Association Between Essential Metal Elements and the Risk of Autism in Chinese Han Population

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Abstract

Essential metal elements (EMEs) have essential roles in neurological development and maintenance of human homeostasis. We performed a case-control study to explore association between the risk of autism spectrum disorder (ASD) and the 11 EMEs [Calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), manganese (Mn), selenium (Se), cobalt (Co), Molybdenum (Mo), copper (Cu), zinc (Zn), and iron (Fe)] in serum. Ninety-two autistic subjects (cases) and age-sex-matched healthy subjects (controls = 91) from Beijing, China were recruited. In addition, totally 109 mothers of recruited children participated in this study. ICP-AES and ICP-MS were applied to determine the concentration of 11 EMEs in serum. The concentrations of Ca, K, and Mg were significantly higher in the cases than in the controls (OR [95% CI]: 1.031 [1.006–1.058] for Ca; 1.081 [1.046–1.118] for K; 1.161 [1.012–1.331] for Mg), while the concentrations of Zn and Cu were significantly lower (0.997 [0.995–0.999] for Cu; 0.996 [0.992–1.000] for Zn). Clear dose-response relationships between EMEs concentrations and the risk of ASD, as well as the correlation between EME concentrations and the severity of ASD were observed for most of the above EMEs. Six and seven specific correlated pairs between mothers and children were found in the cases and controls separately. The overall profiles of the EMEs were changed in the cases as compared to the controls. This study suggested that the higher levels of Ca, K, and Mg and lower levels of Zn and Cu may be associated with an elevated risk of ASD.

Keywords Macro essential metal elements · Zn · Autism · Serum · Risk

Introduction

Autism spectrum disorder (ASD) is a developmental disorder associated with a strong genetic component as well as other causes, characterized by social communication deficits and repetitive sensory-motor behaviors [25]. The World Health Organization (WHO) reported that the prevalence of ASD in children is 0.76% based on around 16% of the world population, while the prevalence was estimated as 0.95% in boys and 0.30% in girls in China [52]. In recent years, the incidence of ASD has been increasing and has caused serious public health concern [7, 26, 29, 31, 38, 45].

A large number of studies have investigated the potential physiopathology of ASD. But the causes and risk factors of this disease remain unclear [10, 28, 32, 33, 42]. The imbalance of metal elements, which may result in biological malfunctions

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and nervous system impairment is thought, however, to be associated with the pathophysiology of ASD [2, 4, 13].

Essential metal elements (EMEs) participate in a number of immune and metabolic processes of the human body and are reported to have an important role and influence in the development of the nervous system [51, 53]. Substantial studies have suggested association between the lack or excess concentration of EMEs and the risk of mental disease, including ASD. It has been reported that zinc (Zn) as a constituent metal of superoxide dismutase, participates in the antioxidant process and binding process of neural ligand protein and Shank3 protein which are most closely related to ASD in the shank family [17]. Also, some studies have shown that abnormal concentrations of sodium (Na), calcium (Ca), and potassium (K) in the blood are associated with oxidative stress and implicated in the pathogenesis of ASD [13, 23]. Moreover, several studies suggested that the concentration of EMEs was related to the clinical features of autism. For example, a study conducted in Italy found the concentration of Mn was inversely correlated with cognitive level in ASD individuals [14]. Another study performed with Bengali showed Mg was associated with hyperactivity and irritability of ASD children [22]. Significant association between the concentration of Zn and severity of autistic symptoms (including fear, nervousness, and more impairment in creativity) was found both in Europeans and Asians [8, 14]. However, the associations between metal elements and ASD found by studies are not consistent. For example, a study conducted with Arizona subjects in America indicated that the concentration of K in autistic children was significantly lower than that in normal children [2], while another two studies performed with subjects in the Sultanate of Oman and Saudi Arabia separately suggested the inverse results, i.e., the concentration of K was higher in autistic children than in healthy controls [4, 13]. In addition, the sample size of previous studies was relatively small, and there was no strict control of the treatment or drugs provided to the children, and this may have impacted the concentration of EMEs and caused bias. Moreover, despite the increasing prevalence of ASD in the Chinese population, few studies have been conducted with Chinese. In addition, the concentration of EMEs in the mother's body had a great influence on children and mothers very likely had been exposed to the same environment as the children. Therefore, it is worthwhile to analyze the EMEs in the mother's body simultaneously with that of the children. Therefore, large-scale and more comprehensive studies performed with drug naive ASD children and their mother are needed.

In this study, we conducted a pilot study on the relation of 11 EMEs (i.e., Ca, K, Mg, Na, Mn, Se, Co, Mo, Zn, Cu, and Fe) and the risk of ASD. We believe that these findings could help to clarify factors related to ASD and support investigation of the relationship between the serum concentration of these 11 EMEs and ASD in the Han Chinese population.

