

# Low Maternal Education, Gastrointestinal Problems and High Blood Lead Level: Risk Factors Related to the Severity of Autism Spectrum Disorder in Northeast China

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## Research article

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# Abstract

**Background:** The prevalence of autism spectrum disorder (ASD) has increased rapidly in recent years. Environmental factors may play an important role in the pathogenesis of ASD. These factors may include socioeconomic factors, nutritional factors, heavy metal exposure, air pollution, etc. Our aim is to analyze possible environmental risk factors associated with the severity of ASD.

**Methods:** All participating children were divided into two groups (mild and severe) according to the severity of their symptoms, as determined by their Childhood Autism Rating Scale (CARS) scores. The socioeconomic and demographic factors that may affect the severity of ASD and the nutritional factors that were correlated with ASD symptoms were included in the logistic regression to analyze whether they were risk factors that affected the severity of ASD.

**Results:** Logistic regression showed that maternal education ( $P=0.038$ ,  $OR=1.694$ , 95% CI: 1.029-2.789), gastrointestinal problems ( $P=0.045$ ,  $OR=1.770$ , 95% CI: 1.012-3.097) and a high serum concentration of lead ( $P=0.001$ ,  $OR=1.038$ , 95% CI: 1.016-1.060) were statistically significantly associated with ASD severity.

**Conclusion:** Many environmental factors affect the severity of ASD. We concluded that maternal education, gastrointestinal problems and serum concentration of lead were risk factors that affected the severity of ASD in northeast China.

## Background

Autism spectrum disorder (ASD) was originally defined by Leo Kanner in 1943 and is characterized by persistent deficits in social communication and interaction and stereotyped or repetitive patterns of behavior, interests or activities. ASD is classified as a neurodevelopmental disorder in the Diagnostic and Statistical Manual of Mental Disorders 5th Edition (DSM-5). The prevalence of ASD is increasing so rapidly that it has led to the development of many social issues and placed a heavy burden on families.

Currently, the cause of ASD remains unclear. It is suggested that ASD is the consequence of both genetic factors and environmental factors. In recent years, the prevalence of ASD has increased significantly. This increase is partly due to changes in diagnostic criteria, reductions in the misdiagnosis rate, and increases in the consultation rate, but the reasons for the increase are not limited to these factors. In addition, large-scale mutations that generate ASD-related pathogenic genes in a short time are obviously unlikely, and we have reason to believe that environmental factors may play an important role in the pathogenesis of ASD. These factors may include nutritional factors, heavy metal exposure, air pollution, socioeconomic factors, etc. [1].

Nutritional factors include omega-3 fatty acids [2-4], vitamin A [5, 6], vitamin D [7, 8], folic acid [9, 10], iron [11, 12] and other micronutrients. Abnormal metabolism may be linked to neurological and behavioral disorders and ASD [13], providing ideas for the nutritional treatment of ASD. In recent years, problems related to environmental toxicants have become increasingly prominent. Heavy metals, especially lead and mercury, are among the environmental toxicant risk factors for ASD [14-16]. Global air pollution is a very

serious issue that has caused many developmental problems, including neurodevelopmental disorders and ASD. Traffic-related air pollution includes polycyclic aromatic hydrocarbons (PAHs) [17, 18] and PM (particulate matter) 2.5 and 10[18]. Some studies have reported relationships between ASD and organic toxicants [19] and pesticides [20]. Socioeconomic factors include parental education [21, 22], family income [23], caregivers, birth order [24], siblings [25], etc. An understanding of socioeconomic factors can be very helpful for the management of autism and for social work, including social welfare practices.

