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RESEARCH ARTICLE

# Association between 10 urinary heavy metal exposure and attention deficit hyperactivity disorder for children

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## Abstract

Attention deficit hyperactivity disorder (ADHD) is associated with heavy metal exposure during adolescent development. However, the direct clinical evidence is limited. To investigate the possible association between environmental heavy metal exposure and ADHD, a case-control study was conducted with children aged 6–14 years in Guangzhou, China. Results showed that median concentrations of chromium (Cr), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), molybdenum (Mo), tin (Sn), barium (Ba), and lead (Pb) in the urine of the case group were significantly higher than those of the control group. Children with ADHD had significantly higher levels of 8-OHdG and MDA compared with those from the control group. In addition, correlations between urinary Co, Ni, Cu, Mo, and Sn were significantly correlated with 8-OHdG and MDA concentrations in urine. After the case and control groups were combined together and the first quartile was used as the reference category, odds ratios (ORs) of ADHD for children increased significantly with the quartile increasing of urinary Co, Cu, and Sn. Our study provides a clinical evidence that Co, Cu, and Sn exposure, particularly Sn exposure, may be an environmental risk of the incurrence of ADHD for children. Furthermore, Co, Ni, Cu, Mo, and Sn exposures were significantly correlated with DNA and lipid damage.

**Keywords** Heavy metals · ADHD · 8-OHdG · DNA damage · MDA · Lipid damage

## Introduction

Attention deficit hyperactivity disorder (ADHD) is one of the most common neurodevelopmental diseases with an incidence of 6.26% in children of China (Li et al. 2018; Wang et al. 2017), which is characterized by hyperactivity, impulsive behavior, an inability to pay persistent attention, low frustration tolerance, and is associated with various grades of functional damage (Polanczyk et al. 2014). Compared with healthy ones, children with ADHD have poorer interpersonal relationships and lower learning abilities, resulting in a lack of self-confident, low self-evaluation, negative emotions, and other negative effects (Barkley et al. 2002; Lecendreux et al. 2011).

The prevalence of ADHD is approximately 7.2% across the world and keeps a rapid growth tendency (Patros et al. 2016; Polanczyk et al. 2007; Thomas et al. 2015). For example, the percentage of children aged 4–17 years in the USA with parent-reported ADHD increased by 21.8% from 2003 to 2007 (He et al. 2019). Reasons for ADHD risk factors are various, including single nucleotide polymorphisms, various pre- and peri-natal factors, toxins, dietary factors, psychosocial risks, and heavy metal exposure (He et al. 2019). Among them, heavy metal exposure has attracted considerable public attentions and concerns.

As heavy metals, particularly lead (Pb), can affect children's neurodevelopment, they are the important environmental factors inducing the incurrence of ADHD. For instance, the toxic effect of Pb can affect the development and the growth

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of almost all body systems (Mitra et al. 2012). Human blood Pb concentrations higher than 100 μg/dL can exert severe toxicities and result in vomiting, mental retardation, cerebral palsy, seizures, and even death (Hubbs-Tait et al. 2005). Remarkably, the Center for Disease Control Advisory Committee on Childhood Lead Poisoning Prevention found there is no safe Pb level for children (He et al. 2019) because even low-level Pb exposure over a lifetime can exert severe negative effects on health (Needleman 2004). For example, it is reported that blood Pb lower than 5–10 μg/dL can affect the development of ADHD disease (He et al. 2019) via influencing brain morphology and impairing attention and impulse control (Willcutt et al. 2012).

Besides Pb exposure, organic mercury (Hg, e.g., methyl Hg) is identified to be toxic for the development of the nervous system (Winneke 2011). Its neurotoxicity is well-documented as tremor and psychomotor impairment (Winneke 2011). Hg deposition in the III, IV, and V layers of the human cerebral cortex can alter human behavior by influencing neuron function (Kiecolt-Glaser et al. 2008; Talarovicova et al. 2007). In addition, the potential neurotoxicity of aluminum (Al) was first identified by cases of dialysis dementia and subsequent neurobehavioral observations in vivo (Winneke 2011). An association between copper/zinc (Cu/Zn) SOD serum levels and ADHD children with high serum Cu is observed as Cu/Zn superoxide dismutase (SOD-1) is a key enzyme in the dismutation of superoxide radicals resulting from cellular oxidative metabolism. Hence, Al, Hg, and Cu may be one of the risk factors of ADHD (Russo 2010). In addition to high heavy metal levels, lack of some metal is also associated with ADHD. For example, blood Zn is inversely associated with ADHD and this study observed ADHD children with a lower Zn level (Zhou et al. 2016; Akhondzadeh et al. 2004; Bilici et al. 2004) and Zn supplementation can reduce ADHD symptoms in children with low Zn levels.

