

## ARTICLE



# Neurometabolite levels in the brains of patients with autism spectrum disorders: A meta-analysis of proton magnetic resonance spectroscopy studies ( $N = 1501$ )

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Evidence suggests that neurometabolite alterations may be involved in the pathophysiology of autism spectrum disorders (ASDs). We performed a meta-analysis of proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS) studies to examine the neurometabolite levels in the brains of patients with ASD. A systematic search of PubMed and Web of Science identified 54 studies for the meta-analysis. A random-effects meta-analysis demonstrated that compared with the healthy controls, patients with ASD had lower N-acetyl-aspartate-containing compound (NAA) and choline-containing compound (Cho) levels and NAA/(creatine-containing compound) Cr ratios in the gray matter and lower NAA and glutamate + glutamine (Glx) levels in the white matter. Furthermore, NAA and gamma-aminobutyric acid (GABA) levels, NAA/Cr ratios, and GABA/Cr ratios were significantly decreased in the frontal cortex of patients with ASD, whereas glutamate (Glu) levels were increased in the prefrontal cortex. Additionally, low NAA levels and GABA/Cr ratios in the temporal cortex, low NAA levels and NAA/Cr ratios in the parietal and dorsolateral prefrontal cortices, and low NAA levels in the cerebellum and occipital cortex were observed in patients with ASD. Meta-regression analysis revealed that age was positively associated with effect size in studies analyzing the levels of gray matter NAA and white matter Glx. Taken together, these results provide strong clinical evidence that neurometabolite alterations in specific brain regions are associated with ASD and age is a confounding factor for certain neurometabolite levels in patients with ASD.

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## INTRODUCTION

Autism spectrum disorder (ASD) refers to a group of brain-based mental diseases, including autism, Asperger's syndrome, and pervasive developmental disorders, not otherwise specified, that affect over 1% of the global population [1]. As a representative neurodevelopmental disorder, ASD is behaviorally characterized by deficits in social interactions, impaired verbal communication, and repetitive behavior [2]. Despite considerable efforts in this field, the heterogeneity of the illness features that characterize ASD makes it challenging to elucidate the underlying pathology of the illness and its treatment. Therefore, it is important to identify reliable biomarkers for the early screening and treatment of this disorder.

The direct measurement of neurometabolites is a potential strategy for identifying individuals with psychiatric disorders [3]. Proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS) is a noninvasive and simultaneous neuroimaging tool that allows for in vivo quantification of brain metabolites in patients [4]. An increasing number of <sup>1</sup>H-MRS studies have explored the pathophysiology of ASD. The commonly investigated metabolites include N-acetyl-aspartate-containing compounds (NAA), creatine-containing compounds (Cr), choline-containing compounds (Cho), glutamate + glutamine (Glx), myo-inositol (ml), and gamma-aminobutyric acid

(GABA). The NAA signal, which is the most prominent spectral peak, reflects the neuronal density and health [5]. As an important regulator of glutamatergic neurotransmission [6], N-acetyl-aspartyl-glutamate generates a small peak in the brain, which is difficult to distinguish from the N-acetyl-aspartate peak in the <sup>1</sup>H-MRS spectrum. Therefore, the NAA signal measured in this study was contributed by both N-acetyl-aspartate and N-acetyl-aspartyl-glutamate. Cr plays an essential role in central nervous system (CNS) energy homeostasis, representing the most stable cerebral metabolite [7]. The benefit of the concentration of creatine or phosphocreatine is relatively similar throughout the brain, and it is often used as an "internal standard" to normalize signals from other metabolites (for example, the NAA/Cr ratio for the measurement of NAA). Typically, creatine and phosphocreatine levels cannot be reliably distinguished using <sup>1</sup>H-MRS. In this study, we used the term "Cr" used in the context of <sup>1</sup>H-MRS measurements to refer to the combined signal from creatine and phosphocreatine. Cho is often interpreted as a measure of the membrane turnover rate because choline is a constituent of cell membranes [8]. The Cho signal observed by <sup>1</sup>H-MRS is composed of phosphatidylcholine metabolites, especially phosphocholine and glycerol-phosphocholine. As an osmolyte in the CNS [9], ml is a key component of the secondary messenger system, is believed

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to be an essential requirement for cell growth, and has been proposed as a glia-specific marker [10]. Glutamate (Glu) is one of the most abundant mobile metabolites in the brain, while glutamine (Gln) is involved in glutamate recycling and the regulation of brain ammonia metabolism [11]. However, because of the lack of methyl groups in Glu, it produces broad complex peaks that do not generate a prominent single peak in the  $^1\text{H}$ -MRS spectrum [7]. Unless optimized  $^1\text{H}$ -MRS methods are used (e.g., a high-field scanner with a short echo time and long acquisition time or a specialized J-editing or J-resolved sequence), the measurements obtained are generally considered to reflect the combined signal from Glu and Gln [12]. Thus, Glx represents overall Glu and Gln levels and their functioning in the brain. In contrast to Glu, GABA is the major inhibitory neurotransmitter in the brain and plays an important role in maintaining the balance of neural circuits [13].

Although  $^1\text{H}$ -MRS shows promise for profiling ASD by providing chemical compositions of a variety of brain regions, the findings across studies are inconsistent. Taking frontal NAA as a reference, some investigators demonstrated that NAA levels were decreased in patients with ASD compared to those in healthy controls (HCs) [14–16], whereas another study documented that NAA levels were increased in patients with ASD [17]. No significant differences in NAA levels were found in any brain region between patients with ASD and HCs [18]. Similarly, inconsistent findings have been reported for other neurometabolites in patients with ASD. These differences may be attributed to differences in the regions of interest (ROIs), MRS methodologies, stages or severity of illness, or medications.

Therefore, we conducted a systematic review and meta-analysis to analyze the neurometabolite levels in different regions of the brain of patients with ASD. We also explored the influence of age, sex, full-scale intelligence quotient (FSIQ), and magnetic field strength on the neurometabolite levels in the brains of patients with ASD.

## METHOD

We conducted a systematic review and meta-analysis of ASD in accordance with the guidelines recommended by the Preferred Reporting Items for Systematic Reviews and Meta-Analysis statement [19].

### Data sources and study selection

We performed a systematic search of peer-reviewed publications in the English language using the PubMed and Web of Science databases through October 14, 2022, without year limitations. We used the search terms "Autism" AND "MRS" OR "magnetic resonance spectroscopy". In addition, the references of the relevant articles were manually searched to identify additional studies. Studies utilizing  $^1\text{H}$ -MRS to examine neurometabolite levels in patients with ASD and HCs were included. Studies were excluded in the following conditions: (1) review articles, (2) MRS was measured in animal models, (3) lack of HCs, (4) samples overlapped with other studies, (5) in vitro data, (6) lack of necessary data, (7) participants suffering from serious complications, (8) postmortem samples, (9) non-English publications, and (10) articles with individual metabolites studied in < 3 articles.

