 0.007$) between intervention group and waitlist group. RC, accuracy; RT, reaction time; $^{**}P < 0.01$.

including deficits in response inhibition, planning, working memory, interference control, and error correction (49). As a consequence, many children with ADHD have trouble in forgetting and impairment in planning. Studies have shown that increased engagement in cognitively challenging activities could promote brain development as well as improve core symptoms of ADHD (50). Considering the participation and interest, our GEF training used functional tasks to target multiple EF components to promote neural and cognitive growth.

Significant functional improvements brought by GEF-OPT are shown in BRIEF scores, including inhibition and emotional control, the metacognition index consisting of working memory, planning/organization, monitor, and total score. The possible reason may be that after a children's training course, we would start the corresponding OPT courses. Parents knew the content of EF courses, conducted practice, and followed behavioral management training at home. For example, children were asked to do tasks in a timetable including EF trainings such as visual tracking task, cancellation test, and other tasks, homework, and real-life activities (e.g., daily chores) under the guidance of parents. Parents used behavior management strategies such as obey training, positive reinforcement method, and token economy to enable children to make positive responses and choices. After that, parents could recognize that the children's behavior was getting better during these processes.

On the other hand, results showed insignificant improvements in the task shifting, initiation, and organization of materials, which were parts of behavioral flexibility and planning and reflect an individual's ability to carry out a certain task independently (51). This may be for the following reasons: our GEF training is a form of group interaction, designed to strengthen the child's ability to hold and manipulate multiple pieces of information, to process information flexibly and the child's team skills. The program was carried out in strict accordance with the study protocol by qualified professionals. The severity of symptoms across the involved children was not identical, and we did not require parents to keep daily completion records in parent–child family tasks. This is why the effect of similar at-home parental training was not as notable. In addition, the duration of training time in each EF lesson might not be enough. Qian et al. (20) reported that the second round of EF training in ADHD students was well-accepted and had positive effects in a 1-year follow-up; this is because children's EF was enhanced by structured, repeated training that extended to early adulthood or even older. Thus, it would be necessary to do fidelity checks and increase the time duration of GEF training as needed.

Gioia et al. (52) proposed that it should be a combination of neurophysiological experiments and ecological assessment tools that fully reflects the subject's EF level. Therefore, the Go/No-Go task was employed to investigate response inhibition, which is a fundamental aspect of every organized cognitive or behavioral response. We were able to find an improvement in the RC and RT of both groups. This was similar to the findings by Monden et al. (53) who found that performance was significantly improved in the post-drug treatment session. Although our intervention was not pharmacological, it showed effectiveness. Defective inhibition processes profoundly affect daily life, leading to impulsive behavior, which is usually detrimental for an individual (54) and has been strongly associated with ADHD. Our research provides a reference for improving inhibition to suppress impulsivity.

We found changes in only a few subscales in the WFIRS-P. One possible explanation of this outcome was that our broad intervention program might have trained all these functions to some extent, leading to a significant improvement of part or overall functions—as found on the learning and school function and total score of WFIRS-P—but not enough for apparent changes on separate functional subscales (55).

As predicted, we found that there were significant differences in parenting distress, dysfunctional interaction, the difficult child, and the total score of PSI between intervention and waitlist groups. This result extended the findings of Franke et al. (33) by offering both EF intervention and using online technology to carry out parent training in families of younger students; in contrast, the former study afforded online parenting intervention only. We demonstrate that the GEF-OPT program frees parents from traffic and time constraints. As a result, this program not only increased the involvement of parents but also increases the efficiency of training lessons. As expected, the parental involvement in this study is higher compared to the traditional GEF program, and the attendance rates for each session were close to 100%. In addition, pediatricians could give precise guidance to families directly. Taken together, this study demonstrates that GEF-OPT offered by professional pediatricians can support parents in managing the stress of raising a school-aged child with ADHD and enhance parent–child communication.

STRENGTHS AND LIMITATIONS

The GEF-OPT program provided a multimodal treatment of GEF-OPT for children and parents. This treatment addressed important areas of functional impairment in school-aged students and was led by healthcare professionals. The program reduced barriers for taking part in the intervention and facilitated collaborative treatment efforts with good short-term effects.

The limitation in this study should be noted. This is a short-term effect study without long-term follow-up, so we cannot know whether the intervention can produce long-term improvements. Further study will extend the follow-up time. Additionally, the results would be more robust if the control group took part in a more traditional face-to-face parent–child training intervention. We are planning to improve our study design and gather more evidence to confirm the benefits of the GEF-OPT program to ADHD children in the future.

CONCLUSION

In summary, our study provides an evidence of the effectiveness of the GEF-OPT program in decreasing school-aged students' core ADHD symptoms, mitigating executive deficits, and improving learning ability and parental well-being. These findings highlight the potential benefits of the combination of field and online trainings in ADHD intervention.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Shanghai Children's Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

LC, GY, and YW contributed to the conception and design of the study. LC, PZ, and LP designed the intervention plan and participated in the training program. CM, LP, and LS contributed to data collection and organization. LC, PZ, and DW performed the statistical analyses and wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

FUNDING

This work was supported by the project of the Shanghai Health and Hygiene Commission on Aging and Maternal and Child Health (2020YJZX0203), a 3-year action plan for the construction of Shanghai public health system (GWV-10.1-XK19,GWV-10.1-XK14), Shanghai Shenkang Hospital Development Center Critical Disease Multi-center Clinical Research Project (SHDC2020CR1047B), Medical Guidance Science and Technology Support Project of Shanghai Science and Technology Commission (19411969000), and Shanghai Natural Science Foundation Project (19ZR1477700).

ACKNOWLEDGMENTS

We are grateful to the families, teachers, and other volunteers involved in this project and to Sam Holtzen for his linguistic assistance during the preparation of this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.813305/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor FL declared a shared parent affiliation with the authors at the time of the review.

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 29 March 2022
doi: 10.3389/fped.2022.851109

Effect of the Early Start Denver Model on Children With Autism Spectrum Disorder Syndrome of Different Traditional Chinese Medicine Types in Northeast China

*Lili Wang†, Junyan Feng†, Yu Zhang and Tiantian Wang**

Developmental Behavioral Pediatrics, The First Hospital of Jilin University, Jilin, China

Background: The clinical presentation of children with autism spectrum disorder (ASD) is heterogeneous, and there are little data available on the treatment of children with different types of ASD. We sought to explore which traditional Chinese medicine (TCM) syndrome type was more effective for children with ASD after 3 months of Early Denver Model intervention and to analyze the reasons for its efficacy from the perspective of TCM.

Methods: This was a retrospective study. The subjects were children with ASD who were first diagnosed at the Developmental Behavioral Pediatrics, the First Hospital of Jilin University, between December 2018 and September 2019. Eighty-nine children were divided into a kidney jing deficiency group, a liver qi stagnation group, and a group with deficiency of both the heart and spleen.

Results: After treatment, the total Autism Behavior Checklist (ABC), Autism Treatment Evaluation Checklist, and Childhood Autism Rating Scale scores were significantly reduced in the three groups ($p < 0.05$) compared to before treatment. Significant improvements were seen in all five domains of the Griffiths Development Scales-Chinese version in the LQ group ($p < 0.05$). After intervention, the LQ group showed greater improvements compared to the other two groups in the language, eye–hand coordination, body and object use, social and self-help, and total ABC scores.

Conclusion: Our study showed that Early Denver Model intervention is effective in the treatment of three syndrome types of children with ASD, with the LQ group experiencing the most significant effects.

Keywords: autism spectrum disorder, early start denver model, traditional Chinese medicine types, kidney jing deficiency, liver qi stagnation

OPEN ACCESS

Edited by:
Tingyu Li,
Chongqing Medical University, China

Reviewed by:
Francesca Felicia Operto,
University of Salerno, Italy
Arlette Setiawan,
Universitas Padjadjaran, Indonesia

***Correspondence:**
Tiantian Wang
tiantian20@mails.jlu.edu.cn

†These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Child and Adolescent Psychiatry, a section of the journal Frontiers in Pediatrics

Received: *09 January 2022*
Accepted: *03 March 2022*
Published: *29 March 2022*

Citation:
Wang L, Feng J, Zhang Y and Wang T (2022) Effect of the Early Start Denver Model on Children With Autism Spectrum Disorder Syndrome of Different Traditional Chinese Medicine Types in Northeast China. Front. Pediatr. 10:851109. doi: 10.3389/fped.2022.851109

INTRODUCTION

Autism spectrum disorder (ASD) is a heterogeneous group of neurodevelopmental conditions characterized by the presence of impaired social communication and reciprocity and a restricted and stereotyped pattern of behaviors and interests. In the last few decades, the prevalence of ASD has increased dramatically, appearing as a sort of "epidemic," affecting 1 in 59 children in

the United States and significantly influencing the quality of life of children and their families because of the core developmental disability and associated medical and behavioral symptoms (1). In Jilin City of China, 77 cases of autism were identified from a total population of 7258, equating to a prevalence of 108 per 10,000 (2). Effective therapies for ASD core symptoms have not yet been established. Evidence-based first-line treatments are represented by behavioral therapies. The Early Start Denver (ESDM) model is an intervention for pre-school–aged children, which incorporates behavioral, developmental, and relationship-based strategies within a naturalistic teaching framework (3). The ESDM is specifically designed for children aged 12–60 months and is a developmental- and relationship-focused intervention that incorporates techniques designed to foster positive relationships between parent and child and to increase the child's motivation to engage in social interactions (4, 5). At present, ESDM has been applied in the intervention of children with ASD and achieved satisfactory clinical effects (6, 7). However, there are still some children with ESDM intervention whose efficacy is not significant, which is related to a variety of factors, such as intervention scenarios and differences in educational concepts. Due to the heterogeneity of clinical symptoms, we observed that the clinical manifestations of children with ASD of the same severity were inconsistent. Some children were irritable, while others avoided eye contact. This variation may be associated with different subtypes, yet there are no relevant clinical subtypes of ASD. From the point of view of traditional Chinese medicine (TCM), the different types of syndrome differentiation represent reasons for the inconsistent clinical symptoms and manifestations of these children. At present, there is no study that has observed the therapeutic effect of ESDM by TCM syndrome–differentiation analysis in children with ASD. The hypotheses of this study are as follows: first, children with different types of ASD will experience different therapeutic effects of ESDM; second, for children with autism, a single treatment method is not enough, and more comprehensive therapy plans are needed for joint treatment, which may have a better effect.

MATERIALS AND METHODS

Ethics Statement

The study protocol was approved by the ethics committee of the First Hospital of Jilin University (approval no. 20170107) and was registered with the Chinese Clinical Trial Register Center (registration no. ChiCTR1800019702) on November 24, 2018. The parents of the study participants provided written informed consent for inclusion in this study before enrollment.

Study Participants

This was a retrospective study. Participants of the study were children with ASD who were first diagnosed at the Developmental Behavioral Pediatrics, the First Hospital of Jilin University, between December 2018 and September 2019. Inclusion criteria were as follows: first, mild to moderate ASD Children. The children were diagnosed by a multidisciplinary team following the American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 criteria (8); second, the participant was aged 24–60 months; and, third, the parents/caregivers understood the content of the study and agreed to participate in it, to receive 3 months of ESDM intervention and 10 sessions of parent skills training after having a conversation with the researchers, and to sign the informed consent form prior to enrollment. Conversely, individuals with Rett syndrome, fragile X syndrome, Angelman syndrome, Prader-Willi syndrome, tuberous sclerosis, or another syndrome caused by known genetic defects or inherited metabolic diseases and those with brain injuries and physical or sensory disabilities were excluded. Parents who did not provide home videos as assigned 3 times across the 3 months also prompted the exclusion of their children from this study. All potential participants were selected based on the criteria listed above.

Study Protocol (Syndrome Differentiation Type)

The pathologic involvement of ASD is in the brain (in Chinese medicine, the brain usually refers to brain dysfunction), relating to the heart, liver, spleen, and kidney sin TCM theory (9). The concept of syndromes (zhengs) is unique to Chinese medicine. Syndromes are identifiable from a holistic understanding of a patient's clinical presentation using the four Chinese medicine diagnostic methods: observation, listening/smelling, questioning, and pulse analyses. At present, there is no standardized of TCM syndrome types for ASD children, and the reports are inconsistent. In this study, TCM syndrome types for ASD children were classified according to the clinical manifestations, and based on **Table 1** (10). When ASD children have multiple clinical manifestations, the most important clinical manifestation is used as the basis of TCM syndrome types assessment. A total of 89 ASD children were classified by TCM, each child were performed by two experienced TCM attending physicians independently. If the results are inconsistent, the disagreements were resolved through discussions with another senior physician.

Kidney Jing Deficiency

From the perspective of TCM, the process of development involves the gradual filling of the kidney qi. When the kidney qi is deficient, it will affect the growth of children. The kidney qi rules over long-term memory, so a kidney jing deficiency will result in poor mental development. In a personal translation and commentary of the texts of Wu et al. from the Qing dynasty, Fredes (11) stated that a lack of communication in small children is related to impaired heart qi, allowed by insufficient kidney qi, inherited from parents with weak qi and blood. This supports the idea of a genetic origin of the condition. Clinical manifestations of a kidney jing deficiency include low intelligence; sluggish expression; insensitivity; the ability to hear but not respond; a whitish tongue; and a deep, thready pulse. For treatment, it is recommended to invigorate the kidney, replenish the essence, nourish the liver, and strong bones.

TABLE 1 | TCM syndrome differentiation of ASD.

Type	Clinical manifestation
Kidney jing deficiency	Low intelligence, sluggish expression, insensitivity, can hear but not respond
	A whitish tongue; deep, thready pulse
Liver qi stagnation	Firing of the heart and liver, impulsivity, quick temper, rash actions, red face, thirsty
	Red tongue or red tip of the tongue, thin and yellow tongue, a wiry pulse
Deficiency of both the heart and spleen	Speaking less, speaking mistakenly, not speaking, making no acknowledgment of relatives or strangers, apathy, no willingness to participate in social communication, can hear but does not respond, speaks repetitively, words are hard to understand
	Whitish tongue, thready pulse

Liver Qi Stagnation

The liver is an unyielding viscus organ, storing blood and governing tendons. The liver controls activities, stores the ethereal soul, and corresponds to anger in emotion and shouting in sound. Additionally, the liver advocates dredging, when the liver's dredging function is normal, the person's qi is smooth, and they are in a happy mood. However, if liver function is lost, a person's emotions will be affected and they will appear uninterested in talking or unhappy. Autisms are often rejected by their parents, teachers, or peers because of their problematic behavior. Children with autism exist in a state of poor mood for a long time, will appear internal fire, irritability, insomnia, and other symptoms, which can affect the overall development of the child. At the same time, the liver is tied to the eyes, and the function of the liver can also be reflected in the activity of the eyes. Children with autism exhibit a lack of eye contact or active avoidance of eye contact, which can also be considered to be closely related to reduced liver function. Clinical manifestations of liver qi stagnation include heat in the heart and liver, impulsivity, a quick temper, rash actions, a red face and thirst, a red tongue or red tip of the tongue, a thin and yellow tongue, and a wiry pulse, For treatment, it is recommended to soothe the liver and resolve depression.

Deficiency of Both the Heart and Spleen

The heart is the master of the zang-fu, which governs the blood, harbors the spirit, and controls mental and emotional activities. The existence of sufficient heart-yin and heart-blood moisten and nourish the spirit and keep it at peace. Balance in the heart is another key element because a heart-blood or -yin deficiency, as well as heart fire, will lead to abnormal psychological activity of the spiritual consciousness and thinking or a reluctance to communicate, manifested by lethargy and quietness, fidgety restlessness, or aggressive behaviors. The spleen stores an individual's intentions, attention, and intelligence and corresponds to thinking in cognition. The nature of the spleen is quiet. Clinical manifestations of a deficiency in both the heart and spleen include speaking less, speaking mistakenly, not speaking, making no acknowledgment of relatives or strangers, apathy, an unwillingness to participate in social communication, showing the ability to hear but not respond, speaking repetitively, speaking words that are hard to understand, a whitish tongue, and a thready pulse. For treatment, it is recommended to invigorate the spleen and nourish the heart.

Sample Size Calculation

According to previous data published by Li et al. (12), the main measurement indicator, the Autism Behavior Checklist (ABC), was decreased by 15 points to be effective, and was set as unilateral $\alpha = 0.05$, $\beta = 0.2$. The sample size was calculated based on the following equation: $n = (Za/2 + Z\beta)2 \cdot (\sigma 12 + \sigma 22)/\delta 2$. After calculation, the sample size for each group was 11 cases, and we estimated a 20% dropout rate, so the final sample size was 14 cases per group. A total of 42 subjects were required.

Intervention

A total of 116 subjects signed the informed consent form to participate in this study; they were required to fill a demographic information sheet (including age, gender, parents' age, financial income, and parents' education level). Finally, 89 children were enrolled, divided into the kidney jing deficiency group (KJ group, $n = 17$), liver qi stagnation group (LQ group, $n = 46$), and the deficiency of both the heart and spleen group (HS group, $n = 26$) based on TCM syndrome differentiation. The three groups received intensive training in ESDM for 3 months, and the intervention was conducted by therapists trained in ESDM in our department. The intervention time was 2 h per day,6 days per week. The intervention was centered on the children, and the children's social skills, comprehension and expressive communication, joint attention, imitation, cognition, gross and fine motor skills, and self-care abilities were improved through games. In addition, parents of children received 10 sessions of parent skills training, once a week for 3.5 h. This training was divided into two areas. First, there was a theoretical part, for a total of 10 sessions, covering how to seize the child's attention, feel the fun of social conventions, establish back-and-forth interaction patterns, non-verbal communication, imitation, the antecedent–action–outcome relationship, joint attention, functional and symbolic games, and the development of speech and independent living. A theoretical knowledge assessment was conducted after class to monitor the learning quality. Second, there was the family video presentation, with two videos provided for training at home, each of which was3–5 min long, and which mainly focused on the intervention plan formulated by the therapist. The videos were reviewed by a therapist who has received advanced ESDM training. The main purposes of the videos were to ensure the quality and execution of parents' training at home and to offer timely guidance and suggestions.

Baseline (T1) Assessments

At baseline, developmental and behavioral medical history, demographic factors, and family characteristics, including age, gender, maternal age, paternal age, and parents' education degrees, were collected. Additionally, the following assessments were completed.

The Griffiths Development Scales-Chinese version (GDS-C) is a standardized developmental assessment tool used for children from birth to 8 years old in China. There are five domains (locomotion, personal–social, language, eye–hand coordination, and performance [A–E]) for toddlers < 2 years old and one more domain (practical reasoning [F]) for children aged 2–8 years old. The GDS-C was localized and validated from the extended and revised version of the Griffiths Mental Development Scales. A child's developmental age is determined based on the norms for their numerical age, and developmental quotients (DQs) are calculated by the following equation: developmental age/chronological age × 100. DQs for domains have a mean of 100 points (standard deviation = 15 points).

The ABC is applicable to individuals >18 months old, with a total of 57 items, including 5domains of sensation, communication, somatic movement, language, and self-care, with a total possible score of 158 points. The higher the ABC score, the more severe the ASD symptoms; the score for normal children is <53 points (13).

The Childhood Autism Rating Scale (CARS) is applicable to children >2 years old. There are 15 items, each of which is scored 1–4 points to evaluate the social communication, behavior, emotion, and sensory perception abnormalities of children. The typically developing child should score <30 points, and the higher the total CARS score, the worse the ASD symptoms (14, 15).

Finally, the Autism Treatment Evaluation Checklist (ATEC) is applicable to children >2 years old, covering language, social ability, sensory, and behavioral factors. The total score ranges from 0 to 180 points and consists of four subscales: speech/language communication, sociability, sensory/cognitive awareness, and health/physical behaviors. The higher the ATEC score, the more severe the ASD symptoms (16).

Post-intervention (T2) Assessments

All measures were re-administered to the three groups of participants at 3 months.

Raters

All professionals who administrated the above mentioned assessments were trained and blinded to the group assignment of each participant.

Statistical Analysis

All data collected were analyzed using the SPSS version 20.0 software program (IBM Corporation, Armonk, NY, United States). The normality of the data was analyzed using the Kolmogorov–Smirnov test.

Continuous data were means ± SDs or P50 (P25, P75) (i.e., median, 25th percentile, and 75th percentile measures), whereas categorical data were given as frequencies with percentages. Chi-square test were used to compare the distributions of demographic data among the three groups. Additionally, non-parametric tests, and one-way analysis of variance were used to compare the developmental outcomes and ASD symptoms of children in the three groups. An α value of ≤0.05 was accepted as the level of statistical significance.

RESULTS

Subject Enrollment and Type Flowchart

To identify the final group of participants, we set a protocol, including steps of enrollment and ASD types, to screen eligible and initial participants in accordance with the inclusion and exclusion criteria. Finally, 89 subjects were identified as eligible to join this clinical trial (**Figure 1**).

Patient Demographic Characteristics

Collecting the baseline characteristics of patients was important for performing comparisons between the three groups. We needed to collect all possible demographic characteristics at baseline to describe subject homogeneity. Since age, gender, maternal age, paternal age, and parents' education degrees are potential influencing factors in terms of the effect of intervention, these demographic characteristics were recorded in this study. There were 17 cases in the KJ group, including 15 boys and 2 girls, aged 25–58 months. There were 46 cases in the LQ group, including 40 boys and 6 girls, aged 24–50 months. Finally, there were 26 cases in the HS group, including 23 boys and 3 girls, aged 24–51 months. The main demographic characteristics of children, parents, and families in the KJ, LQ, and HS groups are presented in **Table 2**. Participants across the groups were well matched with respect to all demographic variables, although

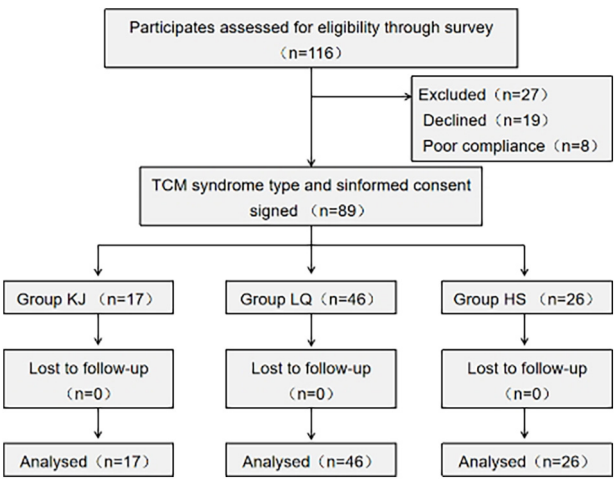

FIGURE 1 | Flow diagram of the subject: screening, grouping, and intervention. KJ group, kidney jing deficiency group (n = 17); LQ group, Liver Qi stagnation group (n = 46); HS group, group with deficiency of both the heart and spleen (n = 26).

TABLE 2 | Patient demographic characteristics in the three groups.

Characteristics	KJ group	LQ group	HS group	χ^2	*p*
n	**17**	**46**	**26**		
Gender				0.042	0.979
Male	15 (88%)	40 (87%)	23 (88%)		
Female	2 (12%)	6 (13%)	3 (12%)		
Age, months				1.560	0.458
24–36	2 (12%)	4 (9%)	3 (12%)		
37–48	12 (71%)	36 (78%)	18 (69%)		
49–60	3 (17%)	6 (13%)	5 (19%)		
Education of the mother				0.872	0.929
Primary	3 (17.6%)	7 (15.3%)	4 (15.4%)		
Secondary	12 (70.6%)	33 (71.7%)	19 (73.1%)		
Tertiary	2 (11.8%)	6 (13%)	3 (11.5%)		
Education of the father				0.334	0.988
Primary	3 (17.65%)	6 (13%)	4 (15.4%)		
Secondary	11 (64.7%)	33 (71.7%)	18 (69.2%)		
Tertiary	3 (17.65%)	7 (15.3%)	4 (15.4%)		
Age of the mother, years				1.297	0.523
16–24	0 (0)	1 (2%)	0 (0)		
25–34	16 (94%)	43 (94%)	24 (92%)		
35–44	1 (6%)	2 (4%)	2 (8%)		
Age of the father, years				1.486	0.476
16–24	1 (6%)	0 (0)	0 (0)		
25–34	14 (82%)	41 (89%)	23 (88%)		
35–44	2 (12%)	5 (11%)	3 (12%)		

the LQ group included the most children among the three groups (51.7%).

Comparison of the Clinical Efficacy of the Three Groups Between Before and After Treatment

There was no significant difference in GDS-C, ABC, CARS, and ATEC scores among the three groups before treatment ($p > 0.05$). After treatment, compared to before treatment, the total ABC, ATEC, and CARS scores were significantly reduced in all three groups ($p < 0.05$). Excluding the locomotor domain in the KJ group, there were significant improvements in other areas. In the HS group, there was no improvement in the language field, but significant improvements were observed in the other four fields. Significant improvements were also seen in all five domains of the GDS-C in the LQ group ($p < 0.05$). At T2, the DQs in the personal–social domain, language domain, eye–hand coordination domain, and performance domain of the GDS-C showed the most significant differences among the three groups ($p = 0.011$, $p = 0.007$, $p = 0.002$, and $p = 0.007$). In the locomotor domain, although the LQ group showed relatively obvious progress, there was no significant difference between the three groups ($p = 0.062$). See **Table 3**.

The change scores of DQ in the language domain and eye–hand coordination domain showed the most significant differences, with improvements of 15.5 and 12.8 points in the LQ group compared to 4.2 and 4.4 points in the HS group ($p = 0.007$ and $p = 0.023$). There was also no statistical difference in the ATEC change scores between the three groups. Also, though the ABC and CARS scores demonstrated a decreasing trend after the intervention, no significant difference was found between the three groups after intervention. However, the change scores for body/object use, social/self-help, and total ABC showed a significant difference among the three groups ($p = 0.025$, $p = 0.029$, and $p = 0.002$; **Table 4**).

DISCUSSION

Our study showed that ESDM can alleviate the core symptoms of children with ASD, but ASD has more heterogeneity and different efficacies. To our knowledge, this study is the first to observe the effects of ESDM intervention in children with ASD subtypes classified by TCM. The current study offers three main findings. First, the number of children in the LQ group was highest among the three groups. Second, ESDM was effective in the treatment of three syndrome types of children with ASD. Third, children in the LQ group performed better than those in the KJ group or the HS group under the ESDM intervention for 3 months.

The number of children in the LQ group was greatest (51.7%), which might be related to the climate characteristics and eating habits of northeast China, where a temperate monsoon climate reigns, but, because of the higher latitude, the warm summer is short and the cold winter is long. Here, food that can produce heat to help the consumer keep warm is given priority in the diet,

TABLE 3 | Comparison of the GDS-C, ABC, CARS, and ATEC scores between T2 and T1.

	KJ group (*n* = 17)		LQ group (*n* = 46)		HS group (*n* = 26)			
	T1	**T2**	**T1**	**T2**	**T1**	**T2**	***p* value (among three groups T1)**	***p* value (among three groups T2)**
GDS-C								
A: Locomotor	72.6 ± 15.8	75.3 ± 15	78.2 ± 16.2	82.3 ± 15.1#	74.1 ± 15.7	74.5 ± 13.9#	0.499	0.062
B: Personal–social	50.5 ± 10.5	60.4 ± 14.2#	56.4 ± 19.7	72.2 ± 19.4#	50.2 ± 15.6	59.9 ± 19.3#	0.249	0.011*
C: Language	35(23,60)	54(39.5,62.5)#	40(35,59.3)	64(42.3,84)#	35(28,60)	35.5(26,65.3)	0.183	0.007*
D: Eye–hand coordination	57(41,63.5)	67(58,70.5)#	61.5(49.8,80)	80(65,86.5)#	61(50.5,74.8)	62.5(54.8,76.3)#	0.099	0.002*
E: Performance	60.4 ± 17.7	68.2 ± 16.8#	71.6 ± 20.5	83.6 ± 19.2#	62.3 ± 18.4	71.5 ± 22.2#	0.053	0.007*
ABC								
Sensory	7(3.5,12)	6(2.5,9)#	9(7,13)	8(4,9.3)#	8.5(5,12.5)	8(5,9.5)	0.139	0.237
Relating	15.7 ± 5.7	12.6 ± 5.3	15.8 ± 4.6	10.3 ± 5.6	16.3 ± 4.3	12.7 ± 6.1	0.878	0.138
Body and object use	10(4.5,15)	6(4,12.5)	10(7,16.3)	6.5(2,9.3)#	9.5(4,14.3)	7.5(4,10)#	0.413	0.252
Language	9.4 ± 5.2	8.8 ± 5.3	9.4 ± 4.8	8.8 ± 5.3#	10.9 ± 4.3	9.0 ± 6.2#	0.363	0.985
Social and self-help	10.6 ± 5.2	8.9 ± 4.4#	12.9 ± 5.1	8.3 ± 4.4#	12.6 ± 3.1	10.6 ± 4.5	0.219	0.114
Total score	53.3 ± 15.3	43.4 ± 13.9#	59.8 ± 14.4	40.4 ± 15.4#	58.6 ± 13.9	47.9 ± 13.9#	0.287	0.12
ATEC								
Speech/language communication	21(11,25)	17(9,24)#	14(9,22)	9(5.8,17.3)#	21(10,25)	15.5(7.8,24)#	0.139	0.019*
Sociability	15(11.5,19.5)	13(7.5,17.5)	14(7,20)	7(4,14.3)#	18.5(9,23.3)	13(7.3,17)#	0.204	0.035*
Sensory/cognitive awareness	16.6 ± 6.9	15.5 ± 7.0#	14.8 ± 8.2	11.2 ± 6.7#	17.4 ± 8.5	15.0 ± 8.3#	0.381	0.035*
Health/physical behaviors	11.6 ± 5.5	10.3 ± 4.4	11.4 ± 8.0	7.9 ± 6.2#	12.7 ± 8.8	9.3 ± 7.2#	0.83	0.374
Total score	64(43.5,74.5)	57(32.5,70.5)#	53(29.5,78.5)	36.5(20,57.5)#	70(35.8,85.8)	52.5(29.8,76)#	0.261	0.027*
CARS	32.0 ± 4.2	31.0 ± 4.8#	32.5 ± 3.8	29.3 ± 4.8#	33.1 ± 3.6	31.7 ± 3.5#	0.653	0.086

#*T1 vs T2 ($p < 0.05$). *$p < 0.05$.*

TABLE 4 | Comparison of ΔGDS-C, ΔABC, Δ CARS, and ΔATEC between the three groups after 3 months of treatment.

	KJ group (*n* = 17)	LQ group (*n* = 46)	HS group (*n* = 26)	*p* value (among three groups Δ)
	Δ	Δ	Δ	
GDS-C				
A: Locomotor	2.7 ± 13.5	4.1 ± 14.1	0.4 ± 12.7	0.534
B: Personal–social	10(3.5,18)	17(4.8,24.8)	8.5(4,17)	0.088
C: Language	12.0 ± 15.7	15.5 ± 14.9[b]	4.2 ± 11.2[c]	0.007*
D: Eye–hand coordination	9.7 ± 11.3	12.8 ± 13.1[b]	4.4 ± 10.5[c]	0.023*
E: Performance	7.8 ± 14.9	11.9 ± 16.2	9.2 ± 13.2	0.567
ABC				
Sensory	2.2 ± 4.3	3.1 ± 4.4	1.2 ± 4.5	0.203
Relating	3.1 ± 5.9	5.5 ± 6.2	3.6 ± 6.8	0.282
Body and object use	2.3 ± 7.3	5.6 ± 5.7[b]	2.0 ± 5.2	0.025*
Language	0.65 ± 6.2	0.5 ± 5.6	1.9 ± 5.5	0.588
Social and self-help	1.7 ± 4.5	4.6 ± 4.8[ab]	1.9 ± 4.8	0.029*
Total score	9.9 ± 12.5	19.3 ± 12.3[ab]	10.6 ± 9.1	0.002*
ATEC				
Speech/language communication	2.5 ± 4.5	3.9 ± 5.9	2.8 ± 4.9	0.564
Sociability	2.0 ± 6.4	4.7 ± 5.0	4.5 ± 7.4	0.281
Sensory/cognitive awareness	1.1 ± 6.4	3.6 ± 5.7	2.4 ± 5.9	0.327
Health/physical behaviors	1.3 ± 5.1	3.5 ± 5.8	3.4 ± 6.6	0.408
Total score	6.9 ± 14.1	15.6 ± 16.6	13.0 ± 20.3	0.220
CARS	1.0 ± 3.7	3.1 ± 4.4	1.4 ± 3.4	0.087

[a]*Between the KJ group and LQ group ($p < 0.05$).*
[b]*Between the LQ group and HS group ($p < 0.05$).*
[c]*Between the KJ group and HS group ($p < 0.05$).*
*$p < 0.05$.

and this tends to create an excess amount of "fire" that remains in the body. In the Guide to Clinical Practice with Medical Records: Synopses of Pediatrics, Ye Tianshi said, "the constitution of infants belongs to pure yang, so they are likely suffering from heat disease." Thus, combined with the physiological characteristics of children, it is easy for an excess syndrome to form, which arises as the liver qi stagnation type in children with ASD. In this study, the participants with ASD were all permanent residents in northeast China, and their dietary habits were basically the same, involving food capable of the abovementioned effect.