In China, some studies have focused on the influence of various environmental factors on the severity of autism symptoms. Liu [26] conducted a case-control study with 81 children with ASD and showed that maternal occupational toxicant exposure, diseases during pregnancy, and living in an impoverished area the time of birth may be specific risk factors associated with ASD. Shen [27] collected a basic medical history and information regarding maternal prepregnancy and pregnancy conditions and reported that maternal prepregnancy BMI might not be associated with autism risk. Zhang [28] conducted a case-control study of 190 Han children and showed that 9 prenatal and perinatal risk factors were associated with ASD. Our team's previous research also found that vitamin D levels had some relationship with ASD symptoms [29].

However, there is still a lack of comprehensive analysis of the influence of socioeconomic factors, nutritional factors, and heavy metal elements on the severity of ASD symptoms in a large sample of children with ASD in northeast China. These factors are clearly related to race, region, and social environment. Therefore, regional research may be more meaningful for understanding the role of environmental risk factors for ASD in the pathological processes of ASD in our own region. For these reasons, we conducted this retrospective study to identify possible environmental risk factors related to the severity of ASD.

## Methods

### 2.1 Participants

We retrospectively analyzed 512 children with ASD (417 boys and 95 girls) ranging in age from 2 to 13 years ( $3.32 \pm 2.15$ ). All the children were diagnosed according to the DSM-5 criteria for the first time and were confirmed to not have fragile X syndrome, Rett syndrome or other severe neurological diseases, such as epilepsy, by developmental and behavioral pediatricians in the First Hospital of Jilin University from October 2017 to January 2020. All the children were divided into two groups according to the severity of their symptoms, as determined by their Childhood Autism Rating Scale (CARS) scores. The average CARS score of the children with ASD was  $30.9 \pm 4.8$  (22-47) points. Children with CARS scores below 30.9 were included in the mild group, and children with CARS scores greater than 30.9 were included in the severe group. The mild group included 249 children (208 boys and 41 girls), and the severe group included 263 children (209 boys and 54 girls). The mean CARS scores of these groups were  $27.0 \pm 2.78$  (22-30.5) and  $34.6 \pm 3.1$  (33-47), respectively.

All parents were informed about the study and provided written consent. The ethics committee of our hospital approved this research program.

## 2.2 Evaluations and measurements

All children in this study were assessed via socioeconomic and demographic profile surveys, symptom evaluation scales and blood tests.

The socioeconomic and demographic information that was collected included name, sex (male or female), age, birth date (year, month and day), place of residence (urban or rural area), caregivers (parents, grandparents or both), siblings, age of parents during pregnancy, education level of parents, household income, family history of mental illness, vitamin intake during pregnancy (none, folic acid or multivitamins), mode of delivery (eutocia or cesarean), presence of eating problems, presence of sleeping problems, presence of gastrointestinal problems, and comorbidity with attention deficit hyperactivity disorder (ADHD).

The symptom evaluation scales included the Autism Behavior Checklist (ABC), the CARS, and the Autism Treatment Evaluation Checklist (ATEC). The ABC is a 57-item screening checklist for autism containing 5 subscales (body behavior, sensory, self-care, language and social interaction). The CARS was developed by Schopler and Reichler and is used as a diagnostic scale. The CARS consists of 15 scales, and each scale is scored on a continuum from normal to severely abnormal. The ATEC was designed to measure treatment effects and has four subscales: speech/language communication, sociability, sensory/cognitive awareness and health/physical/behavior; the ATEC is usually used to evaluate treatment effects in children with ASD. The reliability and validity of the ABC, CARS and ATEC are sufficiently good, reflecting the scales' usefulness for clinical diagnosis and the evaluation of ASD symptoms [30]. The survey was conducted by doctors and families together, including children and their parents. The ABC and ATEC scales are designed to be administered via parent interviews. The CARS requires the observation of children with ASD in a consulting room.

Blood tests included measurements of vitamins A, D, and E; copper; zinc; iron; and lead. The serum concentrations of vitamins A, D and E were detected by high-performance liquid chromatography (HPLC). The serum concentrations of copper, zinc, iron, and lead were detected by graphite furnace atomic absorption spectrometry (AAS). All samples were tested by Guangzhou KingMed Diagnostics Group Co., Ltd. (KingMed Diagnostics, SSE 603882).