Though several in vivo and epidemiological studies have shown the relationship between some heavy metals and ADHD (Yousef et al. 2011), the direct clinical evidence is still limited and we need more cases to strengthen the relationship between heavy metal exposure and ADHD. Moreover, previous studies focused on individual metal and correlation of ADHD with co-exposure to ten heavy metals is few. Our target is to investigate the possible association between 10 environmental heavy metal exposure and ADHD, as well as to screen the potential, unreported heavy metals that may be related to ADHD.

## Materials and methods

### Equipment

An Agilent 7800 ICP-MS (USA) was used to determine heavy metals in urine. A UV2300 spectrophotometer (Techcomp, China) was used to determine urinary creatinine. A 30A UPLC (Shimadzu, Japan)/API 4000 mass spectrometer (AB, SCIEX, USA) was used to determine 8-OHdG concentration in urine. An enzyme-labeled instrument (PerkinElmer, USA) was used to determine urinary MDA concentration.

### Reagents

Pure water was purchased from Wahaha Company, China. Sixty-five percent suprapur®nitric acid (optical grade) was from Merck Company (Germany). The mixed standard solutions of 10 heavy metals were from National Center of Analysis and Testing for Nonferrous Metals and Electronic Materials (Beijing, China), including chromium (Cr), manganese (Mn), cobalt (Co), nickel (Ni), Cu, molybdenum (Mo), cadmium (Cd), tin (Sn), barium (Ba), and Pb who are reported with negative health effects. The mixed internal standard solution of 5 elements (i.e., scandium (Sc), germanium (Ge), yttrium (Y), indium (In), and bismuth (Bi)) were from SPEX CertiPrep (USA). Large quantities of previous studies in vitro and in vivo have identified Cu and Pb with neurotoxicity (Buck 2004; Chang et al. 1982; Dyer et al. 1982; Feldman et al. 1993; Hubbs-Tait et al. 2005; Segal 1988; Mitra et al. 2012). Hence, they are chosen as our target heavy metals from the beginning of this study. However, other heavy metals, such as Sn, have been reported to be closely related to nerve injury (Balaban et al. 1988; Chang et al. 1982; Zhang et al. 2019; Zhou et al. 2017). To investigate the relationship of ADHD and heavy metal exposure and to screen more metal with potential neurotoxicity, Cr, Mn, Co, Ni, Mo, Cd, and Ba are included in this study.

### Subjects recruited

One hundred seventy-eight children aged 6–11 years who were diagnosed with ADHD by two doctors from the Department of Psychology of Guangzhou Women and Children's Medical Center (Guangzhou, China) were recruited as the case group. The diagnosis of ADHD was basically according to the Statistical Manual of Mental Disorders-5th Edition (DSM-5) (Achenbach, Association 2013). In addition, Child Behavior Checklist (CBCL) (Achenbach 1983; Buck 2004) and Conners' Parent Symptom Questionnaire (CPSQ) as well as the Conners' Teacher Rating Scale (CTRS) completed by children' teacher (Goyette et al. 1978) were referred. Children with other mental disorders (e.g., Asperger's syndrome and Tourette's syndrome) were excluded from this study. One hundred six healthy children aged 6–14 years from two primary schools located in Guangzhou were recruited as the control group. Children who have cough, cold, bronchitis, pneumonia, asthma, poor learning and memory ability, mood control disorders (e.g., anxiety, mania, depression), and CVD were excluded from the study. To avoid possible exposure to

heavy metals or other pollutants from the environment, children who live close to the factory or highway will be excluded. Additionally, children whose parents have mental health disorders will be excluded from the study as well.

This study was approved by the Research Ethics Committee of South China Normal University. The parent or guardian of each participant signed an informed consent and completed a questionnaire including gender, age, height, weight, lifestyle habits, and physical condition before sampling. As diet contaminated by heavy metals is an important exposure source for humans, diet habit and material of tableware in households were inquired, including consumption frequencies of semi-manufactured foods/beverages (Table 1) and materials of tableware (data not shown).

## Collection of urine samples

All morning urine samples were collected from May 2014 to November 2019. Then, they were sealed in polyethylene plastic bottles and shipped to the lab within 2 h. Fresh urinary creatinine was determined by using the Jáffee method to avoid the effect of urine dilution. Subsequently, all urine samples were stored at − 20 °C until pretreatment and instrumental analysis.

## Urine sample preparation, calibration curve, recovery, and precision

Firstly, 98 mL purified water and 2 mL concentrated nitric acid solution were added into polyethylene plastic bottles to prepare 2% (V/V) diluted nitric acid. Then, 1 mL mixed internal standard solution with 100 μg/L of Sc, Ge, Y, In, and Bi were added into 1 mL of urine sample and diluted to 10 mL by adding 8 mL 2% (V/V) dilute nitric acid. Therefore, concentrations of the mixed internal standard solution (i.e., Sc, Ge, Y, In, and Bi) were diluted to 10 μg/L. Ten concentrations of Cr, Mn, Co, Ni, Cu, Mo, Cd, Sn, Ba, and Pb prepared in 2% (V/V) diluted nitric acid for calibration curve ranged from 0.01 to 100 μg/L. The urine sample was prepared by adding 1 mL mixture of internal standard and 8 mL 2% (V/V) diluted nitric acid into 1 mL urine sample.