The ESDM draws from teaching practices developed in the original Denver Model, such as relationship-based aspects of the therapist's work with the child, using play as a foundation for learning, and using communication intervention principles from the field of communication science (3). Consistent with the results of other clinical studies, this study found that ESDM can effectively benefit children with ASD, and ESDM was effective in the treatment of three syndrome types of ASD. In addition, in the classification of TCM, the effect on children with the LQ type of ASD is more obvious. There are several reasons why this may be. First, in the intervention of ESDM, emotional problems of children with ASD can be easily observed clinically. The liver qi stagnation type of ASD often shows impulsivity, quick temper and rash actions. ESDM is based on children's interests and carried out in the natural routine of daily play and care, which can well relieve the emotions of children with ASD and guide correct behaviors. Second, many studies (17–19) have shown that the parents of ASD children are under greater parenting stress. This kind of negative psychology of parents will also have a great negative impact on the family interaction mode and the intervention effects of children (20). Therefore, parents of ASD children need more information about ASD as well as emotional and social support (21). The main source of parental pressure is that children with ASD will have more behavioral problems due to difficulties in language communication and emotional regulation, while parents simultaneously lack corresponding skills, so they face great challenges in both nursing and intervention. This causes parents of children with ASD to lose control of their emotions and show anger when confronted by their children, leading to an increase in behavioral and emotional problems. When the emotion of children with ASD is relieved, the pressure of parents will be reduced, so that they can better interact with children. The liver qi stagnation type of ASD has prominent emotional problems. When the emotional problems are relieved, the progress of children can be seen immediately. Therefore, the effect of ESDM intervention on the improvement of children with the liver qi stagnation type of ASD is more obvious.

Syndrome differentiation aims to divide patients into several types according to their clinical symptoms and signs, which is essential for TCM. In TCM theory, the persistent emotional stimulus affects the function of the liver free flow and causes stagnation of the liver qi. Dysfunction of the liver free flow is

often found in the early stage of ASD, which is characterized by mental depression and an apathetic expression. Depressed Liver qi transforms into fire, which is characterized by agitation and anger, a red face and eyes, constipation, and yellow urine. ASD children disregard other people, fail to look at each other, and avoid one's eyes. Dysfunction in the liver free flow may also be observed because, from a TCM perspective, liver function is reflected in the activity of the eyes. From another point of view, this relates to the physiology and pathology of children on the one hand because a child's "liver qi is not full" (The function of the liver is not fully developed) and, on the other hand, the "liver often has excess." So, for liver regulation function in children, an understanding of the external environment is different from that in adults, and this is the main cause of mental behavior disorders in children. Therefore, the clinical manifestations of the liver qi stagnation type of ASD tend to be more centered in the areas of emotional problems and digestive problems (e.g., constipation and yellow urine). These problems are often more easily observed by parents and therapists, and the clinical symptoms may be improved through active intervention, which is more prominent in the score changes of ABC, CARS, and ATEC. On the other hand, the children in the KJ and HS groups show a deficiency syndrome. With clinical observations, we found that the pace of treatment and improvement of deficiency syndrome is lower than excess syndrome. The results of this study may be because the course of treatment was not long enough, in that 3 months of intervention did not produce a significant change in deficiency syndrome. In addition, the symptoms of deficiency syndrome (e.g., sluggish expressions, speaking less, and apathy) have not received enough attention because many parents think these are innate characteristics and timely intervention is not pursued, which is one of the reasons why deficiency syndrome is not easy to correct.

Some studies using TCM techniques other than behavioral therapies in ASD have already been performed and have reported beneficial effects, including acupuncture (10, 22, 23), Tuina (24–26), Qigong (27, 28), and herbal medicine (29). There is an inadequacy in the description of ASD in TCM in ancient documents. With the acknowledgment of relative disease, ASD exists in the grouping of "slow speaking," "weak fetus," and "wuchi" ("five retardations"). In TCM, zang-fu is a term for the organs of the human body. Many of the organ names are familiar terms, which refer not only to a physical organ but also to the energetic functions of that organ. Each organ relates to an emotional response, sensory organ, and soft tissue. Individuals with ASD experience difficulties with sensory integration. In the treatment of ASD, the four primary organ systems of concern are the heart, spleen, liver, and kidneys; these organ systems are associated with speech, taste, vision, and hearing. There is no single direct cause-and-effect relationship for ASD in Chinese medicine, though there are a set of cofactors that must be present. This forms the manifestation of different syndromes. TCM emphasizes performing treatment based on syndrome differentiation, which is the core technology that can embody the characteristics and advantages of TCM diagnosis and treatment. However, in the treatment of ASD, limited articles discussing treatment based on syndrome differentiation exist.

Based on the yin/yang theory, TCM views disease within the framework of energy balance. Therefore, the diet of children with ASD in northeast China should be adjusted mainly to reduce the intake of calories, and proper outdoor exercise should be prescribed to facilitate the release of residual fire from the body in order to achieve the goal of a balance between yin and yang in the body of children with ASD. Some studies have shown that acupuncture and massage have a certain clinical efficacy in improving gastrointestinal function and sleep by regulating the qi and blood, thereby restoring homeostasis and offering relief from many of the behavioral and regulatory symptoms commonly found in children with ASD (30–32). One of the principles of Chinese medicine emphasizes the connection and harmony of the body in which the external "skin" is closely related to the internal "organs." Therefore, stimulation of the skin has been used as a way to stimulate internal organs to restore balance in the body. In future research, we will adopt dietary structure, exercise, acupuncture, and massage methods as clinical interventions for ASD, aiming to tend to the balance of yin and yang in children to improve their overall symptoms.

This research project has some limitations. For example, fewer measurement scales were used to measure the main clinical symptoms and accompanying symptoms in children with ASD. More comprehensive assessments including ADOS, ADIR, 6-Gastrointestinal Severity Index (6-GSI), Parenting Stress Index (PSI), Child Behavior Checklist (CBCL), and Children's Sleep Habits Questionnaire (CSHQ) should be performed in our future investigations (33, 34). In the meantime, we hope to further explore the efficacy of traditional Chinese medicine treatment on autism, especially adolescent autism, by carrying out a larger research (35).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of The First Hospital of Jilin University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

LW and JF wrote the manuscript. YZ reviewed the literature and contributed to writing the manuscript. TW conceived the review and provided final approval of the version to be published. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the National Natural Science Foundation of China (Grant Number: 81973054), Key Scientific and Technological Projects of Guangdong Province (Grant Number: 2018B030335001), and Joint Fund Bethune Medical Special Project of Jilin Province (Grant Number: 20200201507JC).

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frontiers | Frontiers in Psychiatry

ORIGINAL RESEARCH
published: 09 June 2022
doi: 10.3389/fpsyt.2022.870374

Correlation Between Sialidase NEU1 mRNA Expression Changes in Autism Spectrum Disorder

*Haiqing Zhang[1], Yuhang Gu[2], Wenxiang He[1], Fengyi Kuo[3], Yiran Zhang[1], Duan Wang[1], Li He[1], Ying Yang[4], Hepeng Wang[5] and Yanni Chen[1,5]**

[1] Department of Child Healthcare, Xi'an Children's Hospital, Xi'an, China, [2] Department of Pediatrics, Ankang Maternal and Child Health Hospital, Ankang, China, [3] LIH Healthcare, Beijing, China, [4] Shaanxi Institute for Pediatric Diseases, Xi'an Children's Hospital, Xi'an, China, [5] Department of Pediatrics, Shaanxi University of Chinese Medicine, Xianyang, China

Abnormal alterations in enzymes functioned in sialic acid modifications may be associated with ASD. In order to study the differences in peripheral blood sialidase (neuraminidase 1; NEU1) mRNA expression between autism spectrum disorder (ASD) children and healthy control, and to examine the correlation between NEU1 mRNA expression and the main behavioral phenotypes in children with ASD, we performed RT-qPCR to measure NEU1 mRNA expression in peripheral blood of 42 children with ASD and 42 healthy controls. In addition, we used the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) to measure and evaluate the behavioral phenotypes of children with ASD. Our results showed that NEU1 mRNA in the ASD group was significantly higher than in the control group ($P < 0.0001$). In addition, the ADOS-2 diagnostic scores of 42 children with ASD were correlated with their NEU1 mRNA expression results ($R = 0.344$, $P = 0.0257$). Moreover, in general, NEU1 mRNA expression was also positively correlated with the Social Affect (SA) of ADOS-2 ($R = 0.3598$, $P = 0.0193$) but not with the Restricted and Repetitive Behavior (RRB) ($R = 0.15$, $P = 0.3432$). Our results indicated that sialidase NEU1 mRNA was significantly increased in children with ASD, and its expression was correlated with the SA of children with ASD, which suggested that sialidase NEU1 may affect the SA of ASD. Our data highlighted the potential of NEU1 expression change may play an important role in ASD disease and lay the foundation for further studies on the relationship between NEU1 and ASD.

Keywords: autism spectrum disorder, sialidase NEU1, gene expression, Language and Communication, ADOS-2

OPEN ACCESS

Edited by:
Yiping Shen,
Harvard Medical School,
United States

Reviewed by:
Simone Diestel,
University of Bonn, Germany
Kazuhiro Shiozaki,
Kagoshima University, Japan

***Correspondence:**
Yanni Chen
chenyannichil@163.com

Specialty section:
This article was submitted to Child and Adolescent Psychiatry, a section of the journal Frontiers in Psychiatry

Received: *06 February 2022*
Accepted: *05 May 2022*
Published: *09 June 2022*

Citation:
Zhang H, Gu Y, He W, Kuo F, Zhang Y, Wang D, He L, Yang Y, Wang H and Chen Y (2022) Correlation Between Sialidase NEU1 mRNA Expression Changes in Autism Spectrum Disorder. Front. Psychiatry 13:870374. doi: 10.3389/fpsyt.2022.870374

INTRODUCTION

Autism spectrum disorder (ASD) is a complicated neurodevelopmental disorder with clinical characterizations of social and communication deficits, as well as repetitive and stereotyped behaviors (1). According to the Centers for Disease Control and Prevention (CDC), the latest prevalence rate of ASD in 8-year-old children in the United States is 1/44 (~2.27%), which has become a social problem (2). Despite the high prevalence rate, the pathophysiology of ASD remains elusive. Previously, multiple studies suggested abnormal glycosylation as an emerging research direction for the etiology of ASD (3–5). Sialylation belongs to one of the types of glycosylation, it functions by adding sialic acid to growing glycan chains on glycoproteins and glycolipids (6, 7).

In our previous study, we used lectin microarrays and lectin-magnetic particle conjugate-assisted liquid chromatography with tandem mass spectrometry (LC-MS/MS) analyses and found that sialic acid modification was abnormal in the serum glycoprotein group of children with ASD (8). Enzymes related to sialylation are mainly divided into two categories: sialyltransferase, which catalyzes the sialic acid modification of sugar chains, and sialidase, which removes the sialic acid modification on sugar chains (9, 10). One of the recent studies reported that sialyltransferase ST3GAL5 deficient mice exhibit ASD-like behavior (11). However, the role of sialidase in ASD has not been systematically studied yet. Thus, in this study, we aim to examine the expression of sialidase in children with ASD.

There are four types of sialidases present in mammalians: neuraminidase 1 (NEU1), neuraminidase 2 (NEU2), neuraminidase 3 (NEU3), and neuraminidase 4 (NEU4) (12, 13). NEU1 is the most abundant mammalian sialidase; it primarily presents in lysosomes and acts on glycopeptides and oligosaccharides. NEU1 plays an essential role in the degradation of N-glycans (14, 15). Sialidase NEU1 deficiency has been found to affect sialic acid deposition (16–18). Moreover, NEU1 also participates in the immune system and exhibits immunomodulatory effects (19, 20).

Only a few studies focus on NEU1 expression changes in the peripheral blood in children with ASD. Therefore, we aimed to examine the NEU1 mRNA expression level in peripheral blood of children with ASD and analyze the association between NEU1 mRNA expression and ASD phenotypes to explore the relationship between NEU1 and ASD.

TABLE 1 | Demographic and clinical variables (Means and Standard Deviations).

Variable	ASD	Control	*P*
Gender	37 male, 5 female	38 male, 4 female	1.0000
Age	4.050 ± 0.1039	4.083 ± 0.1708	0.8664

P-values were calculated using the Chi-square test. ASD, autism spectrum disorder children; Control, healthy control children.

TABLE 2 | DNA Sequences of primers used for qPCR.

Genes primer	primer sequence
NEU1 F	5′GCACATCCAGAGTTCCGAGT3′
NEU1 R	5′CAGGGTTGCCAGGGATGAAT3′
β-actin F	5′CCTTCCTGGGCATGGAGTC3′
β-actin R	5′TGATCTTCATTGTGCTGGGTG3′

Primer sequences of sialidase (neuraminidase 1; NEU1) and the internal reference gene β-actin were designed in NCBI prime BLAST. F, forward primer; R, reverse primer.

MATERIALS AND METHODS

Subject Selection

We collected 42 children with ASD and 42 healthy controls with age and gender-matched. Patients were enrolled in the Child Healthcare Department of Xi 'an Children's Hospital from May to December 2019. Two experienced pediatricians made diagnoses based on DSM-5, the American Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (1) and Autism Diagnostic Observation Schedule, Second Edition (ADOS-2). According to the parents' report, none of the control children had psychiatric disorders and no family history with ASD. In order to clarify whether the included children and the grouping were reasonable, a statistical analysis of the gender and age of the included children in each group was performed using statistical methods, and the results were not statistically different (**Table 1**). Consent forms were obtained from the parents of all participating children. This study was approved by the Ethics Committee of Xi'an Children's Hospital.

Blood Sampling and RT-qPCR

The classical Trizol method for total RNA extraction from peripheral blood was performed in this experiment (21). Sterility and enzyme-free consumables are guaranteed throughout the experiments. A total of 2 ml peripheral blood was collected in the EDTA anticoagulation tube from elbow veins. After transferring an appropriate amount of blood sample into a 1.5 ml Eppendorf tube, 1 ml of Trizol was added and mixed well. All blood samples were collected at the same time and under the same storage conditions. Total RNA concentration and purity measurements were performed on a Agilent BioTek Take3 Micro-Volume Plate (BioTek, Vermont, USA). Subsequently, the cDNA Reverse transcription amplification (LongGene A300 PCR, Hangzhou, China) was performed following the Goldenstar RT6 cDNA Synthesis Kit (Tsingke Biotechnology Co., Ltd, Beijing, China). The amplification procedure (BIOER FQD-96A, Hangzhou, China) was performed according to the operation of 2×T5 Fast qPCR Mix (SYBR Green I, Tsingke Biotechnology Co., Ltd, Beijing, China). The melting and amplification curves, as well as Ct values generated by the reaction, were collected, recorded, and statistically analyzed. Triplicates were performed for each sample, and the relative changes in gene expression were quantified using 2-ΔΔCt values. Primer sequences for sialidase NEU1 and the internal reference gene β-actin were designed in NCBI prime BLAST by intron spanning (**Table 2**) and synthesized by Tsingke Biotechnology Co., Ltd (China) with a primer concentration of 10 μM.

ADOS-2 Evaluation

Autism Diagnostic Observation Schedule, Second Edition is a semi-structured, standardized assessment of communication, social interaction, play/ imaginative use of materials, and restricted and repetitive behaviors for individuals who have been referred because of possible ASD. The ADOS-2 has been referred to as the "gold standard" observational assessment for diagnosing ASD. ADOS-2 contains five assessment modules, which are relevant to the diagnosis of ASD at different developmental levels and chronological ages. Each module contains five domains, which are A (Language and Communication), B (Reciprocal Social Interaction), C (Play), D (Stereotyped Behaviors and Restricted Interests), and E (Other Abnormal Behaviors). Sub-entries include diagnostic items and observation items. The SA domain includes items pertaining to "Communications" and "Reciprocal Social Interactions". The RRB domain includes items pertaining to "Restricted and Repetitive Behaviors" (22). A total

of four modules of the ADOS-2 (T, 1, 2, and 3 modules) were used in this study, and the appropriate module was selected according to the age and language level of the individual. Children with ASD were evaluated by two certified developmental pediatricians using ADOS-2. Each assessment was conducted under the supervision of the child's parent or guardian. The correlation between NEU1 mRNA and ADOS-2 total diagnostic score, as well as SA and RRB score in children with ASD, were statistically analyzed.

Statistical Analysis

To analyze the obtained data, IBM SPSS Statistics 20 was applied. A test for normality and removal of discrete values is required for each set of data from the raw results. NEU1 mRNA values that did not conform to normal distribution were expressed as the median M (range), and the nonparametric Mann-Whitney test was used for NEU1 mRNA comparison in peripheral blood of healthy controls and children with ASD. The area under the ROC curve was calculated. Pearson correlation analysis was performed to determine whether there was a correlation between NEU1 mRNA and ADOS-2 in children with ASD.

RESULTS

Gene Expression Result and Receiver Operating Characteristic Analysis

Neuraminidase 1 mRNA expression in peripheral blood in the ASD group [4.19 (1.588–5.767)] was significantly higher than in the Control group [1.198 (0.745–1.597)] ($P < 0.0001$) (**Table 3** and **Figure 1**). We then calculated the ROC curve of NEU1 for all ASD samples and control samples to assess the predictive power of NEU1 levels in differentiating children with ASD from healthy controls. The analysis showed an AUC of.868 ($P < 0.0001$) with high sensitivity (73.81%) and specificity (83.33%). These results indicated the feasibility of NEU1 as a potential clinical diagnostic indicator for ASD (**Figure 2**).

TABLE 3 | NEU1 mRNA expression between groups (*p*-values) [M (Q1∼Q3)].

Gene	ASD	Control	*P*
NEU1	4.19 (1.588–5.767)	1.198 (0.745–1.597)	<0.0001

*Statistical analysis of NEU1 expression between groups. P-values were calculated using the Mann-Whitney U test. ***, P < 0.001 vs control.*

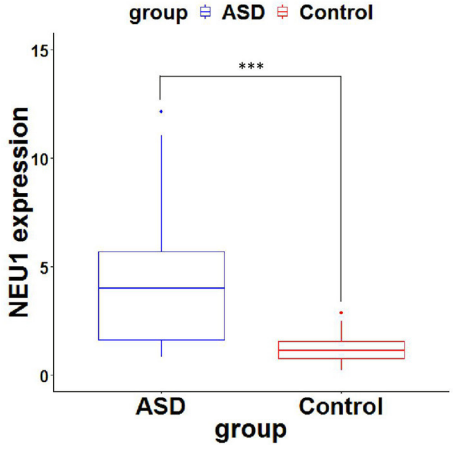

FIGURE 1 | NEU1 mRNA expression ($2^{-\Delta\Delta}$Ct values) of ASD and Control. *P*-values were calculated using the Mann-Whitney U test. ***, significant difference between ASD group and control group.

Correlation of ASD Phenotype With NEU1 mRNA

To investigate the relationship between NEU1 mRNA and symptoms in children with ASD, we performed and recorded ADOS-2 scores in 42 children with ASD. The diagnostic scores of ADOS-2 and NEU1 mRNA expression levels were analyzed by Pearson correlation. We found that the ADOS-2 diagnostic score in children with ASD was correlated with the expression of NEU1 mRNA ($R = 0.344$, $P = 0.0257$) (**Figure 3A**). Furthermore, in order to further examine the correlation between the increased expression of NEU1 and the behavioral performance in children with ASD, we calculated the correlation of NEU1 mRNA expression level with SA and RRB in ADOS-2. The results indicated that increased expression of NEU1 mRNA was positively correlated with SA ($R = 0.3598$, $P = 0.0193$) (**Figure 3B**), but not with RRB ($R = 0.15$, $P = 0.3432$) (**Figure 3C**). The correlation of NEU1 gene expression results with ADOS-2, SA, and RRB data is listed in **Table 4**.

DISCUSSION

In this study, we showed that the expression of NEU1 mRNA in peripheral blood was significantly increased in children with ASD compared to healthy controls. Furthermore, we demonstrated that NEU1 mRNA expression in peripheral blood

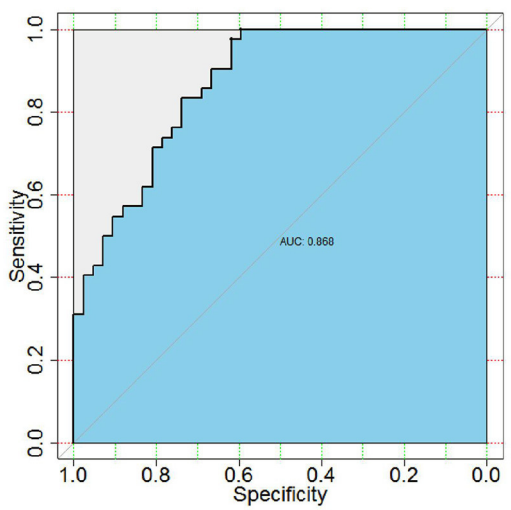

FIGURE 2 | Receiver operating characteristic (ROC) curve between clinical sensitivity and specificity for every possible cut-off. ROC curves of ASD and control with NEU1 expression. AUC was 0.868 ($P < 0.0001$), sensitivity was 73.81% and specificity was 83.33%.

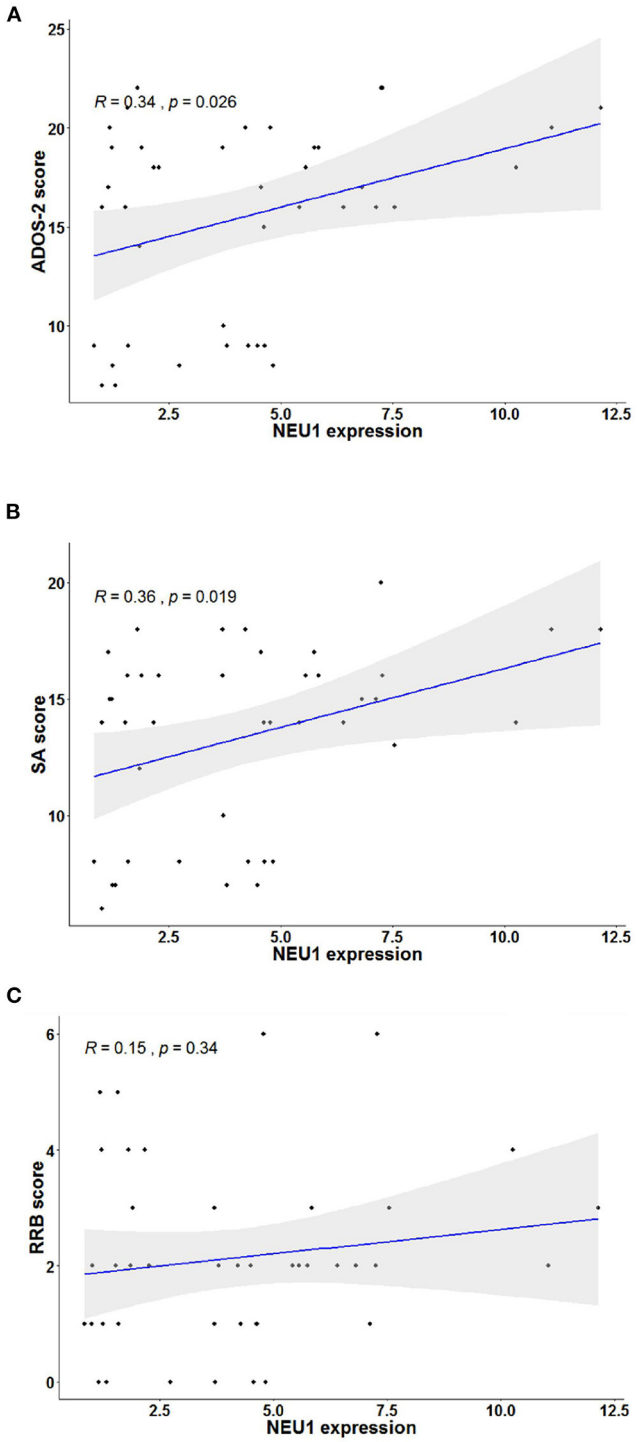

FIGURE 3 | Correlation analysis in children with ASD's blood NEU1 expression with ADOS-2, SA, and RRB scores. Statistical calculation of correlation was performed on each pair to obtain the Pearson correlation result, and the sig two-tailed probability *P*-value < 0.05 represented correlation. **(A)** Correlation of NEU1 gene expression results with ADOS-2 score. **(B)** Correlation of NEU1 gene expression results with SA score. **(C)** Correlation of NEU1 gene expression results with RRB score. **(A,B)** show that the elevated expression of NEU1 was positively correlated with ADOS-2 and SA. **(C)** shows that no correlation was found with the RRB score.

TABLE 4 | Correlation of NEU1 gene expression results to Autism Diagnostic Observation Schedule, Second Edition (ADOS-2), Social Affect (SA), and Restricted and Repetitive Behavior (RRB) in children with ASD.

Gene expression	ADOS-2 score	SA score	RRB score
NEU1	$r = 0.344$ $P = 0.0257$	$r = 0.3598$ $P = 0.0193$	$r = 0.15$ $P = 0.3432$

Statistical calculation of correlation was performed on each pair to obtain the Pearson correlation result, and the sig two-tailed probability P-value <0.05 represented correlation.

could effectively distinguish children with ASD from healthy controls. In addition, we showed that the increased expression of NEU1 mRNA was positively correlated with both ADOS-2 total diagnostic score and SA score, but not with RRB score, suggesting that NEU1 alteration may be associated with ASD behavioral phenotypes, especially in social interaction deficits. Our results suggest that NEU1 may play an important role in ASD disease and lay the foundation for further studies on the relationship between NEU1 and ASD.

Our previous study focused on Maackia amurensis lectin-II (MAL-II) to study the serum proteome and serum glycoproteome in children with ASD. We found that the glycoprotein sialic acid modification in the serum of children with ASD was increased (8). In this study, our data suggested that sialidase NEU1 mRNA expression is increased in children with ASD, we speculate that the underlying mechanism is a feedback regulation on the increase of protein sialylation level caused by increased glycoprotein sialic acid modification, thereby upregulating sialidase NEU1 mRNA expression. In order to test our hypothesis, we plan to generate a mouse model to increase NEU1 expression. We will measure the protein sialylation level as well as check for ASD-related behaviors in the future. Here, we reported that NEU1, one of the sialidases responsible for removing sialic acid modifications on sugar chains, was highly expressed in ASD. One recent study also showed that the expression of plasma sialic acid is significantly reduced in ASD (23), which may affect the function of sialidase. Together, all of these recent findings support the idea that abnormal modification of glycoprotein sialylation may be one of the essential factors in the occurrence of ASD.

It has been shown that immune dysregulation is closely associated with ASD (19, 24). Sialylation plays an important role in the immune system as well (25). One recent study showed that sialyltransferase ST3GAL5 deficient mice exhibit ASD-like behaviors and dysregulated inflammatory responses. Moreover, NEU1 has been shown to functionally remove the sialic acid on TLR4, so that it will not be recognized for degradation, thereby increasing the immune response (26–28). Furthermore, sialic acid modification of TLR4 can regulate the nuclear factor kappa-B (NF-κB) signaling pathway and lead to altered the protein expression level of cytokines (29, 30). Significantly increased serum NF-κB concentration was found in children with ASD (31). Animal experiments have shown that TLR4 expression is increased in mice with maternal LPS exposure, and their offspring exhibit ASD-like behaviors (32). This may be related

to the activation of microglia by TLR4 stimulation, which eventually leads to neuroinflammatory damage and neuronal death (33). Moreover, the neuroimmune response has been shown to cause ASD-like behaviors (30, 34). Overall, these results suggest that abnormal NEU1 expression may affect ASD behavior by regulating immune responses.

In addition, we tested the performance of NEU1 as a potential clinical diagnostic marker for ASD. The result of the ROC curve suggested that NEU1 mRNA increased expression in peripheral blood exhibited high sensitivity (73.81%) and specificity (83.33%) for ASD, and the area under the ROC curve was 0.868 ($P < 0.0001$). We found that the amount of NEU1 mRNA increased expression positively correlated with the severity of ASD symptoms in the diagnosed children. Studies in zebrafish reported that NEU1-KO zebrafish developed behavioral traits opposite to ASD, including excessive exploratory/boldness behavior on various tests. Furthermore, anxiety induction upregulated NEU1 expression in zebrafish (35), which is consistent with our findings.

LIMITATIONS AND FUTURE DIRECTIONS

This study found that the increase of NEU1 mRNA expression was statistically significant with SA ($P < 0.05$), but the correlation was not strong ($R = 0.3598$). Therefore, we need to further expand the sample size in the future. Meanwhile, in order to explore whether the abnormal expression of NEU1 is specific to ASD, related studies can be carried out on other common neurodevelopmental disorders in children. In addition, future research on NEU1 can explore its crucial role and related signaling pathways involved in ASD from different cells and animal models.

CONCLUSION

We showed that NEU1 mRNA expression is significantly increased in children with ASD. And there is a correlation between the increased expression of NEU1 and social dysfunction in children with ASD.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Xi'an Children's Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

YC, LH, YY, and FK designed the project. HZ, YG, LH, HW, and DW to conduct experiments and collect data. FK, HZ, WH, and YZ for the ASD entry evaluation. YC, HZ, and YY analyzed the data and interpreted the results and wrote the manuscript. All authors participated in the revision of the manuscript, and all read and approved the submitted version. All authors provide approval for publication of the content and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

FUNDING

This study was jointly supported by a grant from Shaanxi Province Key R&D Program (2020GXLH-Y-013), TCM combination with Modern Medicine of prevention and treatment of developmental brain disorders innovation team, Shaanxi University of Chinese Medicine (2019-YL07), National Natural Science Foundation of China (NSFC 81371900), The Shaanxi Provincial Science and Technology Research and Development Program (213ST2-09), Shaanxi Province Key R&D Program (2021SF-194), Xi'an Science and Technology Plan Project [20YXYJ0006(4)], and Natural Science Basic Research Program of Shaanxi (2022JQ-979).

ACKNOWLEDGMENTS

We are grateful to the families and children who participated in the research.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2022.870374/full#supplementary-material

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Conflict of Interest: FK was employed by LIH Healthcare.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Psychiatry

TYPE Original Research
PUBLISHED 22 July 2022
DOI 10.3389/fpsyt.2022.893226

OPEN ACCESS

EDITED BY
Fei Li,
Shanghai Jiao Tong University, China

REVIEWED BY
Lijie Wu,
Harbin Medical University, China
Marc Woodbury-Smith,
Newcastle University, United Kingdom

*CORRESPONDENCE
Yan Hao
haoyaner@163.com

SPECIALTY SECTION
This article was submitted to Child and Adolescent Psychiatry, a section of the journal Frontiers in Psychiatry

RECEIVED 10 March 2022
ACCEPTED 27 June 2022
PUBLISHED 22 July 2022

CITATION
Chen S, Zhao J, Hu X, Tang L, Li J, Wu D, Yan T, Xu L, Chen M, Huang S and Hao Y (2022) Children neuropsychological and behavioral scale-revision 2016 in the early detection of autism spectrum disorder.
Front. Psychiatry 13:893226.
doi: 10.3389/fpsyt.2022.893226

Children neuropsychological and behavioral scale-revision 2016 in the early detection of autism spectrum disorder

Shuling Chen[1,2], Jinzhu Zhao[1], Xiaolin Hu[1], Lina Tang[1], Jinhui Li[1], Dandan Wu[1], Tian Yan[1], Lu Xu[1], Min Chen[1], Shan Huang[1] and Yan Hao[1]*

[1]Division of Child Healthcare, Department of Pediatrics, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [2]Department of Child Health Care, Wuhan Children's Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Background: The Children Neuropsychological and Behavioral Scale-Revision 2016 (CNBS-R2016) is a widely used developmental assessment tool for children aged 0–6 years in China. The communication warning behavior subscale of CNBS-R2016 is used to assess the symptoms of autism spectrum disorder (ASD), and its value of >30 points indicates ASD based on CNBS-R2016. However, we observed that children with relatively lower values were also diagnosed with ASD later on in clinical practice. Thus, this study aimed to identify the suitable cutoff value for ASD screening recommended by the communication warning behavior of CNBS-R2016.

Materials and methods: A total of 90 typically developing (TD) children and 316 children with developmental disorders such as ASD, developmental language disorder (DLD), and global developmental delay (GDD; 130 in the ASD group, 100 in the DLD group, and 86 in the GDD group) were enrolled in this study. All subjects were evaluated based on the CNBS-R2016. The newly recommended cutoff value of communication warning behavior for screening ASD was analyzed with receiver operating curves.