## 2.3 Statistical analysis

We used the Statistical Package for the Social Sciences (SPSS) 19.0 (SPSS for Windows, SPSS Inc. Chicago) to analyze the data.

We compared the socioeconomic and demographic profiles of the mild group and severe group to determine whether there were factors that differed between the two groups. Continuous variables with normal distributions are represented as means  $\pm$  standard deviations (SDs) and were compared by Student's t-test. Continuous variables with nonnormal distributions are represented as medians (P25-P75) and were compared using Wilcoxon's rank-sum test. Categorical variables are represented as frequencies (percentages) and were compared using the  $\chi^2$  test.

Correlations between the symptom evaluation scales and blood test results were detected by the Pearson correlation test (normal distribution) or the Spearman correlation test (nonnormal distribution) because the variables were normally distributed.

The socioeconomic, demographic and nutritional factors that had correlations with ASD symptoms were included in the logistic regression to analyze whether these factors affected the severity of ASD.

## Results

Table 1 shows the comparison of the socioeconomic and demographic profiles of the mild group and the severe group. The mild group consisted of 208 boys (83.5%) and 41 girls (16.5%), and the severe group consisted of 209 boys (79.5%) and 54 girls (20.5%). A statistically significant difference was found for the following 6 factors: age ( $P=0.01$ ), place of residence ( $P=0.034$ ), caregivers ( $P=0.046$ ), maternal and paternal education ( $P=0.034$  and  $0.008$ , respectively) and gastrointestinal problems ( $P=0.03$ ). The children in the mild group were older than those in the severe group. Regarding place of residence, more families in the mild group lived in urban areas; the parents of children in the mild group also had a higher degree of education than the parents of children in the severe group. The children in the mild group were more likely to have parents as their main caregivers and had a lower rate of gastrointestinal problems than those in the severe group.

The serum concentration of lead in the children with ASD enrolled in our study was  $10.4\text{-}172\ \mu\text{g/L}$  ( $39.09 \pm 15\ \mu\text{g/L}$ ), the vitamin D concentration was  $3.8\text{-}56\ \text{ng/ml}$  ( $26.46 \pm 9.03\ \text{ng/ml}$ ), and the copper concentration was  $6.96\text{-}31.4\ \mu\text{mol/L}$  ( $18.40 \pm 4.21\ \mu\text{mol/L}$ ). Table 2 shows the correlations between symptoms and blood test results. Autism symptoms were related to the serum concentrations of vitamin D, copper and lead. There was a negative correlation between the ATEC speech/language communication score and vitamin D concentration ( $r=-0.149$ ,  $P=0.008$ ), and the ABC social interaction score had a positive correlation with copper concentration ( $r=0.159$ ,  $P=0.007$ ). Lead concentration had positive correlations with the total ABC score ( $r=0.173$ ,  $P=0.004$ ), the ABC sensory subscale score ( $r=0.157$ ,  $P=0.008$ ), the ABC self-care subscale score ( $r=0.161$ ,  $P=0.007$ ), the ABC social interaction subscale score ( $r=0.128$ ,  $P=0.032$ ), the total CARS score ( $r=0.202$ ,  $P=0.001$ ) and the ATEC sociability subscale score ( $r=0.139$ ,  $P=0.037$ ).

Therefore, we entered age, place of residence, caregivers, parental education level, gastrointestinal problems, and serum concentrations of vitamin D, copper and lead into the logistic regression model to determine whether these were risk factors for the severity of ASD. The categorical variables were coded as follows: place of residence (Urban=0, Rural=1), caregivers (Parents=0, Grandparents and others=1), parental education level (Junior college or above=0, Senior high school or below=1), and gastrointestinal problems (No=0, Yes=1). The results showed that maternal education ( $P=0.038$ , OR=1.694, 95% CI: 1.029-2.789), gastrointestinal problems ( $P=0.045$ , OR=1.770, 95% CI: 1.012-3.097) and serum concentration of lead ( $P=0.001$ , OR=1.038, 95% CI: 1.016-1.060) were statistically significant, indicating that low maternal education, the presence of gastrointestinal problems and a high serum concentration of lead were risk factors related to the severity of ASD symptoms.