### Data extraction

Two investigators independently extracted data from the included articles. Primary outcomes of sample size, mean neurometabolite levels, standard deviation (SD), and *P*-values were collected to generate the effective size (ES) from eligible studies. Data on the last name and country of the first author, publication year, mean age, sex distribution (male %), FSIQ, and criteria and diagnosis of the participants were extracted for potential moderator analyses. The ROIs were as follows: (1) cerebellar cortex, (2) prefrontal cortex, (3) dorsolateral prefrontal cortex (DLPFC), (4) frontal cortex, (5) occipital cortex, (6) parietal cortex, (7) temporal cortex, including the hippocampus, (8) thalamus, and (9) BG. When data from the bilateral lobes were reported separately, the left lobe was used because it has been examined in most studies. The demographic and clinical characteristics of the included studies are summarized in eTable.

## Statistical analysis

The Comprehensive Meta-Analysis Version 3 software (Biostat, Englewood, NJ, USA) was used for all statistical analyses. In most cases, ES was obtained using sample size, mean neurometabolite levels, and SDs. If neurometabolite levels and SDs were not available, sample sizes and *P*-values were used to obtain the ES. ES was calculated as the standardized mean difference in brain metabolite levels between patients with ASD and HCs and then converted to Hedges' *g*, and a random-effects model was employed. Hedges' *g* statistic provides a correction factor to address potential biases resulting from the sample size. If between-study heterogeneity exists, the random-effects model can allocate the study weight based on the inverse of the total variance, producing a wider 95% confidence interval than the fixed-effects model [20].

Between-study heterogeneity was assessed using the Cochrane *Q* test and  $I^2$  statistics. For the Cochrane *Q* test,  $P < 0.10$  was considered to be statistically significant. The  $I^2$  index determined the inconsistencies across the original studies and subgroups and evaluated the impact of heterogeneity.  $I^2$  values of 0%, 25%, 50%, 75%, and 100% indicated no, small, moderate, and high heterogeneity, respectively. We then performed subgroup analyses and unrestricted maximum-likelihood random-effects meta-regression of ES to address the between-study heterogeneity.

We first performed a visual inspection of funnel plots to investigate publication bias. Funnel plots were plotted by the effect sizes against precision (inverse of SE). We also used the Egger's test to evaluate statistical significance.

The current meta-analyses included studies with considerable heterogeneity and confounders, including categorical variables (assessed brain region, magnetic field strength, and medication status) and continuous variables (sample size, age, sex, publication year, course of the disease, and disease severity). Therefore, we performed meta-regression analyses to investigate the influence of these potential sources in studies showing significant differences in metabolites between patients with ASD and HCs.

We considered  $P < 0.05$  to be statistically significant unless otherwise noted; a *P*-value below 0.1 was reported as a trend.

## RESULTS

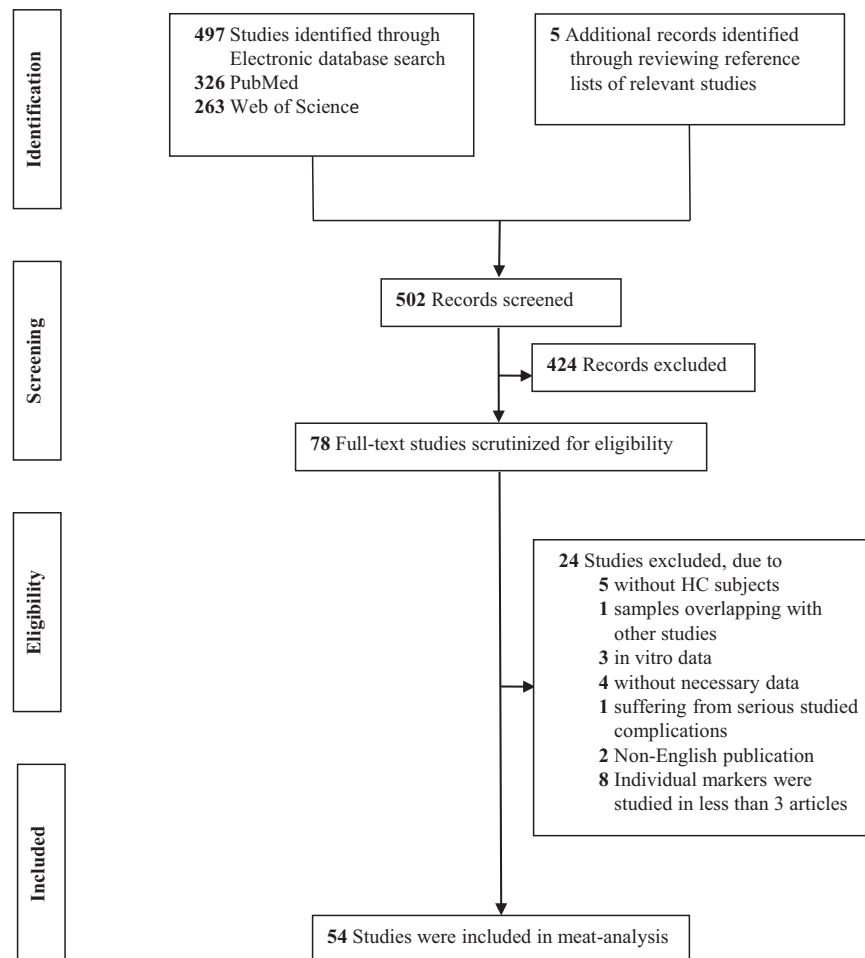
### Characteristics of the included studies

We performed a systematic search, which produced 244 records from PubMed, 253 records from Web of Science, and 5 additional records from the reference lists of the retrieved articles. After scanning the titles and abstracts, 78 articles relevant to the present meta-analysis were identified for a full-text review. A total of 54 studies (including 1,501 patients and 1,294 HCs) that met the criteria were included in this meta-analysis (Fig. 1) [14–18, 21–69].

Most studies were conducted on children ( $N = 34$ , 63%). The sample sizes ranged from 8 to 114 for patients with ASD and 8 to 70 for HCs. The average of each study ranged from 1.9 to 40.54 for patients with ASD and from 2.0 to 41.08 for HCs. Twenty-six (48%) studies were performed at a magnetic field strength of 3 T, while 27 (50%) studies were performed at 1.5 T.  $^1\text{H}$ -MRS protocols and methodological information, including measurement techniques and parameters, are described in Table 1.

### Meta-analysis for neurometabolite level measurements

We compared seven neurometabolite levels across the gray and white matter of the brain between patients with ASD and HCs. Our research revealed a statistically significant decrease in NAA concentrations in both the gray matter and white matter of patients with ASD compared with those in controls (gray matter: nine studies, Hedges'  $g = -3.03$ , 95% CI =  $-0.603$  to  $-0.003$ ,  $P = 0.047$ ; white matter: six studies, Hedges'  $g = -0.690$ , 95% CI =  $-1.168$  to  $-0.213$ ,  $P = 0.005$ ; Table 1). A significant decrease in the NAA/Cr ratio was observed in the gray matter of patients with ASD (five studies, Hedges'  $g = -0.302$ , 95% CI =  $-0.569$  to  $-0.034$ ,  $P = 0.027$ ; Table 1; Fig. 2A). For Glx, there was a significant decrease in the white matter in patients with ASD, while a trend for a significant difference in the gray matter was observed between cases and controls (white matter: five studies, Hedges'  $g = -0.743$ , 95% CI =  $-1.288$  to  $-0.197$ ,  $P = 0.008$ ; gray matter: eight studies, Hedges'  $g = -0.263$ , 95% CI =  $-0.567$  to  $0.041$ ,



**Fig. 1 PRISMA flowchart of the literature search.** A systematic search of Pubmed, Web of Science and relevant references identified 54 articles to be included in the meta-analysis.