Results: Children in the ASD group presented with lower developmental levels than TD, DLD, and GDD groups in overall developmental quotient assessed by CNBS-R2016. We compared the consistency between the scores of communication warning behavior subscale and Autism Behavior Checklist (ABC), Childhood Autism Rating Scale (CARS), Autism Diagnostic Observation Schedule, second edition (ADOS-2), and clinical diagnosis for the classification of ASD at a value of 30 based on the previously and newly recommended cutoff value of 12 by the CNBS-R2016. The *Kappa* values between the communication warning behavior and ABC, CARS, ADOS-2, and clinical diagnosis were 0.494, 0.476, 0.137, and 0.529, respectively, with an agreement rate of 76.90%, 76.26%, 52.03%, and 82.27%, respectively, when the cutoff point was 30. The corresponding *Kappa* values were 0.891, 0.816, 0.613,

and 0.844, respectively, and the corresponding agreement rate was 94.62%, 90.82%, 90.54%, and 93.10%, respectively, when the cutoff point was 12.

Conclusion: The communication warning behavior subscale of CNBS-R2016 is important for screening ASD. When the communication warning behavior score is 12 points or greater, considerable attention and further comprehensive diagnostic evaluation for ASD are required to achieve the early detection and diagnosis of ASD in children.

KEYWORDS

Children Neuropsychological and Behavioral Scale-Revision 2016, communication warning behavior, autism spectrum disorders, screen, early detection

Introduction

Autism spectrum disorder (ASD) is a life-long neurodevelopmental disorder characterized by persistent impairments of social communication and restricted and repetitive behavior (1), with incidences rapidly increasing worldwide. According to Centers for Disease Control and Prevention of American, the prevalence of ASD is as high as 1/54 in children before the age of 8 (2). The latest national survey shows that the prevalence of ASD in children aged 6–12 years in China is 0.70% (95% CI: 0.64–0.74%) (3), which is generally lower than that in the United States, indicating the possibility of many unidentified cases.

Autism spectrum disorder is a serious disease that affects children's social adaptability. A national sample survey has shown that ASD is the leading cause of disability among disabled children aged 6 or younger in China (4). ASD not only affects children's families, but also causes a huge economic burden to the society (5, 6). Studies have shown that early behavioral treatment can largely improve the cognitive and adaptive abilities of children with ASD (7, 8). In general, the earlier the intervention, the better the outcomes (9–12). Early screening and early diagnosis play a key role in the prognosis of this disease (13, 14). Signs of ASD can occur very early, and the symptoms could usually be captured before the age of 2 (14–17), such as the lack of social smile at the age of 6 months, the lack of orientation to his or her name at the age of 12 months, and inability to point at things at the age of 15 months (18–21). However, at present, the diagnosis of ASD is performed around the age of 4–5 years on average (22, 23). A delay can be observed between the onset of ASD symptoms and diagnosis. Therefore, research about early screening, particularly the screening tools, can continue to effectively optimize and accelerate diagnostic procedures.

Some imported tools, such as the Checklist for Autism in Toddlers, Modified Checklist for Autism in Toddlers, Autism Behavior Checklist (ABC), and Clancy Autism Behavior Checklist, have been used for screening ASD (24, 25). Although these tools have been commonly applied in municipal maternity and children's healthcare/(tertiary) hospitals and primary medical institutions in some large cities in China, these scales are rarely utilized in community health service centers and district maternity and child healthcare hospitals (24) as well as difficult to use as routine well-child visit items because of culture and cost factors (26–28). Moreover, the existing screening tools in clinical practice are suitable for a limited age group, whereas the age range of target assessment objects varies greatly, and the screening stages used are not the same. Some scale copyrights are more restricted. Therefore, at present, effective screening tools in Chinese are lacking.

The Children Neuropsychological and Behavioral Scale is an indigenous development assessment tool with Chinese norms that was developed by the Capital Institute of Pediatrics of China (29) since the early 1980s. Researchers designed the test items in accordance with developmental rules and behavioral characteristics of Chinese infants. These items were verified and completed in a cross-sectional study of 1,275 children aged 0–4 years and further standardized in 15,053 children from 12 representative provinces and cities through strict nationwide sampling. Finally, 177 items were included in the children neuropsychological and behavioral scale for young ones aged 0–4 years. The five subscales, namely, gross motor, personal social, language, fine motor, and adaptive behavior, were consistent with the relevant subscales in Gesell (30) and demonstrated adequate reliability in children with typical development. The scale was revised from 2005 to 2016 to include new items and expand the age range and standardized sample size and then named the Children Neuropsychological and Behavioral Scale-Revision 2016 (CNBS-R2016). The CNBS-R2016 includes 294 items, and the test age was expanded to 6 years. A new subscale called communication warning behavior was added apart from the five subscales to assess the

symptoms of ASD. Communication warning behavior contains 33 items, including social communication disorder, restricted and repetitive behavior, language, sensory abnormalities, physiological disorder, intelligence, and abnormal behavior. CNBS-R2016 is a widely used developmental assessment scale at various levels of medical institutions in China, particularly in maternal and child healthcare and primary care hospitals, as a part of routine well-child visits.

Some of developmental behavioral pediatricians or child healthcare physicians in China have screened and detected ASD based on the communication warning behavior. A point of the communication warning behavior over than 30 points indicates ASD as suggested by CNBS-R2016. However, we observed that children with lower values of communication warning behavior were also diagnosed with ASD later on in clinical practice. Therefore, we aimed to study the suitable cutoff value for screening ASD and provide suggestions about the application of CNBS-R2016 in the early detection of ASD in children.

Materials and methods

Participants

Children aged 2–5 years who visited the outpatient Division of Child Healthcare, Department of Pediatrics, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology from March 2019 to December 2021 were enrolled in this study. Children with developmental disorders and typically developing (TD) children were recruited. Developmental disorders included ASD, developmental language disorder (DLD), and global developmental delay (GDD). All participants completed developmental assessment of the CNBS-R2016. ASD and GDD groups were diagnosed based on the ASD criteria of American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-V) (31) and further confirmed by the Autism Diagnostic Observation Schedule, second edition (ADOS-2). GDD refers to children aged under 5 years with profound delay of ≥2 standard deviations below the mean in two or more developmental domains (32), and children who showed abnormal results in gross motor and fine motor domains without any other backward domain were excluded in this study. The DLD group was diagnosed based on the diagnostic criteria of the ICD-11 (33) and backward only in language. Children in the TD group were those without any developmental disorders and with normal results of CNBS-R2016 and who were recruited in the same period from routine well-child visits. The parents or caregivers of the children who agreed to participate in this study were provided with informed consent.

Instruments

CNBS-R2016

All children included in this study participated in the developmental assessment by the CNBS-R2016. The mean value of the general developmental quotient (DQ) and the five subscale quotients of the CNBS-R2016 is 100. A subscale quotient of less than 70 points (<2 standard deviations [SDs]) indicates a developmental delay; a quotient between 70 and 79 points is slightly below the threshold for developmental delay, and a quotient greater than or equal to 80 points showed no developmental delay (29). The communication warning behavior subscale of CNBS-R2016 has a total of 33 items, including the core characteristics of ASD such as reducing social interaction ability; inappropriate communication styles; repetitive behavior; the lack of shared attention, sympathy, and imagination; and physiological disorder in infant. It is assessed through questioning or interactive observation. Items that affect social interaction function are assigned with higher values, which are similar to the Autism Behavior Checklist. Based on the original opinion, a score less than 7 points shows less possibility of ASD; a score between 7 and 12 points indicates a need for follow-up; a score between 12 and 30 points indicates a risk of communication and interaction disorder, and a score greater than 30 points indicates a high possibility of ASD.

ABC and childhood autism rating scale

The ABC is a behavior questionnaire that is completed by child's parents or caregivers. The questionnaire covering five aspects of autism symptoms: sensory, relating, body concept and object use, language, social and self-care. Items are scored on a 4-point scale, ranging from 0 (no problem) to 3 (severe problem). The higher the score, the more serious the problem (34). The standard cutoff value was 53, and a score above 53 points indicated high probability of ASD (35). The Childhood Autism Rating Scale (CARS) is a clinician-completed tool to rate the presence and severity of ASD by incorporating information from caregivers' reports and direct observation. A score of ≥30 points indicates a possible diagnosis of ASD (36, 37). The ABC and CARS are commonly used scales in clinical practice and ASD research. The higher the scores on the two scales, the more severe the autism symptoms.

Autism diagnostic observation scale-2

The Autism Diagnostic Observation Scale-2 (ADOS-2) is a standardized and partly structured tool that provides a standardized assessment of ASD symptoms. It is a play-based, semi-structured assessment tool used to assess communication, social interaction, and restricted and repetitive behavior in individuals with ASD, which forms the part of the recommended "gold standard" for the diagnosis of ASD (38). ADOS-2 can be used as a diagnostic assessment for children aged 12 months and above with possible ASD. It includes five modules (module

T and module 1–4), and the selection of different modules depends on the age and language expression level. Based on DSM-V, ADOS-2 is composed of two parts: social affect (SA) and repetitive behavior (RRB) (39). The total score (TA) is the combined score of these two parts by following a specific algorithm. In eliminating the effect of age, TA will be transferred to the corresponding calibrated severity (CSS) score in accordance with a standardized conversion table provided by ADOS-2. The CSS of each module has a cutoff point corresponding to the diagnostic criteria. The higher scores of ADOS-2 CSS, the more severe of the autism symptoms.

Procedure

We introduced this project to 685 parents and children, 434 of which agreed to participate in this project, including 341 children with developmental disorders and 90 typically developing children. Of the 341 children with developmental disorder, 25 were excluded because of incomplete medical records, and they did not meet the inclusion criteria.

These subjects were recruited in accordance with a standardized process. During the first visit to the hospital, children suspected of developmental delay received an initial inquiry approximately 20 min by an outpatient developmental behavioral pediatrician. This process collected information about children's current health condition, developmental status, and family history. For children highly suspected of ASD, the outpatient pediatrician would schedule the evaluation, including ABC, CARS, CNBS-R2016, and ADOS-2. The parents completed the ABC by following the instruction of another developmental pediatrician; meanwhile, this pediatrician completed the CARS by observing children's behavior and interviewing their parents or guardians. A trained and qualified developmental pediatrician would complete the CNBS-R2016 during children's first visit. The ADOS-2 was scheduled within 1 week by a certified developmental pediatrician. It was completed in an assessment room approximately 20 m^2 in size, and approximately 1 h was needed for each child. DLD and GDD were recruited simultaneously in accordance with the corresponding diagnostic criteria. All DLD and GDD children recruited in this study were assessed by CNBS-R2016, ABC, and CARS. Some diagnoses could not be distinguished from ASD, and ADOS-2 was scheduled as necessary. The later scale evaluators were blinded to the initial diagnosis of outpatient pediatrician to avoid bias. The hearing and vision of all children with developmental disorders were examined to exclude disabilities caused by serious hearing and vision loss. Brain MRI, EEG, molecular genetics, and metabolic test were scheduled optionally. TD children were recruited as the control group. They did not have any developmental problems, and they were assessed in accordance with the CNBS-R2016 as a part of the routine physical examination.

The final diagnoses were established by integrating data from parent interviews, developmental status, medical records, information provided by other caregivers and teachers, and direct observation and interaction with children during at least two assessment visits. All diagnoses were confirmed by two developmental and behavioral pediatricians. Participants were excluded if they had any diseases of the nervous system, deafness, selective mutism, or other difficulties with known biomedical conditions such as metabolic or genetic diagnoses, and so on. The recruitment and diagnosis of participants are shown in **Figure 1**.

Data analysis

Data analyses were performed using SPSS 22.0 and GraphPad Prism 6.0. The Kolmogorov–Smirnov test was used to determine the distribution of the analyzed variable before analysis. Continuous normal variables are described as means ± SDs. Non-normal distribution of variables is described as median (P25 and P75). Categorical variables are described as frequencies and percentages. One-way analysis of variance (ANOVA) was performed to compare age, subscale, and DQ scores among all groups (TD, ASD, DLD, and GDD), and the least significant difference (LSD) *post hoc* test was performed for multiple comparison. In addition, the Kruskal–Wails H test was conducted to compare communication warning behavior scores of four groups. The chi-squared test (χ^2) was used for categorical variables. Spearman's correlations were conducted to examine the relationship between the communication warning behavior scores and established autism scales (ABC, CARS, and ADOS-2). $P < 0.05$ was considered statistically significant.

The ability of the communication warning behavior to predict the diagnostic category for each of the cutoffs was examined by receiver operating characteristic (ROC) analysis. In evaluating the accuracy of the diagnostic instrument, the area under the curve (AUC) was used. Based on the criteria of Swets et al. (40), the AUC value was interpreted as low diagnostic accuracy for AUC < 0.7, moderate diagnostic accuracy for AUC ranging from 0.7 to 0.9, and high diagnostic accuracy for AUC > 0.9. For each optimal cutoff point, the false-negative rate (FNR; proportion of true positive that is mistook as negative), false-positive rate (FPR; proportion of true negative that is mistook as positive), positive predictive value (PPV; proportion of a positive test result that is true positive), and negative predictive value (NPV; proportion of a negative test result that is true negative) were calculated. The consistency among the communication warning behavior of CNBS-R2016 and ASD screening tools (ABC and CARS), ASD diagnostic tool ADOS-2, and clinical diagnosis for the classification of ASD was expressed as *Kappa* value and agreement rate (AR; proportion of true and negative results). *Kappa* ≤ 0.2, *Kappa* ranging from 0.4–0.6, and

TABLE 1 Demographics and developmental levels of participants.

	ASD (a) (n = 130)	DLD (b) (n = 100)	GDD (c) (n = 86)	TD (d) (n = 90)	$X^2/F/H$	Overall group comparison P	*Post hoc* comparisons
Male (n%)	104 (80.00)	78 (78.00)	69 (80.23)	69 (76.67)	0.501*	>0.05	
Age (year)	3.09 ± 0.73	3.01 ± 0.66	3.08 ± 0.65	3.17 ± 0.66	0.936**	0.423	
Gross motor	77.30 ± 15.64	93.31 ± 9.89	67.27 ± 10.54	104.87 ± 9.15	177.827**	<0.001	c <a < b < d
Fine motor	54.02 ± 14.55	78.14 ± 10.29	60.43 ± 10.32	96.36 ± 10.73	258.113**	<0.001	a <c < b < d
Adaptive behavior	61.62 ± 17.73	86.75 ± 11.66	67.63 ± 10.22	110.52 ± 12.65	252.655**	<0.001	a <c < b < d
Language	40.08 ± 15.48	47.98 ± 9.86	56.15 ± 9.26	106.42 ± 12.32	570.711**	<0.001	a <b < c < d
Personal-social	54.56 ± 13.13	75.25 ± 8.99	61.03 ± 8.24	107.96 ± 11.99	458.398**	<0.001	a <c < b < d
Developmental quotient	57.52 ± 12.49	76.36 ± 5.96	62.20 ± 5.43	105.21 ± 5.69	625.364**	<0.001	a <c < b < d
Communication warning behavior	24 (16, 32.25)	2 (0, 4)	8 (4, 13)	0 (0, 0)	303.251***	<0.001	d <b < c < a

TD (a), typically developing children; ASD (b), autism spectrum disorder; DLD (c), developmental language disorder; GDD (d), global developmental delay. Data of communication warning behavior were shown in the format of median (P25, P75). *Data analyzed with chi-squared test. **Data analyzed with one-way ANOVA test. ***Data analyzed with Kruskal–Wails H test. The overall group comparison serves as an omnibus test comparing the means, medians, or ratio between four groups. *Post hoc* comparisons with step-up LSD correction. Two-sided at significance level of 0.05.

Kappa ≥ 0.6 were interpreted as poor, moderate, and excellent consistency, respectively (41). With regard to ABC, children with a score ≥ 68 points were classified as ASD. With regard to CARS, children with a score ≥ 30 points were classified as ASD. As for ADOS-2, children who were given moderate or severe attention based on module T and who obtained ASD diagnosis based on modules 1–4 were deemed as positive results and classified as ASD.

Results

Characteristics of the study population

A total of four hundred and six cases aged 2–5 years were included in final analyses. Among them, 130 children fulfilled the diagnostic criteria of ASD, 100 children of DLD, 86 children of GDD, and 90 TD children. Table 1 presents the demographic characteristics and developmental levels of participants assessed by CNBS-R2016 in each of these four groups. No significant differences in gender and age were observed among these four groups. With regard to developmental levels assessed by CNBS-R2016, four groups differed significantly with one another in six subscales of DQs and overall DQs (Table 1, $p < 0.05$).

Distribution of communication warning behavior score in different groups

The distribution of communication warning behavior scores of all four groups is shown in Figure 2. The communication warning behavior scores in CNBS-R2016 in children with ASD, DLD, GDD, and TD ranged from 1 to 72, 0 to 15, 0 to 44, and 0 to 5, respectively, and the median score of four groups was 24, 8, 2, and 0, respectively. The ASD group scored dramatically higher than the other three groups (Table 1, $p < 0.05$).

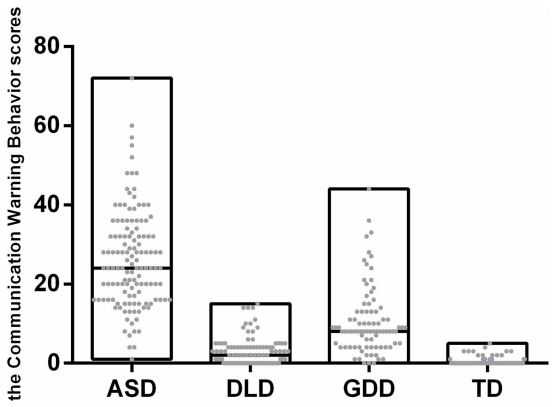

FIGURE 2
Communication warning behavior score distribution of different groups.

The correlation between quotients of communication warning behavior and established autism scales in ASD children is presented in Table 2. Correlation coefficients between

TABLE 2 Correlations between the communication warning behavior scores and established autism scales (n = 130).

	ABC	CARS	ADOS-2 CSS
Mean ± SD	67.16 ± 26.46	33.73 ± 8.40	17.97 ± 4.64
r	0.812	0.761	0.821
p	<0.05	<0.05	<0.05

ABC, Autism Behavior Checklist; CARS, Childhood Autism Rating Scale; ADOS-2 CSS, calibrated severity score of ADOS-2. $P < 0.05$ stands for significant correlation.

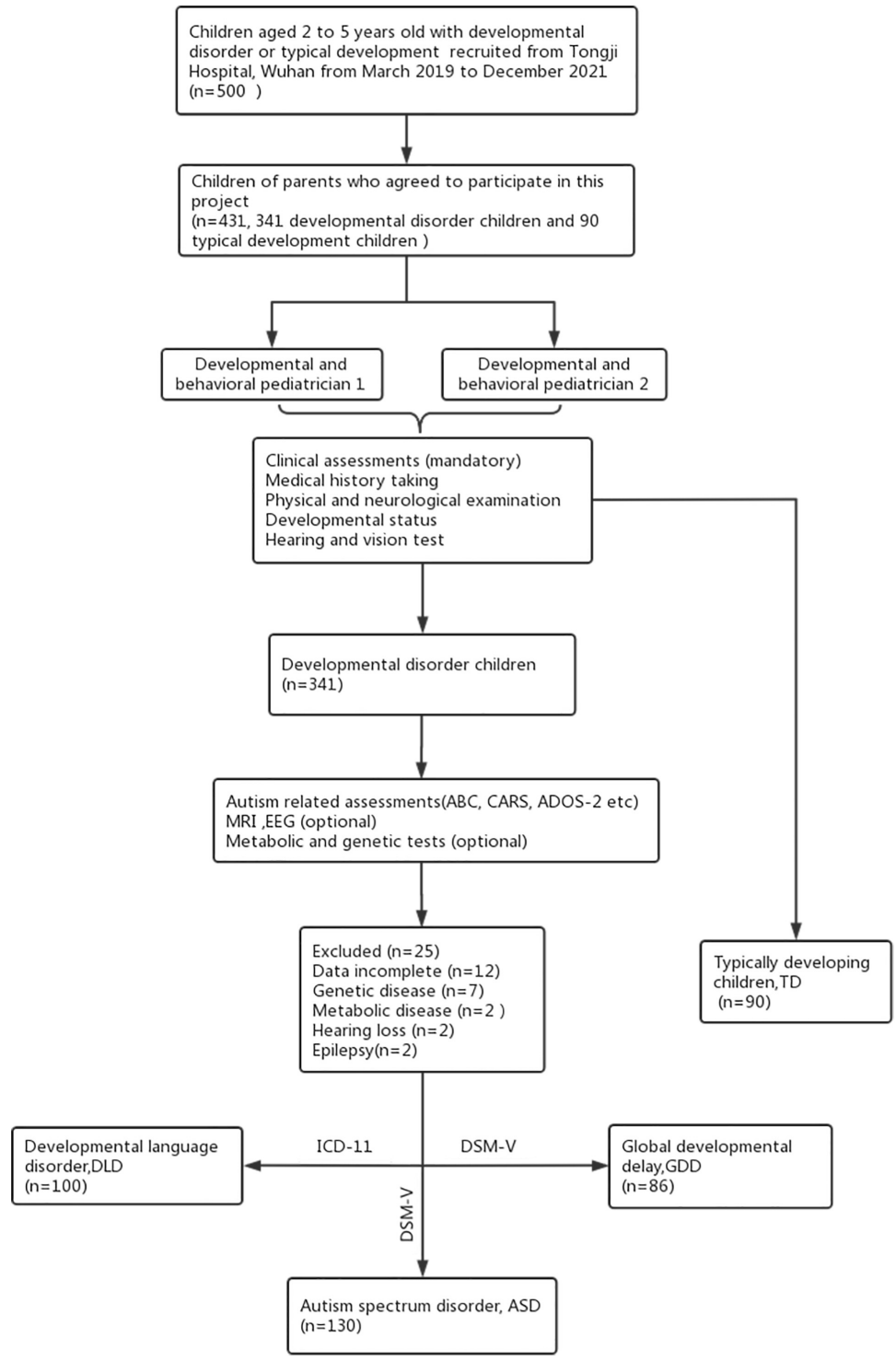

FIGURE 1
Recruitment and diagnosis of participants.

communication warning behavior scores and ABC, CARS, and ADOS-2 CSS were 0.812, 0.761, and 0.821, respectively, which were all positive and significant ($p < 0.05$).

The communication warning behavior of CNBS-R2016 score of >30 points was selected for the prediction of ASD, and consistency with ABC, CARS, ADOS-2, and clinical diagnosis

is shown in Table 3. Consistency among them was moderate except for ADOS-2, which was limited by a number of children who completed an ADOS-2 assessment. However, almost all children with 30 points and above were classified within ASD, and only three children were diagnosed as non-ASD.

Early detection of ASD based on the communication warning behavior of CNBS-R2016

The diagnostic validity (value for diagnostic classification) of CNBS-R2016 was analyzed by ROC analysis for ASD vs. TD, ASD vs. DLD, ASD vs. GDD, ASD vs. TD, and combination of DLD and GDD. The ROC curve plotted for the communication warning behavior scores (Figures 3A–D) determined the cutoff score on communication warning behavior that maximized sensitivity and specificity based on *Youden*'s index. Table 4 shows the corresponding AUC, sensitivity, specificity, FNR, false-positive rate, positive predictive value, and negative-predictive value for each measure. The AUC indicates the ability of the tests to correctly classify individuals with and without ASD. Excellent values of AUC were obtained in this study at each cutoff score. When we selected the communication warning behavior of CNBS-R2016 score of $\geq$12 points as the prediction of ASD, its consistency index with ABC, CARS, ADOS-2, and clinical diagnosis is shown in Table 5.

Discussion

As a native child developmental assessment tool in China, CNBS-R2016 has a unique role in cultural adaptability, and it has been widely used in child care department as a development assessment tool for routine well-child visit in Mainland China. Tang et al. had proven that the CNBS-R2016 and Griffiths Mental Development Scales (GMDS) showed good consistency in the developmental assessment of children with ASD (42). Compared with GMDS, CNBS-R2016 is more time efficient (an experienced psychologist can complete the CNBS-R2016 in 30–50 min). The communication warning behavior subscale was added to assess autism symptoms, which indicates that CNBS-R2016 not only has the potential function of screening for ASD but also has a comprehensive developmental level of children with ASD. Given the three-level child healthcare system in China, primary-level pediatricians transfer children with abnormities to higher-level medical institutions. Integrating ASD screening into routine well-child visits is helpful for the systematic monitoring of early ASD symptoms and the promotion of early diagnosis and intervention (43).

This study primarily explored whether a lower cutoff value for ASD screening is more recommended for referral in accordance with the Communication Warning Behavior of CNBS-R2016.

Children with ASD, GDD, DLD, and TD were different from one another in the developmental assessment of CNBS-R2016

We compared the developmental level of ASD, GDD, DLD, and TD groups assessed by CNBS-R2016. With regard to the overall developmental level, the ASD group had the lowest overall DQ, followed by GDD and DLD, and they were all significantly lower than the TD group. Children in the four above mentioned groups differed from one another in the subscale of CNBS-R2016. ASD showed the lowest score in language subscale probably because speech and language problems were the main reasons that encouraged caregivers to initially seek for treatment in preschool ASD population. In addition, children in the ASD group were generally normal in gross motor but delayed in fine motor, adaptive behavior, language, and personal-social domains. The DLD group was only delayed in language, whereas the GDD group showed developmental delays in all domains. This study showed that children with different developmental disorders varied in developmental profile of CNBS-R2016. The results of CNBS-R2016 were in line with the clinical presentation of children with ASD, DLD, and GDD.

Communication warning behavior reflected core symptoms of ASD

The communication warning behavior subscale contains 33 items. Among which, 14 items were related to social communication, 5 to restricted and repetitive behavior, 3 to language, 3 to sensory, 3 to physiological disorder in infants, 2 to intelligence, and 3 to abnormal behavior. These items served as checklists of common symptoms of ASD and referred to DSM-V (29). We revealed in our study that children with the maximum communication warning behavior scores are those with ASD. Clinical subgroups, such as GDD and DLD children, may manifest a few signs of autism symptoms, with notably higher mean communication warning behavior scores than those of the TD group. Median scores of ASD, GDD, DLD, and TD groups were 24, 8, 2, and 0, respectively, and significantly different from one another ($p < 0.05$). Li et al. (42) reported a significant positive correlation between the CNBS-R2016 communication warning behavior subscale

TABLE 3 Consistency between the communication warning behavior (cutoff value = 30) of CNBS-R2016 and ABC, CARS, ADOS-2, and clinical diagnosis for the classification of ASD.

		ABC		CARS		ADOS-2		Clinical diagnosis	
		ASD (*n* = 135)	Non-ASD (*n* = 181)	ASD (*n* = 133)	Non-ASD (*n* = 183)	ASD (*n* = 130)	Non-ASD (*n* = 180)	ASD (*n* = 130)	Non-ASD (*n* = 276)
Communication warning behavior	>30	63 (19.94)	1 (0.31)	61 (19.30)	3 (0.95)	61 (41.22)	2 (1.35)	61 (15.02)	3 (0.74)
	≤30	72 (22.79)	180 (56.96)	72 (22.79)	180 (56.96)	69 (46.62)	16 (10.81)	69 (17.00)	273 (67.24)
	Kappa	0.494		0.476		0.137		0.529	
	AR	76.90%		76.26%		52.03%		82.27%	

Data are presented as n (%). TD, typically developing children; ASD, autism spectrum disorder; DLD, developmental language disorder; GDD, global developmental delay; AR, agreement rate.

TABLE 4 Cutoff score, sensitivity, specificity, AUC, FNR, FEP, PPV, and NPV based on ROC curve analysis to discriminate ASD and TD control, as well as ASD and non-ASD clinical groups.

	Cut-off	Sensitivity	Specificity	AUC	FNR	FPR	PPV	NPV
ASD vs. TD	3.5	0.992	0.978	0.998*	0.008	0.022	0.985	0.989
ASD vs. DLD	6.5	0.977	0.890	0.985*	0.023	0.110	0.920	0.957
ASD vs. GDD	12.5	0.892	0.849	0.910*	0.108	0.167	0.906	0.841
ASD vs. TD, DLD, and GDD	12.0	0.923	0.920	0.966*	0.080	0.082	0.833	0.962

TD, typically developing children; ASD, autism spectrum disorder; DLD, developmental language disorder; GDD, global developmental delay; AUC, area under the curve; FNR, false-negative rate. *$P < 0.05$, statistically significant.

TABLE 5 Consistency between the communication warning behavior (cutoff value = 12) of CNBS-R2016 and ABC, CARS, ADOS-2, and clinical diagnosis for the classification of ASD.

		ABC		CARS		ADOS-2		Clinical diagnosis	
		ASD (*n* = 135)	Non-ASD (*n* = 181)	ASD (*n* = 133)	Non-ASD (*n* = 179)	ASD (*n* = 130)	Non-ASD (*n* = 18)	ASD (*n* = 130)	Non-ASD (*n* = 276)
Communication warning behavior	≥12	130 (41.14)	12 (3.80)	125 (39.56)	17 (5.38)	120 (81.08)	4 (2.70)	120 (29.56)	18 (4.43)
	<12	5 (1.58)	169 (53.48)	8 (2.53)	162 (51.27)	10 (6.76)	14 (9.46)	10 (2.46)	258 (63.55)
	Kappa	0.891		0.816		0.613		0.844	
	AR	94.62%		90.82%		90.54%		93.10%	

TD, typically developing children; ASD, autism spectrum disorder; DLD, developmental language disorder; GDD, global developmental delay; AR, agreement rate.

quotient and the total ABC ($r = 0.821$, $p < 0.001$) and the total CARS ($r = 0.734$, $p < 0.001$) scores in children with ASD, respectively. ASD of preschoolers with low neurodevelopmental levels presented high scores of ABC, SRS, and CARS and a high communication warning behavior score (44). High communication warning behavior scores of children with severe autism symptoms were also verified in ASD children with sleep disorders and developmental regression (45, 46). In this study, Spearman analysis positively showed the correlations between communication warning behavior subscale quotients and scores of ABC, CARS, and ADOS-2 of the ASD group children, which were 0.812, 0.761, and 0.821, respectively ($p < 0.05$), and this result was consistent with conclusion of Li et al. (42). The communication warning behavior not only has a good correlation with ASD screening tools but also with gold standard diagnostic tool of ADOS-2. We first examined the relevance of communication warning behavior and ADOS-2 in this study and showed that communication warning behavior reflected autism core symptoms well. It could be used as an efficient ASD screening tool.

Communication warning behavior score of 12 points is the recommended cutoff value for screening of ASD

Children with a communication warning behavior score of over than 30 points are highly suspected of ASD based on the CNBS-R2016. However, we found that some children with a communication warning behavior score of less than 30 were also diagnosed with ASD later on. In this study, children with

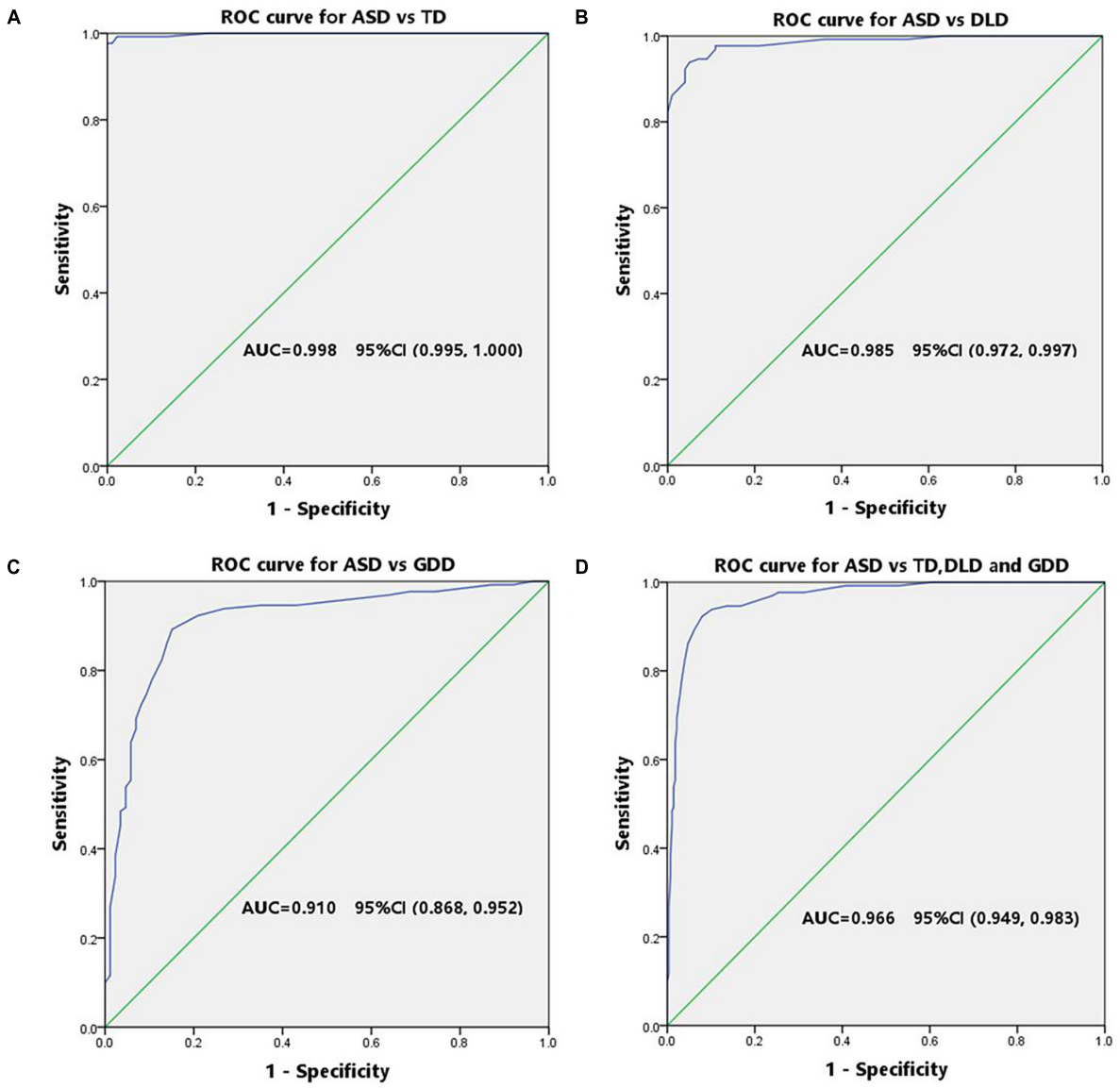

FIGURE 3
ROC curve of the communication warning behavior for screening ASD. **(A)** Receiver operator curve of ASD vs. TD. **(B)** Receiver operator curve of ASD vs. DLD. **(C)** Receiver operator curve of ASD vs. GDD. **(D)** Receiver operator curve of ASD vs. the combination of DLD and GDD.

a communication warning behavior score of below 30 points accounted for 53% of all the diagnoses of ASD. We calculated the consistency of a communication warning behavior score of over 30 points and ABC, CARS, ADOS-2, and clinical diagnosis for the prediction of ASD. Consistency indicators, including the *Kappa* value and agreement rate, were low to moderate (**Table 3**). Excellent specificity of 98.9% was obtained, but sensitivity of 46.9% was poor. Therefore, we aimed to explore an appropriate lower communication warning behavior score to ensure the best predictive effect of ASD. The ROC curve was used for this analysis. A cutoff point of 12 was achieved for distinguishing ASD from non-ASD, and the corresponding area under the ROC curve was 0.966, with sensitivity of 0.923 and 0.920, respectively.