Material and Methods

Characteristics for Study Population

In this case-control study, sex-age matched 92 children who had ASD (cases), and 91 were healthy subjects (controls) were recruited from September 2015 to September 2016 in the Autism Rehabilitation Institution in Beijing, China. All autistic children were evaluated by expertise clinicians in evaluating ASD and satisfied the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th Revision (DSM-IV) for ASD and with a score of Childhood Autism Rating Scale (CARS) over 30. At the same time, a total of 109 mothers of children was recruited (81 mothers of children with ASD V.S. 28 mothers of healthy controls). Detailed inclusion criteria for autistic subjects were as follows: 2–8 years of age, drug-naïve, and no other nervous system diseases. Age- and sex-matched control subjects were recruited during the same time in Shibalidian Kindergarten in Beijing (closest kindergarten to Autism Rehabilitation Institution). A clinical specialist confirmed that all subjects had (1) not received mineral or hormone supplements and other drugs that might have affected metal concentration; (2) not suffered from a cold, asthma and other diseases; (3) not experienced fragile X syndrome, nodular sclerosis, Down's syndrome, and other genetic diseases.

Demographic information was collected face-to-face by trained local health workers, including age, sex ("male," "female"), family income ("< 5000", "≥ 5000"), birth information (weight, father age, mother age), prenatal situation [X-ray ("no," "yes"), active smoking ("no," "yes"), drinking ("no," "yes"), psychological trauma ("no," "yes"), times of pregnancy ("< 3," "≥ 4"), mood ("happy for most of the time," "sad for most of the time," "for a certain time unhappy").

CARS was used by psychiatrists to assess the severity of ASD [39]. This scale contains 15 items, i.e., relating to people (RTP); imitation (IMT); emotional response (ER); body use (BU); object use (OU); adaptation to change (ATC); visual response (VR); listening response (LR); taste smell and touch response and use (TSTRU); fear or nervousness (FON); verbal communication (VC); nonverbal communication (NC); activity level (AL); level and consistency of intellectual response (LCIR); and general impressions (GI). For each item, the assignment ranges from 1 (no symptom) to 4 (most serious). The total score of CARS ranges from 15 to 60, and over 30 is the cutoff for diagnosis of ASD.

The study protocol was reviewed and approved by the Ethics Review Committee of the Health Science Center, Peking University (IRB00001052-13064). The guardians of the children participated in the project gave written informed consent in accordance with the Declaration of Helsinki. All methods were used in accordance with relevant guidelines and regulations.

Serum Sample Preparation and ICP Analysis

A blood sample (~ 3 mL) was collected through venous puncture after an overnight fast. Each blood sample was stored in a vacuum blood collection vessel (BD Vacutainer® SST™ II Advance) and kept for 1 h at room temperature. All the samples were centrifuged at 2000 rpm for 10 min and then the supernatant was transferred into a 500-μ LEP tube and stored at − 20 °C until analysis.

For the measurement of Ca, K, Mg, and Na, the serum sample (0.05 mL) was mixed with 4.85 mL 1% ultrapure nitric acid (Merck) and 0.1 mL yttrium (0.2 μg/mL, Yttrium Standard for ICP-MS, Merck). Afterwards, a concentration of above four metals was confirmed by an inductively coupled plasma atomic emission spectrometry (ICP-AES, iCAP-6300, Thermo, USA). For the measurement of Mn, Se, Co, Mo, Zn, Cu, and Fe, a 0.1 mL serum sample was mixed with 0.1 mL rhodium (20 ng/mL, Merck), 0.1 mL indium (2 ng/mL), and 1.7 mL 1% nitric acid, and then the above 7 metals were measured by an inductively coupled plasma mass spectrometry (ICP-MS, ELAN DRCII, PerkinElmer, USA). Instrument condition and parameter of ICP-AES and ICP-MS are shown in Supplementary Table 1 and Supplementary Table 2. For quality control, ClinChek® Serum Control (Level I, REF: 8880, Germany) and Pork Liver Reference Material (GBW10051, China) were applied as standards.

The instrument limit of elements of ICP-AES/ICP-MS is presented in Supplementary Table 3. The measured and standard concentrations of these certified reference materials are shown in Supplementary Table 4.

Statistical Analysis

For the continuous variables, normal distribution data were summarized as the mean and standard deviation and the independent t-test was used to test statistical significance between groups. Non-normal distribution data were summarized as median (inter-quartile range) and the Mann-Whitney U test was used to compare differences of groups. The categorical variables were summarized as the frequencies and proportions and the Chi-squared ($\chi 2$) test was used for analyzing the difference of the categorical variable. A logistic regression model was applied to determine the association between ASD and essential metals. Birth information of children (i.e., weight, father age, mother age) and the prenatal mood of the mother were adjusted in the above model. Variables significantly different between case and control were adjusted. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were estimated. Spearman correlation was used to calculate the correlation between variables. A two-sided $p < 0.05$ was considered statistically significant. All analyses were performed using R 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria, 2019).

Table 1 Baseline characteristics of the autistic subjects (cases) and healthy subjects (controls)