$P = 0.090$ ; Table 1; Fig. 2B). Additionally, although we found a significant decrease in Cho (six studies, Hedges'  $g = -0.701$ , 95% CI =  $-1.297$  to  $-0.106$ ,  $P = 0.021$ , Fig. 2C) and a trend for a significant difference in Cr (six studies, Hedges'  $g = -0.425$ , 95% CI =  $-0.859$  to  $-0.009$ ,  $P = 0.055$ ) in the gray matter of children with ASD, subgroup analysis showed that there was no difference between any specific brain regions (Table 2).

Next, we performed a region-specific meta-analysis and found that patients with ASD had significant reductions in NAA in most parts of the brain (DLPFC, frontal cortex, occipital cortex, parietal cortex, and temporal cortex) (DLPFC, 5 studies: Hedges'  $g = -0.349$ , 95% CI =  $-0.694$  to  $-0.004$ ,  $P = 0.048$ ; frontal cortex, 24 studies: Hedges'  $g = -0.234$ , 95% CI =  $-0.419$  to  $-0.048$ ,  $P = 0.014$ ; occipital cortex, 4 studies, Hedges'  $g = -0.667$ , 95% CI =  $-1.026$  to  $-0.309$ ,  $P < 0.001$ ; parietal cortex, 8 studies: Hedges'  $g = -0.318$ , 95% CI =  $-0.535$  to  $-0.102$ ,  $P = 0.004$ ; temporal cortex, 11 studies: Hedges'  $g = -0.354$ , 95% CI =  $-0.672$  to  $-0.037$ ,  $P = 0.029$ ; Table 2; Fig. 3A, B). Notably, NAA level changes in patients with ASD were age-dependent, as we found that NAA level reductions in the cerebellum, frontal cortex, and temporal cortex were specific to children with ASD (cerebellum, 5 studies: Hedges'  $g = -0.305$ , 95% CI =  $-0.558$  to  $-0.053$ ,  $P = 0.018$ ; frontal cortex, 13 studies: Hedges'  $g = -0.288$ , 95% CI =  $-0.534$  to  $-0.043$ ,  $P = 0.022$ ; temporal cortex, 6 studies: Hedges'  $g = -0.514$ , 95% CI =  $-0.917$  to  $-0.111$ ,  $P = 0.012$ ; Table 2). In line with the age-dependent NAA alterations in specific brain regions, analyses of the NAA/Cr ratio in the frontal and parietal cortices revealed a reduced NAA/Cr ratio (frontal

cortex, 12 studies: Hedges'  $g = -0.383$ , 95% CI =  $-0.625$  to  $-0.141$ ,  $P = 0.002$ ; parietal cortex, 3 studies: Hedges'  $g = -0.341$ , 95% CI =  $-0.666$  to  $-0.016$ ,  $P = 0.040$ ) in patients with ASD when compared with that in controls, and the NAA/Cr ratio (nine studies: Hedges'  $g = -0.406$ , 95% CI =  $-0.687$  to  $-0.124$ ,  $P = 0.005$ ) remained low in the frontal cortex of children but not in that of adults with ASD (Table 2).

Moreover, the comparison of metabolic differences in Glx across various brain regions revealed no statistically significant differences between the cases and controls. Although the number of related studies was limited, we found that Glu levels increased significantly in the prefrontal cortex of patients with ASD (five studies: Hedges'  $g = 0.461$ , 95% CI =  $0.043$  to  $0.880$ ,  $P = 0.031$ ; Table 2; Fig. 4A), which was not observed in the BG (three studies: Hedges'  $g = -0.065$ , 95% CI =  $-0.644$  to  $0.515$ ,  $P = 0.827$ ; Table 2). The results further showed that the concentration of GABA (six studies: Hedges'  $g = -0.418$ , 95% CI =  $-0.773$  to  $0.064$ ,  $P = 0.021$ ) as well as the GABA/Cr ratio (four studies: Hedges'  $g = -0.702$ , 95% CI =  $-1.064$  to  $0.340$ ,  $P < 0.001$ ) decreased significantly in the frontal cortex of patients with ASD; a decreased GABA/Cr ratio (three studies: Hedges'  $g = -0.862$ , 95% CI =  $-3.086$  to  $-0.638$ ,  $P = 0.003$ ) was also found in the temporal cortex of patients with ASD (Table 2, Fig. 4B-D).

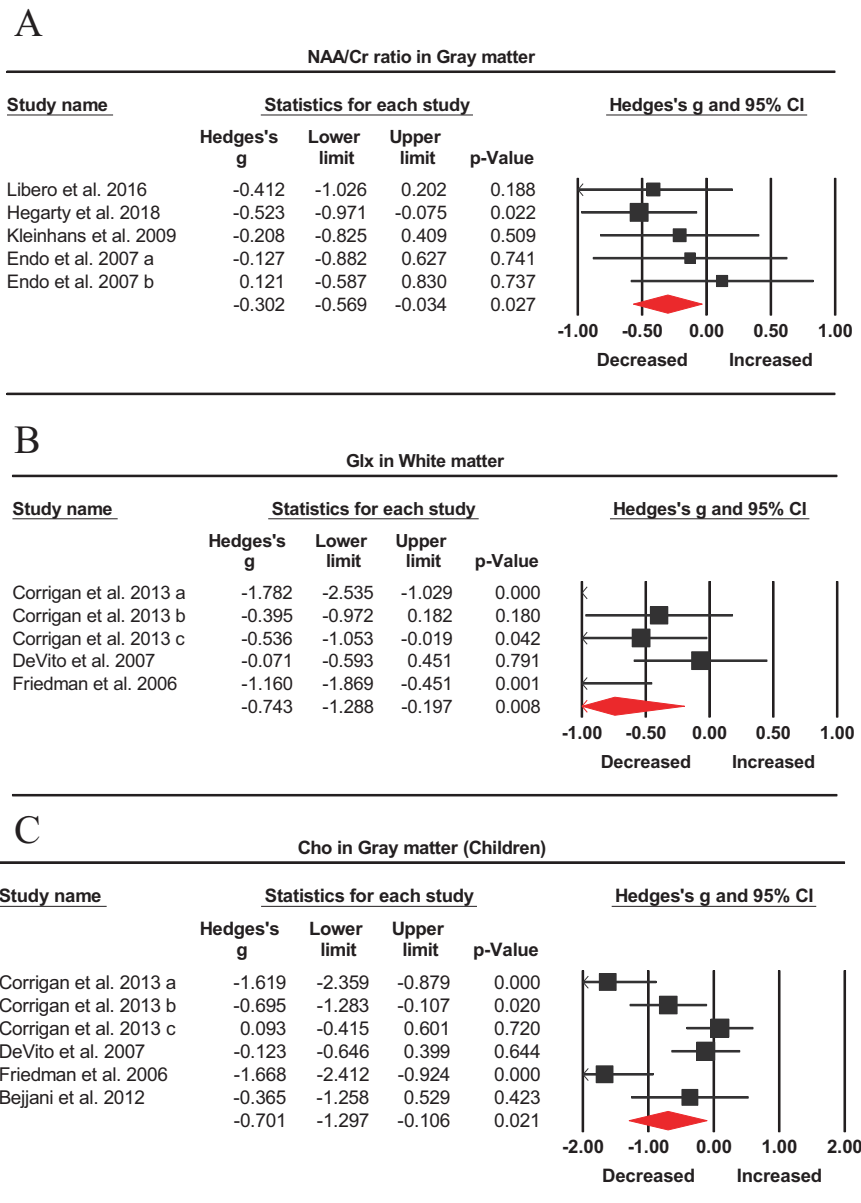
#### Investigation of heterogeneity

Most of the between-study heterogeneities were at low-to-moderate levels in the meta-analysis; next, we performed meta-regression analyses, as shown in eTable 2.