# Discussion

ASD onset occurs in early infancy; ASD is a chronic neurodevelopmental disorder and is now regarded as the consequence of both genetic and environmental factors. In past decades, evidence from twin sibling studies has shown that ASD has a strong inherited tendency [31-33]. Unfortunately, the relationship between genotype and phenotype is not as clear, and copy number variation may be associated with not only ASD but with many other kinds of mental disorders, such as ADHD or schizophrenia. Some disorders are associated with epigenetics, which also indicates the importance of the environment. In recent years, environmental factors have been viewed as increasingly important in the pathogenesis of ASD.

Our results showed that low maternal education, gastrointestinal problems and high blood lead levels are risk factors related to the severity of autism spectrum disorder in northeast China.

## 4.1 Maternal education

Higher maternal and paternal education levels were protective factors against ASD severity. Parents with higher education levels can pay more attention to prenatal care, provide a good family environment, and adopt a reasonable parenting style. These factors can offset the poor performance of children with serious genetic susceptibility to ASD, and parents with higher education levels can recognize earlier that their children are not typically developing. Many domestic studies have reported this phenomenon. Mandell et al. reported that a high parental education level is a protective factor against ASD [34]. In our study, maternal and paternal education affected the severity of ASD symptoms in a single-factor analysis, but paternal education level was not included in the logistic regression model. This suggests that high maternal education is a more protective factor against ASD. High maternal education may be a more protective factor because in most families, the education and rearing of young children is mainly the responsibility of women, whereas men are more often responsible for external issues, such as maintaining the family's economic status [35].

## 4.2 Gastrointestinal problems

Gastrointestinal symptoms were strongly correlated with ASD symptom severity. Children with severe symptoms are likely to have a much higher proportion of accompanying gastrointestinal problems [36]. These gastrointestinal problems may be the result of different intestinal bacteria and may mediate inflammation and immunological processes that affect the brain [37]. We also concluded that gastrointestinal problems are risk factors for ASD symptom severity and observed that the severe symptom group had a much higher proportion of gastrointestinal problems. There are various gastrointestinal symptoms, including constipation, diarrhea, foul-smelling stool, flatulence and abdominal pain. However, we simply analyzed whether the children had gastrointestinal problems and did not perform a detailed evaluation of the kinds and severity of symptoms. In addition, we did not conduct a detailed analysis of gastrointestinal probiotics, immune- and inflammatory-related indicators or the mechanisms related to their relationships with the nervous system. These factors may constitute a future research direction. For human mental health, an important and popular dictum is "fix your gut, fix your brain." [38].

### 4.3 Lead

Toxic heavy metals, such as lead and mercury, may affect the developing brain. Lead has been identified as a main neurotoxicant environmental trigger for ASD because it induces neuroinflammation and autoimmunity [39]. Afaf reported that significantly elevated mercury and lead levels were found in the red blood cells of patients with ASD compared with healthy controls [40]. However, related findings have been inconsistent, and Li reported that higher levels of only mercury and arsenic were observed in children with ASD and that their lead levels were not different [41]. The research of Rahbar showed a higher geometric mean blood lead concentration for typically developing controls than for individuals with ASD (2.73 µg/dL vs. 2.25 µg/dL;  $p < 0.05$ ) [42]. Furthermore, studies have found that the severity of autism is also related to an increase in urinary porphyrins (a biological marker related to lead toxicity) [43]. Our results indicated a relationship between blood lead concentration and ASD symptoms, and lead level was included in the final equation, which illustrated that lead level was an environmental factor that affected the severity of ASD.