Characteristics	Cases ($N^a = 92$)	Controls ($N = 91$)	P
Age (years)			
2–5	79 (86.8)	71 (78.0)	0.119[b]
6–8	12 (16.2)	20 (22.0)	
Sex			
Male	77 (89.0)	81 (84.6)	0.381[b]
Female	14 (11.0)	10 (15.4)	
Family income			
< 5000	44 (51.2)	36 (42.4)	0.513[b]
≥ 5000	42 (48.8)	49 (57.6)	
Birth information[d]			
Weight (kg)	3.4 ± 0.7	3.5 ± 0.5	<0.001[c]
Father age	29.8 ± 3.8	27.6 ± 3.8	<0.001[c]
Mother age	29.9 ± 3.7	27.3 ± 3.9	<0.001[c]
Prenatal situation[e]			
X-ray			0.497[b]
No	88 (96.7)	85 (94.4)	
Yes	3 (3.3)	5 (5.6)	
Active smoking			
No	88 (96.7)	88 (97.8)	1.000[b]
Yes	3 (3.3)	2 (2.2)	
Drinking			
No	88 (96.7)	85 (94.4)	0.497[b]
Yes	3 (3.3)	5 (5.6)	
Psychological trauma			
No	83 (95.4)	88 (97.8)	0.439[b]
Yes	4 (4.6)	2 (2.2)	
Times of pregnancy			
< 3	70 (80.5)	70 (78.7)	0.766[b]
≥ 4	17 (19.5)	19 (21.3)	
Mood			
Happy for most time	60 (67.4)	74 (87.0)	0.007[b]
Sad for most time	9 (10.1)	2 (2.4)	
Certain time unhappy	20 (22.5)	9 (10.6)	

[a] Number of subjects

[b] Pearson's chi-square test

[c] Student t test

[d] Data record the information at the children birth. Weight was for the children

[e] Data record the information of mother

Results

Population Characteristics

In this study, at total of 183 subjects was recruited with an age range of 2 to 8 years old. Of these, 92 were cases, and 91 were controls. Significant differences between cases and controls showed in the birth information of the children, including weight ($p < 0.001$), father and mother's age when the children born ($p < 0.001$), as well as the mood of the mother during

pregnancy (p = 0.007). However, there was no significant difference observed for other demographic characteristics. Details of the baseline information are presented in Table 1.

Serum concentration of EMEs

The 11 EMEs were 100% detected from serum in this study. The concentrations of K and Mg were significantly higher in the case group than the control group (p < 0.05), while the concentrations of Se, Cu, and Zn were significantly lower in the case group than in the control group (p < 0.05). There was no significant difference between cases and controls for the concentrations of Ca, Na, Mn, Co, Mo, and Fe (Table 2). Significant differences for the mother's group only showed in the Mo (p < 0.05) (Table 2).

Associations Between EMEs Concentrations and ASD Risk

The strength of the association between the risk of ASD and the concentrations of EMEs were quantified by odds ratios (ORs) calculated from logistic regression. Results indicated that lower concentrations of Cu and Zn and higher concentrations of Ca, K, and Mg were associated with an increased risk of ASD with and without adjusting potential confounders. Adjusted ORs for the above EMEs were 1.031 [95% CI: 1.006–1.058] for Ca, 1.081 [95% CI: 1.046–1.118] for K, 1.161 [95% CI: 1.012–1.331] for Mg, 0.997 [95% CI: 0.995–0.999] for Cu, and 0.996 [95% CI: 0.992–1.000] for Zn (Table 3).

The dose-response relationships between 11 EMEs and the risk of ASD were investigated by calculating the ORs of each

quartile of EMEs. The risk of ASD increased with increasing levels of Ca, K, and Mg, as well as with decreasing levels of Cu and Zn. The detailed dose-response relationships between the ASD risk associated EMEs concentration (i.e., Ca, K, Mg, Cu, and Zn) are depicted in Fig. 1 and Supplementary Table S5.

Correlation Between EME Concentrations and ASD CARS Scores/EME Concentrations of Children and Mothers

To test whether EME concentrations correlate with ASD severity, the CARS was adopted to represent ASD severity. Nine significant negative correlations were found separately between 3 EMEs and 3 items of CARS (i.e., Ca and LR, Ca and TSTRU, Ca and FON, Mg and LR, Mg and TSTRU, Mg and FON, Na and LR, Na and TSTRU, Na and FON). In addition, two significant positive correlations were observed between concentrations of Cu and 3 items of CARS separately (i.e., RTP, OU, and GI). (Supplementary Table S6). In addition, inter-correlation among the 11 EMEs showed more positively and strongly in the control group than in the case group (Fig. 2 and Table 4).

For mothers and children, the overall correlation trends for the case and control groups differed significantly. Six specific correlated pairs appeared in the case group (i.e., Se in mothers' serum and Se in children's serum, Cu in mothers' serum and Co in children's serum, Cu in mothers' serum and Cu in children's serum, Zn in mothers' serum and K in children's serum, Zn in mothers' serum and Zn in children's serum, Fe in mothers' serum and K in children's serum) and 7 specific correlated pairs appeared in the control group which are

Table 2 Serum concentrations of EME in autistic subjects (cases) and healthy subjects (controls)