**Table 1.** Summary of comparative outcomes for measurements of gray matter and whiter matter.

1a. Global differences in Gray matter absolute metabolite concentrations alterations in ASD												
Metabolites	No. of Studies	No. With ASD/ Controls	Main Effect			Heterogeneity			Publication Bias			
			Hedges g (95% CI)	Z Score	P Value	Q Statistic	df	P-Value	I <sup>2</sup> Statistic	Egger Intercept	P-Value	
NAA (total)	9	248/170	-0.303 (-0.603 to -0.003)	-1.982	<b>0.047</b>	16.984	8	0.030	52.898	-1.44055	0.68589	
NAA (children)	6	184/106	-0.527 (-0.835 to -0.220)	-4.015	<b>0.001</b>	7.158	5	0.209	30.152	/	/	
NAA (adults)	3	64/64	0.118 (-0.223 to 0.459)	0.677	0.498	1.233	2	0.540	0.000	/	/	
Cr (total)	9	248/170	-0.292 (-0.621 to -0.037)	-1.737	0.082	20.195	8	0.010	60.386	-5.99236	0.04022	
Cr (children)	6	184/106	-0.425 (-0.859 to 0.009)	-1.918	0.055	13.916	5	0.016	64.071	/	/	
Cr (adults)	3	64/64	-0.063 (-0.615 to 0.490)	-0.222	0.824	5.148	2	0.076	61.151	/	/	
Cho (total)	9	248/170	-0.388 (-0.860 to 0.083)	-1.616	0.106	41.056	8	< 0.001	80.514	-6.99692	0.13242	
Cho (children)	6	184/106	-0.701 (-1.297 to -0.106)	-2.309	<b>0.021</b>	25.808	5	< 0.001	80.626	/	/	
Cho (adults)	3	64/64	-0.200 (-0.253 to 0.653)	0.866	0.386	3.464	2	0.177	42.267	/	/	
Glx (total)	8	228/151	-0.263 (-0.567 to 0.041)	-1.696	0.090	13.553	7	0.060	48.349	5.87952	0.04191	
ml (total)	8	228/150	-0.078 (-0.423 to 0.267)	-0.445	0.656	17.458	7	0.015	59.903	-5.75833	0.08944	
ml (children)	5	184/106	-0.150 (-0.726 to 0.426)	-0.512	0.609	16.620	4	0.002	75.932	/	/	
ml (adults)	3	64/64	-0.018 (-0.361 to 0.326)	-0.101	0.920	0.835	2	0.659	0.000	/	/	
NAA/Cr (total)	5	112/102	-0.302 (-0.569 to -0.034)	-2.212	<b>0.027</b>	2.724	4	0.605	0.000	3.35433	0.06199	
Cho/Cr (total)	5	112/102	0.117 (-0.247 to 0.480)	0.630	0.529	6.959	4	0.138	42.522	2.13396	0.55056	
1b. Global differences in White matter absolute metabolite concentrations alterations in ASD												
Metabolites	No. of Studies	No. With ASD/ Controls	Main Effect			Heterogeneity			Publication Bias			
			Hedges g (95% CI)	Z Score	P-Value	Q Statistic	df	P-Value	I <sup>2</sup> Statistic	Egger Intercept	P-Value	
NAA (children)	6	198/116	-0.690 (-1.168 to -0.213)	-2.837	<b>0.005</b>	18.678	5	0.002	73.231	-10.46915	0.05303	
Cr (children)	6	198/116	-0.328 (-0.794 to 0.138)	-1.379	0.168	18.360	5	0.003	72.769	-7.11707	0.31623	
Cho (children)	6	198/116	-0.006 (-0.424 to 0.411)	-0.030	0.976	14.944	5	0.011	66.543	-0.39652	0.95415	
Glx (children)	5	179/96	-0.743 (-1.288 to -0.197)	-2.669	<b>0.008</b>	16.306	4	0.003	75.470	-11.00020	0.04191	
ml (children)	5	179/96	-0.089 (-0.716 to 0.537)	-0.280	0.780	22.145	4	0.000	81.937	-13.80366	0.03002	

df Degrees of freedom, ASD Autism Spectrum Disorder, NAA N-acetyl-aspartate/N-acetyl-aspartyl-glutamate, Cr Creatine/Phosphocreatine, Cho Choline-containing compounds, Glx Glutamate/ Glutamine, ml Myo-Inosito. Bold values represent statistical significance  $p < 0.05$ .



**Fig. 2 Forest plot for random-effects meta-analysis for NAA/Cr, Glx and Cho in gray matter and white matter.** Forest plot for random-effects meta-analysis on differences in various brain neurometabolite (A, NAA/Cr ratio in gray matter; B, Glx in white matter; C, Cho in gray matter) concentrations between autism spectrum disorder (ASD) patients and healthy controls. The sizes of the squares are proportional to the study weight. The diamond marker indicates the pooled effect size. CI Confidence interval.

The current meta-regression for NAA in the gray matter revealed that the mean age of the participants was positively associated with ES (coefficient [95% CI], 0.0337 [0.0113–560];  $P = 0.0032$ ), but not with sex, FSIQ, or publication year (eFig. 1A). For Glx in the white matter, the results showed that mean age was significantly associated with ES (coefficient [95% CI], 0.1914 [0.0504–3324];  $P = 0.0078$ ), whereas sex and publication year had no moderating effect on the outcome of the meta-analysis (eFig. 1B). Furthermore, publication year had a moderating effect on the outcome of the meta-analysis (coefficient [95% CI], 0.1799 [0.0446–3152];  $P = 0.0092$ ) for studies measuring frontal cortex GABA levels, but not mean age, as shown in eFig. 1C. There were no associations between any covariates and NAA or NAA/Cr ratios in the specific brain regions (eTable 2).

#### Publication bias

Publication bias was assessed quantitatively and qualitatively using linear regression analysis and visual inspection of the funnel

plots for each group and brain region. No significant publication bias was detected for most region-specific metabolites, except for Glx in the white matter ( $P = 0.04191$ , Table 1), NAA in the DLPFC ( $P = 0.00125$ , Table 2), and GABA in the frontal cortex ( $P = 0.01774$ , Table 2).

#### DISCUSSION

We conducted a comprehensive systematic review and meta-analysis to compare commonly investigated neurometabolites, such as NAA, Cho, Cr, Glu, Glx, ml, and GABA, in specific brain regions between patients with ASD and controls, while also considering factors that could affect metabolite levels, such as age and intelligence quotient. Our study provided the results of the quantitative synthesis of data from 54  $^1\text{H}$ -MRS studies, of which 1501 were patients with ASD and 1294 were HCs. Our main findings were as follows.

