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Letter to the Editor

Wen-jun Tu[a], Chang-hao Yin[a], Yan-qin Guo, Si-ou Li, Hui Chen*, Yan Zhang, Yong-lu Feng and Bao-hua Long

Serum homocysteine concentrations in Chinese children with autism

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[a]Wen-jun Tu and Chang-hao Yin contributed equally to this work.
***Corresponding author: Hui Chen,** Department of Clinical Laboratory, China Rehabilitation Research Center, No. 10, Jiao men North Road, Fengtai District, Beijing 100068, P.R. China, Phone: +86 10 87569188. E-mail: zsf0508@163.com
Wen-jun Tu: Department of Clinical Laboratory, China Rehabilitation Research Center, Beijing, P.R. China
Chang-hao Yin: Department of Neurology, Hongqi Hospital, Mudanjiang Medical University, Mudanjiang, P.R. China
Yan-qin Guo: Department of Neurology, Hongqi Hospital, Mudanjiang Medical University, Mudanjiang, P.R. China
Si-ou Li: Department of Neurology, Hongqi Hospital, Mudanjiang Medical University, Mudanjiang, P.R. China
Yan Zhang: Autism Treatment Center, China Rehabilitation Research Center, Beijing, P.R. China
Yong-lu Feng: Department of Clinical Laboratory, China Rehabilitation Research Center, Beijing, P.R. China
Bao-hua Long: Autism Treatment Center, China Rehabilitation Research Center, Beijing, P.R. China

Autism is a neurodevelopmental disability usually diagnosed before age 3 years. Autism is characterized by deficits in social reciprocity and in language skills that are associated with social interaction, difficulty with communication, and restrictive and repetitive behaviors [1]. The need to understand the causes of autism and the underlying pathophysiology has become more acute since the number of diagnosed cases has risen markedly in recent years [2].

A number of factors, such as genetic, environmental, gastrointestinal dysfunction and inflammatory biomarkers have been implicated in the etiology of autism [3, 4]. However, the underlying mechanism or a specific metabolic target relevant to autism has not yet been identified. Several recent reviews and research studies lend support to the hypothesis that abnormalities involving the folate-dependent homocysteine methylation reactions, oxidative stress, and genetic predisposition have been implicated as potential causes [3, 5–9].

Homocysteine (Hcy) is an excitatory amino acid which markedly enhances the vulnerability of neuronal cells to excitotoxicity and oxidative injury. Epidemiological studies show a positive, dose-dependent relationship between mild-to-moderate increases in plasma total Hcy concentrations and the risk of neurodegenerative diseases, such as Alzheimer's disease, vascular dementia, cognitive impairment or stroke [10]. An increased vulnerability to oxidative stress and a decreased capacity for methylation may contribute to the development and clinical manifestation of autism. Higher concentrations of Hcy have been reported in the serum, plasma and urine of autistic children [5, 6]. No such research is available for autistic and normal children in the Chinese population. Our study is the first one to our knowledge to report the presence of serum Hcy, folate and vitamin B12 concentrations in normal and autistic Chinese children.

A cross-sectional study was conducted over the period from December 2010 to December 2011. A total of 60 2–6-year-old Chinese children (30 confirmed autism cases and 30 age and gender matched control subjects) participated in this study. The diagnosis of autism was based on the criteria for autistic disorder as defined in the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) and by a diagnostic interview conducted by a developmental pediatrician. All enrolled patients with autism were newly diagnosed. All subjects with autism exhibited symptoms were within the typical triad of autistic traits: communication impairment, social deficits, and ritualistic interests. All the eligible control subjects were defined as children 2–6 years old who were not known to be autistic or to have any other neurodevelopmental delay disorder that could be related to autism. In order to exclude the possibility that the controls could have any sub-clinical autistic features, all control subjects were also clinically examined by the pediatricians.

Exclusion criteria for both groups included a diagnosis of malnutrition, the presence of active infection, or known genetic disease. The protocol and informed consent for this study were reviewed and approved by the

Institutional Review Board at the China Rehabilitation Research Center. The details of the study were explained to the parents of the participating children, and written informed consent was obtained from the parents.