EMEs	Cases		Controls		P^a	
	Children	Mother	Children	Mother	Children	Mother
Ca[b]	105.0 (93.4–118.5)	85.1 (81.6–88.9)	100.3 (94.8–107.9)	83.7 (81.9–86.5)	0.061	0.258
K[b]	176.6 (169.9–188.7)	151.5 (147–156.8)	167.4 (159.6–173.2)	154.1 (149.4–158.8)	<0.001	0.142
Mg[b]	20.7 (19.3–23.3)	18.8 (17.8–19.6)	20.0 (19.1–21.6)	18.9 (18.2–19.5)	0.041	0.591
Na[b]	3362.5 (3272.5–3504.3)	3091.0 (3035.0–3181.0)	3338.0 (3284.0–3425.0)	3102.5 (3067.0–3135.5)	0.338	0.635
Mn[c]	1.2 (1.1–1.3)	1.0 (0.9–1.1)	1.3 (1.1–1.4)	1.0 (0.9–1.1)	0.107	0.822
Se[c]	137.9 (120.2–155.1)	138.4 (130.8–152.3)	150.2 (135.9–165.7)	151.3 (132.7–160.6)	0.003	0.116
Co[c]	0.9 (0.8–1.0)	1.0 (0.9–1.2)	1.0 (0.9–1.0)	0.9 (0.8–1.0)	0.068	0.098
Mo[c]	1.8 (1.6–2.0)	1.6 (1.3–1.8)	1.8 (1.6–2.0)	1.4 (1.2–1.6)	0.919	0.036
Cu[c]	1152.4 (1056.0–1309.4)	888.8 (821.7–996.5)	1234.1 (1142.6–1378.3)	903.3 (846.8–1023.3)	0.007	0.431
Zn[c]	825.1 (767.3–907.9)	754.5 (697.6–806.7)	897.8 (806.5–948.9)	745.8 (705.6–803.6)	0.002	0.942
Fe[c]	1756.2 (1352.9–1977.2)	1538.3 (1364.1–1746.0)	1603.7 (1397.8–1852.7)	1361.7 (1106.7–1663.0)	0.222	0.078

[a] In comparison with the median of controls by Mann-Whitney U test

[b] Serum concentrations of EMEs showed in μg/mL

[c] Serum concentrations of EMEs showed in ng/mL

Table 3 Associations between the prevalence of ASD and the concentrations of EME

EMEs	Median (IQR)[a]	Univariate OR (95%CI)[d]	P[d]	Adjusted OR (95%CI)[e]	P[e]
Ca[b]	102.5 (94.2–113.3)	1.027 (1.003–1.052)	0.021	1.031 (1.006–1.058)	0.020
K[b]	171.1 (163.6–181.5)	1.078 (1.047–1.110)	<0.001	1.081 (1.046–1.118)	<0.001
Mg[b]	20.4 (19.1–22.7)	1.153 (1.021–1.302)	0.023	1.161 (1.012–1.331)	0.034
Na[b]	3340.0 (3277.5–3476.0)	1.001 (0.999–1.003)	0.135	1.002 (1.000–1.004)	0.068
Mn[c]	1.2 (1.1–1.3)	0.319 (0.074–1.367)	0.124	0.433 (0.087–2.158)	0.307
Se[c]	145.8 (125.8–160.8)	0.995 (0.987–1.003)	0.258	0.994 (0.984–1.004)	0.229
Co[c]	1.0 (0.9–1.0)	0.277 (0.034–2.271)	0.231	0.203 (0.018–2.328)	0.200
Mo[c]	1.8 (1.6–2.0)	1.120 (0.590–2.125)	0.729	1.259 (0.617–2.569)	0.528
Cu[c]	1200.4 (1079.5–1332.9)	0.998 (0.996–1.000)	0.006	0.997 (0.995–0.999)	0.006
Zn[c]	860.8 (787.7–932.9)	0.996 (0.994–0.998)	0.003	0.996 (0.992–1.000)	0.005
Fe[c]	1682.3 (1376.7–1934.0)	1.000 (1.000–1.000)	0.153	1.001 (1.001–1.001)	0.210

[a] IQR, inter-quartile range

[b] unit: μg/mL

[c] unit: ng/mL

[d] Calculated by an unconditional Logistic regression model

[e] Adjusted OR and 95%CI were calculated by an unconditional Logistic regression model adjusting for the potential confounders, including birth information (i.e., weight, father age, mother age) and prenatal mood of mother

highlighted with yellow (i.e., Mo in mothers' serum and Ca, K, Mg, Se, Co, Mo, and Cu in children's serum) (Fig. 3).

Discussion

This case-control study evaluated the association between 11 EMEs in serum and ASD in a Chinese population. All 11 EMEs were detected in all subjects. Results suggest that lower concentrations of Cu and Zn and higher concentrations of Ca,

K, and Mg are separately associated with an elevated risk of ASD, and most of the above EMEs are found correlated with the severity of certain disease symptoms (CARS score). In addition, the inter-correlation analysis suggested that the distribution and profile of EMEs are different between cases and controls. Six and seven specific correlated pairs between mothers and children were found in the case and controls separately.