**Table 2.** Summary of comparative outcomes for measurements across various brain regions.

Metabolite	Brain regions	No. of Studies	No. With ASD/Controls	Main Effect		Heterogeneity				Publication Bias		
				Hedges g (95% CI)	Z Score	P-Value	Q Statistic	df	I <sup>2</sup> Statistic	Egger Intercept	P-Value	
NAA	Cerebellum (total)	8	193/164	-0.185 (-0.509 to 0.139)	-1.120	0.263	14.843	7	0.038	52.841	0.51988	0.79560
	Cerebellum (children)	5	139/110	-0.305 (-0.558 to -0.053)	-2.369	<b>0.018</b>	3.526	4	0.474	<0.001	/	/
	Cerebellum (adults)	3	54/54	0.144 (-0.700 to 0.989)	0.335	0.738	8.906	2	0.012	77.544	/	/
	PFC (total)	11	245/253	-0.195 (-0.453 to 0.064)	-1.474	0.141	20.419	10	0.026	51.027	-1.02588	0.72000
	PFC (children)	7	155/131	-0.250 (-0.631 to 0.130)	-1.291	0.197	15.107	6	0.019	60.283	/	/
	PFC (adults)	4	90/122	-0.113 (-0.464 to 0.238)	-0.632	0.527	4.848	4	0.183	38.119	/	/
	DLPFC (total)	5	92/84	-0.349 (-0.694 to -0.004)	-1.980	<b>0.048</b>	5.462	4	0.243	26.771	-10.19998	0.00125
	Frontal (total)	24	572/531	-0.234 (-0.419 to -0.048)	-2.467	<b>0.014</b>	52.394	23	<0.001	56.102	-1.10058	0.46458
	Frontal (children)	13	362/287	-0.288 (-0.534 to -0.043)	-2.299	<b>0.022</b>	27.471	12	0.007	56.317	/	/
	Frontal (adults)	11	210/244	-0.166 (-0.460 to 0.127)	-1.112	0.266	24.135	10	0.007	58.566	/	/
	Occipital (total)	4	78/79	-0.667 (-1.026 to -0.309)	-3.654	< <b>0.001</b>	3.754	3	0.289	20.082	-2.86305	0.58770
	Parietal (total)	8	162/159	-0.318 (-0.535 to -0.102)	-2.879	<b>0.004</b>	3.448	7	0.841	<0.001	1.52689	0.13872
	Temporal (total)	11	280/210	-0.354 (-0.672 to -0.037)	-2.187	<b>0.029</b>	26.818	10	0.003	62.711	1.71751	0.33591
	Temporal (children)	6	205/139	-0.514 (-0.917 to -0.111)	-2.502	<b>0.012</b>	13.808	5	0.017	63.789	/	/
	Temporal (adults)	5	75/71	-0.138 (-0.584 to -0.309)	-0.603	0.546	7.602	4	0.107	47.379	/	/
	Thalamus (total)	6	106/111	-0.244 (-0.511 to 0.024)	-1.786	0.074	5.116	5	0.402	2.268	0.18828	0.95256
	Cerebellum (total)	6	130/105	0.008 (-0.249 to 0.266)	0.064	0.949	1.492	5	0.914	<0.001	1.30922	0.36580
	Cerebellum (children)	3	76/51	-0.076 (-0.434 to 0.282)	-0.415	0.678	0.180	2	0.914	<0.001	/	/
	Cerebellum (adults)	3	54/54	0.098 (-0.272 to 0.468)	0.519	0.603	0.874	2	0.646	<0.001	/	/
	PFC (total)	12	265/273	-0.165 (-0.423 to 0.092)	-1.259	0.208	24.007	11	0.013	54.181	-4.22678	0.08975
PFC (children)	7	155/131	-0.338 (-0.772 to 0.095)	-1.531	0.126	19.258	6	0.004	68.845	/	/	
PFC (adults)	5	110/142	0.000 (-0.248 to 0.248)	0.002	0.998	2.654	4	0.617	<0.001	/	/	
DLPFC (total)	4	72/64	-0.345 (-1.063 to 0.373)	-0.942	0.346	13.168	3	0.004	77.217	-14.19969	0.14076	
Frontal (total)	21	489/452	-0.077 (-0.256 to 0.103)	-0.835	0.403	36.975	20	0.012	45.910	-0.86646	0.59811	
Frontal (children)	12	307/236	-0.131 (-0.402 to 0.141)	-0.944	0.345	25.852	11	0.007	57.450	/	/	
Frontal (adults)	9	182/216	-0.026 (-0.259 to 0.206)	-0.222	0.824	10.923	8	0.206	26.758	/	/	
Occipital (total)	5	93/96	-0.291 (-0.682 to 0.100)	-1.457	0.145	7.440	4	0.114	46.235	1.23651	0.84141	
Parietal (total)	7	107/108	-0.116 (-0.377 to 0.145)	-0.871	0.384	2.055	6	0.915	<0.001	-2.92718	0.31240	
Temporal (total)	9	217/151	-0.028 (-0.416 to 0.361)	-0.139	0.889	24.650	8	0.002	67.546	-0.42591	0.87087	
Temporal (children)	4	142/80	-0.147 (-0.424 to 0.131)	-0.139	0.889	1.518	3	0.678	<0.001	/	/	
Temporal (adults)	5	75/71	-0.078 (-0.691 to 0.848)	0.199	0.842	21.190	4	<0.001	81.124	/	/	
Thalamus (total)	7	135/142	-0.137 (-0.397 to 0.123)	-1.032	0.302	7.283	6	0.296	17.611	-2.17717	0.41715	
Cerebellum (total)	7	138/113	-0.067 (-0.315 to 0.181)	-0.530	0.596	1.919	6	0.927	<0.001	0.76022	0.52551	
Cerebellum (children)	4	84/59	-0.104 (-0.438 to 0.231)	-0.607	0.544	0.951	3	0.813	<0.001	/	/	
Cerebellum (adults)	3	54/54	-0.023 (-0.392 to 0.347)	-0.120	0.905	0.867	2	0.648	<0.001	/	/	
PFC (total)	10	225/239	0.031 (-0.198 to 0.261)	0.269	0.788	13.686	9	0.134	34.239	-3.08075	0.22940	
PFC (children)	5	113/97	0.028 (-0.339 to 0.396)	0.151	0.880	6.916	4	0.140	42.164	/	/	
PFC (adults)	5	112/142	0.028 (-0.299 to 0.354)	0.165	0.869	6.768	3	0.149	40.900	/	/	
DLPFC (total)	5	92/84	-0.031 (-0.508 to 0.447)	-0.126	0.900	10.431	4	0.034	61.651	-13.68222	0.07231	
Frontal (total)	22	497/466	0.066 (-0.106 to 0.238)	0.754	0.451	36.600	21	0.019	42.622	-0.73531	0.64323	
Frontal (children)	10	265/202	0.037 (-0.189 to 0.263)	0.319	0.750	12.840	9	0.170	29.909	/	/	
Frontal (adults)	12	232/264	0.093 (-0.170 to 0.356)	0.696	0.486	23.601	11	0.015	53.391	/	/	
Occipital (total)	4	78/79	-0.141 (-0.816 to 0.534)	-0.410	0.681	13.346	3	0.004	77.522	-3.19736	0.73930	
Parietal (total)	7	107/108	-0.201 (-0.463 to 0.061)	-1.506	0.132	3.012	6	0.807	<0.001	-4.16711	0.19710	