The achieved cutoff point of 12 coincides with CNBS-R2016's previous conclusion that 12–30 points indicate the potential of communication and interaction disorder. Approximately 73–80% children with moderate to severe social communication disorder were diagnosed with ASD. Other reasons include GDD or intellectual disability (GDD/ID), hearing loss, and metabolic or genetic diseases (47–49). A total of three children with a communication warning behavior score over 12 points were diagnosed with GDD but not with ASD in this study. In addition to typical abnormal behaviors, children

with ASD often experience comorbidities, such as GDD/ID (25). Notably, children diagnosed with severe GDD/ID may also show autism-like symptoms (49). A rigorous diagnostic evaluation for ASD needs to be initiated to identify the diagnosis for those children. A total of ten children with a communication warning behavior score of less than 12 points were also diagnosed with ASD in this study. Further conditional analysis of these 10 children showed that three suffered from developmental regression at around 2.5 years old, without evident disorders before this age. A total of two children presented mild symptoms, and the five other ASD children were high functioning. Developmental regression is a warning behavior of ASD that requires further investigation in children with developmental regression. Although high functioning ASD children or those with mild symptoms are difficult to detect in the early stage because of the negative results of common ASD screening scales, the condition of these children is usually only recognized by experts. Therefore, it is no wonder that these seven children had lower communication warning behavior scores.

The sensitivity of 12 points was more excellent than 30, with no significant decrease in specificity. In clinical practice, the communication warning behavior is generally used to indicate children for further ASD diagnostic evaluation, thereby requiring high sensitivity. A cut off score of 12 points could serve this purpose well.

We also analyzed the best cutoff values for distinguishing ASD vs. TD, ASD vs. DLD, and ASD vs. GDD using the same method, which were 3.5, 6.5, and 12.5, respectively, and corresponding AUC value was all above 0.85, which indicated high sensitivity and specificity. Children with DLD, GDD, and ASD have a communication disorder with varying degrees. This study could be used as a reference for clinical classification among ASD, DLD, GDD, and TD.

Recommendations

The communication warning behavior subscale of CNBS-R2016 is important for the early detection and differential diagnosis of ASD. In this study, the communication warning behavior score of 12 points was the best cutoff for screening ASD children. Therefore, we suggest that when the communication warning behavior scores are 12 points or greater, considerable attention is needed, and further comprehensive diagnostic evaluation of ASD is required in institutions that are qualified to diagnose ASD. Moreover, in primary care hospital or institutions that are not qualified to diagnose ASD, children with a communication warning behavior score of 12 points or greater should be referred to qualified institutions for diagnosis.

Conclusion

Our study sublimated the original explanation about communication warning behavior of CNBS-R2016 and provides specific and feasible recommendations for children with communication warning behavior scores over than 12 points. A recommended cutoff point of 12 for further comprehensive diagnostic evaluation for ASD can better assist the early detection and diagnosis of ASD in China.

Limitations and further directions

The limitations of this study must be noted to provide a comprehensive understanding of the results. First, the sample children were only recruited from Wuhan, China, and this study had a single-center design. Second, the size of the control group was smaller than that of the ASD group, and different groups were not matched in numbers. In addition, the control samples except for the TD group primarily recruited those with language disorders, and most ASD children had intellectual disabilities, which may limit the power of this study. Therefore, muti-centered and larger population with more different kinds of developmental disorders is necessary to verify the reliability of the results.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethical Committee of Tongji Hospital Affiliated Tongji Medical College, Huazhong University of Science and Technology. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

SC, XH, JZ, and YH designed the study. SC, JL, DW, TY, LT, LX, MC, SH, and YH recruited subjects and collected medical information. SC carried out the analysis

and wrote the manuscript. JZ instructed the statistical method and writing idea. XH and LT revised the manuscript. YH guided and supervised all work. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the grants from the Ministry of Science and Technology of China (grant number: G2021154019L), the Huazhong University of Science and Technology Emergency Technology Research Project (grant number: 2020kfyXGYJ020), and the Key Project of Independent Innovation Research Fund of Huazhong University of Science and Technology (grant number: 2017KFYXJJ100).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Pediatrics

TYPE Original Research
PUBLISHED 12 August 2022
DOI 10.3389/fped.2022.860947

OPEN ACCESS

EDITED BY
Fei Li,
Shanghai Jiao Tong University, China

REVIEWED BY
Jie Chen,
Children's Hospital of Chongqing Medical University, China
Francesca Felicia Operto,
University of Salerno, Italy

*CORRESPONDENCE
Xiaojing Yue
yuexj@jlu.edu.cn

SPECIALTY SECTION
This article was submitted to Child and Adolescent Psychiatry, a section of the journal Frontiers in Pediatrics

RECEIVED 24 January 2022
ACCEPTED 29 July 2022
PUBLISHED 12 August 2022

CITATION
Wang T, Feng J, Xue Y, Shan L, Jia F and Yue X (2022) Feeding problems, age of introduction of complementary food and autism symptom in children with autism spectrum disorder. *Front. Pediatr.* 10:860947. doi: 10.3389/fped.2022.860947

Feeding problems, age of introduction of complementary food and autism symptom in children with autism spectrum disorder

Tiantian Wang, Junyan Feng, Yang Xue, Ling Shan, Feiyong Jia and Xiaojing Yue*

Department of Developmental and Behavioral Pediatrics, The First Hospital of Jilin University, Jilin University, Changchun, China

In this cross-sectional study, 84 children with autism spectrum disorder (ASD) and 77 healthy subjects showing typical development (TD) were reviewed. Parents reviewed the age of introduction of complementary foods (CFs), completed a demographic, diet behavior questionnaire and the Autism Behavior Checklist (ABC). The results showed that the age of introduction of CFs was later in children with ASD than their TD counterparts. The age of introduction of CFs in ASD group was positively correlated with feeding problem. While the correlation was not observed in TD group. Children in the ASD group had higher total scores of the diet behavior questionnaire and all four subdomains (poor eating ability, mealtime eating behavior, food selectivity, and parental feeding behavior). ASD symptoms were clearly associated with feeding problems. The sensory subdomain score in ABC was positively correlated with poor eating ability, mealtime behavior and total score of the diet behavior questionnaire. The social self-care subdomain score was positively correlated with food selectivity. The interaction subdomain score was negative correlated with parental feeding behavior and total score of the diet behavior questionnaire. Further studies are required to establish the utility of delayed CFs introduction and/or early feeding problems as potential indicators of ASD.

KEYWORDS

autism, children, complementary foods, age, feeding problem

Introduction

Autism spectrum disorder (ASD) is a heterogeneous neurological developmental disorder typically characterized by difficulties in communication and socialization, frequently presenting traits including fewer interests, abnormal sensory processing, and repetitive behaviors (1). The prevalence of ASD is 1 in 44 in 8-year-old children with an estimated male–female ratio of 4.2:1 (2). Feeding and eating problems are prevalent in children with ASD with a range from 40.3 to 96% (3–6). Food selectivity, food refusal and poor eating behaviors are prevalent in children with ASD (7–9).

Eating is a primary function in the human development. The period of early childhood is key in nutrition. Especially during the infancy phase, the children learn to ingest the fluid and solid food. It is not only crucial to the nutritional programming or metabolic programming, but also important to intellectual potential and physical fitness. The children should learn to ingest specific food and accept new tastes at specific age. Disruption of the process of learning to eat and accept new tastes during the critical "window" of opportunity can result in both oral-sensory and oral-motor dysfunction (10). The physical process of feeding can be disrupted through neurodevelopmental disabilities.

At present, the researches on feeding problem of ASD children mostly focus on children over 3 years old. Research on early feeding disorders in ASD has been limited. Brzóska et al. (11) highlighted that feeding problems might show up very early in the course of ASD, and dietary problems are more common during the 1st year of life from the time of introduction of complementary foods (CFs). Emond et al. (12) reported late introduction of solid foods in infants with ASD after 6 months, described as "slow feeders" at 6 months. Owing to the prevalence of feeding problems in ASD children and associated negative consequences, clinicians should be alert to the presence of these symptoms early in life. However, limited empirical information is available on the age of introduction of CFs in ASD infants. The current study was conducted to determine the potential interrelationships between age of introduction of CFs, feeding problems and ASD symptoms. A comparison group of children showing typical development (TD) was included to assess the differences in feeding problems and age of introduction of CFs relative to subjects with ASD. The main aims of the study were to: (1) compare the age of introduction of CFs between children with and without ASD, (2) compare feeding problems between children with and without ASD, and (3) examine the relationships between age of introduction of CFs, feeding problems and symptoms of ASD.

Methods

Participants

This was a cross-sectional study of children with ASD ($N = 84$) and healthy controls ($N = 77$). The data were collected from clinical notes of children between the ages of 2 and 5 who were diagnosed with ASD in the Department of Developmental and Behavioral Pediatrics (The First Hospital of Jilin University, Changchun, China) from October 2018 to April 2019. They were diagnosed by a multidisciplinary team following the American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 criteria. Regarding ASD severity level, 42 children (50%) had mild ASD, 33 children (39%) had moderate ASD, 9 children (11%) had severe ASD. Seventy-nine children (94%) had coexisting global developmental delay. The data of healthy controls that were collected from the clinical notes of the healthy children who were routine check-ups in the same hospital during the same time. Children with a history of other neurological and severe somatic disease, head trauma and uncontrolled seizures were excluded from study. The study protocol was approved by the ethics committee of the First Hospital of Jilin University and informed consent obtained from all parents.

Measures

Parents completed a demographic questionnaire on general information (age, gender, date of birth, birth weight, BMI, early feeding pattern, parents' educational level, family income and age of introduction of CFs). Parents also completed a diet behavior questionnaire developed by Zhou et al. based on the Children's Eating Behavior Inventory (CEBI), Mealtime Behavior Questionnaire (MBQ), Children's Eating Behavior Questionnaire (CEBQ), Chinese version of the Identification and Management of Feeding Difficulties (IMFED), and adjusted according to Chinese dietary habits (13). Cronbach's alpha coefficient with the diet behavior questionnaire of Zhou and co-workers was 0.86, suggesting robust internal consistency (13). The questionnaire consists of 29 items and measures four main factors: poor eating ability, mealtime eating behavior, food selectivity, and parental feeding behavior. The poor eating ability scale mainly measures whether children have difficulties in eating, such as mastication or swallowing. Mealtime behavior mainly measures children's bad behavior when eating, such as leaving the table. Food selectivity measures whether children have food choices, such as eating the same food repeatedly or rejecting a certain food. Parental feeding behavior measures parents' feeding behavior, such as chasing their children to feed, or always worrying about their children eating less. A 5-point Likert scale was used. For each item, frequency was assessed based on response options of: never (0), rarely (1), sometimes (2), often (3), and always or almost always (4). The Autism Behavior Checklist (ABC) was applied to assess ASD symptoms. The ABC is a list of 57 "yes" or "no" questions, with each question corresponding to a specific symptomatic category whereby five categories are evaluated: sensory, social and self-help, interaction, stereotypes and object use, and language. The scores of single items are rated 1 to 4, with a total score of $\geq$53 used as a cutoff value for suspected autism (14).

Statistical analysis

SPSS23.0 software was used for statistical analysis of data. Continuous variables with normal distributions are represented as the means $\pm$ standard deviations (SDs) and the two independent samples t-test used for comparison between

TABLE 1 Demographic data of TD and ASD subjects.

	TD ($N = 77$)	ASD ($N = 84$)	$t/\chi^2/U$	p
Gender			11.104	0.001*
Male	46 (59.7%)	70 (83.3%)		
Female	31 (40.3%)	14 (16.7%)		
Age (month)	35 (28–44)	33 (28–41)	−0.962	0.364
BMI (kg/m^2)	17.03 ± 1.90	17.01 ± 2.50	0.048	0.962
Birth weight (kg)	3.46 ± 0.49	3.46 ± 0.58	−0.063	0.95
Feeding pattern			1.705	0.192
BF	21 (27.3%)	31 (36.9%)		
FF	56 (72.7%)	53 (63.1%)		
Maternal education			0.023	0.879
Primary	44 (57.1%)	49 (58.3%)		
High	33 (42.9%)	35 (41.7%)		
Paternal education			0.287	0.592
Primary	49 (63.6%)	49 (59.5%)		
High	28 (36.4%)	35 (40.5%)		
Family income (RMB)	5000 (3333–6667)	6667 (3333–8333)	−1.652	0.099

BF, Breastfeeding; FF, formula feeding; TD, typical development; ASD, autism spectrum disorder; CFs, complementary feeding, *p < 0.05.

TABLE 2 Comparison of the age of introduction of CFs in the ASD and TD groups.

	B	SE	Waldχ^2	p
Age of CFs				
TD group	−0.740	0.2824	6.872	0.009*
ASD group	-	-	-	-
Sex (Male)	0.257	0.3144	0.666	0.414
Sex (Female)	-	-	-	-

ASD, autism spectrum disorder; TD, typical development; CFs, complementary foods, *p < 0.05.

groups. Continuous variables with abnormal distributions are represented as the medians (P25–P75) and the Mann-Whitney U test used for comparison between groups. Categorical variables are represented as frequencies (percentages) and the $\chi 2$ test used for comparison between groups. Correlation tests were performed using Spearman's correlation analysis. Differences were considered statistically significant at $P < 0.05$.

To manage possible differences between the two groups, we used generalized linear model (GLM) for further group comparisons to minimize the impact of other factors. We used the age of introduction of CFs and the scores of the diet behavior questionnaire as dependent variables, respectively, included categorical (group, sex) on which the two groups differed significantly as factors and covariates, respectively and ran the regression models.

Results

Comparison of clinical baseline data between the ASD and TD groups

The study included 84 children with ASD and 77 TD children. No significant differences in chronological age, body mass index (BMI), birth weight, feeding patterns, family income, and degree of maternal and paternal education were observed between ASD and TD groups. The ASD group contained more boys than the TD group (83.3 and 59.7%, respectively, $p = 0.001$) (Table 1).

The age of introduction of CFs in the ASD and TD groups

We performed GLM in order to account for the significant baseline differences in our groups as we examined the age of introduction of CFs. For these analyses we used the age of introduction of CFs as dependent variables. The sex which the groups significantly differed at baseline, was included as covariates when analyzing group difference. The sex has no significant effect on the age of introduction of CFs ($P > 0.05$). The age of introduction of CFs of the TD group were lower than those of the ASD group ($B = -0.740$, $P < 0.05$) (Table 2).

TABLE 3 Comparison of feeding problems between ASD and TD groups.

		B	SE	Waldχ^2	*p*
PEA					
	TD group	−1.750	0.4531	14.913	<0.0001*
	ASD group	-	-	-	-
	Sex (Male)	−0.829	0.5044	2.704	0.1
	Sex (Female)	-	-	-	-
MB					
	TD group	−2.453	0.8198	8.593	0.003*
	ASD group	-	-	-	-
	Sex (Male)	−2.388	0.9126	6.848	0.009*
	Sex (Female)	-	-	-	-
FS					
	TD group	−1.910	0.6082	9.864	0.002*
	ASD group	-	-	-	-
	Sex (Male)	−1.381	0.6770	4.161	0.041*
	Sex (Female)	-	-	-	-
PFB					
	TD group	−2.270	0.6163	13.564	<0.0001*
	ASD group	-	-	-	-
	Sex (Male)	−1.419	0.6861	4.280	0.039*
	Sex (Female)	-	-	-	-
Total					
	TD group	−8.178	1.9271	18.008	<0.0001*
	ASD group	-	-	-	-
	Sex (Male)	−6.291	2.1451	8.600	0.003*
	Sex (Female)	-	-	-	-

PEA, poor eating ability; MB, mealtime behavior; FS, food selectivity; PFB, parental feeding behavior, *p < 0.05.

TABLE 4 Relationships between age of introduction of CFs and eating and feeding problems in subjects of TD and ASD group.

		PEA	MB	FS	PFB	Total
TD	r	−0.113	−0.005	0.035	−0.01	−0.031
	p	0.329	0.965	0.761	0.93	0.791
ASD	r	0.171	0.328	0.113	0.098	0.25
	p	0.119	0.002*	0.307	0.374	0.022*

PEA, poor eating ability; MB, mealtime behavior; FS, food selectivity; PFB, parental feeding behavior, *p < 0.05.

Eating and feeding problems between ASD and TD groups

To address the issue of group differences in eating and feeding problems, we examined the scores obtained with the diet behavior questionnaire. We performed GLM in order to account for the significant baseline differences in our groups as we examined the scores obtained with the diet behavior questionnaire. For these analyses we used the scores obtained with the diet behavior questionnaire as dependent variables. The sex which the groups significantly differed at baseline, was included as covariates when analyzing group difference. After adjusting for the effect of baseline sex, total scores of the TD group were lower than those of the ASD group ($B = -8.178$, $P < 0.05$). Furthermore, subjects in the TD group had significantly lower scores for all four subdomains (poor eating ability, mealtime behavior, food selectivity, and parental feeding behavior) compared to those in the ASD group ($P < 0.05$; Table 3).

TABLE 5 Relationships between age of introduction of CFs and symptoms in subjects with ASD.

rs	Total	BU	Sensory	SS	Language	Interaction
r	−0.011	−0.011	0.002	−0.017	−0.137	0.009
p	0.92	0.92	0.986	0.876	0.215	0.937

BU, Body and object use; SS, social and self-help.

TABLE 6 Relationships between eating and feeding problems and symptoms in subjects with ASD.

		Total ABC	BU	Sensory	SS	Language	Interaction
PEA	r	0.083	0.087	0.278	0.210	−0.044	−0.21
	p	0.453	0.431	0.01*	0.055	0.690	0.055
MB	r	0.050	0.117	0.217	0.037	0.062	−0.160
	p	0.649	0.289	0.047*	0.74	0.576	0.145
FS	r	0.054	0.028	0.212	0.223	0.010	−0.152
	p	0.627	0.8	0.053	0.041*	0.927	0.167
PFB	r	−0.117	0.002	0.171	0.067	−0.167	−0.344
	p	0.29	0.983	0.119	0.546	0.129	0.001*
total	r	0.007	0.091	0.274	0.132	−0.056	−0.288
	p	0.952	0.411	0.012*	0.233	0.613	0.008*

PEA, poor eating ability; MB: mealtime behavior; FS, food selectivity; PFB, parental feeding behavior; BU, Body and object use; SS, social and self-help, *$p < 0.05$.

Relationships between age of introduction of CFs and eating and feeding problems in subjects of TD and ASD group

No statistically significant correlation was observed between the age of introduction of CFs and eating and feeding problems in the TD group ($P > 0.5$). The age of introduction of CFs was positively correlated with the total diet behavior questionnaire score in the ASD group. Correlation analysis was further performed to determine the association between age of introduction of CFs and the four subdomains of the diet behavior questionnaire in the ASD group. Statistically significant correlation was observed only for mealtime behavior. Specifically, the age of introduction of CFs was positively correlated with the score for mealtime behavior (Table 4).

Relationships between age of introduction of CFs and symptoms in subjects with ASD

Correlation analysis between the age of introduction of CFs and ABC scores used to assess ASD symptoms was performed in the ASD group. No significant associations were identified, either for total ABC scores or scores of the five subdomains (sensory, social and self-help, interaction, stereotypes and object use, and language; Table 5).

Relationships between eating and feeding problems and symptoms in subjects with ASD

Correlation analysis was further performed for the associations between five subdomains of ABC and four subdomains of the diet behavior questionnaire in the ASD group. The sensory subdomain score was positively correlated with poor eating ability, mealtime behavior, and total score of the diet behavior questionnaire. No significant correlation was found for food selectivity and parental feeding behavior. The social and self-help subdomain score was positively correlated with food selectivity. No significant correlation was found for poor eating ability, mealtime behavior, parental feeding behavior and total score. The interaction subdomain score was negatively correlated with parental feeding behavior and total score of the diet behavior questionnaire. No significant correlation was found for poor eating behavior, mealtime behavior and food selectivity. Body and object use subdomain, language subdomain and total ABC scores were not significantly correlated with scores of the total diet behavior questionnaire and its subdomains (Table 6).

Discussion

In the present study, eating and feeding problems and age of introduction of CFs were compared between children with and without ASD. We additionally examined the potential

associations between the age of CFs introduction, eating and feeding problems and ASD symptoms. The main findings were as follows: (1) compared to subjects with TD, most children with ASD were introduced to CFs at a later stage, (2) age of introduction of CFs was positively correlated with total score of the diet behavior questionnaire and mealtime behavior in the ASD group, and (3) children with ASD showed more feeding problems than the TD group, which were associated with symptoms of ASD.

Compared to children showing TD, those with ASD were introduced to CFs later, inconsistent with the report of Huxham et al. (15) showing that the mean age of introduction of CFs was earlier at 5 months in children with ASD. This difference may be due to the different definitions of CFs between the reports. Formula milk was not included as CFs in our study (16). Huxham et al. (15) examined infants who were exclusively breastfed and included formula in the definition of CFs. Their study showed that 38.8% subjects with ASD struggled to ingest spoon-fed pureed foods and 55.6% had difficulties with lumpy foods consistent with our findings. Zobel et al. (17) reported that ASD participants consumed pureed foods at 6.6 months relative to 5.89 months for TD participants, with no significant group mean differences. The group of Emond 2010 observed that children with ASD had difficulty in accepting solids after 6 months and proposed that this could be an early symptom of problems with accepting change by autistic subjects (12). Brzóska et al. (11) also found a delayed introduction of foods with solid and lumpy structure. These results also present delayed introduction of CFs consistent with our findings. The reason why children with ASD have delayed introduction of CFs we analyze may be the following aspects: (1) Introduction to CFs is one of the means by which infants interact with their parents. Responsive feeding is an important contributory factor to the success of introduction to CFs, emphasizing the interaction and emotional communication between parents and infants during feeding, which encourages infants to signal hunger and satiety and mothers to identify these cues and provide timely and appropriate responses (18). A core feature of ASD is social skill deficits. Poor emotional communication between parents and infants may make responsive feeding difficult, leading to delayed introduction of CFs. (2) In addition, children with ASD often show the typical sameness of routine, inflexibility, and fear of novelty, which may also be a potential reason for delayed introduction of CFs. The delay in acceptance of CFs in infants may be an early signal of ASD. (3) Moreover, the process of eating is associated with multiple sensations. Abnormalities in sensory processing processes to the environment are associated with ASD (19). Earlier reports suggest that infants diagnosed later with ASD are more perceptually sensitive to environmental stimuli (20–22). Infants with ASD may therefore be more sensitive to novel dietary experiences, leading to refusal to accept these foods and consequential delay in introduction of CFs in their diet.

We observed that the age of introduction of CFs was positively correlated with the total diet behavior questionnaire and eating behavior scores in the ASD group. While this correlation was not observed in TD group. Further investigation of the potential relationship between age of CFs introduction and symptoms of ASD (assessed via ABC scores) revealed no significant correlations. Delayed introduction of CFs may also be an early sign of eating and feeding problems in children with ASD.

In our study, children with ASD presented with more eating and feeding problems than their same-age non-ASD peers. Total scores along with scores for all four subdomains (poor eating ability, mealtime eating behavior, food selectivity, and parental feeding behavior) of children in the ASD group were consistently higher relative to the TD group, in keeping with empirical findings reported in the literature (17, 23–28). Further analysis of the correlation between feeding problems and autism symptoms showed that the sensory score was positively correlated with poor eating ability, mealtime behavior and total score of the diet behavior questionnaire. Our data on feeding problems support previous findings (8, 17, 19). Sensory impairments are frequent in children with ASD (29). Recent studies suggested that feeding problem in children with ASD may be related to sensory processing dysfunction (17, 19). Atypical sensory processing, such as sensitivity to color, taste, smell, and/or texture of food may lead children with ASD to refuse to food (30). Sensory impairments in children with ASD may be one factor that interferes with mealtime behavior (17). We did not detect a correlation between sensory score and food selectivity inconsistent with previous study, possibly since sensory behaviors were analyzed via subdomains of the ABC scale, which is not a professional sensory scale and incorporates a lower number of items for evaluation of food selectivity. However, we found the social self-care subdomain score was positively correlated with food selectivity. The reason may be children score higher in social self-care subdomain often cannot take food by themselves. Their parents may find more food selectivity problems when feeding them, compared with parents whose children can take food independently. However, some of our results were inconsistent with previous studies, which found no association between eating and feeding problems and interaction impairment severity (27). The interaction subdomain score was negatively correlated with parental feeding behavior and total score of the diet behavior questionnaire. One possibility to explain these associations is that children displaying better interactions respond to their parents more clearly that they do not want to eat the food their parents supply through expression, gesture or language, making it easier for parents to detect problems of their child. In this case, parents use more commands or distraction tactics by talking or coaxing the child to eat, which represent disruptive parental feeding behavior. Another reason maybe our sample size is small. This correlation may not exist in large sample

studies because the correlation coefficient in our study is very small.

To our knowledge, this study is one of the first to report a significant association between age of introduction of CFs and feeding issues in children with ASD. The age of CFs introduction was positively associated with feeding problems in ASD group, not associated with feeding problems in TD group. The feeding problems were positively associated with symptoms of ASD. There may be some correlation between age of CFs introduction and symptoms of ASD. But our study did not find the correlation between age of CFs introduction and symptoms of ASD. It requires further investigation. Previous studies suggest that detection of early-life feeding problems is relevant for early diagnosis of ASD and could be potentially included as an ASD-specific screening tool (24). Our results may provide a preliminary point that the introduction of CFs can be included in detection of early-life feeding problems. Not just the time of introduction of CFs, but details of introduction of CFs. It requires further exploration. Feeding problems are additionally associated with symptoms of ASD. Accordingly, in clinical practice, the impact of ASD symptoms on feeding problems should be considered in comprehensive assessment and intervention approaches for children with ASD (6).

Our study has several limitations that should be taken into consideration. Firstly, the sample size was relatively small and differences in the male-female ratio were observed between the two sample groups. Since ASD is generally more prevalent in boys, our clinical sample was not equally distributed between the sexes. Although we made adjustments in statistics, future studies including the sex matched control group will be better. Secondly, we only analyzed the age of introduction of CFs and did not include more detailed information. Thirdly, our study was performed at a single center and involved a relatively small number of subjects. Further detailed multicenter, large-scale clinical studies on samples with well-matched sex ratios are warranted. In addition, we did not include parents' eating habits which also affect feeding. Further studies including factors of parents' eating habits are needed.

Conclusion

Our data reveal a delayed time of introduction of CFs in children with ASD. The age of CFs introduction is associated with later feeding problems. ASD subjects present more feeding problems than TD subjects, which are clearly associated with symptoms of ASD. Based on the collective findings, we propose that clinicians should pay attention to infants presenting with difficulties in adjusting to introduction of dietary CFs who may have more feeding problems later and give the parents some guidance.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the First Hospital of Jilin University. The parents of participants provided written informed consent to participate in this study.

Author contributions

XY and TW devised the project and the main conceptual ideas, worked out almost all of the technical details, and performed statistical analyses with assistance from FJ. XY, TW, LS, YX, and JF performed data collections and measurements. TW wrote the manuscript, with assistance from XY. All authors contributed to the article and approved the submitted version.

Funding

The study was supported by the National Nature Science Foundation of China (81973054), Key Scientific and Technological Projects of Guangdong Province (2018B030335001), Effect of vitamin D regulating glutamate NMDA receptor on symptoms of autism rat model and its mechanism (20200201507JC).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Psychiatry

TYPE Original Research
PUBLISHED 09 November 2022
DOI 10.3389/fpsyt.2022.977879

OPEN ACCESS

EDITED BY
Yiping Shen,
Harvard Medical School, United States

REVIEWED BY
Sitong Chen,
Victoria University, Australia
Weizhao Lu,
Shandong First Medical University, China
Say How Ong,
Institute of Mental Health, Singapore

*CORRESPONDENCE
DaLi Lu
childpsy2012@163.com
Jin Jing
jingjin@mail.sysu.edu.cn

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to ADHD, a section of the journal Frontiers in Psychiatry

RECEIVED 25 June 2022
ACCEPTED 24 October 2022
PUBLISHED 09 November 2022

CITATION
Wu J-B, Yin X-N, Qiu S-Y, Wen G-M, Yang W-K, Zhang J-Y, Zhao Y-F, Wang X, Hong X-B, Lu D and Jing J (2022) Association between screen time and hyperactive behaviors in children under 3 years in China. *Front. Psychiatry* 13:977879. doi: 10.3389/fpsyt.2022.977879

Association between screen time and hyperactive behaviors in children under 3 years in China

Jian-Bo Wu[1†], Xiao-Na Yin[1†], Shuang-Yan Qiu[1], Guo-Ming Wen[1], Wei-Kang Yang[1], Jing-Yu Zhang[1], Ya-Fen Zhao[1], Xin Wang[2], Xiao-Bing Hong[3], DaLi Lu[1*] and Jin Jing[4*]

[1]Shenzhen Longhua Maternity and Child Healthcare Hospital, Shenzhen, China, [2]Key Laboratory of Brain, Cognition and Education Science, Ministry of Education, Institute for Brain Research and Rehabilitation, Guangdong Key Laboratory of Mental Health and Cognitive Science, South China Normal University, Guangzhou, China, [3]School of Management, Jinan University, Guangzhou, China, [4]Department of Maternal and Child Health, School of Public Health, Sun Yat-sen University, Guangzhou, China

Background: Screen time during early life has increased dramatically among Chinese children. Excessive screen time has raised growing concerns about the neuropsychological development of children. The effects of screen exposure on early life and the boundary between screen time and hyperactive behaviors are well worth investigating. We examined associations between screen time and hyperactive behaviors in children under the age of 3 years using data from the Longhua Children Cohort Study (LCCS).

Methods: A cross-sectional study was conducted among 42,841 3-year-old children from Longhua District, Shenzhen. Information on socio-demographic characteristics, children's annual screen time since birth, and hyperactive behaviors (measured by the Conners Parental Symptom Questionnaire) was collected through self-administered structured questionnaires completed by the primary caregiver. A series of logistic regression models assessed the association between screen time and hyperactive behaviors.

Results: The average daily screen time of children under the age of 3 years was 55.83 ± 58.54 min, and screen time increased with age. Binomial logistic regression analysis found that the earlier the screen exposure, the greater the risk of hyperactive behaviors. Using binary logistic regression model, after controlling for confounding factors, the study found that more screen time was more associated with hyperactive behaviors. For children aged 0–3 years with daily screen time exceeding 90, 120, 150, and 180 min, the risk values for hyperactive behaviors were 1.98 [95% confidence interval (CI): 1.05, 3.78), 2.71 (95%CI:1.38, 5.30), 3.17 (95% CI: 1.50, 6.65), and 4.62 (95% CI: 2.45, 8.71)], respectively.

Conclusion: Early screen exposure may be associated with hyperactive behaviors in children under the age of 3 years. More than 90 min of screen time per day in children under 3 years was associated with hyperactive behaviors. The findings support the importance of screen time interventions for children under 3 years.