Imbalance in macro EMEs may play an important role in central nervous system and cognitive function, interfering
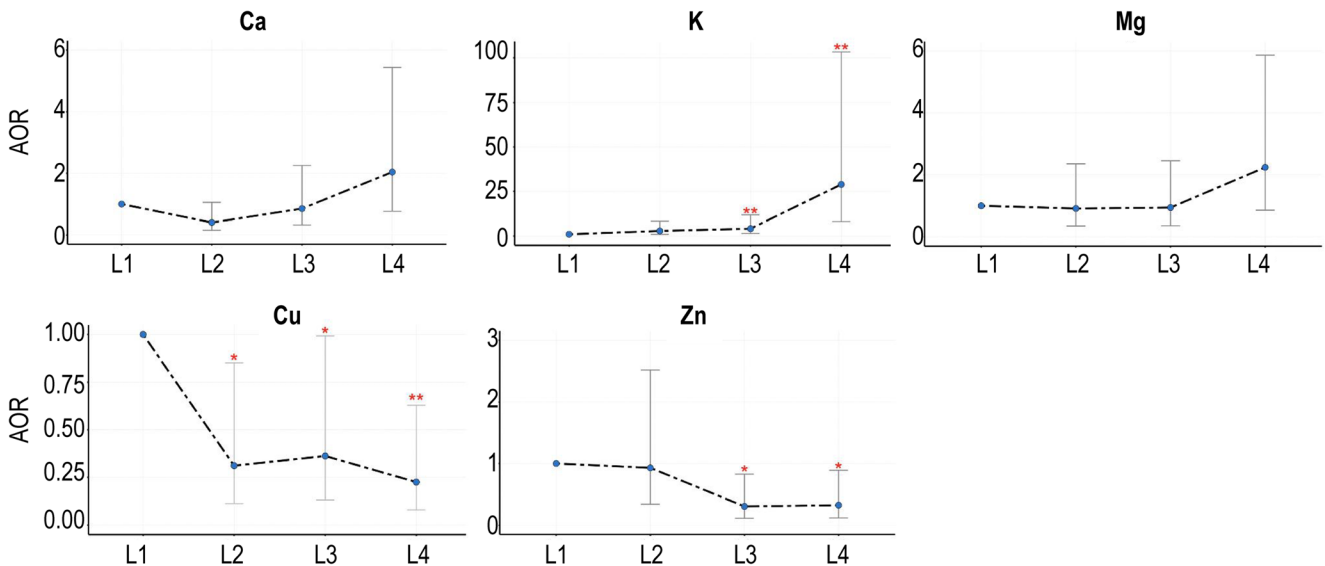

Fig. 1 Dose-response relationship between the risk EMEs (Ca, K, Mg, Zn, and Cu) and risk of ASD. Adjusted odds ratios (AORs) for ASD associated with 4 concentration quartiles are represented by the blue dots. Error bar indicates the 95% CI. The four quartiles were calculated from all the 183 subjects according to the concentration of each EME and indicated from low to high as L1, L2, L3, and L4; * $p < 0.05$ and ** $p < 0.01$

Table 4 Coefficients of Spearman correlation between CARS scores and EMEs (r)

EMEs	CARS categories[a]															TS[b]
	RTP	IMT	ER	BU	OU	ATC	VR	LR	TSTRU	FON	VC	NC	AL	LCIR	GI	
Ca	-0.060	0.084	0.121	-0.041	0.134	-0.143	-0.100	-0.408**	-0.324**	-0.297**	-0.081	0.079	-0.206	0.021	-0.016	-0.190
K	-0.015	0.141	-0.083	0.011	0.000	0.071	0.108	0.077	0.075	0.055	0.063	-0.063	-0.167	-0.028	0.020	0.038
Mg	-0.024	0.149	0.030	-0.093	0.046	-0.142	-0.097	-0.328**	-0.251*	-0.338**	-0.104	-0.017	-0.092	-0.039	-0.079	-0.200
Na	-0.022	0.123	0.182	-0.124	0.183	-0.159	-0.092	-0.368**	-0.368**	-0.252*	-0.104	0.096	-0.063	0.068	0.011	-0.132
Mn	0.046	0.004	0.131	-0.155	0.031	-0.006	0.031	-0.189	-0.132	-0.130	0.140	0.007	-0.175	0.199	0.132	0.005
Se	0.009	-0.133	-0.102	-0.020	-0.086	0.078	-0.032	0.134	0.171	0.121	-0.087	-0.092	0.191	0.052	-0.022	0.044
Co	-0.007	-0.122	0.113	-0.145	0.046	-0.149	0.066	-0.066	-0.185	-0.154	-0.004	0.025	-0.073	0.120	0.058	-0.018
Mo	-0.104	0.101	-0.015	-0.033	-0.010	0.136	-0.050	0.144	0.146	-0.039	-0.176	-0.089	0.093	0.002	-0.100	0.003
Cu	0.212*	-0.043	0.100	-0.162	0.215*	-0.131	0.043	-0.102	-0.158	-0.146	0.161	0.097	0.003	0.166	0.254*	0.083
Zn	-0.069	0.014	0.016	-0.129	0.060	0.026	0.004	-0.013	-0.073	-0.072	0.063	-0.001	0.094	0.118	0.128	0.037
Fe	-0.067	0.016	-0.039	-0.091	0.073	-0.106	0.048	0.078	0.061	-0.173	-0.031	-0.113	0.027	0.039	0.055	-0.037