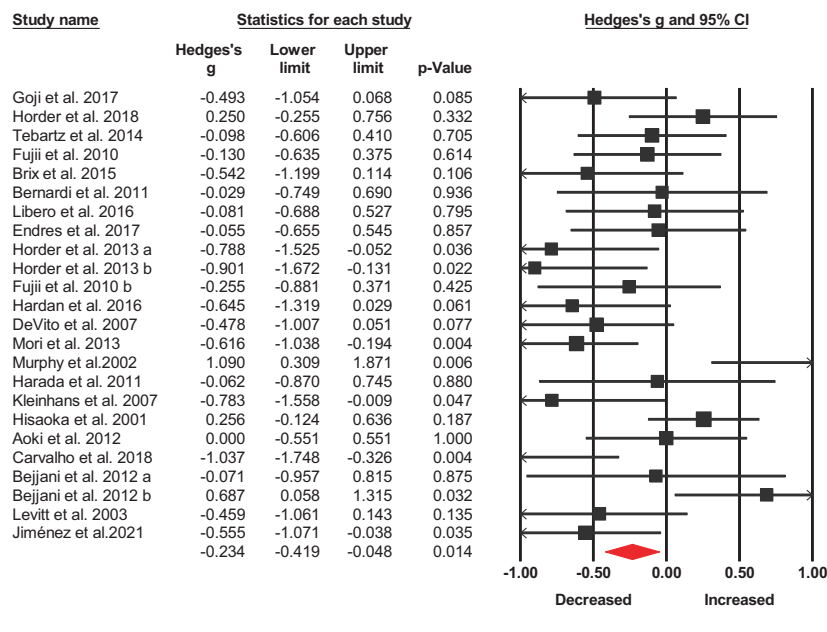
Table 2. continued

Metabolite	Brain regions	No. of Studies	No. With ASD/Controls	Main Effect		Heterogeneity				Publication Bias		
				Hedges g (95% CI)	Z Score	P-Value	Q Statistic	df	P-Value	I <sup>2</sup> Statistic	Egger Intercept	P-Value
Glu	Temporal (total)	10	225/159	0.044 (-0.358 to 0.447)	0.215	0.830	31,003	9	< 0.001	70.971	1.87790	0.43838
	Temporal (children)	5	150/88	0.026 (-0.380 to 0.433)	0.127	0.899	7,960	4	< 0.001	49.750		
	Temporal (adults)	5	75/71	-0.002 (-0.799 to 0.796)	-0.004	0.997	22,683	4	< 0.001	82.366		
	Thalamus (total)	6	106/111	-0.142 (-0.608 to 0.324)	-0.598	0.550	14,820	5	0.011	66.262	-6.16755	0.21129
	BG (total)	3	79/81	-0.065 (-0.644 to 0.515)	-0.219	0.827	6,705	2	0.035	70.170	2.06801	0.73949
	PFC (total)	5	118/153	0.461 (0.043 to 0.880)	2.161	<b>0.031</b>	9,913	4	0.042	59.647	2.29480	0.34045
Glx	Cerebellum (total)	3	78/70	-0.117 (-0.551 to 0.317)	-0.528	0.598	3,507	2	0.173	42.973	6.01322	0.50773
	PFC (total)	11	240/243	-0.050 (-0.243 to 0.144)	-0.501	0.616	11,359	10	0.330	11.967	1.30329	0.54203
	PFC (children)	5	102/83	-0.104 (-0.565 to 0.357)	-0.548	0.583	9,428	4	0.051	57.571	/	/
	PFC (adults)	6	138/160	0.012 (-0.217 to 0.241)	0.104	0.917	1,185	5	0.946	< 0.001	/	/
	DLPCF (total)	4	72/66	-0.210 (-0.539 to 0.119)	-1.249	0.212	1,820	3	0.611	< 0.001	-6.94769	0.00977
	Frontal (total)	15	302/310	-0.127 (-0.293 to 0.040)	-1.490	0.136	15,174	14	0.366	7.738	-0.59977	0.71510
	Frontal (children)	7	140/122	-0.187 (-0.516 to 0.142)	-1.114	0.265	10,373	6	0.110	42.159	/	/
	Frontal (adults)	8	162/188	-0.068 (-0.278 to 0.141)	-0.641	0.522	4,141	7	0.763	< 0.001	/	/
	Occipital (total)	3	75/89	-0.143 (-0.557 to 0.271)	-0.678	0.498	3,615	2	0.164	44.675	9.06574	0.42018
	Parietal (total)	5	88/100	-0.168 (-0.451 to 0.114)	-1.166	0.244	2,914	4	0.572	< 0.001	-2.26413	0.35372
	Temporal (total)	4	88/96	0.325 (-0.419 to 1.070)	0.857	0.391	17,931	3	< 0.001	83.269	9.20697	0.12764
	Thalamus (total)	3	44/40	-0.272 (-0.692 to 0.148)	-1.271	0.204	1,510	2	0.470	< 0.001	7.96170	0.37486
	Frontal (total)	6	140/137	-0.418 (-0.773 to 0.064)	-2.314	<b>0.021</b>	10,268	5	0.068	51.305	-5.42153	0.01774
	Cerebellum (total)	5	160/100	0.024 (-0.232 to 0.279)	0.183	0.855	2,369	4	0.668	< 0.001	0.12495	0.91476
	PFC (total)	3	136/96	0.025 (-0.433 to 0.482)	0.106	0.916	3,785	2	0.151	47.163	-0.14142	0.96585
DLPCF (total)	3	59/56	-0.726 (-1.098 to -0.354)	-3.829	< <b>0.001</b>	1,668	2	0.434	< 0.001	-20.66423	0.05109	
GABA	Frontal (total)	12	354/292	-0.383 (-0.625 to -0.141)	-3.103	<b>0.002</b>	22,589	11	0.020	51.303	-1.47876	0.24433
	Frontal (children)	9	301/233	-0.406 (-0.687 to -0.124)	-2.819	<b>0.005</b>	17,559	8	0.025	54.438	/	/
	Frontal (adults)	3	53/59	-0.314 (-0.904 to 0.276)	-1.043	0.297	5,030	2	0.081	60.239	/	/
	Parietal (total)	3	68/78	-0.341 (-0.666 to -0.016)	-2.055	<b>0.040</b>	1,568	2	0.456	< 0.001	8.07828	0.25111
	Temporal (total)	7	98/99	-0.372 (-0.964 to 0.221)	-1.230	0.219	25,433	6	< 0.001	74.409	-3.47306	0.60066
	Temporal (children)	4	48/55	-0.277 (-0.678 to 0.123)	-1.356	0.175	24,082	3	< 0.001	87.542	/	/
	Temporal (adults)	3	50/44	-0.475 (-1.633 to 0.682)	-0.805	0.421	1,185	2	0.553	< 0.001	/	/
	Frontal (total)	4	56/66	-0.702 (-1.064 to 0.340)	-3.802	< <b>0.001</b>	3,001	3	0.392	0.025	-11.43606	0.17160
	Temporal (total)	3	60/55	-0.862 (-3.086 to -0.638)	-2.981	<b>0.003</b>	14,840	2	0.001	86.523	-3.33252	0.89620

df Degrees of freedom, ASD Autism spectrum disorder, BG Basal ganglia, HC Healthy controls, NAA N-acetyl-aspartate/ N-acetyl-aspartyl-glutamate, Cr Creatine/ Phosphocreatine, Glx Glutamate/ Glutamine, PFC Prefrontal cortex, DLPCF Dorsolateral prefrontal cortex. Bold values represent statistical significance  $p < 0.05$ .