KEYWORDS

hyperactive behaviors, screen time, early life, boundary, developmental sensitivity

Introduction

Attention-deficit hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders that often affects an individual's educational achievement and peer relationships, and is associated with an increased risk of adverse life events, such as antisocial activity and illicit drug use (1). Hyperactive behaviors are the main clinical manifestations of ADHD (2) and are one of the most common neurobehavioral conditions in 3-year-old children. In addition, hyperactive behaviors also exert long-term economic burden upon families and the entire country (3). Hyperactive behaviors can emerge in early childhood and continue into adulthood, which may lead to a lifetime dysfunction without effective treatment or prevention (4). The causes of hyperactive behaviors are still unknown, but results from a wide range of epidemiological studies have established a relationship with prenatal exposure to environmental factors. Screen choice has increased significantly in the last two decades (5), and there is growing concern that screen time may have a negative impact on mental health (6). The effects of screen exposure on early life and the boundary between screen time and hyperactive behaviors are well worth investigating.

A Canadian study found that children aged 3–5 years who spent more than 2 h per day in front of a screen had a 7.7 times higher risk of developing hyperactive behaviors than those who spent less than 0.5 h a day in front of a screen (7). A cross-sectional study of school-age children has shown that an increased TV time is associated with hyperactive behaviors (8). Screen time should be considered a risk factor for ADHD symptoms, according to a study examining sedentary behavior in adolescents (9). One review looked at the association between screen time and hyperactive behaviors over nearly four decades and found a slightly significant statistical association (10). Some studies have used theoretical models to explain the relationship between screen exposure and hyperactive behaviors, and estimated that the rapid conversion of electronic screen images would increase children's excitability and cause hyperactive behaviors (11). Previous studies have mostly focused on children and adolescents, and there have been few studies on the relationship between screen time and hyperactive behaviors in children under the age of 3. There were no studies on the threshold between screen time and hyperactive behaviors in children under the age of 3. However, we do know that early screen time has a greater impact on children's neuropsychological development because the brains of children under 3 years of age will be undergoing rapid development (12, 13). Therefore, we used data from the Chinese Longhua Children Cohort Study (LCCS) to investigate the association of early screen exposure and longer screen time on hyperactive behaviors in children under 3 years of age.

Materials and methods

Study population

Participants in this study were from the 2019 to 2020 LCCS survey. The LCCS is an ongoing prospective cohort study of preschoolers in Longhua, Shenzhen, China, which aims to assess the influence of the family environment on early childhood psychobehavioral development. During 2019–2020, children aged around three and their parents were enrolled from 250 kindergartens in Longhua District of Shenzhen, China. Exclusion criteria were children with serious physical illness or mental disorder. A total of 51,520 questionnaires were sent out from 2019 to 2020, and 47,113 were returned with a recovery rate of 91.45%. The sample size of the study was 42,841 questionnaires (excluding 2,111 that provided incomplete exposure and outcome information). This study was approved by the Ethics Committee of the Longhua Maternal and Child Health Hospital of Shenzhen (ethics license number: 2016102501) and the School of Public Health of Sun Yat-sen University (ethics license number: 2015–016). All participants provided informed written consent to participate in this study.

Data collection

To create a more standardized survey, members of the research team trained kindergarten principals and doctors twice a year. Each kindergarten held a parents' meeting in school and invited primary caregivers of 3-year-old children (generally

the mother) to attend. After obtaining informed consent from the primary caregiver, a questionnaire survey was conducted. This survey used the electronic questionnaire of WeChat small program following other studies (14, 15) WeChat as the product of Tencent company is a widely used social communication app with more than 1.2 billion users in China and WeChat small program is easily obtained and spread in WeChat, which has an excellent user experience. The electronic questionnaire was set up in logical conditions, and if missed, the system would send automatic prompts. Information was obtained via questionnaire which contained screen time for children under 3 years of age, hyperactive behavior status, general information about the children (such as sex, age), parents' socio-demographic characteristics (including age at birth, educational level, monthly family income, and marital status).

Measurement of exposure to electronic screen (primary exposure variable)

Caregivers of children were investigated retrospectively by questionnaire. The aim was to investigate screen exposure and screen time in children under 3 years of age. The following is an excerpt from the questionnaire for 0–1-year-olds (see **Table 1**).

Measurement of hyperactive behaviors (outcome variable)

Children's hyperactive behaviors were measured using the hyperactivity index (HI) of Conners' Parent Rating Scale-48 item version (CPRS-48). CPRS-48 is an internationally transmitted and validated screening tool for assessing behavioral difficulties in children aged 3–16 years (16). This tool has been translated into Chinese and has shown good reliability and validity (17). CPRS-48 Cronbach α coefficient was 0.83, the composite reliability was 0.94, and the average variance extracted was 0.77. The results of a confirmatory factor analysis revealed that HI had good construct validity (most of the items had factor loadings above 0.60, CFI = 0.91, GFI = 0.95, RMSEA = 0.08) (18). These findings indicated that HI is a reliable and validated tool for the measurement of Chinese children's hyperactive behaviors. HI is comprised of 10 items. The specific contents of the 10 items are as follows: excitable and impulsive; cries easily or often; restless in a squirmy sense; restless, always up, and on the go; destructive; fails to finish things; high distractibility or low attention span; quick and drastic mood changes; easily frustrated; and disturbs other children. Each item is rated on a scale of 0–3 depending on the extent to which each statement is true of the children's behaviors, i.e., never (for a score of 0), sometimes (score of 1), often (score of 2), and frequently (score of 3). The measurement of hyperactive behaviors was originally a continuous variable ranging between 0 and 3, where a higher score indicated a higher level of hyperactive behavior. The items were summed and then divided by 10 to get the mean score. The HI score $\geq$ 1.5 was used as the cutoff for establishing hyperactive behaviors in Chinese children (19). It was also treated categorically in previous literature, using a cut-off score of 1.5 to identify the children with and without hyperactivity behaviors (20). In the current study, the measurement of hyperactive behaviors was treated in both categorical and continuous formats for analysis.

Covariates

The following confounding covariates were included in the analysis: child's age and sex, parents' age at child's birth, parents' education level, family's monthly income, and parental marital status.

Statistical analysis

For continuous variables, we reported the mean and standard deviation (SD), and for categorical variables, numbers and proportions were presented. Chi-square tests and analysis of variance were applied to compare the socio-demographic characteristics between children with and without hyperactive behaviors. To examine the associations between exposure to

TABLE 1 Questions and options for screen exposure (0–1 years of age as an example).

No.	Questions	Options
Q1	Did your child watch television at age 0–1 years?	A = "no" B = "yes"
Q1.1	If "yes" was chosen for (1.1), how long on average did your child spend watching television per day at age 0–1 years?	Minutes
Q2	Did your child use handheld electronic devices (e.g., mobile phone, tablet computer PAD, game console, etc.) at age 0–1 years?	A = "no" B = "yes"
Q2.1	If "yes" was chosen for (2.1), how long on average did your child spend using handheld electronic devices (e.g., mobile phone, tablet computer PAD, game console, etc.) per day at age 0 1 years?	Minutes
Q3	Did your child watch computer, notebook at age 0–1 years?	A = "no" B = "yes"
Q3.1	If "yes" was chosen for (3.1), how long on average did your child spend watching computer, notebook per day at age 0–1 years?	Minutes

For example, if YES is selected for Q1, Q1.1 problems will be displayed, otherwise Q1.1 will be hidden.

electronic screens (i.e., initial age of screen exposure, daily average screen time) and hyperactive behaviors, a series of logistic regression models were fitted after adjusting for the covariates. Moreover, we conducted further analyses to probe into the sensitive period and cumulative effect between exposure to electronic screen and hyperactive behaviors. Firstly, the age sensitivity of screen time was analyzed. Binomial logistic regression analyses were used to model the association between early screen exposure and hyperactive behaviors based on annual exposure (Yes) and non-exposure (No) across the children's three age groups of 0–1, 1–2, and 2–3 years. Secondly, referring to recommendations for children's screen time in the United States, Canada and previous studies of screen time on children's behavioral boundaries of the recommendations for children's screen time (21, 22), we divided the average daily screen time of children into eight subgroups (no screen exposure, < 30 min, 31–60 min, 61–90 min, 91–120 min, 121–150 min, 151–180 min, > 181 min). By finely dividing screen time, we can more accurately guide parents in the proper use of electronic screens. Binary logistic regression was used to analyze the relationship between screen time and hyperactive behaviors in each subgroup. The results were presented as odds ratio (OR) with 95% confidence intervals (*CI*). Statistical significance was set at a two-tailed test with $P < 0.05$. Data management and statistical analysis were performed using Statistical Package for the Social Sciences (version 25.0; SPSS Inc., Chicago, IL, USA).

Results

Social characteristics and hyperactive behaviors

Table 2 shows an overview of the sociodemographic characteristics of the participants. Of the 42,841 children in the study, 345 (0.81%) had hyperactive behaviors. The number of male participants 0.93% was higher than that of female participants 0.68% ($P < 0.01$). This result supports conclusions of previous studies (23, 24). Low educational level of parents, low economic income, and single-parent families are risk factors for hyperactive behaviors. See Table 2 for details.

Distribution of screen time by age for children under 3 years of age

Figure 1 shows the comparison of screen time of children with hyperactive behaviors and children without hyperactive behaviors at different ages. Children with hyperactive behaviors have higher screen time than children without hyperactive behaviors at every age. Screen time for children under 3 years of age increased with age. Screen time for children under the age of 1 year was 34.12 ± 53.87 min. Screen time for children under 2 years old was 51.12 ± 65.77 min. Screen time for children under 3 years of age was 82.27 ± 83.47 min. Screen time for children under 3 years of age was 55.83 ± 58.54 min.

Effects of early screen exposure on children under 3 years of age

Table 3 shows that screen exposure at ages 0–1 and 1–2 is associated with hyperactive behavior. Screen exposure at 2–3 years of age was not significantly associated with hyperactive behavior (Model 1). The binomial logistic regression model (Model 2) included screen exposure and hyperactivity in three different age groups and found that 0–1 year old screen exposure was more associated with hyperactivity. The results held after adjusting for relevant covariates (model 3).

Chi-square test and conditional logistic regression analysis of screen time and hyperactive behaviors (primary outcome)

As shown in Table 4, there was a statistical difference between screen time and the chi-square test of hyperactive behaviors. After controlling for confounding factors using an unconditional logistic regression analysis, the risk of hyperactive behaviors increased as average daily screen time increased. After average daily screen time exceeded 90 min, the risk of hyperactivity increased rapidly with increased screen time.

Discussion

Screen time for children under the age of 3 years

With the rapid development of technology and economy, almost every family has electronic screen products. Many caregivers regard electronic products as "electronic babysitters" to reduce their children's disturbance (25). In addition, the constant emergence of educational video programs has increased screen exposure for children under 3 years of age (26). Our research has found that screen time increases as children age. Low parental education is an important risk factor for screen exposure, which is consistent with previous studies (27, 28). This suggests that the most important intervention group to reduce screen time in children is the population with low educational level. The survey found that 1-year-olds in the region spent 34 min, 2-year-olds spent 51 min and 3-year-olds spent 82 min in front of a screen.

The proportion of children with screen exposure before 1 year old was as high as 57.1%, while the proportion of

TABLE 2 Social characteristics and children's hyperactive behaviors.

Characteristics	Total (N = 42,841)	Hyperactive behaviors		χ^2/t	*P-value*
		No (N = 42,496)	Yes (N = 345)		
Child's age [mean ± SD (years)]	42,841	3.28 ± 0.61	3.25 ± 0.58	1.08	0.27
Child's sex [*n* (%)]					
Male	22,901	22,690 (99.07)	211 (0.93)	8.29	<0.01
Female	19,940	19,806 (99.32)	134 (0.68)		
Single child status [*n* (%)]					
Yes	26,014	25,831 (99.29)	183 (0.71)	8.60	<0.01
No	16,827	16,665 (99.03)	162 (0.96)		
Maternal age at child s birth [mean ± SD (years)]	42,481	28.11 ± 3.10	27.22 ± 3.44	28.02	<0.01
Paternal age at child s birth [mean ± SD (years)]	42,481	30.83 ± 4.90	29.47 ± 4.83	26.38	<0.01
Maternal education level [*n* (%)]					
Junior high school or lower	5,844	5,778 (98.87)	66 (1.13)	12.78	<0.01
High school	9,360	9,275 (99.09)	85 (0.91)		
College	26,163	25,978 (99.29)	185 (0.71)		
Master's degree or above	1,474	1,465 (99.39)	9 (0.61)		
Paternal education level [*n* (%)]					
Junior high school or lower	4,930	4,867 (98.72)	63 (1.28)		
High school	8,875	8,799 (99.14)	76 (0.86)		
College	26,682	26,491 (99.28)	191 (0.72)		
Master's degree or above	2,354	2,339 (99.36)	15 (0.64)		
Monthly household income [*n* (%))]					
≤¥10,000	9,592	9,481 (98.84)	111 (1.16)	21.13	<0.01
¥10,000–20,000	15,194	15,078 (99.23)	116 (0.77)		
¥20,000–30,000	9,163	9,098 (99.29)	65 (0.71)		
>¥30,000	8,892	8,839 (99.40)	53 (0.60)		
Parental marital status [*n* (%)]					
Married	41,556	41,231 (99.21)	325 (0.78)	12.20	<0.01
Unmarried/divorced/	1,228	1,210 (98.53)	18 (1.47)		
Widowed/remarried	57	55 (96.49)	2 C3.51)		

χ^2; was chi-square test: t, Student's *t*-test; SD, standard deviation; N (%), quantity (proportion).

children without screen exposure before 2 years old was only 22.40%. This significantly exceeds the screen guidelines recommended by the American Academy of Pediatrics in 2016. The screen guidelines recommend that children under 18 months should not use screens except for chatting, that children between 18 and 24 months should have limited exposure to screens, and that children between 2 and 5 years old should not spend more than 1 h a day on screen (29).

Effects of screen time on hyperactive behaviors

Our analysis of big data from birth cohorts found that the longer the children's daily screen time, the greater the risk of an increase in hyperactive behaviors. Children with hyperactive behaviors had more screen time. We found that the earlier the age of screen exposure, the more associated with hyperactive behaviors. Children under the age of 3 who spend more than 90 min a day in front of screens was associated with hyperactive behaviors, which is largely consistent with recommendations from the American Academy of Pediatrics' 2016 screen guidelines (29).

A Canadian study found that children aged 3–5 who watched electronic screens for more than 2 h a day were more likely to develop hyperactive behaviors than those who watched screens for just 30 min a day (7). Yet a British study reports that screen time is not an increased risk factor for behavioral problems in children as young as 5 (30). The difference may be related to the weaker effect of screen time on older children. This study helps clarify this question. Our study found that the earlier the exposure to electronic screens, the more likely it was to associate with hyperactive behaviors. It is possible that in

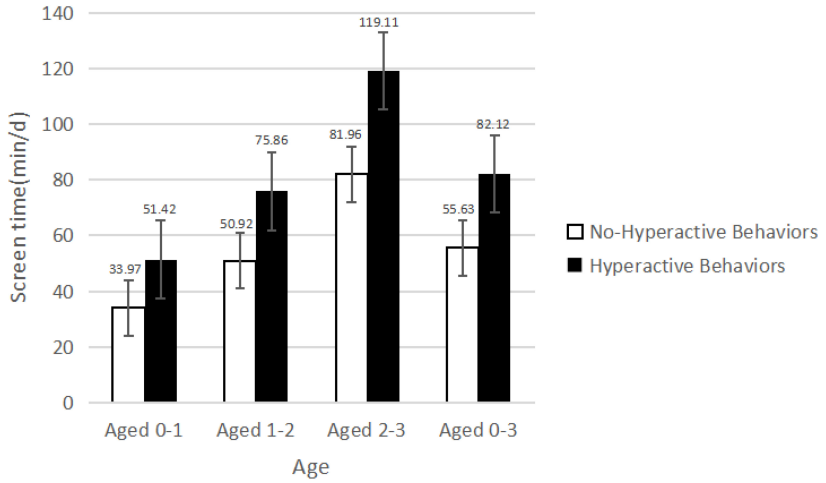

FIGURE 1
Comparison of screen time at different ages between children with hyperactive behaviors and children without hyperactive behaviors.

TABLE 3 The relationship between early screen exposure and hyperactive behaviors (N = 42,841).

Annual exposure (yes) or non-exposure (no)	Model 1‡ OR (95% *CI*)	Model 2§ OR (95% *CI*)	Model 3¶ OR (95% *CI*)
Age 0–1	1.66 (1.32–2.08)***	1.51 (1.18–1.95)**	1.48 (1.15–1.91)**
Age 1–2	1.55 (1.20–2.01)**	1.24 (0.92–1.69)	1.23 (0.90–1.66)
Age 2–3	1.31 (0.92–1.87)	1.08 (0.73–1.58)	1.05 (0.71–1.55)

‡The first model independently analyzed the association between screen exposure and hyperactive behaviors at ages 0–1, 1–2, and 2–3 years.
§The second model included screen exposure at each age into a binomial logistic regression model to analyze the association between screen exposure at each age and hyperactive behaviors.
¶The third model analyzed the association between screen exposure and hyperactivity at each age after adjusting for child's sex, number of children in the family, maternal and paternal age at child's birth, maternal and paternal education level, monthly household income, and parental marital status.
CI: Confidence intervals. **$P < 0.01$; ***$P < 0.001$.

TABLE 4 Chi-square test and conditional logistic regression analysis of screen time and hyperactive behaviors.

Daily screen time by age 0–3	No. of children	Cases (N%)	AOR (95% *CI*)	χ^2/*P-value*
No screen exposure	2,952	13 (0.44)		84.28/ < 0.001
Screen time < 30 min	12,284	84 (0.68)	1.59 (0.88–2.87)	
Screen time 31–60 min	12,071	76 (0.63)	1.41 (0.78–2.55)	
Screen time 61–90 min	7,329	54 (0.74)	1.57 (0.78–2.55)	
Screen time 91–120 min	3,613	35 (0.97)	1.98 (1.05–3.78)*	
Screen time 121–150 min	1,919	26 (1.35)	2.71 (1.38–5.30)**	
Screen time 151–180 min	974	16 (1.64)	3.17 (1.50–6.65)**	
Screen time > 181 min	1,699	41 (2.41)	4.62 (2.45–8.71)***	

AOR, Adjusted odds ratio. OR with adjustment for child's sex, number of children in the family, maternal and paternal age at child's birth, maternal and paternal education level, monthly household income, and parental marital status. *CI*, Confidence intervals; Ref, Reference. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

terms of developmental susceptibility, young children are more susceptible to the effects of electronic screens than children and adolescents (31). Young children are less able to control their arousal level when watching electronic screens, and the effects of electronic screens may be stronger in young children than in children and adolescents (32).

Previous studies have focused on the link between screen time and hyperactive behaviors in children older than 3, while few studies have looked at the effect of screen time on hyperactive behaviors in children younger than 3. A Japanese study found that ADHD at 30 months was positively correlated with time spent watching TV at 18 months, while prosocial behavior was negatively correlated with time spent watching TV, even after adjustment. However, at 30 months, there was no significant difference in the strength and difficulty questionnaire subscale based on daily TV viewing time (33).

This study is consistent with our own evidence that screen time is developmentally sensitive to the effects of hyperactive behaviors. The study also demonstrated an association between screen time and hyperactive behaviors, but this study had a high rate of lost to follow-up and had a small sample size. We used an empirically validated result to measure hyperactive behaviors in young children. The large sample size allowed us to observe the association between screen time and hyperactive behaviors, controlling for multiple confounding factors.

In 2018, a review of screen time in ADHD in the recent 40 years published in the proceedings of the National Academy of Sciences, related research were analyzed, regarding its the potential mechanism (10, 11): First, it was assumed that it was based on the excitation reaction condition allowing the child to repeatedly update electronic screen orientation response and cause increased wakefulness. Accustomed to being on a fast pace, children's baseline arousal levels may decrease, eventually leading to hyperactive behaviors. Second is the scanning and diversion hypothesis, which is based on the role of cognitive response states and believes that watching electronic screens prevents children from developing attention skills (34, 35). Because children with hyperactive behaviors have difficulty engaging in developmentally appropriate play tasks that require sustained attention, children may prefer screen devices to play-based activities because they tend to offer more multisensory and diverse stimulation (36). In addition, for children with hyperactive behaviors, parents may give them more screen time, which seems to reduce hyperactive behaviors. However, as screen time increases, these children may be more likely to miss out on real-world learning opportunities and may replace developmentally beneficial interactions, which may further exacerbate hyperactive behaviors (37, 38).

The main advantage of our study is the detailed breakdown of screen time, which gives a threshold for the effect of screen time on hyperactive behaviors. We also analyzed several potential confounding factors through a large sample of data, further supporting the relationship between screen time and hyperactive behaviors in children under 3 years of age. We also looked at earlier screen exposure, with greater risk of hyperactive behaviors. Our study reminds parents to control screen time in early childhood. More than 90 min of screen time per day may be associated with hyperactive behavior. This study has limitations. First, although the sample size of this study is large, longitudinal studies are still needed to further verify the hypothesis of causality. Second, the data collected on electronic screen exposure is retrospective and relies on parental reporting accuracy, which may be prone to recall bias. Third, this study only assessed screen time, and screen content may modulate the effects of screen exposure (39, 40). Fourth, this study was mainly performed in Longhua District of Shenzhen. Parents may have regional characteristics in terms of education level, which cannot fully represent the situation in China. Further nationwide research is needed.

Conclusion

Overall, our study found that early screen exposure may be associated with hyperactive behaviors in children under 3 years of age. More than 90 min of screen time per day in children under 3 years was associated with hyperactive behaviors in 3-year-olds. The findings support the importance of screen time interventions for children under 3. This study provides preliminary guidance for screen time use in children under 3 years of age.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

This study was approved by the Ethics Committee of the Longhua Maternal and Child Health Hospital of Shenzhen (ethics license number: 2016102501) and the School of Public Health of Sun Yat-sen University (ethics license number: 2015–016). Written informed consent to participate in this study was provided by the participants or their legal guardian/next of kin.

Author contributions

DL and JJ: conceptualization, project administration, supervision, and validation. J-BW and J-YZ: data curation. J-BW and DL: formal analysis. J-BW and W-KY: funding acquisition. X-BH and Y-FZ: investigation. S-YQ and XW: methodology. J-BW: resources. G-MW: software. J-BW and X-NY: visualization and writing – original draft and review and editing. All authors contributed to the article and approved the submitted version.

Funding

This work was funded by the National Natural Science Foundation of China (81872639), the Key-Area Research and Development Program of Guangdong Province (2019B030335001), the Shenzhen Science and Technology Innovation Committee (JCYJ20210324122609025), and the Longhua District Medical And Health Institutions Regional Scientific Research Project (2022086).

Acknowledgments

We would like to show our gratitude to the families who participated in the study, the doctors from Longhua Maternal and Child Healthcare Center, and the kindergarten teachers who took part in the investigation.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Psychiatry

TYPE Clinical Trial
PUBLISHED 13 February 2023
DOI 10.3389/fpsyt.2023.987093

OPEN ACCESS

EDITED BY
Tingyu Li,
Chongqing Medical University, China

REVIEWED BY
Weizhao Lu,
Shandong First Medical University, China
Katya Rubia,
King's College London, United Kingdom

*CORRESPONDENCE
Yi-chao Wang
✉ 12118263@zju.edu.cn

SPECIALTY SECTION
This article was submitted to ADHD, a section of the journal Frontiers in Psychiatry

RECEIVED 05 July 2022
ACCEPTED 13 January 2023
PUBLISHED 13 February 2023

CITATION
Wang Y-c, Liu J, Wu Y-c, Wei Y, Xie H-j, Zhang T and Zhang Z (2023) A randomized, sham-controlled trial of high-definition transcranial direct current stimulation on the right orbital frontal cortex in children and adolescents with attention-deficit hyperactivity disorder. *Front. Psychiatry* 14:987093. doi: 10.3389/fpsyt.2023.987093

A randomized, sham-controlled trial of high-definition transcranial direct current stimulation on the right orbital frontal cortex in children and adolescents with attention-deficit hyperactivity disorder

Yi-chao Wang [1,2]*, Jun Liu[2], Yan-chun Wu[2], Yan Wei[2], Hong-jing Xie[2], Tao Zhang[2] and Zhen Zhang[2]

[1]Affiliated Mental Health Center & Hangzhou Seventh People's Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China, [2]Zhenjiang Mental Health Center (The Fifth People's Hospital of Zhenjiang City), Zhenjiang, Jiangsu, China

Objective: This study aimed to find out the clinical and cognitive effects of high-definition transcranial direct current stimulation (HD-tDCS) on the right orbital frontal cortex (OFC) in the treatment of attention deficit hyperactivity disorder (ADHD).

Methods: A total of 56 patients with ADHD were recruited as subjects and completely and randomly divided into the HD-tDCS group and the Sham group. A 1.0 mA anode current was applied to the right OFC. The HD-tDCS group received real stimulation, while the Sham group received sham stimulation in 10 sessions of treatment. ADHD symptom assessment (the SNAP-IV Rating Scale and the Perceived Stress Questionnaire) was carried out before treatment, after the 5th and 10th stimuli, and at the 6th week after the end of all stimulations, while the cognitive effect was assessed by the Integrated Visual and Auditory Continuous Performance Test (IVA-CPT), the Stroop Color and Word Test (Stroop), and the Tower of Hanoi (TOH). Repeated-measure ANOVA was used to find out the results of both groups before and after treatment.

Results: A total of 47 patients completed all sessions and evaluations. Their SNAP-IV score, their PSQ score, the mean visual and auditory reaction times by IVA-CPT, the interference RT of Stroop Color and Word, and the number of completed steps of TOH did not change with intervention time before and after treatment ($P > 0.0031$). However, the integrated visual and audiovisual commission errors and the TOH completion time results of the HD-tDCS group were significantly decreased after the 5th intervention, the 10th intervention, and the 6th week of intervention follow-up compared to the Sham group ($P < 0.0031$).

Conclusion: This study draws cautious conclusions that HD-tDCS does not significantly alleviate the overall symptoms of patients with ADHD but leads to significant improvements in the cognitive measures of attention maintenance. The study also attempted to fill in the gaps in research studies on HD-tDCS stimulation of the right OFC.

Clinical trial registration: ChiCTR2200062616.

KEYWORDS

attention-deficit hyperactivity disorder (ADHD), orbital frontal cortex (OFC), high-definition transcranial direct current stimulation (HD-tDCS), executive function (EF), IVA-CPT

Introduction

Attention deficit hyperactivity disorder (ADHD) is a common neurodevelopmental disease among children and adolescents. Its core defects are mainly characterized by attention disorder, hyperactivity, impulsivity, and other clinical symptoms. Many children and adolescents with ADHD are associated with learning difficulties (1), with some having marked difficulties with emotional control (2). Neuropsychologists have found that there are performance defects in psychological processes with ADHD (3). Although ADHD is a complex and heterogeneous disorder (4), children and adolescents with ADHD often show performance impairments on tasks that measure some form of executive processes (5). ADHD is associated with deficits across a range of cognitive domains, such as arousal, executive functions, behavioral inhibition, motivation, set-shifting, and working memory (6). A recent meta-meta-analysis involved 34 meta-analyses of neurocognitive ADHD profiles (all ages) concerning 12 neurocognitive domains. Patients with ADHD have moderate impairments in multiple domains, including working memory, reaction time variability, inhibitory control, cognitive flexibility, intelligence/achievement, and planning/organization (7).

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation therapy. Weak current (0.5–2.0 mA) affects specific brain regions through the scalp, which acts as electrodes. Anodal transcranial direct current stimulation (ATDCS) refers to electrical current flow from the anodic electrode to the target brain region, which increases the target brain region excitation. Cathodal transcranial direct current stimulation (CTDCS) refers to electrical current flowing from the cathode electrode to the target brain region, which decreases the target brain region excitation (8). This excitability change is caused by the change in the resting membrane potential in the relevant region (9). There was a prolonged effect after 30 min of the tDCS stimulation (10), and the effect even lasted several months after repeated tDCS stimulation (11). From fMRI observation, tDCS stimulation of the prefrontal lobe improved network connectivity at rest (12). The weak current of tDCS regulates cortical excitability and spontaneous neural activity by stimulating the corresponding cerebral cortex region, thus improving the functional abnormalities of the corresponding brain regions and showing good safety and tolerability (13).

Among the existing tDCS research, there are many studies on the dorsolateral prefrontal cortex (DLPFC), some of which have achieved positive results. Allenby believes that ATDCS can reduce the reaction time of Stop-Signal Tasks, which leads to the conclusion that tDCS can alleviate subject impulsivity and delayed gratification difficulties (14). Nejati suggested that the stimulation of the right DLPFC with ATDCS could improve persistent inhibition and partial interference control (15). A combined stimulation of the DLPFC and the orbitofrontal cortex (OFC) can reduce reaction time, improve cognitive flexibility, and improve working memory (16). According to recent research, Leffa et al. performed daily sessions of 30 min of home-based tDCS for 4 weeks on 64 participants with ADHD, totaling 28 sessions, with 2.0 mA anodal-right and cathodal-left prefrontal stimulations with 35-cm^2 carbon electrodes. The efficacy results assessed by the inattentive scores of the clinician-administered versions of the Adult ADHD Self-report Scale (CASRS-I) show decreased symptoms of inattention in the active tDCS group over the three assessments compared to the sham tDCS group (17). However, according to several research studies, there is no evidence that tDCS can improve the response inhibition ability (18) and the sustained attention (19) of patients with ADHD. Some scholars suggested that the clinical efficacy and cognitive effects of tDCS in the treatment of ADHD, whether the inferior frontal gyrus (IFG) or the DLPFC, still need to be further verified in future studies (20, 21).

Traditional tDCS stimulation results in the diffusion and distribution of current in a wide range of brain regions, which may not be able to display the maximum current density directly below the electrode, leading to inaccurate positioning of tDCS stimulation. In recent years, the new high-definition tDCS (HD-tDCS) has been proposed to solve the problem of traditional tDCS in affecting the stimulation target to the extra brain regions. The HD-tDCS stimulation current is limited to the region below the electrode and thus improves the accuracy. This ensures high-current density in the main target region, minimizes the stimulation of the non-target regions, and reduces the risk of side effects. This shows that the same effect can be achieved by stimulating the corresponding brain regions with less current than conventional tDCS. Researchers stimulated the right IFG of 15 subjects with ADHD aged 10–16 years with HD-tDCS of 0.5 mA and evaluated the stimulation by the N-back test and event-related potential P300/N200. The results showed that, compared with the traditional tDCS stimulation of 1.0 mA, HD-tDCS also improved working memory and inhibitory control (22). Some researchers believe that HD-tDCS should be set as a further topic to study (23).

Orbito-frontal cortico-striato-thalamo-cortical (OFCSTC) loops, also known as the impulse/force loop, are applied in the control of impulsive behavior (24). The nerve fibers of the loop originate from the OFC and extend into the inferior caudate nucleus, then travel to the thalamus, and finally return to the OFC. The inactivation of this circuit leads to impulse control difficulties and emotional processing disorders. The OFC dysfunction was significantly associated with the severity of impulsive (25) and obsessive behavior (24). Impulsive symptoms of ADHD include excessive speech, unthinking interruptions, blurting out words, and unwillingness to wait in order, all of which involve this loop. The cortical thickness of the OFC in patients with ADHD was significantly lower than that in healthy controls (26). A Structural Covariance Network (SCN) analysis shows that the volume of gray matter on the right side of the OFC of patients with ADHD decreased significantly (27). In addition, structurally, a meta-analysis of whole-brain voxel-based morphometry (VBM) showed disorder-specific gray matter volume (GMV) abnormality in the OFC in ADHD (28). The fMRI scans showed that right OFC activation significantly decreased in patients with high-risk behavioral tendencies in the Go/NO-Go tasks (29). In addition, fMRI showed that the activation of the right OFC was associated with emotion-based risk tasks in negative emergencies, reflecting that risk-taking was associated with the ability of emotion-based risk control (30). Boys with ADHD showed disorder-specific underactivation in the OFC (31). High-resolution fMRI showed that adolescent patients with ADHD display enhanced OFC signaling of future rewards and that these increased reward-related responses were correlated with the severity of hyperactivity/impulsivity (32). Decreased cognitive capacity related to hyperactivity and impulsivity was associated with reduced OFC activity during reward expectation in patients with ADHD (28).

Although OFC was not a sufficiently activated region to be underactivated in recent fMRI meta-analyses of ADHD (33–35), there was evidence from the aforementioned studies for OFC underactivation, mainly in terms of rewards or emotions. Furthermore, impulse control difficulties and emotion-processing disorders based on OFCSTC could affect cognitive performance. In other words, relatively higher cognitive ability was associated with normalized OFC responses (32). Therefore, in this study, we hypothesized that HD-tDCS of the right OFC could alleviate the clinical symptoms, impulse control difficulties, and emotion processing of patients with ADHD to further improve their performance on cognitive tasks such as maintaining attention and inhibitory control and then test this hypothesis using a randomized, sham-controlled study.