[a] All the CARS categories are listed. *RTP*, Relating to people; *IMT*, Imitation; *ER*, Emotional response; *BU*, Body use; *OU*, Object use; *ATC*, Adaptation to change; *VR*, Visual response; *LR*, Listening response; *TSTRU*, Taste smell and touch response and use; *FON*, Fear or nervousness; *VC*, Verbal communication; *NC*, Nonverbal communication; *AL*, Activity level; *LCIR*, Level and consistency of intellectual response; *GI*, General impressions
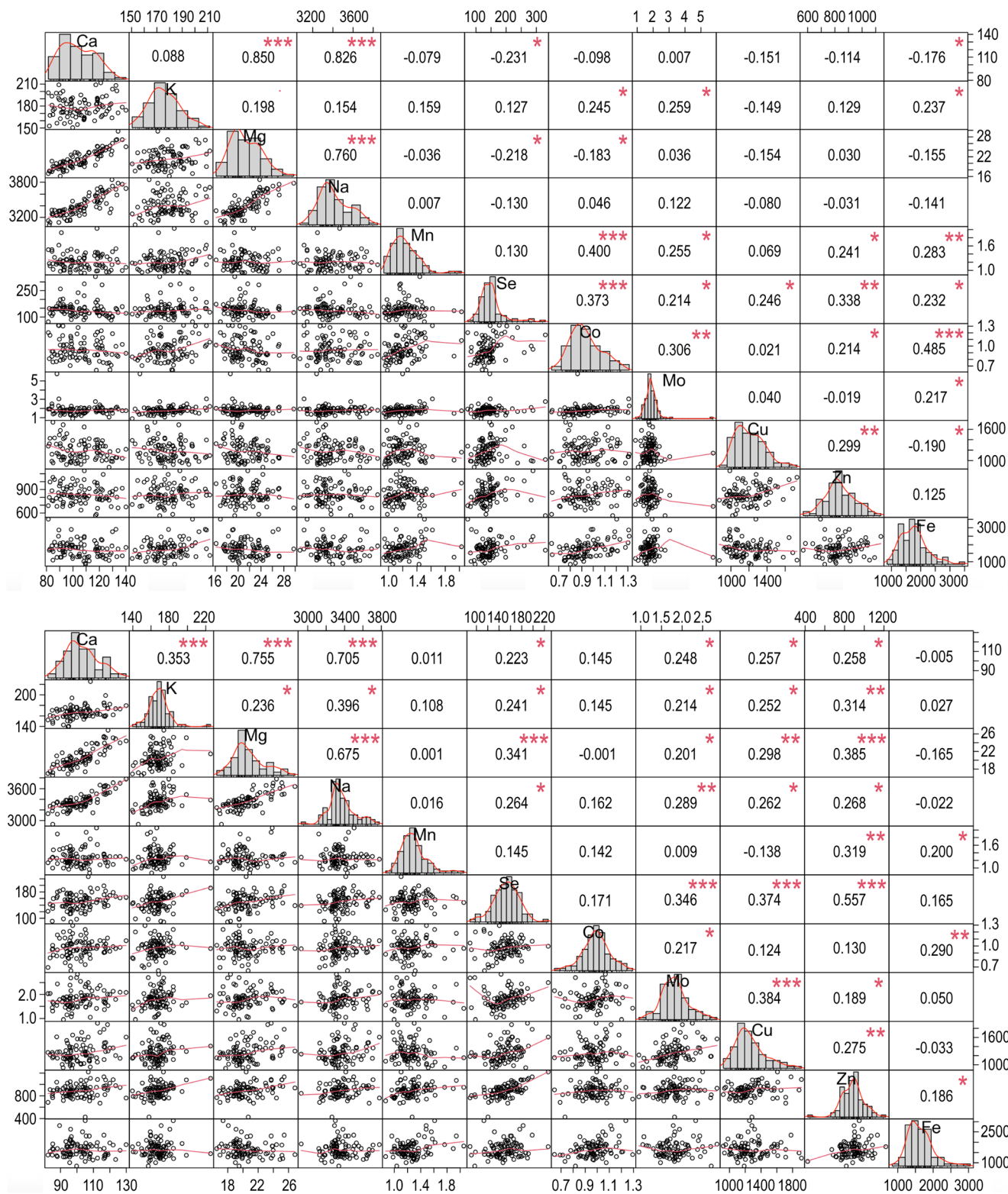
[b] *TS*, Total score

* $P < 0.05$. ** $P < 0.01$

Fig. 2 Spearman correlations between EME in cases and controls. Cases are shown on the left (Figure 2A) and controls shown on the right(Figure 2B). The distribution of each variable is shown on the diagonal; On the bottom of the diagonal: the bivariate scatter plots with a fitted line are displayed; On the top of the diagonal: the value of the correlation plus the significance level appear as stars; Each significance level is associated to a symbol: p-values (0.001, 0.01, 0.05, 0.1, 1) <=> symbols ("***", "**", "*", ".", " ")

a

	Ca_M	K_M	Mg_M	Na_M	Mn_M	Se_M	Co_M	Mo_M	Cu_M	Zn_M	Fe_M
Ca	-0.174	0.07	-0.035	-0.119	0.17	0.046	0.018	0.166	0.187	-0.065	-0.175
K	0.129	0.017	0.092	0.07	0.03	-0.08	-0.144	-0.15	0.008	-0.232	0.254
Mg	-0.093	0.103	-0.022	-0.128	0.159	0.086	-0.006	0.136	0.097	-0.105	-0.135
Na	-0.159	0.016	-0.046	-0.135	0.079	0.024	0.006	0.13	0.082	-0.161	-0.191
Mn	-0.024	-0.08	-0.037	-0.012	0.121	-0.046	-0.098	-0.11	-0.199	0.083	0.169
Se	-0.04	-0.121	0.125	-0.058	-0.171	0.231	-0.071	0.009	-0.131	0.055	-0.054
Co	0.068	-0.095	-0.013	-0.02	0.107	0.186	-0.022	-0.059	-0.236	0.081	0.027
Mo	0.106	0.026	0.192	0.12	0.052	0.112	-0.058	-0.082	-0.077	-0.185	-0.001
Cu	0.084	0.019	0.21	0.156	0.084	0.038	-0.047	0.116	0.229	0.105	0.029
Zn	-0.012	-0.155	-0.02	-0.142	0.104	0.116	0.027	0.002	0.013	0.283	0.189
Fe	0.173	0.095	0.076	0.143	0.107	-0.012	0.059	-0.16	-0.164	0.074	0.115