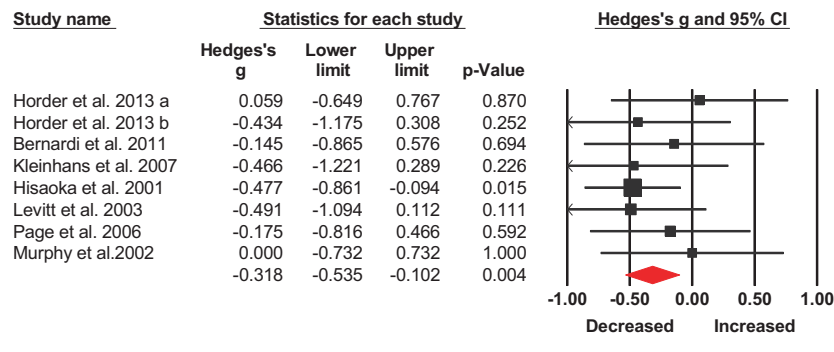
A

## NAA in Frontal cortex



B

## NAA in Parietal cortex



**Fig. 3 Forest plot for random-effects meta-analysis for NAA in frontal cortex and parietal cortex.** Forest plot for random-effects meta-analysis on differences in frontal cortex (A) and parietal cortex (B) NAA concentrations between autism spectrum disorder patients and healthy controls. The sizes of the squares are proportional to the study weight. The diamond marker indicates the pooled effect size. CI Confidence interval.

(1) The comparison of metabolic differences between patients with ASD and HCs across the gray matter of the brain revealed statistically significant differences in NAA levels and NAA/Cr ratios, accompanied by a trend for significant differences between patients with ASD and HCs for Cr and Glx analyses.

(2) In the white matter, there were significant reductions in NAA levels and Glx concentrations between patients with ASD and HCs, and no statistically significant differences were found for Cr, Cho, and ml.

(3) A region-stratified anatomical focus on NAA showed that there were significant reductions in NAA in many brain regions (including the DLPFC and frontal, occipital, parietal, and temporal regions), but not in the prefrontal cortex and thalamus.

(4) Patients with ASD showed a significant decrease in the NAA/Cr ratio in the DLPFC, frontal cortex, and parietal cortex, whereas no differences were observed in the cerebellum, prefrontal cortex, and temporal regions.

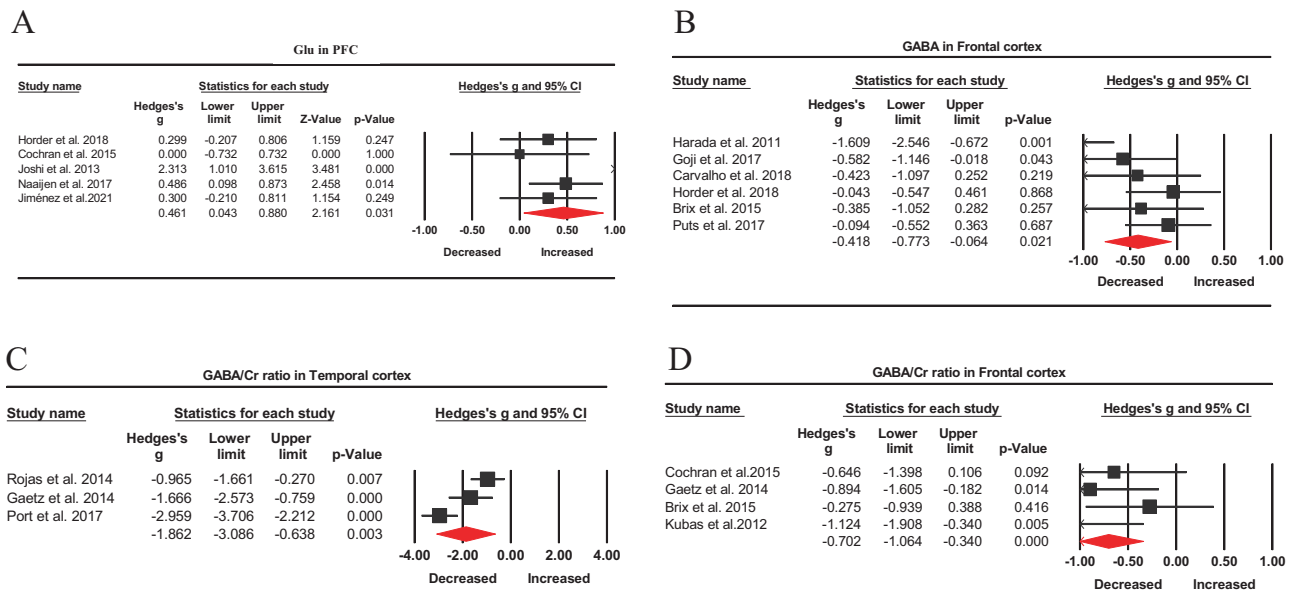
(5) Significant differences were found for GABA and the GABA/Cr ratio between patients with ASD and HCs in the frontal cortex, while the GABA/Cr ratio decreased in the temporal cortex of patients with ASD.

(6) Glu increased significantly in the prefrontal cortex of patients with ASD.

(7) Age was a confounding factor in this meta-analysis.

In addition to ASD, it has been suggested that abnormalities in the neurometabolites in the brain are implicated in the pathophysiology of other neurological diseases such as MDD, Alzheimer's disease, and schizophrenia. Previous meta-analyses have demonstrated that Glx levels are significantly decreased in patients with major depressive disorder [70], which was not observed in our meta-analysis assessing Glx levels in patients with ASD. Another meta-analysis showed that there was a decrease in NAA levels in patients with schizophrenia [71]. Together with our findings of decreased NAA levels in the brains of patients with ASD, this supports the hypothesis that there is a strong association





**Fig. 4 Forest plot for random-effects meta-analysis for Glu, Glx and GABA in various brain regions.** Forest plot for random-effects meta-analysis on differences in various brain neurometabolite (A, Glu in prefrontal cortex; B, GABA in frontal cortex; C, GABA/Cr ratio in temporal cortex; GABA/Cr ratio in frontal cortex) concentrations between autism spectrum disorder patients and healthy controls. The sizes of the squares are proportional to the study weight. The diamond marker indicates the pooled effect size. CI Confidence interval.

between ASD and schizophrenia [72]. Interestingly, the present meta-analysis revealed alterations in Cho, Cr, Glu, and GABA levels in different brain regions between patients with ASD and HCs. It is very likely that patients with ASD have a unique neurometabolite factor profile compared to those with other neuropsychiatric diseases, which may partially explain the pathogenesis of ASD.