Materials and methods

Research subjects

Inclusion criteria

Subjects included patients with ADHD who visited the general outpatient department and the children's outpatient department of the Zhenjiang Mental Health Center between March 2020 and November 2021. The patients were children and adolescents aged 8–18 years. They were diagnosed and reviewed by a senior associate chief physician or a chief physician of the department of psychiatry. All subjects met the diagnostic criteria of ADHD of the validated screening and diagnostic instrument: the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) and The International Classification of Diseases (ICD-10). Simultaneously, the subtypes of ADHD were also classified, such as inattentive, hyperactive-impulsive, or the combined subtype; The Wechsler Intelligence Scale for Children-IV Chinese version was administered to every subject, with a total IQ $\geq$ 80 (36). All children were of Chinese Han origin and were right-handed.

Exclusion criteria

Contraindications for tDCS treatment include patients with metal device implants (such as the cochlear implant, the artery clamp, and the pacemaker); history of brain trauma or cerebrovascular accident, intracranial hypertension, skull defects, epilepsy, and other serious neurological, circulatory, endocrine, and other physical diseases; audio-visual impairments and color blindness, color weakness, or narrow-angle glaucoma. The abovementioned contraindications were excluded by inquiring and collecting medical history, conducting an electrocardiogram (ECG), electroencephalogram (EEG), cranial CT, and blood routine and biochemical examinations. All subjects were evaluated for no comorbidities of other mental disorders with validated screening and diagnostic instruments: DSM-5 and ICD-10, such as substance abuse/dependence, conduct disorders, personality disorders, autism, Tourette's disorder, and obsessive-compulsive disorder. Patients who had used any medication (methylphenidate, atomoxetine, etc.) in the past and recently to treat ADHD or who received other brain stimulation (transcranial magnetic stimulation, electroconvulsive shock, etc.) were also excluded.

To calculate the sample size, we used G*Power (37) with the following settings: effect size $f = 0.25$, α level $= 0.05$, power $= 0.8$, and correlation among repeated measures $= 0.5$. The minimum sample size was found to be $n = 44$. To prevent a potentially large number of dropouts, a total of 56 subjects were recruited, including 33 boys and 23 girls. A completely randomized experimental design was adopted, and the subjects were divided into two groups according to age through a random number table: the HD-tDCS group and the Sham group (Figure 1). A general information questionnaire was developed, including age, gender, educational years, whether the subject comes from a single-parent family, age of onset, and disease. Both the participants and their guardians were informed of this study, and signed informed consent was obtained. The study was approved by the Ethics Committee of Zhenjiang Mental Health Center. This trial was conducted in accordance with the Declaration of Helsinki and the Consolidated Standards of Reporting Trials (CONSORT) guidelines (38).

Methods

While the HD-tDCS group received real stimulation, the Sham group received sham stimulation. Before receiving stimulation (T0), all subjects underwent ADHD symptom assessments (SNAP-IV rating scale, Conners Parents Questionnaire) and cognitive task [Integrated Visual and Auditory Continuous Performance Test (IVA-CPT), Stroop, Tower of Hanoi (TOH)] assessments to collect baseline data. Then, the subjects underwent either HD-tDCS stimulation or sham stimulation. The above ADHD symptom and cognitive task assessments were repeated for all subjects after the 5th stimulus (T1), the 10th stimulus (T2), and at the 6th-week follow-up after the end of all stimuli (T3).

HD-tDCS

HD-tDCS uses a multichannel stimulator (Soterix Medical, 4 × 1-C3A, USA) that uses a constant direct current stimulator of conventional tDCS (Soterix Medical, 1 × 1 Low-Intensity Transcranial DC Stimulator, 1300A, USA), delivers, and converts it to high-definition stimulation. Conventional tDCS produces diffuse brain currents. The electrodes of HD-tDCS are arranged in a 4 × 1 ring on the skull, producing a more concentrated and precise current that is confined to the return electrode ring.

Stimulation site

Five circular Ag/AgCl electrodes with a diameter of 1 cm were placed, and one anode electrode was placed on the center: the right OFC, corresponding to the standard electrode location of the International Electroencephalogram Society 10/20 System: Fp2; four cathode electrodes (i.e., return electrodes) are placed in a square around the anode, about 5 cm away from the anode, and corresponding to Fpz, Afz, AF4, and AF8 (Figure 2). Electric field simulation was performed using the HD-Explorer software (Soterix Medical, USA). The intensity of the simulated field is indicated by the color bar, the arrow points to the direction of the current, and the length represents the current intensity (Figure 3).

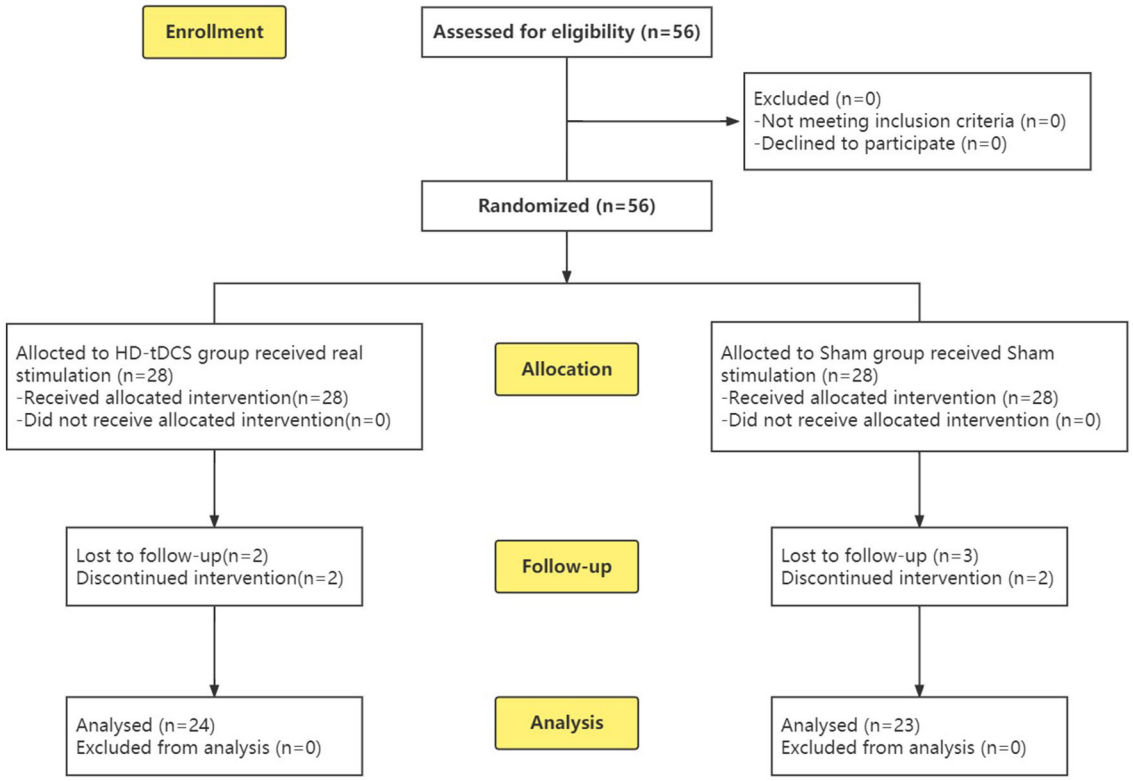

FIGURE 1
CONSORT flow diagram (38) of this RCT from enrolment, intervention allocation, follow-up and analysis.

Stimulation parameters

The HD-tDCS anode current intensity was 1.0 mA, and each stimulation lasted for 20 min during which there was 30 s of current increase time and 30 s of current decrease time, one time a day for five consecutive days, 2 days of rest after five consecutive days of stimulation, and a total of 10 sessions. Most of the previous HD-tDCS studies have shown effective cortical stimulation and inhibition with 2.0 mA. However, several other factors such as the age of the subject and subject-specific skull thickness could have also played a role in the differing outcomes in addition to the current intensity (39). Referring to previous studies, such as that of Breitling et al. (22), on stimulated subjects with ADHD aged 10–16 years with HD-tDCS of 0.25 and 0.5 mA, considering that the subjects are children and adolescents and the skull thickness is different from that of adults, the current intensity is selected as 1.0 mA in this study. The sham group received sham stimulation, in which the subjects of the HD-tDCS group underwent under the same electrode setting. During the stimulation, the current was increased for 30 s, and after reaching 1.0 mA, the current was reduced to 0 in the following 30 s to simulate the skin feeling during HD-tDCS and make the subjects have the same subjective feeling as the real stimulation.

Clinical symptom assessment for ADHD

Swanson, Nolan, and Pelham-IV rating scale

The Swanson, Nolan, and Pelham-IV rating scale (SNAP-IV rating scale) rating scale has good reliability and validity (40, 41). This scale is compiled according to the DSM-IV diagnostic criteria for ADHD, with a total of 18 items that are summarized in two factors: attention deficit (items 1–9) and hyperactivity/impulsivity (items 10–18) were scored on a scale of 4 for symptom severity (none at all 0; A little bit is one point; Not too little is 2 points; and Very many are 3 points), selected by parents according to their children's general impression. The scores are on average.

Conners child behavior scale parent symptom questionnaire

The Conners' Parent Rating Scales (CPRS) revised in 1978 has a total of 48 items (42). Previous research has demonstrated that the Parent Symptom Questionnaire (PSQ) has good reliability in China (Cronbach's $\alpha = 0.93$) and may be used to evaluate Chinese children (43). These include the Conduct factor (items 2, 8, 14, 19, 20, 21, 22, 23, 27, 33, 34, and 39), Learning factor (items 10, 25, 31, and 37), Physical and mental factor (items 32, 41, 43, 44, and 48), Hyperactivity-impulsivity index (items 4, 5, 11, and 13), Anxiety factor (items 12, 16, 24, and 27), and Hyperactivity index (items 4, 7, 11, 13, 14, 25, 31, 33, 37, and 38). Each item was rated on a scale of 4; 0 to 3 by parents based on observation. The score was on average.

Cognitive tasks

Integrated Visual and Auditory Continuous Performance Test

The IVA + CPT is a valuable tool for assessing ADHD (44, 45). The first part of the test is the Visual Attention Test. The visual

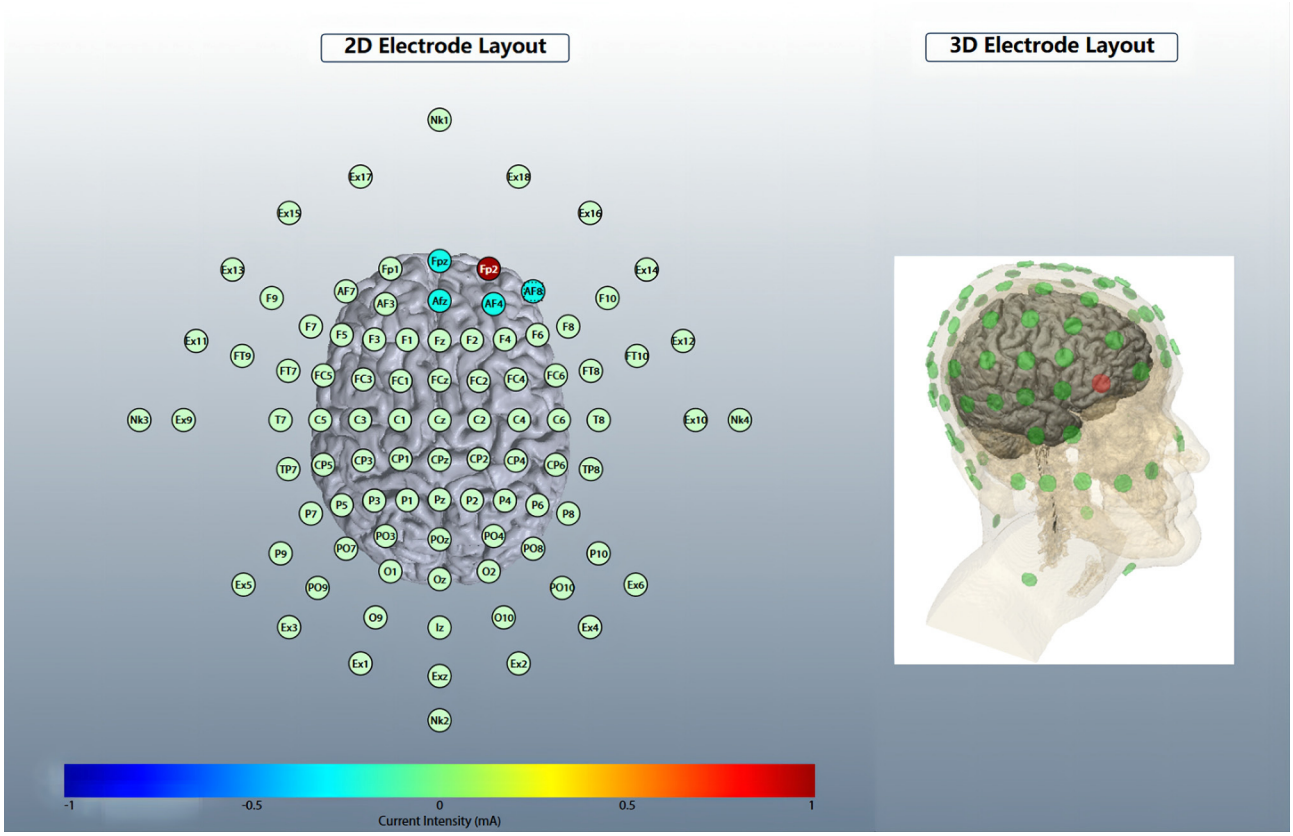

FIGURE 2
HD-tDCS electrode layout. An anodic electrode was placed at the center: right orbitofrontal cortex (OFC), corresponding to Fp2; four cathode electrodes are placed square around the anode, corresponding to Fpz, Afz, AF4, and AF8.

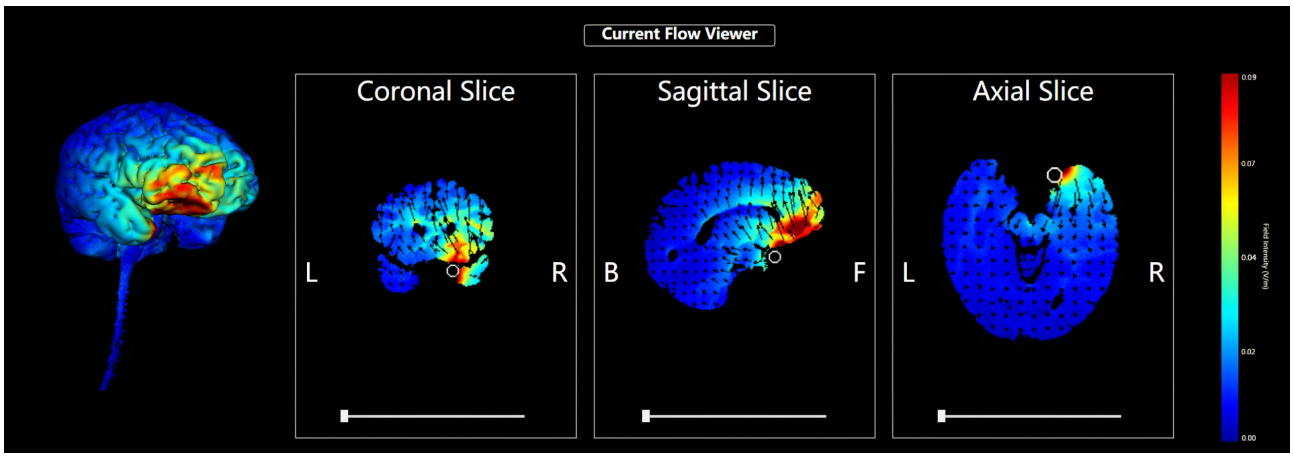

FIGURE 3
HD-tDCS electric field simulation. The intensity of the simulated field is indicated by the color bar, the arrow points to the direction of the current, and the length represents the current intensity.

stimulus numbers 0–9 were presented on the computer monitor screen, and each time, the 10 numbers were randomly arranged on the screen for about 2 s (the time was not fixed to exclude the interference of the subjects' preparatory actions); the subjects were required to find out the specific number (such as "3") then and click the left mouse button to confirm. The test cycle was 12 min.

The second part was the Auditory Attention Test: auditory stimulus numbers 0–9 were played by a computer speaker and a random number was played each time with an interval of about 2 s. The subjects were asked to identify whether the number they heard was a specific number (such as "5") and then click the left mouse button to confirm. The test was repeated several times and lasted for 12 min.

The third part was the Combination of the Audiovisual Attention Tests: whenever a random number 0–9 is displayed on the computer display screen, random numbers 0–9 were displayed on the computer speaker, and when the number displayed on the screen matches the number played by the speaker, the subject was required to confirm by clicking the left mouse button. The test interval was about 2 s and the cycle lasted for 12 min. The evaluation indices included the correct response numbers, the commission errors (false response numbers), the omissions (missed report numbers), and the average reaction time (ms) of the combination of visual, auditory, and audiovisual indices.

Stroop Color and Word Test

This test is a measurement paradigm of interference suppression (46), which is divided into the basic reading part: the Word Test and the Color Test; interference with the reading part: the Word Meaning Interference Test and the Color Meaning Interference Test. In the word test, the subjects are required to read out different characters on the card, including 30 Chinese characters "red, green, blue, and yellow" printed in black on a white background in 3 rows × 10 columns. In the color test, the subjects are required to read out the colors of different color blocks. There are 30 color blocks randomly arranged in 3 rows × 10 columns of "red, green, blue, and yellow." The time (s) of completing the Word and Color Tests are recorded. The Word and Color Tests are automated processes that assess short-term attention and reading speed. In the interference with the reading part, 12 rows × 9 columns are randomly arranged in four colors: red, green, blue, and yellow, showing four kinds of Chinese characters: "red, green, blue, and yellow." A total of 50% of the characters have the same meaning and color and 50% of the characters do not. In the Word Meaning Interference Test, if there is a color word with inconsistent meaning and color, the subjects are required to read the color instead of the Chinese character (for example, "red" is printed in green and "green" is read instead of "red") and name the color. In the Color Meaning Interference test, the subjects are required to read the color words with inconsistent meanings and the color according to the meaning of the word and eliminate the color interference. In the Test order, after the Word Test, namely the establishment of the word dominance response, the Word Meaning Interference Test was conducted. In the same way, after the completion of the Color Test, the color dominance response was established before the Color Meaning Interference Test. The subjects were required to complete the above test quickly within the specified time (2 min). Finally, the RT (reaction time) of the interference effect of Word and Color was calculated: the RT incongruent of color and word—RT congruent of color and word.

The Tower of Hanoi

The mission consists of three vertical wooden poles and a fixed number of disks of different sizes (four disks in this study) with holes in them such that they can be placed on the poles. The goal is to move the disks from a starting position to a target position and arrange them in a pyramid form on the target position (47). Constraint conditions: only one disk can be moved at a time and a larger disk cannot be on top of a smaller disk to complete the task in the process. The disk must either be in the process of moving or on the pole. The image of the disk was displayed on a screen and the subject could move the disk by pressing the corresponding key on a keyboard. The evaluation included the total completion time(s) and the steps taken between the first and last moves.

The cognitive tasks above were completed on the computer with software from Nanjing Vishee Medical Technology Co., Ltd.

Statistical analysis

SPSS 28.0 statistical software was used for data analyses. The measurement scale-data satisfying the normal distribution and homogeneity of variance were expressed as mean ± standard deviation ($M \pm SD$). The nominal-data measurements were expressed as number ± percentage [N (%)]. The independent sample T-test/Chi-square test and the repeated-measures ANOVA were adopted. Then, Mauchly's sphericity test was used to evaluate the sphericity of the data before implementing the repeated measures ANOVA. If a P-value was > 0.05, it indicates that Mauchly's sphericity test was violated and therefore the Greenhouse–Geisser test was performed. If a P-value was < 0.05, it indicates that Mauchly's sphericity test was accepted and therefore Roy's largest root exact test was performed. To test the effects of HD-tDCS, repeated measures ANOVA was performed for the within-subject factor of TIME (T0, T1, T2, and T3), the between-subject factor of CONDITION (the real stimulation and the sham stimulation), and the interaction factor of TIME × CONDITION and the Bonferroni correction test as well as the Bonferroni *post-hoc* test was used. Because ANOVA has 16 variables, after Bonferroni's correction test, test statistics with a P-value of <0.0031 indicate significant results. It means that if the P value is less than alpha 0.31%, then we reject the null hypothesis and consider the result to be statistically significant. GraphPad Prism 9 was used for the diagram.

Results

General demographic information

During the implementation of the experiment, nine cases were lost due to "inconvenient medical treatment, busy study, troublesome treatment and evaluation process, uncomfortable stimulation of the head, and no treatment effect," including four cases in the HD-tDCS group and five cases in the Sham group, which were not included in the statistics. Before the experiment, the subjects were grouped and divided. Finally, 47 subjects completed the experiment and entered the stage of result analysis (Figure 1). There were 24 subjects in the HD-tDCS group, including 14 boys and 10 girls, and their average age was (11.29 ± 2.51) years. There were 23 subjects in the Sham group, including 13 boys and 10 girls; the average age was (11.74 ± 2.59) years. There were no significant differences between the two groups in terms of age, gender, total IQ, educational years, whether they come from a single-parent family, age of onset, course of disease, and ADHD type between the two groups ($P > 0.05$). In addition, SNAP-IV and PSQ were taken as baseline clinical manifestations, and there was no significant difference between the two groups (Table 1).

TABLE 1 Subject's demographic characteristics, intelligence, and clinical manifestations.

Variables	HD-tDCS	Sham	T/χ^2 (P)
Number	24	23	–
Age ($M \pm$ SD)	11.29 ± 2.51	11.74 ± 2.59	−0.601 (0.551)
Gender (%boys)	14 (58.33%)	13 (56.52%)	0.016 (0.900)
Total IQ ($M \pm$ SD)	90.50 ± 5.073	89.39 ± 3.577	0.862 (0.393)
Educational years ($M \pm$ SD)	4.15 ± 2.119	4.91 ± 2.521	−1.131 (0.264)
Whether comes from a single-parent family (%yes)	8 (33.3%)	5 (21.7%)	1.945 (0.378)
Age of onset ($M \pm$ SD)	9.17 ± 2.220	9.37 ± 2.024	−0.327 (0.745)
Course of disease ($M \pm$ SD)	2.125 ± 1.279	2.370 ± 1.693	−0.560 (0.578)
ADHD type			
Combined type (%)	17 (70.8%)	17 (73.9%)	0.122 (0.941)
Inattentive type (%)	3 (12.5%)	3 (13.0%)	
Hyperactive impulsive type (%)	4 (16.7%)	3 (13.0%)	
SNAP-IV ($M \pm$ SD)			
Attention deficit factor	2.217 ± 0.380	2.198 ± 0.330	0.181 (0.858)
Hyperactivity/impulsivity factor	2.120 ± 0.271	2.209 ± 0.211	−1.245 (0.220)
PSQ ($M \pm$ SD)			
Conduct factor	1.614 ± 0.345	1.528 ± 0.355	0.837 (0.407)
Learning factor	1.677 ± 0.486	1.804 ± 0.532	−0.856 (0.397)
Physical and mental factor	1.741 ± 0.384	1.947 ± 0.396	−1.810 (0.077)
Hyperactivity-impulsivity index	1.802 ± 0.312	1.793 ± 0.366	0.087 (0.931)
Anxiety factor	1.677 ± 0.308	1.684 ± 0.370	−0.078 (0.939)
Hyperactivity index	1.629 ± 0.428	1.747 ± 0.378	−1.004 (0.321)

Clinical symptom assessment results from SNAP-IV and PSQ

Repeated measurement ANOVA was performed for the SNAP-IV and PSQ scores of both groups. The results showed no statistical significance ($P > 0.05$) in terms of TIME, CONDITION, and interaction TIME×CONDITION, suggesting that the Attention Deficit and Hyperactivity/Impulsivity factor scores of SNAP-IV, Conduct factor, Learning factor, Physical and mental factor, Hyperactivity-impulsivity index, Anxiety factor, and the Hyperactivity index scores of PSQ did not change with the intervention time in the HD-tDCS group and the Sham tDCS group.

Comparison of cognitive task results

Repeated measurement ANOVA was performed for the correct response number of visual IVA-CPT. There were no statistically significant main effects in terms of TIME ($F_{(3,43)} = 4.916$, $P = 0.005$), CONDITION ($F_{(1,45)} = 0.546$, $P = 0.464$), and interaction effect for TIME × CONDITION ($F_{(3,43)} = 0.006$, $P = 0.083$). Repeated measurement ANOVA was performed for the commission errors of visual IVA-CPT. There were statistically significant main effects for TIME ($F_{(3,43)} = 11.191$, $P < 0.001$), CONDITION ($F_{(1,45)} = 11.512$, $P = 0.001$), and interaction effect for TIME×CONDITION ($F_{(3,43)} = 6.635$, $P < 0.001$). A Bonferroni correction test (*post-hoc*) showed the false response number decreased under the HD-tDCS condition when compared with T0 and T1 ($P < 0.001$), T2 ($P < 0.001$), and T3 ($P < 0.001$). Repeated measurement ANOVA was performed for the omission of visual IVA-CPT. There were statistically significant main effects for TIME ($F_{(3,43)} = 6.486$, $P = 0.001$), but no statistically significant main effect for the CONDITION effect ($F_{(1,45)} = 0.628$, $P = 0.432$) and the interaction effect for TIME×CONDITION ($F_{(3,43)} = 0.702$, $P = 0.556$; Figure 4).

Repeated measurement ANOVA was performed for the correct response number of auditory IVA-CPT. There were statistically significant main effects in terms of TIME ($F_{(2.635,118.573)} = 11.204$, $P < 0.001$), but no statistically significant effect for CONDITION ($F_{(1,45)} = 1.930$, $P = 0.172$) and the interaction effect for TIME × CONDITION ($F_{(2.635,118.573)} = 0.269$, $P = 0.822$). Repeated measurement ANOVA was performed for the commission errors of auditory IVA-CPT. There were statistically significant main effects for TIME ($F_{(3,43)} = 7.360$, $P < 0.001$) and CONDITION ($F_{(1,45)} = 14.210$, $P < 0.001$), but no statistically significant interaction effect for TIME×CONDITION ($F_{(3,43)} = 3.974$, $P = 0.014$). Repeated measurement ANOVA was performed for the omission of auditory IVA-CPT. There were statistically significant main effects for TIME ($F_{(3,43)} = 11.242$, $P < 0.001$), but no statistically significant main effects for CONDITION ($F_{(1,45)} = 3.007$, $P = 0.090$) and the interaction effect for TIME × CONDITION ($F_{(3,43)} = 0.956$, $P = 0.422$; Figure 5).

Repeated measurement ANOVA was performed for the correct response number of the audiovisual combination of IVA-CPT. There were statistically significant main effects for TIME ($F_{(2.891,130.078)}$ =7.092, $P < 0.001$), but no statistically significant main effect for CONDITION ($F_{(1,45)}$ =3.744, $P = 0.059$) and the interaction effect for TIME × CONDITION ($F_{(2.891,130.078)} = 0.010$, $P = 0.998$). Repeated measurement ANOVA was performed for the commission errors of the audiovisual combination IVA-CPT. There were statistically significant main effects for TIME ($F_{(3,43)}$ =12.467, $P < 0.001$), CONDITION ($F_{(1,45)}$ =15.457, $P < 0.001$) and the interaction effect for TIME×CONDITION ($F_{(3,43)}$ =5.469, $P = 0.003$). A Bonferroni correction test (*post hoc*) showed a decrease in the false response number in the HD-tDCS condition compared with T0 and T1 ($P < 0.001$), T2 ($P < 0.001$), and T3 ($P < 0.001$). Repeated measurement ANOVA was performed for the omission of the audiovisual combination IVA-CPT. There were statistically significant main effects for TIME ($F_{(2.865,128.912)} = 12.314$, $P < 0.001$), but no statistically significant main effect for CONDITION ($F_{(1,45)} = 1.879$, $P = 0.177$) and the interaction effect for TIME × CONDITION ($F_{(2.865,128.912)} = 0.440$, $P = 0.716$; Figure 6).

Repeated measurement ANOVA was performed for the mean reaction time of visual IVA-CPT. There were no statistically significant main effects for TIME ($F_{(2.877,129.444)}$ =1.739, $P = 0.162$), CONDITION ($F_{(1,45)}$ =0.471, $P = 0.496$) and the interaction effect for TIME × CONDITION ($F_{(2.877,129.444)} = 0.220$, $P = 0.875$). Repeated measurement ANOVA was performed for the mean reaction time of auditory IVA-CPT. There were no statistically significant main effects for TIME ($F_{(2.878,129.494)} = 2.002$, $P = 0.117$), CONDITION ($F_{(1,45)} = 0.080$, $P = 0.778$) and the interaction effect for TIME × CONDITION ($F_{(2.878,129.494)} = 0.574$, $P = 0.626$). Repeated measurement ANOVA was performed for the mean reaction time of the audiovisual combination IVA-CPT. There were statistically significant main effects for TIME ($F_{(2.681,120.643)} = 10.156$, $P < 0.001$), but no statistically significant main effect for CONDITION ($F_{(1,45)} = 0.165$, $P = 0.687$) and the interaction effect for TIME × CONDITION ($F_{(2.681,120.643)} = 0.500$, $P = 0.679$; Figure 7).

These results suggested that the correct response number of the visual, the mean visual, and the auditory reaction time of both groups did not change with the intervention time, the correct response number of auditory and audiovisual combination, the commission errors of auditory reaction time, the omission of the visual, auditory, and audiovisual combination, and the average reaction time for the audiovisual combination that gradually increased or decreased with the intervention time in both groups, but there was no significant increase or decrease in the HD-tDCS group than in the Sham tDCS group and the commission errors of visual and audiovisual combination in both groups decreased gradually with the intervention time. Furthermore, the HD-tDCS group compared with the Sham tDCS group was more significantly decreased after the 5th intervention, the 10th intervention, and the 6th-week follow-up.

Repeated measurement ANOVA was performed for the Interference RT of Word. There was no statistically significant main effect for TIME ($F_{(2.593,116,707)} = 3.376$, $P = 0.026$) and the interaction effect for TIME × CONDITION ($F_{(2.593,116.707)} = 2.350$, $P = 0.085$). Repeated measurement ANOVA was performed for the Interference RT of Color. There were no statistically significant main effects for TIME ($F_{(3,43)} = 3.454$, $P = 0.025$) and the interaction effect for TIME × CONDITION ($F_{(3,43)} = 3.429$, $P = 0.025$). Figure 8 indicates that the Interference RT of Color and Word in both groups did not change with the intervention time.

Repeated measurement ANOVA was performed for the total completion time of TOH. There were statistically significant main effects for TIME ($F_{(3,43)}$ =13.237, $P < 0.001$) and the interaction effect for TIME × CONDITION ($F_{(3,43)}$ =6.733, $P < 0.001$). A Bonferroni correction test (*post hoc*) showed that the total completion time reduced in the HD-tDCS condition compared to T0 and T1 ($P < 0.001$), T2 ($P < 0.001$), and T3 ($P < 0.001$). Repeated measurement ANOVA was performed for the total completion steps of TOH. There were no statistically significant main effects for TIME ($F_{(3,43)} = 5.194$, $P = 0.004$), CONDITION ($F_{(1,45)} = 9.410$, $P = 0.004$) and the interaction effect for TIME × CONDITION ($F_{(3,43)} = 2.639$, $P = 0.062$). It suggests that the total completion steps of TOH in both groups did not change with the intervention time, but the total completion time of TOH in both groups decreased gradually with the intervention time. Furthermore, the HD-tDCS group compared with the Sham tDCS group was more significantly reduced after the 5th intervention, the 10th intervention, and the 6th-week follow-up (Figures 9, 10).