Fasting blood samples were collected from both the autistic children and controls for measurement of serum Hcy, folate and vitamin B12. Five milliliters of venous blood was drawn from the antecubital vein into potassium ethylenediaminetetra-acetic acid and serum separator tubes. Following centrifugation, the serum was transferred to an Eppendorf tube and stored at −80°C prior to measurements. The serum Hcy was measured using an enzyme cycling method by OLYMPUS AU2700 [OLYMPUS, Tokyo, Japan; Hcy: intra-assay coefficient of variation (CV) 1.9%–3.8%, inter-assay CV 2.1%–4.7%]. Serum folate and vitamin B12 were measured using a chemiluminescence immunoassay method by DPC Immulite 2000 (Diagnostic Products Corporation, CA, USA; folate: intra-assay CV 3.8%–7.1%, inter-assay CV 4.2%–7.7%; vitamin B12: intra-assay CV 3.2%–6.5%, inter-assay CV 3.9%–8.2%).

All statistical analysis was performed with SPSS for Windows, version 19.0 (SPSS Inc., Chicago, IL, USA). Results were presented as means±standard deviations (SD). Student's unpaired t-test was used to compare the values in normal and autistic children. Statistical significance was defined as $p<0.05$

Data of both autism and control groups were presented in Table 1. The two groups were matched for gender and age. All subjects were from the Chinese Han population. Three patients had family history of autism. None of the patients had a prenatal gene diagnosis. Eighty percent [48] of the patients' parents had a high school or higher education diploma and 70% [21] of their income was more than US$14,000 per year (high income). The median length of hospital stay was 98 days (11–336 days). The mean hospitalization expense was US$52.5±4.2 per day.

The results on the serum Hcy concentrations in both autistic children and controls are presented in Table 1 and Figure 1. The results indicated that the mean serum Hcy concentrations were significantly ($p<0.001$) higher in autistic children as compared to controls (8.62±1.33 μmol/L and 6.94±1.02 μmol/L, respectively). In addition, serum folate concentrations were lower in autistic children compared to controls ($p<0.001$) but there were no difference in serum vitamin B12 concentrations between autistic children and control group (Table 1).

Based on the receiver operating characteristic (ROC) curve, the optimal cut-off value of Hcy as an indicator for auxiliary diagnosis of autism was projected to be 8.1 μmol/L which yielded a sensitivity of 80% and a specificity of 80%, the area under the curve was 0.798 (95% CI 0.649–0.946).

These preliminary results indicate that high concentrations of serum Hcy may be associated with the pathophysiology of autism. This finding is expressed by the higher serum Hcy concentrations and lower folate concentrations in the autism group compared with the normal group. In addition, this study suggests that serum Hcy can be used as an auxiliary diagnostic tool for the early detection of autism.

	Autism	Control	p-Value[a]
Patients, n	30	30	–
Male/female, n	25/5	25/5	–
Mean age, years	3.55±1.57	3.55±1.57	1.000
Mean height, cm	97.6±8.6	104.2±10.7	0.038
Mean weight, kg	15.7±2.7	17.8±3.4	0.037
Mean body mass index	15.9±1.3	16.4±1.4	0.303
Mean mothers' age, years[b]	30.1±2.8	28.8±2.7	0.125
Hcy, μmol/L	8.62±1.33	6.94±1.02	<0.001
Folate, μg/L	3.12±0.47	6.27±0.87	<0.001
Vitamin B12, pg/mL	279.1±6.61	283.1±7.36	0.075

Table 1 Comparative data of autism and control group.
Data are presented as mean±SD or numbers. Normal reference values of serum Hcy, folate and vitamin B12 were defined as (4.8–13.6) μmol/L, (4.6–14.2) μg/L and (200–500) pg/mL, respectively. [a]Between groups comparison (Student's unpaired t-test). [b]Age when they delivered.

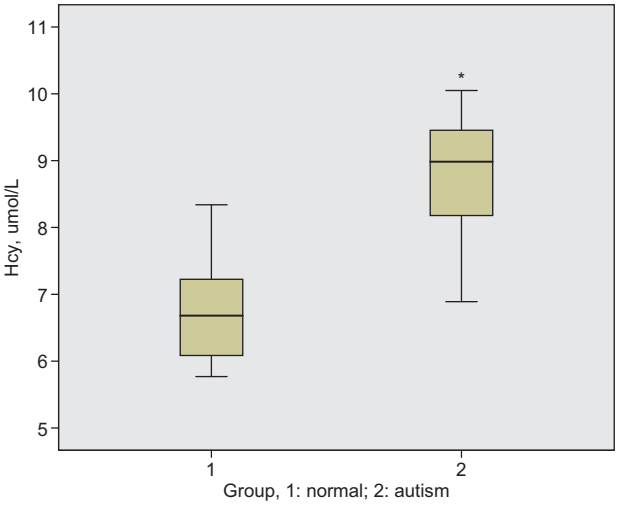

Figure 1 Serum homocysteine concentrations in normal and autism groups.
All data are medians and interquartile ranges (IQR). *Significantly higher in autism as compared to normal group ($p<0.0001$).