## Limitations

Our study had several limitations.

First, we did not recruit typically developing children as a control group. Instead, we reviewed the literature, studied environmental factors that might be risk factors for autism and analyzed which factors were actually associated with the severity of autism symptoms.

Second, we used the CARS to evaluate the severity of ASD symptoms, but the CARS is not a structural scale and is somewhat subjective. We prefer to use the semistructured Autism Diagnostic Observation Schedule (ADOS) or the second version of the CARS.

Third, we analyzed many factors but did not include many pregnancy-related factors, such as maternal obesity, cesarean section and diabetes. However, a meta-analysis illustrated that pregnancy factors are not as important to the severity of ASD [44].

## Conclusion

Many environmental factors affect the severity of ASD. We concluded that low maternal education, gastrointestinal problems and a high serum concentration of lead were risk factors that affected the severity of ASD. The objectives of this study were to identify high-risk populations with potentially severe ASD symptoms and to provide increased knowledge and family training guidance for parents with low maternal education levels, which can promote the early diagnosis and treatment of high-risk children and can result in a better prognosis. Achieving such outcomes may require the efforts of the entire autism prevention system at the government level and all medical workers in related fields. Concerns about gastrointestinal symptoms and blood lead levels in children with ASD warrant further research. Future studies should investigate how these factors affect ASD symptoms and help to further uncover the pathogenesis of autism.

# Abbreviations

ASD: autism spectrum disorder

CARS: Childhood Autism Rating Scale

DSM-5: Diagnostic and Statistical Manual of Mental Disorders 5th Edition

PAHs: polycyclic aromatic hydrocarbons

ADHD: attention deficit hyperactivity disorder

ATEC: Autism Treatment Evaluation Checklist

HPLC: high-performance liquid chromatography

AAS: atomic absorption spectrometry

SPSS: Statistical Package for the Social Sciences

SDs: standard deviations

ADOS: Autism Diagnostic Observation Schedule

# Declarations

## Ethics approval and consent to participate

The study was approved by the institutional ethics committee of the hospital, the First Hospital of Jilin University. The parents or guardians of the eligible children provided written informed consent. An information sheet was provided for the parents or guardians of all the participants.

## Consent for publication

Not applicable.

## Availability of data and materials:

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Competing interests

The authors declare that they have no competing interests.

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### **Authors' contributions:**

Han-Yu Dong☒Analysis and interpretation of data, Drafting the article, Resources☒Investigation☒

Jun-Yan Feng☒Analysis data, Drafting the article (tables)

Hong-Hua Li☒Supervising and editing, Clinical trial registration

Xiao-Jing Yue☒Acquisition of data

Fei-Yong Jia\*☒Concept and design, revising the article critically for important intellectual content, final approval of the version to be published, Funding acquisition