Discussion

This study observed some positive effects. The results showed that the commission errors of the visual and audiovisual combination of IVA-CPT tasks changed significantly in the two groups. Furthermore, comparing real stimulation with sham tDCS, there was a significant improvement in the commission errors after real HD-tDCS intervention, and this effect was even reflected in the follow-up 6 weeks later. The IVA-CPT tasks were not only attention-maintaining tasks but also inhibition-control tasks. The tasks were intended to be mildly boring to produce the omission (i.e., inattention) and commission errors (i.e., impulsivity) through a series of trial sets requiring responding and not responding, respectively. The commission errors of IVA-CPT reflected the inhibition ability of impulse. The subjects had to suppress impulses instead of making mistakes when they received the visual and audiovisual combination stimuli (44, 45, 48). Therefore, it showed that real HD-tDCS can improve the inhibitory control of the subjects in addition to improving attention maintenance. This was similar to previous studies that showed that tDCS tended to improve significantly only in interference control and inhibition, but not in working memory or reaction time variability in the analyses of neuropsychological performance measures (49). This effect is also supported by studies on the mechanism of tDCS, which regulates the concentration levels of the excitatory neurotransmitter glutamate and the inhibitory neurotransmitter γ-aminobutyric acid (GABA). A magnetic resonance spectrum study found that GABA concentration increased after ATDCS stimulation, while glutamate concentration decreased after CTDCS stimulation (50). Some researchers believe that part of the mechanism of tDCS is to regulate the excitatory and inhibitory balance of the cortex (E/I) (51). Some studies suggested that, although the improvement of inhibitory control is assumed to be caused by the enhanced activity of the stimulating region, many experiments do not stimulate the target region alone. For example, the use of large electrodes (35 cm^2 surface area) will cause extensive changes in cortical excitability, which may lead to changes in the overall arousal level of the brain (52). Therefore,

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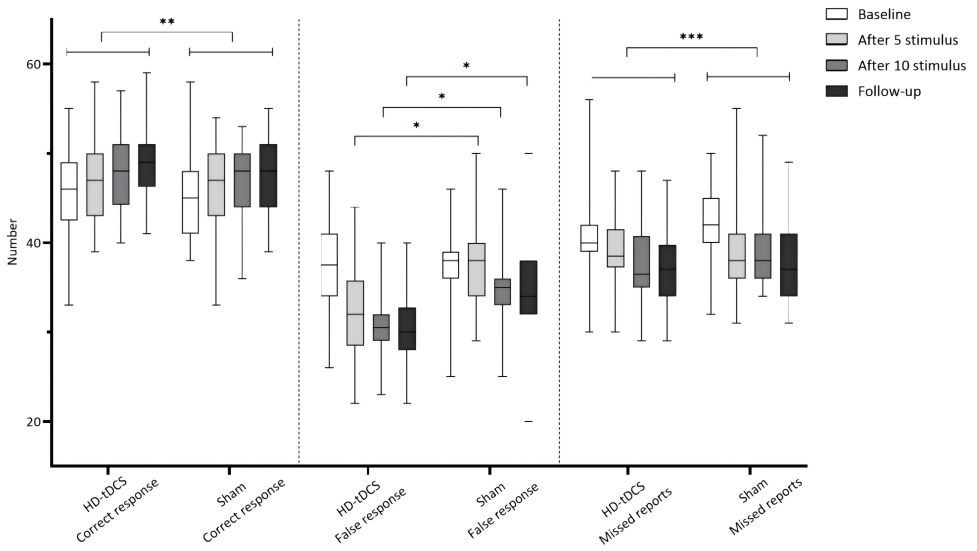
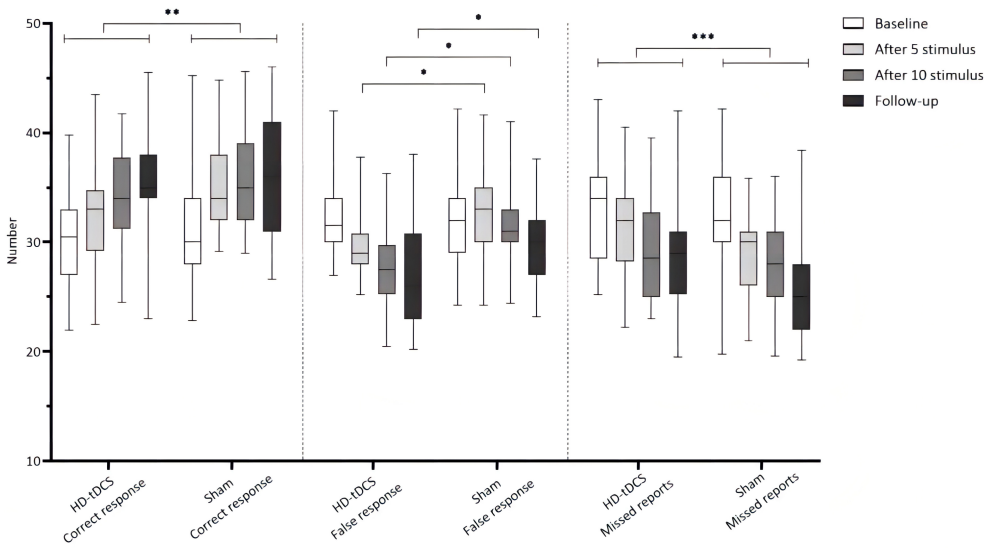


*The false response number of IVA-CPT visual: Mauchly sphericity test: W=0.516, P<0.001. TIME (Roy's largest root exact test: F(3, 43)=11.191, P<0.001, ηp2=0.438), CONDITION (F(1, 45)=11.512, P=0.001, ηp2=0.204),TIME×CONDITION(Roy's largest root exact test: F(3, 43)=6.635, P<0.001, ηp2=0.316).
**The correct response number of IVA-CPT visual: Mauchly sphericity test: W=0.685, P=0.005.TIME (Roy's largest root exact test: F(3, 43)=4.916, P=0.005, ηp2=0.255), CONDITION (F(1, 45)=0.546, P=0.464, ηp2=0.012), TIME×CONDITION(Roy's largest root exact test: F(3, 43)=0.006, P=0.083, ηp2=0.006)
***The missed reports number of IVA-CPT visual: Mauchly sphericity test: W=0.700, P=0.008. TIME (Roy's largest root exact test: F(3, 43)=6.486, P=0.001, ηp2=0.312), CONDITION (F(1, 45)=0.628, P=0.432, ηp2=0.014),TIME×CONDITION(Roy's largest root exact test: F(3, 43)=0.702, P=0.556, ηp2=0.047)

FIGURE 4
Comparison of IVA-CPT visual between the two groups at different time points. *The commission errors of IVA-CPT visual: Mauchly sphericity test: $W = 0.516$, $P < 0.001$ TIME (Roy's largest root exact test: $F_{(3,43)} = 11.191$, $P < 0.001$, ηp2 = 0 438), CONDITION ($F_{(1,45)} = 11.512$, $P = 0.001$, ηp2 = 0.204), TIME × CONDITION(Roy's largest root exact test: $F_{(3,43)} = 6.635$, $P = 0.001$, ηp2 = 0.316). **The correct response number of IVA-CPT visual: Mauchly sphericity test: $W = 0.685$, $P = 0.005$. TIME (Roy's largest root exact test: $F_{(3,43)} = 4.916$, $P = 0.005$, ηp2 = 0.255), CONDITION ($F_{(1,45)} = 0.546$, $P = 0.464$, ηp2 = 0.012), TIME × CONDITION (Roy's largest root exact test: $F_{(3,43)} = 0.006$, $P = 0.083$, ηp2 = 0.006). ***The omission of IVA-CPT visual: Mauchly sphericity test: $W = 0.700$, $P = 0.008$. TIME (Roy's largest root exact test: $F_{(3,43)} = 6,486$, $P = 0.001$, ηp2 = 0.312), CONDITION ($F_{(1,45)} = 0.628$, $P = 0.432$, ηp2 = 0.014), TIME × CONDITION (Roy's largest root exact test: $F_{(3,43)} = 0.702$, $P = 0.556$, ηp2 = 0.047).

it cannot be simply identified as the therapeutic effect produced by a certain area. Sotnikova et al. used ATDCS to stimulate the dorsolateral prefrontal cortex (DLPFC) and analyzed the functional connectivity of the brain through functional magnetic resonance after stimulation. The results showed that, in the N-Back Task, whether the left DLPFC is under the action of the electrode or the left premotor cortex, the left auxiliary motor cortex, and the precuneus are not under the action of the electrode, tDCS-induced activity in these regions, suggesting that anodal tDCS can lead to increased neuronal activation and connectivity, which stimulates not only the brain regions below the electrode but also the possibly other brain regions further away (53). These studies indicate that HD-tDCS is the future direction of research. As HD-tDCS solves the problem of traditional tDCS affecting the stimulation target and the outer brain region, the stimulation current is limited to the region below the electrode, thus improving the accuracy and excluding the interference of non-target region stimulation. However, there are few studies on HD-tDCS and no studies on the right OFC. In the past, only Breitling et al. (22) studied the effect of HD-tDCS on the right IFG in 33 adolescent ADHD subjects for five consecutive days. In this study, HD-tDCS was applied to OFC, and the neuropsychological measures showed that it had a positive effect on attention maintenance and inhibitory control. It was consistent with the role of OFC in the OFCSTC loop, and it also confirmed our hypothesis that tDCS activated OFC to improve impulse control difficulties and emotional processing, thereby further improving the performance of cognitive tasks, such as attention maintenance and inhibitory control.

Another interesting result of this study was that, after the stimulation of real tDCS, the TOH completion time decreases, while the number of TOH completion steps did not change, which seemed to contradict the results of the aforementioned improved inhibitory control. The TOH is a problem that cannot be solved in one step. Subjects need to plan a reasonable sequence of steps to follow the rules and use as few steps as possible. The functions measured by TOH include cognitive planning, problem-solving, attention shifting, and attention maintenance. Inhibitory control also involved solving the TOH problem, in which subjects had to temporarily shift the smaller disk away from where it should end up to place the larger disk in the desired position. Working memory also participates in the whole process of TOH problem-solving, which is inseparable from the spatial memory of the location of the disk, which is undoubtedly a kind of working memory activity to remember the location of a specific disk while moving the disk. If impulsivity control or working memory had been improved in the TOH task, the number of TOH completed steps should have been reduced, but this was not the case. The TOH only increased the time of accomplishment, but not the number of steps required for accomplishment. The possible explanation is that real tDCS improves attention maintenance, and subjects need to increase sustained attention to complete tasks without distraction. In this study, although the time of completing TOH was significantly shortened after real HD-tDCS intervention, there were no significant differences between the two groups in the reaction time, the omission of the IVA-CPT task, and the Interference RT of Stroop Color and Word. The interference effect of Stroop Word and Color reflects attention duration, alertness, and cognitive

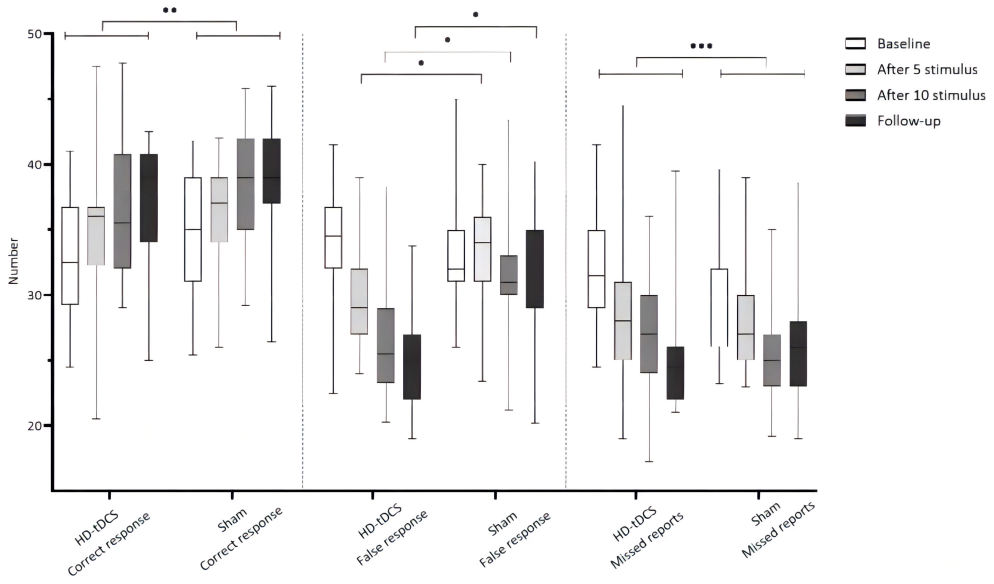


*The false response number of IVA-CPT auditory: Mauchly sphericity test: W=0.697, P=0.008. TIME (Roy's largest root exact test: F(3, 43)=7.360, P<0.001, ηp2=0.339), CONDITION (F(1, 45)=14.210, P<0.001, ηp2=0.240),TIME×CONDITION(Roy's largest root exact test: F(3, 43)=3.974, P=0.014, ηp2=0.217).
**The correct response number of IVA-CPT auditory: Mauchly sphericity test: W=0.785, P=0.061. TIME (Greenhouse- Geisser test: F(2.635, 118.573)=11.204, P<0.001, ηp2=0.199), CONDITION (F(1, 45)=1.930, P=0.172, ηp2=0.041), TIME×CONDITION(Greenhouse- Geisser test: F(2.635, 118.573)=0.269, P=0.822, ηp2=0.006).
***The missed reports number of IVA-CPT auditory: Mauchly sphericity test: W=0.730, P=0.017. TIME (Roy's largest root exact test: F(3, 43)=11.242, P<0.001, ηp2=0.440), CONDITION (F(1, 45)=3.007, P=0.090, ηp2=0.063),TIME×CONDITION(Roy's largest root exact test: F(3, 43)=0.956, P=0.422, ηp2=0.063)

FIGURE 5
Comparison of IVA-CPT auditory between the two groups at different time points. *The commission errors of IVA-CPT auditory: Mauchly sphericity test: $W = 0.697$, $P = 0.008$. TIME (Roy's largest root exact test: $F_{(3,43)=}$ 7.360, $P < 0.001$, $\eta p2 = 0.339$), CONDITION ($F_{(1,45)} = 14.210$, $P < 0.001$, $\eta p2 = 0.240$), TIME × CONDITION (Roy's largest root exact test: $F_{(3,43)} = 3.974$, $P = 0.014$, $\eta p2 = 0.217$). **The correct response number of IVA-CPT auditory: Mauchly sphericity test: $W = 0.785$, $P = 0.061$. TIME (Greenhouse-Geisser test: $F_{(2.635,118.573)} = 11.204$, $P < 0.001$, $\eta p2 = 0.199$), CONDITION ($F_{(1,45)} = 1.930$, $P = 0.172$, $\eta p2 = 0.041$), TIME × CONDITION (Greenhouse-Geisser test: $F_{(2.635,118.573)} = 0.269$, $P = 0.822$, $\eta p2 = 0.006$). ***The omission of IVA-CPT auditory: Mauchly sphericity test: $W = 0.730$, $P = 0.017$. TIME (Roy's largest root exact test: $F_{(3,43)} = 11.242$, $P = 0.001$, $\eta p2 = 0.440$), CONDITION ($F_{(1,45)} = 3.007$, $P = 0.090$, $\eta p2 = 0.063$), TIME × CONDITION (Roy's largest root exact test: $F_{(3,43)} = 0.956$, $P = 0.422$, $\eta p2 = 0.063$).

processing speed. Meanwhile, the subjects need to suppress the automatic processing response to the word meaning or color meaning itself, eliminate the interference of the dominant stimulus attribute, respond to the inferior attribute of the stimulus, and evaluate the inhibition control ability of the subjects. This could be interpreted as that the TOH has higher and more complex difficulties than IVA-CPT and Stroop, requiring more executive function mobilization, which is consistent with previous studies. Gill et al. (54) found that ATDCS stimulation is more effective with a higher working memory load. Another possible explanation, similar to the absence of significant improvement in ADHD symptoms, is that our study may have done too few sessions to observe the effect of the Stoop effect.

In terms of clinical symptoms of ADHD, the results of this study show that there were no significant changes in the SNAP-IV and PSQ scores, and each factor of the two groups before and after the intervention indicates that HD-tDCS, despite real stimulus or sham stimulus, had no obvious immediate effect on the overall symptoms of ADHD. Most of the previous tDCS studies focused on neuropsychological changes, and only a few studies focused on clinical symptoms. Some researchers suggested that there is a dissociation between neuropsychological deficits and clinical symptoms of ADHD, which means that even if there is improvement in the neuropsychological deficits after or during tDCS, such as improvement in inhibitory control and WM, it does not mean that the clinical symptoms have improved as well (55). Meta-analyses of tDCS studies targeting mostly the dorsolateral prefrontal cortex show small effects on cognitive improvements with only two out of three studies showing clinical improvements (21). The systematic retrieval and meta-analysis of tDCS studies showed that most anode tDCS of the left dlPFC had only a very limited trend-level effect in improving inhibition and processing speed, and there was no evidence of alleviation in attention and other clinical symptoms (20). However, other researchers have come up with different conclusions. Brauer et al., by meta-analyzing 13 studies, including 20 study arms, showed that tDCS had an immediate effect on overall symptom severity, inattention, and impulsivity, but not on hyperactivity. The results were significant in children and adolescents. The follow-up data (3 days—4 weeks after stimulation) suggested an ongoing beneficial effect regarding overall symptom severity and a delayed effect on hyperactivity (49). They came to this conclusion on the basis that, although most of these studies did not provide a clinical outcome replacement for assessing the effect of tDCS on cognitive functioning in ADHD, there are several studies that report high correlations between different executive dysfunctions and ADHD core symptoms (56). Soff et al. observed that tDCS could improve the subjects' working memory and memory consolidation ability, thereby alleviating symptoms of inattention and hyperactivity, by following five consecutive anodal tDCS sessions. By the 7th day after the treatment, a long-lasting tDCS effect was implied when applying for repeated sessions (57). Previous studies showed that the tDCS physiological effects might depend on the stimulation duration and current intensity with the potential for long-lasting neuroplastic changes after multiple sessions, likely due to the changes in the synaptic strength induced by long-term potentiation (LTP)-like response and metaplasticity mechanisms (11, 58). However, this delayed effect was not observed for the 10 repeated sessions in this



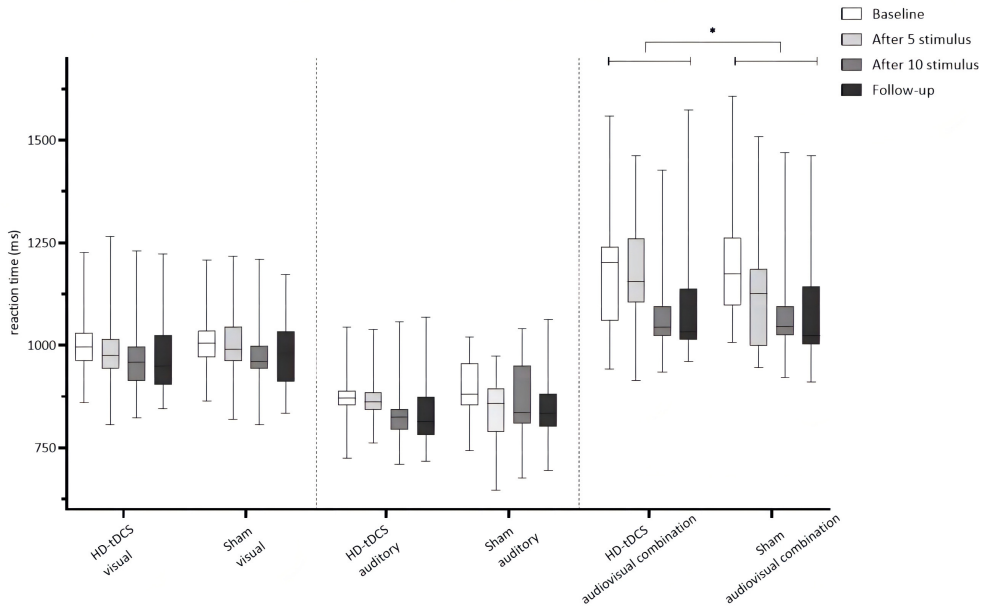
*The false response number of IVA-CPT audiovisual combination: Mauchly sphericity test: W=0.683, P=0.005. TIME (Roy's largest root exact test: F(3, 43)=12.467, P<0.001, ηp2=0.465), CONDITION (F(1, 45)=15.457, P<0.001, ηp2=0.256), TIME×CONDITION(Roy's largest root exact test: F(3, 43)=5.469, P=0.003, ηp2=0.276).
**The correct response number of IVA-CPT audiovisual combination: Mauchly sphericity test: W=0.940, P=0.747. TIME (Greenhouse- Geisser test: F(2.891, 130.078)=7.092, P<0.001, ηp2=0.136), CONDITION (F(1, 45)=3.744, P=0.059, ηp2=0.014),TIME×CONDITION(Greenhouse- Geisser test: F(2.891, 130.078)=0.010, P=0.998, ηp2=0.002).
***The missed reports number of IVA-CPT audiovisual combination: Mauchly sphericity test: W=0.926, P=0.643. TIME (Greenhouse- Geisser test: F(2.865, 128.912)=12.314, P<0.001, ηp2=0.215), CONDITION (F(1, 45)=1.879, P=0.177, ηp2=0.040),TIME×CONDITION(Greenhouse- Geisser test: F(2.865, 128.912)=0.440, P=0.716, ηp2=0.010)

FIGURE 6
Comparison of IVA-CPT audiovisual combination between the two groups at different time points. *The commission errors of IVA-CPT audiovisual combination: Mauchly sphericity test: $W = 0.683$, $P = 0.005$. TIME (Roy's largest root exact test: $F_{(3,43)=}$ 12.467, $P < 0.001$, $\eta p2 = 0.465$), CONDITION ($F_{(1,45)} = 15.457$, $P < 0.001$, $\eta p2 = 0.256$), TIME × CONDITION (Roy's largest root exact test: $F_{(3,43)} = 5.469$, $P < 0.003$, $\eta p2 = 0.276$). **The correct response number of IVA-CPT audiovisual combination: Mauchly sphericity test: $W = 0.940$, $P = 0.747$. TIME (Greenhouse-Geisser test: $F_{(2.891,130.078)} = 7.092$, $P < 0.001$, $\eta p2 = 0.136$), CONDITION ($F_{(1,45)} = 3.744$, $P = 0.059$, $\eta p2 = 0.014$), TIME × CONDITION (Greenhouse-Geisser test: $F_{(2.891,130.078)} = 0.010$, $P = 0.998$, $\eta p2 = 0.002$). ***The omission of IVA-CPT audiovisual combination: Mauchly sphericity test: $W = 0.926$, $P = 0.643$. TIME (Greenhouse-Geisser test: $F_{(2.865,128.912)} = 12.314$, $P = 0.001$, $\eta p2 = 0.215$), CONDITION ($F_{(1,45)} = 1.879$, $P = 0.177$, $\eta p2 = 0.040$), TIME × CONDITION (Greenhouse-Geisser test: $F_{(2.865,128.912)} = 0.440$, $P = 0.716$, $\eta p2 = 0.010$).

study and the effect of HD-tDCS after 6 weeks of follow-up was also insignificant. The likely explanation was that most of the previous studies tested five sessions and only two studies tested larger numbers of sessions. Westwood et al. (59) found no improvement after 15 sessions and Leffa et al. (17) found an improvement after 28 sessions but not after 14 sessions. This suggested that we need more sessions. Our study may have done too few sessions to observe the effect of the clinical symptoms.

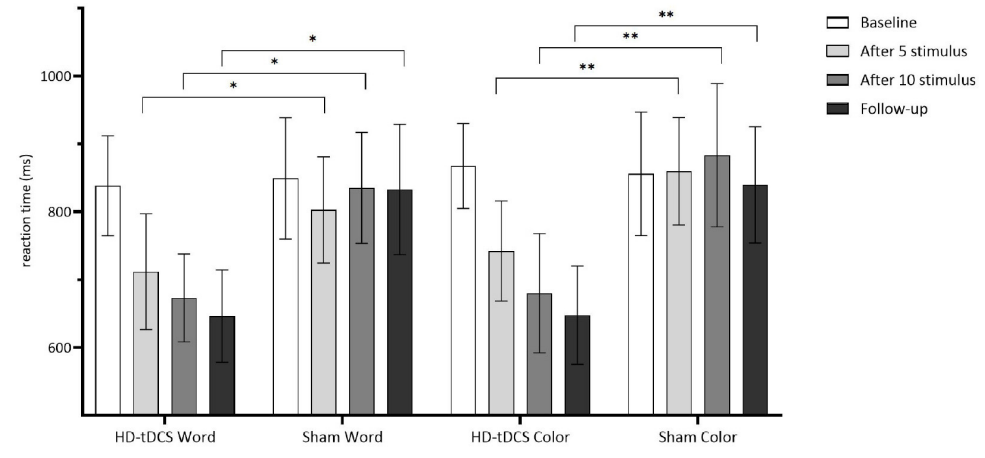
In terms of other neuropsychological indicators, the results of this study showed that, compared with sham stimulus, real HD-tDCS had no significant changes in the correct response number of auditory and audiovisual combination, the commission errors of auditory, the omission of visual, auditory, and audiovisual combination, and the average reaction time of audiovisual combination. The average reaction time of IVA-CPT reflects alertness, cognitive processing speed, and hand-eye-ear coordination. The number of missing reports in IVA-CPT reflects the subjects' attention deficits, that is the intensity and stability of attention. This is partly similar to previous studies. Ouellet et al. evaluated the executive function of healthy subjects by the Iowa Gambling Task, the Stroop Task, the Visual Simulation Scale, the Continuous Work Task, and the Stop Signal Task, among others, after receiving 1.5 mA ATDCS in the left or right OFC. The results showed that subjects receiving ATDCS stimulation of the OFC had more favorable decision-making ability, but tDCS had no effect on attention level (60). The findings of the use of tDCS to improve ADHD cognition were mixed, with some positive results on improving cognition. However, the effect value observed in the meta-analysis is very small. Although the comparability of the results was hampered by the large heterogeneity of the study designs and methods, outcome measures, stimulation parameters, and the sites of anodal and cathodal stimulation (21), there also was heterogeneity in cognitive dysfunction in ADHD (61). Based on current evidence, most of the cognitive effects that have been demonstrated are small and insignificant (20, 21). The results of this study can also be interpreted as the learning effect and the repetition effect of tasks. However, tDCS stimulation might also enhance the learning effect. Sham stimuli that were immediately followed by effective stimuli showed better task performance than expected (62). Jacoby and Lavidor (19) also found that the continuous performance task was not affected by tDCS stimulation, and they believed that the learning effect and the repetition effect of the CPT task itself might have an impact on hyperactivity.

The OFC has also been the target region of tDCS research in recent years, although some results have not been particularly promising. For example, some researchers suggested that tDCS reduces resting blood perfusion in the OFC, which is negatively correlated to risky task behavior (63). The tDCS stimulates the OFC, although it has no effect on the impulsivity, exploration of novel things, and risk-taking behaviors of patients with ADHD.



*The mean reaction time of IVA-CPT audiovisual combination:Mauchly sphericity test: W=0.824, P=0.133.TIME (Greenhouse- Geisser test: F(2.681, 120.643)=10.156, P<0.001, ηp2=0.184), CONDITION (F(1, 45)=0.165, P=0.687, ηp2=0.004), TIME×CONDITION(Greenhouse- Geisser test: F(2.681, 120.643)=0.500, P=0.679, ηp2=0.011)

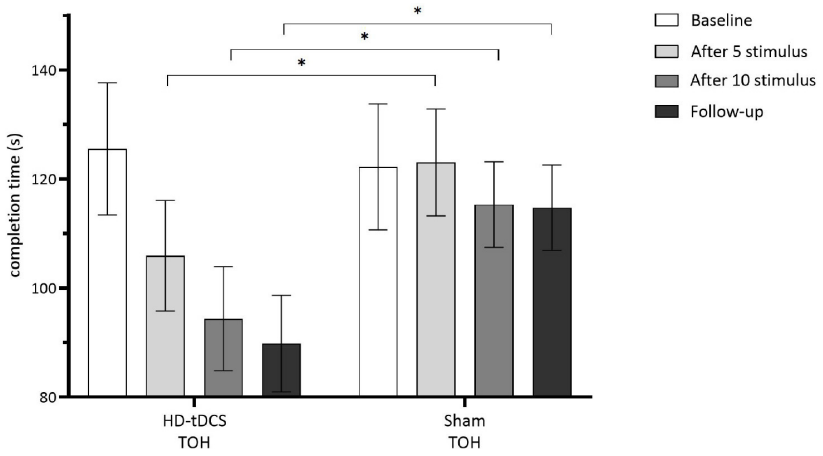
FIGURE 7
Comparison of IVA-CPT mean reaction time between the two groups at different time points. *The mean reaction time of IVA-CPT audiovisual combination: Mauchly sphericity test: $W = 0.824$, $P = 0.133$. TIMF (Greenhouse-Geisser test: $F_{(2.681,120.643)} = 10.156$, $P < 0.001$, ηp2 = 0.184), CONDITION ($F_{(1,45)} = 0.165$, $P = 0.687$, np2 = 0.004), TIME × CONDITION (Greenhouse-Geisser test: $F_{(2.681,120.643)} = 0.500$, $P = 0.679$, ηp2 = 0.011).



*The Interference Effect of Word: Mauchly sphericity test: W=0.785, P=0.060. TIME (Greenhouse- Geisser test: F(2.593, 116,707)=3.376, P=0.026,ηp2=0.070), CONDITION (F(1, 45)=12.790, P<0.001,ηp2=0.221), TIME×CONDITION(Greenhouse- Geisser test: F(2.593, 116.707)=2.350, P=0.085,ηp2=0.050).
**The Interference Effect of Color: Mauchly sphericity test: W=0.689, P=0.006. TIME (Roy's largest root exact test: F(3, 43)=3.454, P=0.025,ηp2=0.194), CONDITION (F(1, 45)=9.532, P=0.003,ηp2=0.175), TIME×CONDITION(Roy's largest root exact test: F(3, 43)=3.429, P=0.025,ηp2=0.194).

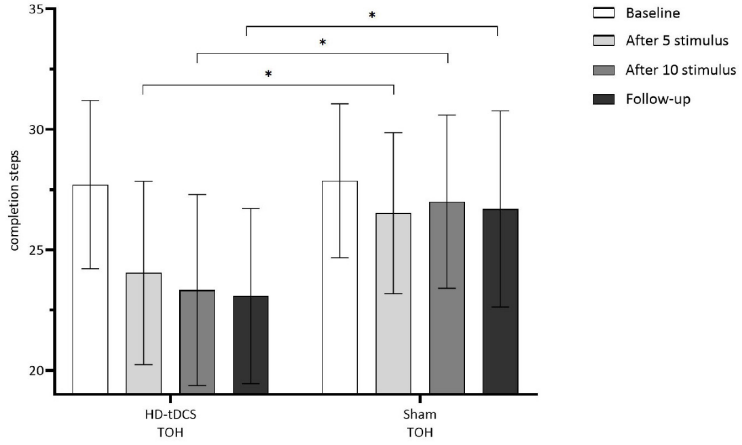
FIGURE 8
Comparison of Stoop Interference effect RT of Word and Color between the two group at different time. *The interference effect of word: Mauchly sphericity test: $W = 0.785$, $P = 0.060$. TIME (Greenhouse-Geisser test: $F_{(2.593,116.707)} = 3.376$, $P = 0.026$, ηp2 = 0.070), CONDITION ($F_{(1,45)} = 12.790$, $P = 0.001$, ηp2 = 0.221), TIME × CONDITION (Greenhouse-Geisser test: $F_{(2.593,116.707)} = 2.350$, $P = 0.085$, ηp2 = 0.050). **The interference effect of color: Mauchly sphericity test: $W = 0.689$, $P = 0.006$. TIME (Roy's largest root exact test: $F_{(3,43)} = 3.454$, $P = 0.025$, ηp2 = 0.194), CONDITION ($F_{(1,45)} = 9.532$, $P < 0.003$, ηp2 = 0.175), TIME × CONDITION (Roy's largest root exact test: $F_{(3,43)} = 3.429$, $P = 0.025$, ηp2 = 0.194).

However, it may benefit the resistance to new things and avoidance behaviors of OCD patients (64). Recently, a strictly double-blind, randomized, sham-controlled trial was conducted to treat 50 boys with ADHD with right frontal hypothalamus (rIFC) anode tDCS (near the OFC stimulation site) for 15 working days, and combined with cognitive training, the results showed no clinical or cognitive improvement. The findings suggested that rIFC stimulation may not be indicated as a neurotherapy for cognitive or clinical remediation for ADHD (59). However, conclusive evidence from previous tDCS studies in ADHD is mixed by remarkable heterogeneity with respect



*The total completion time of TOH: Mauchly sphericity test: W=0.548, P<0.001. TIME (Roy's largest root exact test: F(3, 43)=13.237, P<0.001, ηp2=0.480),CONDITION (F(1, 45)=6.672, P=0.013, ηp2=0.129),TIME×CONDITION(Roy's largest root exact test: F(3, 43)=6.733, P<0.001, ηp2=0.320)

FIGURE 9
Comparison of TOH completion time between the two groups at different time. *The total completion time oh TOH: Mauchly sphericity test: $W = 0.548$, $P < 0.001$. TIME (Roy's largest root exact test: $F_{(3,43)} = 13.237$, $P < 0.001$, ηp2 = 0.480), CONDITION ($F_{(1,45)} = 6.672$, $P = 0.013$, ηp2 = 0.129), TIME × CONDITION (Roy's largest root exact test: $F_{(3,43)} = 6.733$, $P < 0.001$, ηp2 = 0.320.