b

	Ca_M	K_M	Mg_M	Na_M	Mn_M	Se_M	Co_M	Mo_M	Cu_M	Zn_M	Fe_M
Ca	0.302	0.087	-0.09	-0.293	-0.143	0.314	-0.128	0.53	0.117	-0.041	-0.288
K	0.312	0.292	-0.147	0.055	-0.194	0.111	0.008	0.424	0.084	0.107	-0.007
Mg	0.303	0.216	0.033	-0.126	-0.14	0.268	-0.218	0.483	0.188	0.001	-0.337
Na	0.354	0.103	-0.204	-0.238	0.056	0.243	-0.064	0.37	0.163	-0.127	-0.255
Mn	0.008	0.121	0.128	0.317	0.096	-0.088	-0.122	0.219	0.36	-0.033	-0.149
Se	-0.005	-0.011	-0.034	-0.071	-0.282	0.176	0.057	0.564	-0.106	0.024	-0.199
Co	0.193	-0.094	0.18	0.179	-0.002	-0.109	-0.039	0.461	0.051	0.319	-0.143
Mo	0.216	-0.016	-0.236	0.093	0.258	-0.107	0.012	0.131	-0.071	0.119	0.244
Cu	-0.048	-0.039	-0.142	-0.071	-0.066	0.317	0.187	0.452	-0.054	0.007	-0.151
Zn	0.037	0.161	-0.035	0.044	-0.234	-0.008	0.015	0.563	0.213	-0.176	-0.25
Fe	-0.266	0.022	-0.221	-0.11	-0.273	-0.157	0.244	0.255	0.021	-0.201	0.004

Fig. 3 Spearman correlations of EME between mother and children. Cases are shown on the above and controls shown below. Correlation coefficients ® are highlighted with yellow if the correlation was significant ($p < 0.05$). Blue represents positive correlation while red represents negative correlation. The darker the color, the greater the |r|.

with the homeostasis and relating to neuropsychiatric disorders [21]. This study suggested that the higher concentration of 3 macro EMEs (Ca, K, and Mg) were associated with the increased risk of ASD. It has been reported that Ca mainly exited in the extracellular, which played an important role in maintaining the excitability of muscles and nerves [50]. A study performed with 30 Saudi autistic children and 30 age-matching healthy controls found that the concentration of Ca was significantly higher in the ASD group, which is consistent with the result of our study. The above results have also been supported by studies of biological mechanisms. For example, it has been suggested that increased concentration of Ca promoted synthesis of dopamine via a calmodulin-dependent machinery, regulating various brain functions [41]. Moreover, our previous study indicated that the concentration of Ca was positively correlated with several biomarkers related to oxidative stress which associated with the pathogenesis and/or clinical features (i.e., ASD-like behaviors, repetitive and stereotypic activity) of ASD [27, 34].

Multiple studies have investigated the association between K and ASD. The results of two studies conducted with hair and plasma samples separately were echoed in our study, which observed significantly higher concentrations of K in ASD cases than in healthy controls [4, 13]. Moreover, the study performed with plasma samples also found the positive correlation between K and $Na^+ - K^+ - ATP$ ase, suggesting the increased $Na^+ - K^+ - ATP$ ase may be released by damaged brain cells in autistic children and thereby causing neurotoxicity. However, another study performed with hair samples showed the reverse result [2].

Similar to Ca and K, a significantly higher level of Mg in serum of autistic subjects was also observed in our study. To the best of our knowledge, 12 studies have explored the association between Mg and ASD with the samples of hair, serum, plasma, blood, and urine assessed separately. However, the results remain inconsistent even with the same sample type. Specifically, two studies conducted in Canada and Saudi Arabia using blood samples showed that there was no significant difference in Mg concentration between the ASD group and the control group [13, 20] However, one study performed in China found that the concentration of Mg was significantly lower than that of the healthy control group [46]. Two studies carried out with hair samples in Oman and America confirmed our findings, i.e., the concentration of Mg was significantly higher in autistic subjects than in healthy controls [4, 16]. The inconsistence of the results may be caused by different regions, sample types, races, diet, and other factors. Taken together, a relationship may exist between these macro EMEs (i.e., Ca, K, and Mg) and the risk of ASD; however, more direct evidence of the underlying mechanism regarding the association is desirable.