NAA is a reliable marker of mitochondrial activity and is often used as a chemical marker of neuronal integrity [73]. Autopsy reports suggest that a decrease in NAA may activate and increase astroglia with an increased inflammatory response [74, 75] thus negatively affecting tissue damage and nerve repair in the brain [26], which is consistent with our findings of NAA reductions in the brains of children with ASD using <sup>1</sup>H-MRS. Additionally, metabolite differences associated with NAA and the NAA/Cr ratio were found to be age-dependent. A significant decrease in both NAA levels and the NAA/Cr ratio was observed only in the frontal cortex of children with ASD. Children with ASD have an enlarged frontal cortex in their infancy [76] with the presence of inflammation or hypoperfusion, as suggested by signs, including decreased serum levels of adhesion molecules and a correlation with their head circumferences [74, 77]. As a key region of the human brain for communication and cognitive function, the frontal cortex contributes to early developmental defects in children with ASD, including glial activation, migration defects, and abnormal apoptosis [78]. Therefore, it is reasonable to conclude that NAA abnormalities in ASD are age-dependent. This result is consistent with previous studies suggesting premature aging and neurodegenerative processes in autistic brain, as Ivashko-Pachima et al. discovered a genomic autism activity-dependent neuroprotective protein mutation in postmortem Alzheimer's disease brains correlating with increasing tauopathy [79], and data from Grigg further suggested that tauopathy may explain brain imaging results of atrophy in ASD children [80]. Nevertheless, future research should examine NAA longitudinally over the life course, both in humans and rodent models of ASD, to determine the complete profile of the NAA system in ASD.

Another neurometabolite that was significantly decreased in the frontal cortex was GABA, which is consistent with the decrease in GABAergic interneuron subtype cells found in the prefrontal

cortex of postmortem autistic brains [81]. Notwithstanding a previous meta-analysis suggesting that GABA levels are lower in patients with ASD than those in controls [82], our findings further revealed the specific areas of the brain with dysregulated GABA levels in patients with ASD and analyzed the absolute and relative GABA levels. Consistently, various animal models have revealed a correlation between low GABA levels and ASD (for a review, refer [82]). Furthermore, an examination of ASD in post-mortem studies indicated the downregulation of enzymes involved in GABA activation [83, 84]. Clinical studies have demonstrated that the density, number, and protein subunits of GABA receptors are decreased in patients with ASD [85–87]. The low frontal cortex GABA system in ASD could be a result of the loss of GABAergic interneurons [88], indicating that impaired GABA processing could become a novel therapeutic target for autism [89]. Consistent with this hypothesis, we found a significant decrease in the GABA/Cr ratio in the temporal cortex of children with ASD. However, due to the limitations of the included literature, the relationship between GABA and other brain regions requires further study.

Excitatory neurotransmission in the brain is primarily mediated by glutamatergic neurons. The correlation between dysfunction of the glutamatergic system and ASD is widely recognized. Glutamatergic receptor expression, along with its function, is altered in patients with ASD, which is in line with a growing body of evidence from various animal models of ASD (for a review, refer [90]). Glu is a major excitatory neurotransmitter widely distributed throughout the CNS. A meta-analysis suggested that blood Glu levels are higher in patients with ASD than those in controls [91], which is consistent with our results of increased Glu levels in the prefrontal cortex of patients with ASD, suggesting that Glu dysfunction may be regionally specific in ASD. Because GABA levels are decreased in the frontal cortex of patients with ASD, the increase in Glu in these patients may be due to the reduced levels of glutamic acid decarboxylase in the brain that converts Glu into GABA [84, 92]. Therefore, our findings support Fatemi's hyperglutamatergic hypothesis of autism [93]. Genetic studies have suggested that mutations in the *GRIN* gene encoding the N-methyl-D-aspartate receptor are associated with ASD [94, 95]. Thus, further research is needed to explore whether the Glu level

can be used as a potential biomarker for diagnosis in the future. However, Glu and Gln are difficult to separate; therefore, Glx has been used instead in most studies. Our findings revealed low Glx levels in the white matter of children with ASD, and the decrease in Glx may not simply be reflected as a decrease in Glu or Gln because the ratio of Glu to Gln in these patients is unknown.

The strength of this work is that it is the most comprehensive study of neurometabolites in patients with ASD to date. Furthermore, the between-study heterogeneity was not high, suggesting the robustness of our meta-analysis. The potential moderators that we analyzed included the brain region source, magnetic field strength, sample size, publication year, age, and sex. Some unexplained heterogeneity may be due to other variables that were not analyzed, such as medication status, disease severity, and lifestyle. Importantly, our analyses showed a specific decrease in the NAA levels in the frontal and temporal cortices of individuals with ASD, which was not reported in a previous meta-analysis of NAA levels in patients with ASD published in 2012 due to the limited sample size [96].

This meta-analysis has some limitations. First, although we categorized ROIs into different brain areas, the classification might have been oversimplified considering the functional variability within each subarea. Voxel sizes also varied among the studies, and these differences might have skewed the results of the ROI analyses. Second, the field strength was  $\leq 3$  T, which limited the accurate differentiation of homologous neurometabolites. For example, Glx is composed of both Glu and Gln, but Glu accounts for approximately 80% of Glx levels at 1.5 T or 3 T [97]. Moreover, it is difficult to differentiate N-acetyl-aspartyl-glutamate from N-acetyl-aspartate and Glu using *in vivo*  $^1\text{H}$ -MRS because of the similarity in their structures and spectra [98]. Moreover,  $^1\text{H}$ -MRS does not provide information regarding cell types. Future studies should increase the field strength to improve the separation of individual signal spectra. Third, most studies included in the meta-analysis did not consider food or smoking information as potential confounders. Food intake can influence neurometabolic levels in the brain, and a study showed decreased Glx, Cho, NAA, and Cr levels in the human brain after food intake and that the frontal cortex is the most affected by neurometabolite levels [99]. Furthermore, smoking has been reported to directly or indirectly affect Glu and Cho levels in the frontal cortex [100]. Finally, due to the limited number of studies and small sample sizes assessing neurometabolite levels in the DLPFC and occipital and parietal cortices, we did not perform subgroup analyses on these brain regions.

In conclusion, the results of our meta-analysis suggest that compared with HCs, patients with ASD have lower NAA and Cho levels and NAA/Cr ratios in the gray matter, and ASD is associated with decreased levels of NAA in the DLPFC and frontal, occipital, parietal, and temporal cortices. Low levels of Glx were observed in the white matter of children with ASD, whereas Glu levels were found to be increased in the prefrontal cortex. Our study further revealed decreased frontal cortex GABA/Cr and NAA/Cr ratios as manifestations of ASD. These results strengthen the clinical evidence of an abnormal neurometabolite profile in the brains of patients with ASD.

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## AUTHOR CONTRIBUTIONS

YC conceived and designed this study; YD and MCY extracted the data; all authors analyzed and interpreted the data; and YD drafted the manuscript with critical revisions from YC, LC, and MCY.

## COMPETING INTERESTS

The authors declare no competing interests.

## ADDITIONAL INFORMATION

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