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## Tables

**Table 1: Comparison of the socioeconomic and demographic profiles of the mild and severe groups**

<b>Variables</b>	<b>Mild group N (%)</b>	<b>Severe group N (%)</b>	<b>Z/<math>\chi^2</math></b>	<b>P</b>
<b>Sex</b>			1.400	0.237
Male	208 (83.5%)	209 (79.5%)		
Female	41 (16.5%)	54 (20.5%)		
<b>Age</b>	3.5 (3-5)	3 (2.5-4)	-3.250	0.01*
<b>Birth month</b>			2.961	0.398
Nov-Jan	66 (26.5%)	79 (30.0%)		
Feb-Apr	51 (20.5%)	50 (19.0%)		
May-Jul	65 (26.1%)	54 (20.5%)		
Aug-Oct	67 (26.9%)	80 (30.4%)		
<b>Place of residence</b>			4.505	0.034*
Urban	193 (77.5%)	182 (69.2%)		
Rural	56 (22.5%)	81 (30.8%)		
<b>Caregivers</b>			7.986	0.046*
Parents	159 (63.9%)	137 (52.1%)		
Grandparents	66 (26.5%)	93 (35.4%)		
Both parents and grandparents	20 (8.0%)	30 (11.4%)		
Others	4 (1.6%)	3 (1.1%)		
<b>Siblings</b>			1.762	0.184
Yes	52 (20.9%)	68 (25.9%)		
No	197 (79.1%)	195 (74.1%)		
<b>Age of mother during pregnancy</b>	28 (25-30)	28 (24-31.75)	-0.710	0.478
<b>Age of father during pregnancy</b>	29.5 (27-33)	29 (26-33)	-0.297	0.767
<b>Maternal education</b>			7.132	0.008*
Junior college or above	109 (43.8%)	85 (32.3%)		
Senior high school or below	140 (56.2%)	178 (67.7%)		
<b>Paternal education</b>			4.506	0.034*
Junior college or above	104 (41.8%)	86 (32.7%)		

Senior high school or below	145 (58.2%)	177 (67.3)		
<b>Household income (10,000 yuan)</b>	5 (4-7)	5 (4-6)	-1.918	0.055
<b>Family history of mental illness</b>			0.001	0.973
Yes	31 (12.4%)	33 (12.5%)		
No	218 (87.6%)	230 (87.5%)		
<b>Vitamin intake during pregnancy</b>			2.795	0.424
None	29 (11.6%)	43 (16.3%)		
Folic acid	139 (55.8%)	139 (52.9%)		
Multivitamins	57 (22.9%)	53 (20.2%)		
Others	24 (9.6%)	28 (10.6%)		
<b>Mode of delivery</b>			0.026	0.872
Vaginal	85 (34.1%)	88 (33.5%)		
Cesarean	164 (65.9%)	175 (66.5%)		
<b>Eating problems</b>			0.247	0.619
Yes	111 (44.6%)	123 (46.8%)		
No	138 (55.4%)	140 (53.2%)		
<b>Sleeping problems</b>			0.529	0.467
Yes	74 (29.7%)	86 (32.7%)		
No	175 (70.3%)	177 (67.3%)		
<b>Gastrointestinal problems</b>			4.709	0.030*
Yes	53 (21.3%)	78 (29.7%)		
No	196 (78.7%)	185 (70.3%)		
<b>Comorbidity with ADHD</b>			0.250	0.617
Yes	30 (12.0%)	28 (10.6%)		
No	219 (88.0%)	235 (89.4%)		

**Table 2: The correlations of symptoms and blood test results.**

	VD	VA	VE	Zn	Fe	Cu	Pb
	r						
<b>ABC</b>							
Total score	-0.033	-0.009	0.031	0.024	0.036	0.036	0.173*
Body behavior	0.011	0.049	0.034	0.010	0.017	-0.026	0.112
Sensory	-0.065	-0.017	0.009	0.005	-0.036	0.050	0.157*
Self-care	0.027	0.030	0.032	-0.081	-0.022	0.012	0.161*
Language	-0.046	-0.055	-0.010	0.114	-0.062	-0.026	-0.001
Social interaction	-0.021	0.009	0.064	0.062	0.063	0.159*	0.128*
<b>CARS</b>							
Total score	0.049	-0.037	0.027	0.007	-0.029	0.042	0.202*
<b>ATEC</b>							
Total score	0.003	-0.047	-0.017	0.046	-0.006	0.061	0.120
Speech/language/communication	-0.149*	-0.024	0.046	-0.103	0.019	0.126	0.062
Sociability	-0.108	-0.075	-0.057	0.098	0.033	0.080	0.139*
Sensory/cognitive awareness	-0.006	-0.041	-0.090	0.114	0.048	0.097	0.080
Health/physical /behavior	0.045	0.034	0.076	-0.009	-0.015	-0.051	0.086