Zinc is essential for brain development, synaptic transmission and antioxidant process, closely associated with ASD-related protein [17, 43]. In our study, the lower concentration

of Zn was found to be significantly associated with the higher risk of ASD, which is coincident with several similar studies based on blood or hair samples [24, 35, 47, 48]. Deficiency in Zn is widely reported to associate with not only various pathological conditions, but also neurodegenerative diseases and neurodevelopment disorders. Epidemiological studies have shown that the average serum concentration of Zn in autistic children was significantly lower than healthy controls [5]. Furthermore, some studies found that Zn supplementation had a significant effect on the treatment of children with impaired social ability and hyperactivity (better than placebo) [12, 49]. In addition, a large number of animal model studies have suggested that Zn deficiency may lead to the clinical features of ASD in rats or mice, including impairment in social behavior, impairment in learning and memory changes, and depression-like behavior, which enhanced the association between Zn deficiency and ASD [18]. However, some studies showed higher concentration of Zn in the ASD group or no significant difference between the two groups [3, 4, 28, 40, 44]. Besides the factors mentioned above which may lead to the inconsistent results, the age of the included subjects may also act as a confounding factor for Zn. A study in Japan that examined scalp hair concentrations of 26 trace elements for 1967 children with ASD indicated that the group of infants aged 0–3 years old, who need more Zn to maintain growth were more likely to suffer Zn deficiency [48]. Therefore, we assume that studies with an accurate age range regarding the association between the concentration of Zn and the risk of ASD will better clarify this association.

Several studies have suggested the association between the concentration of Cu and the risk of ASD [11, 24, 35]. There are even two studies which explored applying the Zn/Cu ratio as an indicator of ASD [11, 24]. With the optimal cut-off value of 0.665, the area under the curve was 0.968 (95% confidence interval, 0.943–0.993), yielded a sensitivity of 90.0% and a specificity of 91.7% [24]. Our study found an association between lower concentration of Cu and the risk of ASD, which consistent with previous research conducted with US population [15]. As an essential trace element for many physiological functions, Cu plays an indispensable auxiliary role in various enzyme reactions and is an indispensable part of biochemical regulation [50]. The deficiency in Cu was widely reported to result in progressive neurological and cognitive dysfunction and psychosis-like symptoms which are also observed in subjects with ASD [37]. Previous study based on rats' experiment indicated that Cu deficiency resulted in impaired growth and greatly decreased norepinephrine concentrations which related to clinical traits of ASD [36]. However, it should be noted that many studies demonstrated the toxicity of Cu and found higher concentration of Cu in ASD subjects which is contrary to our results [9]. The mechanism of toxicity caused by excessive Cu in ASD

disease has also been expounded, for example, excessive Cu is a risk for synaptic pathology associated with ASD and destroys homeostasis of Zn [6]. In addition, Cu levels are altered in many other disease states and nutritional deficiencies, which also may lead to inconsistent results [9]. Therefore, a proper range for Cu concentration should be estimated based on a large sample size to avoid physiological dysfunction.

Although the dose-response curves for elements are not necessarily linear [19], the toxic or beneficial effects of EME are often more significant at extreme values [30]. In our study, the risk of ASD was the lowest where the quantile of Zn and Cu were at the highest level, while the risk of ASD was the highest where the quantile of Ca, K, and Mg were at the lowest level. For the further investigation of the correlation between EME concentrations and the severity of ASD (i.e., CARS scores), the risk factors (i.e., Ca and Mg) or protective EMEs (i.e., Cu) were correlated with certain items of CARS. These items included listening response, taste smell and touch response and use, fear or nervousness, relating to people, and object use and general impressions, which further illustrated the association of risk EMEs. In addition, we found that the inter-correlation among EMEs in the cases group is less positive and weaker than in the control group. Prenatal exposure of adverse environmental is an important risk factor for the occurrence of ASD. Therefore, it is valuable to explore the EMEs in the mother's body that to some extent reflects the prenatal exposure. Mo was the only EME that showed significant differences between two groups of mothers (i.e., mothers of ASD children and mothers of healthy controls). Although the concentration of Mo was not significant associated with ASD in our study, it has been found that a vitamin/mineral supplement (containing Mo) was able to improve free and total plasma sulfate and prevent the occurrence of ASD [1]. In addition, our study found that correlation between mother and children was also altered in the cases group compared with the control group, where the correlation only showed between Mo in mothers' serum and other seven EMEs in children's serum. Previous study has suggested that there was a positive correlation between Mo and Cu in patients with mental disorders, but few studies explore the relationship between mothers and ASD children [27]. We speculate that the level of Mo in the mothers' body may be related to or even regulate the multiple EMEs in children, but the underlying mechanism needs to be further explored. Overall, both of the above results raised a key question as to whether a change in the overall distribution profile of EMEs rather than a single or partial alter of EMEs is associated with the pathophysiological of ASD, and more epidemiological and mechanistic studies are needed to clarify the above findings.

There were three limitations in the present study. First, as a cross-sectional study, the causal relationship between EMEs concentrations and ASD could not be explored in

this study. Second, whether the EMEs in serum are representative to human brain remains to be further investigated. Third, the blood of mothers and children was collected at the same time, which may not accurately reflect the children's exposure.

Conclusions

Our study analyzed the association between concentrations of EMEs and ASD, and the results suggest that ASD children are more prone to EMEs disturbances and abnormal concentration, including increases, of Ca, K, and Mg, and decreased concentration of Cu and Zn as found in serum samples. Further investigation on dose-response and correlation between EMEs and CARS partly strengthen these findings. Overall, this study indicates that the EMEs have the potential to act as biomarkers of ASD to improve the current methods of diagnosis and treatment.

Declarations

Conflict of Interest The authors declare no competing interests.

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