*The total completion steps of TOH: Mauchly sphericity test: W=0.369, P<0.001.TIME (Roy's largest root exact test: F(3, 43)=5.194, P=0.004, ηp2=0.266), CONDITION (F(1, 45)=9.410, P=0.004, ηp2=0.173), TIME×CONDITION(Roy's largest root exact test: F(3, 43)=2.639, P=0.062, ηp2=0.156)

FIGURE 10
Comparison of TOH completion steps between the two groups at different time. *The total completion steps of TOH: Mauehly sphericity test: $W = 0.369$, $P < 0.001$. TIME (Roy's largest root exact test: $F_{(3,43)} = 5.194$, $P = 0.004$, ηp2 = 0.266), CONDITION ($F_{(1,45)} = 9.410$, $P = 0.004$, ηp2 =0.173), TIME × CONDITION (Roy's largest root exact test: $F_{(3,43)} = 2.639$, $P = 0.062$, ηp2 = 0.156).

to stimulus protocol, sample, ADHD symptoms, and cognitive outcome measures. Therefore, this study draws cautious conclusions that, although HD-tDCS does not significantly improve the overall symptoms of patients with ADHD, it can significantly improve their attention maintenance and other neuropsychological deficits. The results further speculated the effect of HD-tDCS in ADHD, indicating that decision-making and impulse control (cognitive and motor control) are complex and interrelated processes and they depend on neural networks containing multiple cortical and subcortical regions, among which the OFC is particularly important. This study also fills the gap in the research of HD-tDCS stimulation of the right OFC.

Conclusion

This rigorous randomized, sham-controlled trial that had 10 sessions of HD-tDCS was conducted over the right OFC in 47 children and adolescents with ADHD. Although tDCS cannot be recommended as an alternative neurotherapy for ADHD yet, this study draws cautious conclusions that HD-tDCS does not significantly improve the overall symptoms of ADHD patients but leads to significant improvements in cognitive measures of attention maintenance. This study also fills in the research blank of HD-tDCS stimulation of the right OFC.

Limitations

This study has some limitations, for example, the sample size is relatively small. When patients with ADHD and their parents gave informed consent in the clinic, although experimenters have given full information and explanation, they are still sensitive to words like "electrical stimulation" and even mistake them for "electric shock." Therefore, they often refused to join the group, which also added to the difficulty of sample collection. A small sample size poses risks that uncontrollable and confounding variables may be unevenly distributed between groups and, thus, potentially affect the results of the experiment. Another example is the higher depigmentation rate. The depigmentation follow-up was analyzed and it was found that, although they only report mild and transient side effects such as tingling or itching of the scalp, mostly not too good stimulation experience is given, which prompted them to depigmentation experiments. This was also reflected in the fact that some of the subjects who completed the experiment showed impatience when the stimulus was administered. Some subjects even reject repetitive and boring cognitive tasks. They think that the whole experiment process of nearly 2 h is tedious, which is also a big factor for the subjects for whom getting medical treatment is difficult. Finally, the interference items were not excluded as much as possible, and the differences in the age of the subjects (age stratification was not achieved) were not distinguished in the grouping. Among the recruited subjects, there were fewer different ADHD subtypes. For example, in this study, there were more subjects with the combined subtype, but fewer subjects with inattention and hyperactive-impulsive subtypes. Therefore, it was not possible to distinguish the differences among the treatment effects of different subtypes. The learning effect and repetition effect were not excluded, and no crossover experimental design was carried out. All of these factors may overestimate or underestimate the effect of the stimulus. The cortical activity of each subject is also different from their own cognitive level, and these confounding factors are also important reasons for the effect of HD-tDCS. Future studies should systematically evaluate the role of interindividual factors (i.e., ADHD subtype, types of the deficit) and stimulation parameters (i.e., site, polarity, intensity, duration, and repetition rate) on tDCS efficacy in the ADHD population (55).

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The study was approved by the Ethics Committee of Zhenjiang Mental Health Center. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

Y-cWa and JL conceived, designed this study protocol, and wrote this manuscript. Y-cWa, JL, Y-cWu, YW, H-jX, TZ, and ZZ performed this study. Y-cWa, Y-cWu, and ZZ discussed and conducted data analysis. All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Funding

This study was funded by the Zhenjiang Science and Technology Plan (social development guidance) project of the Jiangsu Province of China (No. FZ2020059).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Pediatrics

TYPE Systematic Review
PUBLISHED 20 February 2023
DOI 10.3389/fped.2022.965205

OPEN ACCESS

EDITED BY
Fei Li,
Shanghai Jiao Tong University, China

REVIEWED BY
Xinyue Qi,
Incyte Corporation, United States
Brian Knaeble,
Utah Valley University, United States

*CORRESPONDENCE
Ling-ling Fang
✉ fanglingling2022@126.com
✉ fanglingling@zju.edu.cn

SPECIALTY SECTION
This article was submitted to Child and Adolescent Psychiatry, a section of the journal Frontiers in Pediatrics

RECEIVED 09 June 2022
ACCEPTED 05 December 2022
PUBLISHED 20 February 2023

CITATION
Fang L-l, Zhou Y-y, Jiang H-y and Shi Y-d (2023) Labor epidural analgesia and risk of autism Spectrum disorders in offspring: A systematic review and meta-analysis.
Front. Pediatr. 10:965205.
doi: 10.3389/fped.2022.965205

Labor epidural analgesia and risk of autism Spectrum disorders in offspring: A systematic review and meta-analysis

Ling-ling Fang[1]*, Yuan-yue Zhou[2], Hai-yin Jiang[3] and Yu-dan Shi[4]

[1]Department of Anesthesiology, the Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China, [2]Department of Child Psychiatry, Hangzhou Seventh People's Hospital, Hangzhou, China, [3]Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, the First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China, [4]Department of Chinese Internal Medicine, Taizhou First People's Hospital, Taizhou, China

Background: The effect of labor epidural anesthesia (LEA) on the risk of autism spectrum disorder (ASD) in offspring has been investigated recently, and available results are inconsistent.
Methods: We searched the PubMed and EMBASE databases for relevant studies and performed a systematic review and meta-analysis of the literature. Subgroup analyses were conducted to assess the sources of heterogeneity. Both fixed and random effects models were used was used to estimate overall relative risk.
Results: Our results showed that LEA was associated with an increased risk of ASD in offspring [HR = 1.3, 95% confidence interval (CI): 1.25–1.35; $P < 0.001$] after combining crude estimates from the included studies. This association was gradually reduced, but still statistically significant, when potential confounding factors were considered (HR 1.13, 95% CI 1.03–1.25, $P = 0.014$). However, there was no significant association when we combined data of siblings from other pregnancies (HR = 1.07, 95% CI: 0.99–1.16, $P = 0.076$), implying that the association was due to confounding factors.
Conclusion: The statistically significant association between LEA and ASD in the offspring can be partially explained by unmeasured confounding.
Systematic Review Registration: Identifier CRD42022302892.

KEYWORDS
neurodevelopment, pain, children, meta-analysis, analgesia

Introduction

Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disorder characterized by deficits in social communication and social interaction and the presence of restricted, repetitive behaviors (1). The worldwide population prevalence is about 1% (1). Over the past decade, the incidence of ASD has dramatically increased (2). Although ASD is highly heritable, environmental factors have been shown to be involved in the development of this disorder (3). Thus, recognition of the risk factors for ASD and implementation of appropriate interventions may help to prevent the disorder.

Labor epidural anesthesia (LEA) is the most popular method of pain relief during labor (4). In recent years, growing numbers of women have received some form of neuraxial

procedure during labor (5). Although the effectiveness and safety of LEA for the fetus and newborn have been well described (6), the long-term effects of LEA on the offspring remain unclear. Preclinical studies have demonstrated that standard clinical doses of local anesthetics can alter the normal course of behavioral development in rhesus monkeys (7). Observational studies found that only Cesarean section performed with general anesthesia was associated with an increased risk of ASD compared with vaginal deliveries (8, 9). However, these studies did not evaluate the potential risk associated with the common use of neuraxial anesthesia for routine vaginal delivery. Recently, several epidemiological studies (10–14) have investigated the contribution of LEA to the risk of ASD with varying results. In the earliest study, Qiu et al. (10) reported that LEA was still associated with an increased risk of ASD after taking epidural-related maternal fever into consideration. Meanwhile, one study (11) in Canada also found a significant association between LEA and the risk of ASD in offspring. However, this association was not observed in the latest three studies (12–14). Given that LEA is currently the criterion standard for labor pain management during routine vaginal delivery, it is important to determine whether there is a relation between LEA and the risk of ASD in offspring. We conducted a systematic literature review and meta-analysis to assess the association between fetal exposure to LEA and the subsequent development of ASD.

Methods

This meta-analysis was conducted according to the PRISMA (Preferred Reporting Items of Systematic Reviews and Meta-analysis) guidelines (15). We pre-registered the protocol with PROSPERO (CRD42022302892).

Search strategy

Using the Embase and PubMed Databases, we conducted a search for all studies published in English until January 26, 2022. The search was performed using the terms "labour OR labor" AND "anesthesia OR analgesia" AND "Autism Spectrum Disorder OR Autism OR ASD". To ensure a complete review of the available studies, reference lists of relevant published literature were manually checked to identify additional eligible meta-analyses.

Study selection

Two of the authors (LLF and HYJ) independently evaluated the eligibility of all relevant articles based on the selection criteria until January 28, 2022. Full texts were retrieved after reading the titles and abstracts. Any discrepancies were resolved by discussion with a third author (YYZ). Peer-reviewed studies were included if they met the following (PICO) criteria: (1) types of studies: randomized controlled trials (RCTs), cohort, nested case–control, and case–control studies; (2) type of participant: children exposed or unexposed to LEA; (3) type of intervention: LEA administered during labor and delivery with a valid control group who received no LEA during labor and delivery; (4) types of outcome measures: subsequent ASD development reported in studies with the adjusted ORs or RRs or HRs and 95% confidence intervals (CIs) or provision of adequate data to calculate risk estimates.

Data extraction and quality assessment

Data were extracted independently by YYZ and YDS, and discrepancies were resolved by a third author (HYJ) before the final analysis. The following data were extracted: author, year of publication, data source, study time/period, study design, number of participants, outcome assessment, ascertainment of LEA exposure, and study quality. We assessed the methodologic quality of the included studies using the Newcastle–Ottawa Scale (NOS) as recommended by the Cochrane Collaboration (16). A score >7 points was taken to indicate a high-quality study.

Statistical analysis

All data management and analyses were performed using Stata SE software (ver. 13.0; StataCorp, College Station, TX, USA). Random effects models were used to analyse pooled effects when statistical heterogeneity existed. Otherwise, fixed effects models were used (17). The I^2 statistic was used to assess between-study heterogeneity; studies with I^2 values <25% were considered minimal heterogeneous, values between 25% and 50% indicated moderate heterogeneity, and values ≥50% indicated statistical heterogeneity (18). Publication bias was not assessed because the meta-analysis included fewer than 10 studies (19, 20). All statistical analyses were two-sided, and p-values < 0.05 were considered statistically significant.

Results

Search results

This systematic review identified 74 references from these two databases. After adjusting for duplicates, a total of 52 papers were entered into full-text review, with 38 excluded immediately on inspection of the title and abstract. Two studies (12, 14) used data from the Danish Medical Birth

Register. Although the study period of Ren et al. fully covered that of Mikkelsen et al., Mikkelsen et al. conducted further analyses to test robustness of the overall analysis; hence, Mikkelsen et al.'s study (12) was included in the subgroup-analysis. Finally, five cohort studies (10–14) were identified for inclusion in the review. Some of the excluded studies, together with the reasons for their exclusion, are presented in **Figure 1**.

Characteristics of the included studies

Characteristics of the five studies are presented in **Table 1**. All included studies were published in the past year, and all had large sample sizes, ranging from 123,175 to 624,952. Two studies (11, 13) were performed in Canada, one (10) in the USA, and the remaining two (12, 14) in Denmark. Exposure to LEA was assessed using pharmacy data, and valid

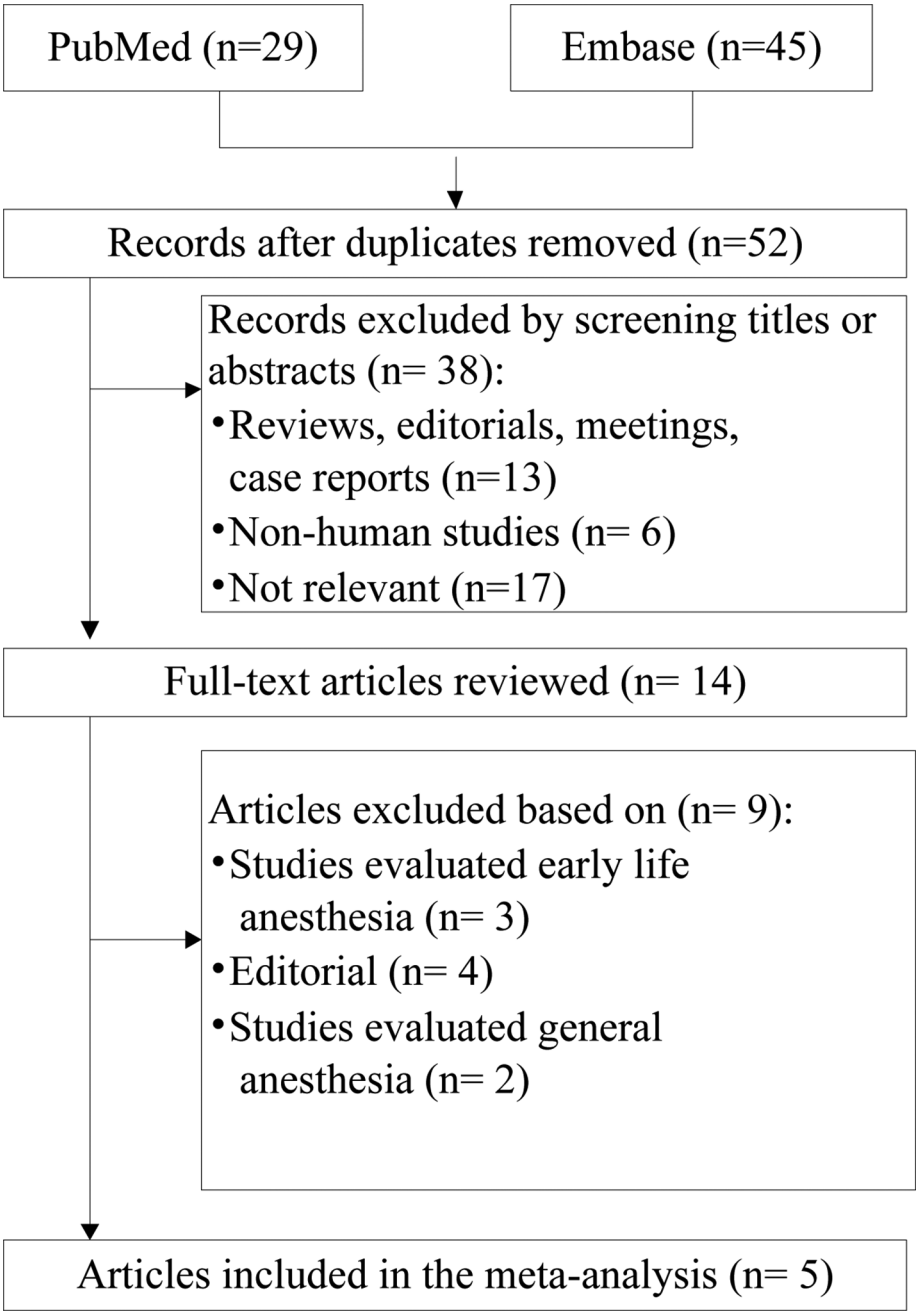

FIGURE 1
Flow chart of the studies considered and finally selected for review.

TABLE 1 Characteristics of the included studies.

Author, year	Location, setting	Study design/ Born period	LEA measurement	ASD assessment	Age at ASD assessment	Age range at the end of study	Number of children	HR, 95%CI				Quality
								Crude	Adjusted[a]	Adjusted[b]	Fully adjusted[c]	
Qiu et al, 2020	USA, population-based	Retrospective cohort study, 2008-2015	Pharmacy data	ICD-9	1.5	3 -11	Unexposed: 485/38,176 Exposed: 2,039/109,719	Overall: 1.48 (1.34-1.65)	NA	NA	Overall: 1.37 (1.22-1.53) Full term: 1.4 (1.25-1.57)	8
Wall-Wieler et al, 2021	Canada (Manitoba), population-based	Retrospective cohort study, 2005-2016	Pharmacy data	ICD-9 or ICD-10	1.5	4.5–15.5	Unexposed: 1,272/76,164 Exposed: 985/47,011	Overall: 1.25 (1.15-1.36)	Overall: 1.28 (1.17-1.4)	Overall: 1.15 (1.04-1.26)	Overall: 1.08 (0.97-1.2) Full term: 1.09 (0.98-1.22) Sibling: 0.97 (0.78-1.22) Restrictive definition: 1.04 (0.91-1.2) First birth only: 1.03 (0.88-1.2)	9
Hanley et al, 2021	Canada (British Columbia), population-based	Retrospective cohort study, 2000-2014	Pharmacy data	ADOS or ADOS and ADI-R	2	2–16.5	Unexposed: 3,482/276,774 Exposed: 1,710/111,480	Overall: 1.32 (1.24-1.4)	Overall: 1.3 (1.22-1.38)	Overall: 1.12 (1.05-1.2)	Overall: 1.09 (1-1.15) Sibling: 1.1 (0.99-1.2)	9
Mikkelsen et al, 2021	Denmark, population-based	Retrospective cohort study, 2006-2013	Pharmacy data	ICD-10	1	4–12	Unexposed: 5,019/386,278 Exposed: 1,409/92,990	Overall: 1.29 (1.21-1.37)	NA	NA	Overall: 1.05 (0.98-1.11) Full term: 1.05 (0.98-1.12) Sibling: 1.05 (0.9-1.21) Restrictive definition: 1.05 (0.96-1.14) First birth only: 1.06 (0.99-1.14)	9
Ren et al, 2021	Denmark, population-based	Retrospective cohort study, 2005-2016	Pharmacy data	ICD-10	1	2 -14	Unexposed: 6,023/508,656 Exposed: 1,648/116,296	Overall: 1.38 (1.31-1.46)	NA	NA	Overall: 1.11 (1.04-1.18) Sibling: 1.03 (0.84-1.27)	9

ADI-R, Autism Diagnostic Interview–Revised; ADOS, Autism Diagnostic Observation Schedule; NA, not available; ICD, International Classification Of Diseases.

[a]Adjusted for maternal sociodemographic covariates.

[b]Adjusted for maternal sociodemographic, pre-pregnancy and pregnancy-related covariates.

[c]Adjusted for maternal sociodemographic, pre-pregnancy and pregnancy-related, and perinatal covariates.

10.3389/fped.2022.965205

diagnostic definitions of ASD were used to identify ASD cases in all studies. The extent of adjustment for potential clinical risk factors varied considerably across studies. Based on the methodological quality assessment scores, all studies were of high quality; their mean score was 8.8. The breakdown of scores is shown in **Supplementary Table S1**.

Meta-analysis

The meta-analysis of the four cohort studies (10, 11, 13, 14) revealed a significant relation between LEA exposure and the risk of ASD (HR 1.33, 95% CI 1.28–1.39, $P < 0.001$; **Figure 2A**) after combing the crude estimates; furthermore, we found moderate heterogeneity across the studies ($I^2 = 27.4\%$). When the analysis was limited to two studies (11, 13) adjusted for only maternal sociodemographic covariates, the pooled HR was 1.29 (95% CI: 1.23–1.36, $P < 0.001$; $I^2 = 0\%$; **Figure 2B**). When the analysis was limited to two studies (11, 13) that were adjusted for maternal pre-pregnancy and pregnancy related covariates, the pooled HR was 1.13 (95% CI: 1.07–1.19, $P < 0.001$; $I^2 = 0\%$; **Figure 2C**). The meta-analysis of the four cohort studies (10, 11, 13, 14) revealed a significant relation between LEA exposure and the risk of ASD (HR 1.15, 95% CI 1.05–1.25, $P = 0.002$; **Figure 2D**) when combining the fully adjusted estimates; however, we found significant heterogeneity across the studies ($I^2 = 82.1\%$). When the analysis was limited to three studies included children older than 12 years at the end of study, the pooled HR was 1.1 (95% CI: 1.05–1.15, $P < 0.001$; $I^2 = 0\%$).

A sibling-matched analysis was conducted in three studies (11, 13, 14) to control for confounding genetic and social factors. As shown in **Figure 3A**, this analysis revealed a nonsignificant difference in the risk of ASD between siblings who were and those who were not exposed to LEA (HR = 1.07, 95% CI: 0.99–1.16, $P = 0.098$; $I^2 = 0\%$). When the analysis was limited to two studies (12, 13) with restrictive definitions of ASD, no significant difference was observed in the risk of ASD (HR = 1.05, 95% CI: 0.97–1.13, $P = 0.215$; $I^2 = 0\%$; **Figure 3B**). When the analysis was limited to two studies (12, 13) evaluating first birth only, no significant difference was observed in the risk of ASD (HR = 1.05, 95% CI: 0.99–1.12, $P = 0.103$; $I^2 = 0\%$; **Figure 3C**). When the analysis was limited to two studies (12, 13) evaluating term birth only, a significant difference was observed in the risk of

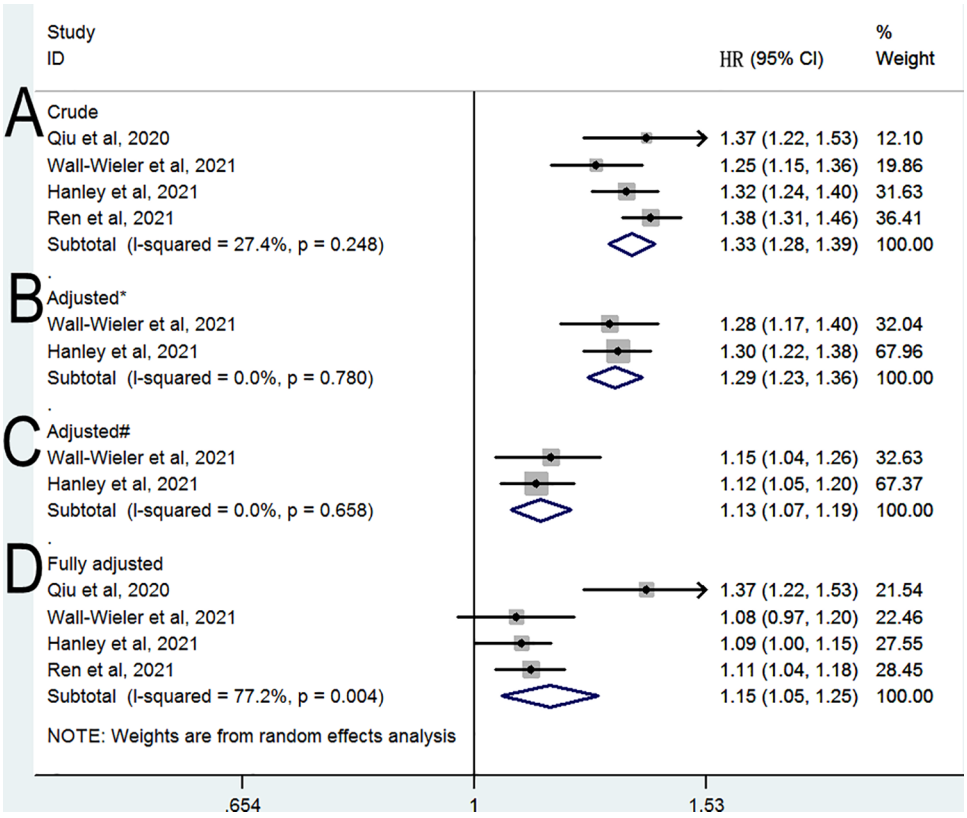

FIGURE 2
Relative risk of subsequent ASD (A) crude HR (B) HR adjusted for * (B) HR adjusted for # (B) fully & adjusted HR. * Adjusted for maternal sociodemographic covariates; # Adjusted for maternal sociodemographic, pre-pregnancy and pregnancy-related covariates; &Adjusted for maternal sociodemographic, pre-pregnancy and pregnancy-related, and perinatal covariates.

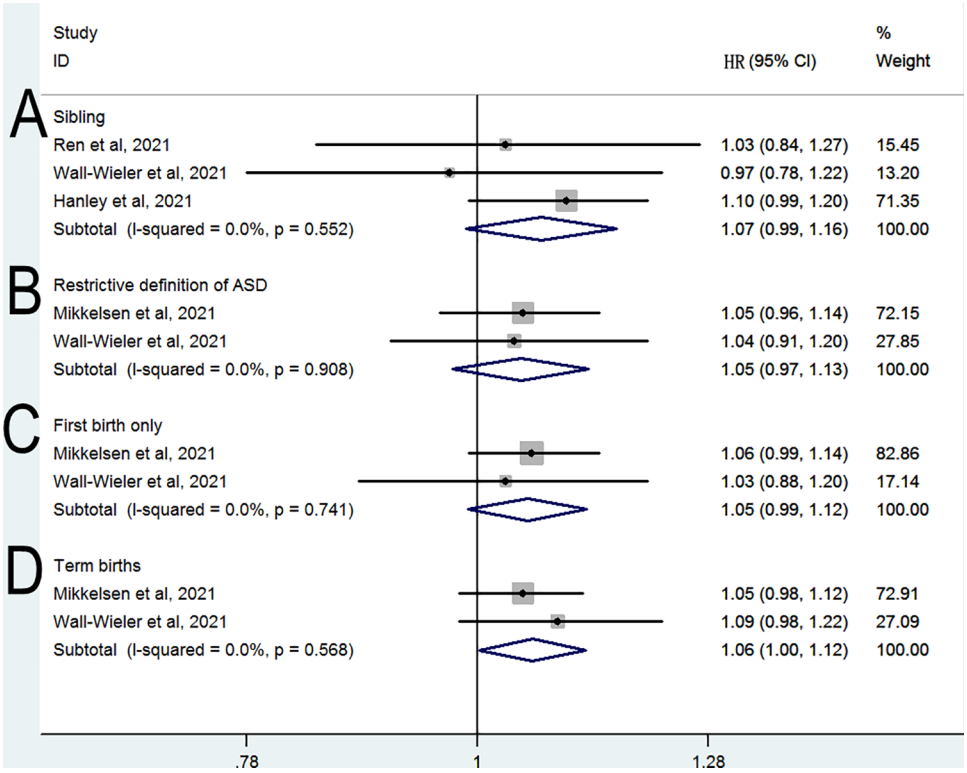

FIGURE 3
Relative risk of subsequent ASD in subgroup analyses (A) sibling (B) restrictive definition of ASD (C) first birth only (D) term births.

ASD (HR = 1.06, 95% CI: 1–1.12, $P = 0.043$; $I^2 = 0\%$; **Figure 3D**).

Discussion

Our findings indicate that LEA exposure was associated with a subsequent risk of ASD in the offspring after combining the crude data. This association was gradually reduced, but still statistically significant, when potential confounding factors were considered step by step. However, results from restrictive definitions of ASD and term birth only suggest that LEA use is not associated with an increased offspring risk of ASD. Furthermore, the sibling-matched analysis showed a nonsignificant effect toward an increased risk of ASD, indicating that genetic and familial confounding factors may largely explain the observed association. Because our review included only a small number of studies, the results should be interpreted with caution.

Our main analysis, based on four observational studies (10, 11, 13, 14), was limited by the existence of residual unknown confounders. All four studies found a positive association between maternal LEA exposure and ASD in the unadjusted model; a significant increased risk of ASD (pooled HR = 1.33) was also observed after we pooled the crude estimates from the included studies. Maternal age, parents' educational background, and economic status were associated with ASD development in the offspring (3); when combined with the estimates adjusted for maternal sociodemographic factors, the risk (pooled HR = 1.29) was comparable to the pooled crude HR. Considering the role of other environmental factors in offspring ASD, two studies (11, 13) gradually added pregnancy-related and perinatal factors and in their adjusted models; the pooled adjusted HR was reduced to 1.13 and 1.15, suggesting that any observed association could be partially explained by potential confounding factors. Also, previous epidemiological studies (21–23) found that a family history of ASD and psychiatric diseases was strongly associated with an increased risk of ASD in the offspring. The study conducted by Qiu et al. (10) reported the highest risk of ASD (adjusted HR = 1.37) among the included studies. However, their findings did not consider the history of mental disorder, and the prevalence of mental disorders was higher in mothers exposed to LEA; thus, the association may be overestimated in this study. Therefore, the ideal control for the unmeasured confounding factors would be a sibling-matched design, which should minimize the effects of familial factors on the observed association. Our analysis based on a sibling-matched design found that the relationship between exposure to

LEA and ASD was not statistically significant, suggesting that any observed association could be a result of genetic factors. It also should be noted that the heterogeneity among the three sibling-matched studies (11, 13, 14) was reduced to 0%. The sample sizes in the sibling-matched studies were small, and further studies are needed to verify these results.

In our main analysis based on fully adjusted estimates, we observed high heterogeneity among the included studies. To explore the clinical heterogeneity and test the robustness of our results, we conducted further subgroup analyses. The studies used various forms of assessment for ASD and different diagnostic definitions of ASD, which could lead to substantially different assessments even in the same study population. To minimize heterogeneity, subgroup analyses based on a restrictive definition of ASD were performed; these found no significant increase in the risk of ASD. Meanwhile, an analysis limited to studies that provided data for first-birth offspring found no difference in ASD risk between children exposed and those unexposed to LEA. This may result for two reasons. First, their sample sizes are small, and their confidence intervals are large. Hence, the results of those studies are inconclusive. Second, it could be that first born individuals are less susceptible to possible adverse effects of LEA. A previous meta-analysis (24) demonstrated that preterm birth was associated with an increased risk of ASD, and three studies (10, 12, 13) included in the present analysis that provided data on term birth revealed a small but significant increase in the risk of ASD (pooled HR = 1.06). The results of our subgroup analyses may be limited by sample size, and further investigation is needed to clarify the effects of these factors on the risk of ASD.

This systematic review and meta-analysis is the first to provide an overall estimate of the effect of maternal LEA exposure on ASD risk in offspring. The strength of our meta-analysis lies in the exclusive use of cohort studies, which are less prone to bias in terms of assessing LEA exposure. In addition, the included studies were of high quality and used valid assessments to evaluate ASD. Another strength of this meta-analysis is the careful consideration of potential confounding factors, especially in step-by-step analyses including the adjustments for confounding factors and subgroup analyses based on sibling-matched studies.

Nevertheless, the study has several limitations. First, the number of included studies in which ASD risk was evaluated was small, especially for sub-group analyses. Second, all reviewed studies were performed with European and North American populations with no subjects from Asian or African countries, which may have affected the generalizability of our findings. Third, limited data were available on the duration of LEA in the included studies; therefore, we could not draw robust conclusions about exposure parameters potentially associated with ASD risk. Finally, controlling confounders in observational studies is a major challenge for causal inference. Future well-designed studies using methods of causal inference (e.g., the use of natural experiments or sensitivity analysis) and considering the duration of LEA are needed to clarify the contribution of LEA to the risk of ASD in children.

Current evidence suggests the associations between LEA and ASD risk in the offspring may be overestimated because previous studies failed to control for genetic confounding factors. Therefore, our findings might not warrant a recommendation to prohibit LEA used pain relief during labor and delivery. Meanwhile, children exposed to LEA do not require additional ASD surveillance.

Conclusion

In conclusion, the findings of our meta-analysis suggest a small but significant link between LEA and ASD risk in the offspring. However, we could not exclude the possibility that this association was overestimated due to potential residual confounders.

Data availability statement

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

Author contributions

LLF and YDS searched the library and wrote the manuscript text. YYZ and HYJ extracted data and reviewed all articles. LLF designed the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by Natural Science Foundation of Zhejiang Province (Grant No. LY20H090012).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.965205/full#supplementary-material.

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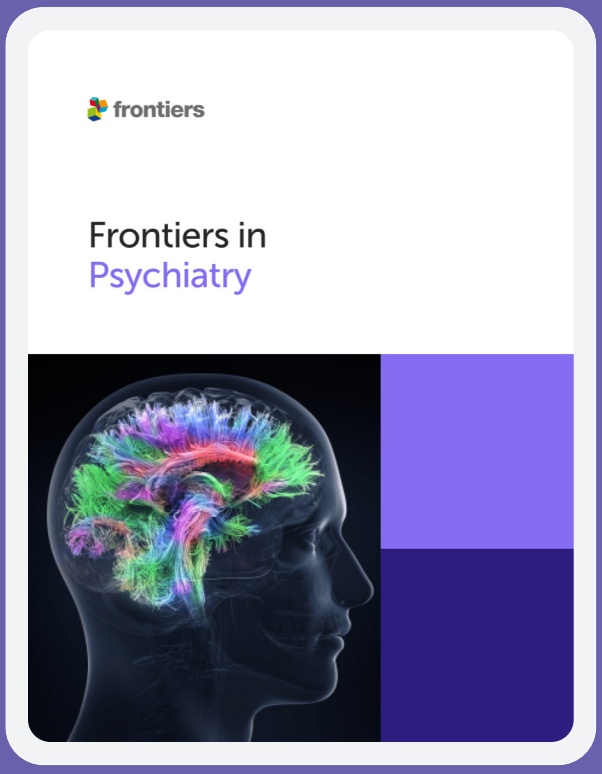

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