The results of serum Hcy are consistent with the previously reported data of Ali et al. [7] who observed significantly higher concentrations of serum Hcy in autistic children as compared to normal children (20.1±3.3 μmol/L and 9.64±2.1 μmol/L, respectively, $p<0.05$). Paşca et al. [8] also reported that plasma Hcy concentrations were significantly higher in autism as compared to controls (9.83±2.75 μmol/L and 7.51±0.93 μmol/L, respectively, $p<0.05$). However, serum Hcy concentrations in children with autism in our region were relatively low at 8.62 μmol/L. Different authors might come from different subspecialties and have different backgrounds; thus, study population and number also could produce a large bias.

Hcy is a powerful excitotoxin. It is toxic to neuronal cells in vitro. Hcy is an endogenous glutamate receptor agonist that acts on NMDA and non-NMDA receptor subtypes. The effect of Hcy on NMDA receptors increases calcium influx and causes apoptosis or changes in cell signaling. Furthermore, Hcy induces neurological dysfunction via oxidative stress [11]. It can cause oxidative stress via a number of mechanisms [12]. Protein-related Hcy metabolism produces Hcy-thiolactone, N-Hcy-protein, and N epsilon-homocysteinyl-lysine (N epsilon-Hcy-Lys), which can cause protein damage and structural changes. These structural changes generate proteins, which are toxic and induce an autoimmune response [13]. High concentrations of serum Hcy and increased oxidative stress may have a pathological role in autism.

Our finding of serum folate concentrations is consistent with Ali et al. [7], however, it is not in agreement with the previously reported data by Paşca et al. [14] who did not show any difference. Parents of children with autism often report gastrointestinal problems as well as picky eating and selective eating in their children. Xia et al. [15] reported that the average intake of vitamins A, B6 and C, folic acid, calcium, and zinc in Chinese children with autism did not meet the national dietary reference intakes (DRI) requirements at all. James et al. [16] suggested that targeted nutritional intervention with folic acid may be of clinical benefit in some children who have autism. We hypothesize that folic acid supplementation can improve the clinical symptoms of autism by reducing the serum Hcy concentrations. 5-Methylenetetrahydrofolate reductase (MTHFR) polymorphism was found in significantly higher frequency in autistic individuals. A consequence of the presence of the polymorphic form of this enzyme during reduced folate status are higher plasma Hcy concentrations than non-carriers and the combination of these factors have been shown in several studies to result in an increase rate of miscarriage via thrombotic events [17]. Folic acid supplementation can reduce the serum Hcy level through above mechanism. Kałużna-Czaplińska et al. [18] also reported that vitamin supplementation reduced the level of Hcy in the urine of autistic children. In addition, meta-analysis of secondary prevention trials showed that B vitamins supplementation caused a decrease in plasma Hcy [10]. Further study needs to accumulate more relevant information.

Based on the ROC curve, we consider that serum Hcy could be used as an indicator of autism. Lentile et al. [19] also hypothesized that higher Hcy concentrations should not only be considered as a mere marker of vitamin deficiency but also as a risk factor or an indicator of disease.

This study contains a number of limitations. Sample sizes are small for both groups. The samples are also geographically limited, potentially limiting the generalizability of our results. This means that the finding should not be overinterpreted. In addition, these experiences relate largely to a single cohort study in one center. It cannot be assumed that they will apply to other centers or populations. Our work is only a small-sample prospective study, a large multicenter approach would be necessary to validate our conclusions in future research.

In conclusion, serum Hcy concentrations were significantly higher in autism as compared to control, suggesting the hypothesis that increased serum Hcy concentrations could be implicated in the pathophysiology and progression of autism in Chinese children. The lower serum folate concentrations observed in children with autism might provide us with a useful way to reduce the serum Hcy concentrations and improve the clinical symptoms of autism. Serum Hcy could also be considered as a potential risk factor of autism.

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Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.
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