Different volumes of mixed standard solution were spiked into the same diluted urine pooled to prepare high (0.050–5.0 μg/L), median (0.10–10 μg/L) and low concentrations (0.50–20 μg/L) of quality assurance and quality control (QA/QC) samples. The limit detection (LOD) for all heavy metal analytes was calculated as three times of standard deviation of blank urine samples which were continuously measured 10 times. And the limits of quantitation (LOQ) were calculated as ten times of standard deviation of blank urine samples. The LOD of 8-OHdG was calculated as 3 times of signal/noise (S/N) in blank urine samples. The LOD of MDA was achieved according to the instruction of commercial kits. The method of recovery and accuracy was evaluated by calculating the relative standard deviations (RSDs) of repetitive measurement of the QC samples at three different concentrations. Recovery was calculated by dividing the measured concentrations of target analytes by the spiked concentration. And their recoveries ranged from 81.7 to 118% with intra- and inter-day coefficient of variation (CV) below 15% (Table 2). The LOD and LOQ of targets are listed in Table 3.

**Table 1** Characteristics of 284 studied subjects from Guangzhou, China

| Variable | *N* (%) or Mean ± SD | | | *p* value[a] |
|---|---|---|---|---|
| | All (*N* = 284) | Cases (*N* = 178) | Controls (*N* = 106) | |
| Gender | | | | < 0.001[b] |
| Male | 214 (75.4) | 161 (90.4) | 53 (50.0) | |
| Female | 70 (24.6) | 17 (9.60) | 53 (50.0) | |
| Age | | | | |
| Mean ± SD (years) | 8.54 ± 1.68 | 8.30 ± 1.77 | 8.93 ± 1.44 | < 0.001[c] |
| Body mass index | | | | |
| Mean ± SD (kg/m$^2$) | 16.9 ± 3.56 | 17.1 ± 3.67 | 16.5 ± 3.34 | 0.1[c] |
| Consumption frequencies of semi-manufactured foods/beverages | | | | |
| Often | 6 (2.10) | 6 (3.40) | 0 (0) | < 0.001[b] |
| Sometimes | 46 (16.2) | 40 (22.5) | 6 (5.70) | |
| Never or rarely | 229 (80.6) | 130 (73.0) | 99 (93.4) | |
| Missing | 3 (1.10) | 2 (1.10) | 1 (0.900) | |

[a] Comparison between the cases and control group

[b] Statistics with a chi-square test

[c] Statistics with a Mann-Whitney *U* test

**Table 2** Results of accuracies and precisions in urine

| Element | Conc. of blank urine (ng/mL) | Spiked concentration (ng/mL) | Recovery (%) | CV% (1) | CV% (2) |
|---|---|---|---|---|---|
| Cr | 0.28 ± 0.038 | 0.10 | 106.0 | 4.20 | 3.90 |
| | | 0.50 | 110.6 | 2.40 | 4.90 |
| | | 1.0 | 96.60 | 3.60 | 6.30 |
| Mn | 0.70 ± 0.029 | 5.0 | 115.8 | 2.60 | 7.90 |
| | | 10 | 96.50 | 1.20 | 6.20 |
| | | 20 | 103.4 | 1.10 | 3.00 |
| Co | $0.059 \pm 8.0 \times 10^{-3}$ | 0.050 | 89.30 | 5.30 | 13.0 |
| | | 0.10 | 95.20 | 3.00 | 13.0 |
| | | 0.50 | 100.6 | 4.00 | 6.30 |
| Ni | 0.36 ± 0.019 | 1.0 | 107.7 | 4.20 | 5.40 |
| | | 5.0 | 100.0 | 3.60 | 3.60 |
| | | 10 | 105.0 | 1.90 | 3.60 |
| Cu | 0.96 ± 0.027 | 0.50 | 92.80 | 1.60 | 8.80 |
| | | 1.0 | 92.70 | 3.00 | 5.20 |
| | | 5.0 | 94.40 | 2.60 | 4.70 |
| Mo | 6.9 ± 0.075 | 1.0 | 85.80 | 0.900 | 2.70 |
| | | 5.0 | 96.20 | 0.600 | 2.30 |
| | | 10 | 102.2 | 0.900 | 1.90 |
| Cd | $0.036 \pm 9.0 \times 10^{-3}$ | 0.050 | 81.70 | 11.2 | 7.80 |
| | | 0.10 | 103.0 | 10.5 | 14.7 |
| | | 0.50 | 103.9 | 5.30 | 7.60 |
| Sn | 0.56 ± 0.12 | 1.0 | 88.60 | 6.40 | 8.90 |
| | | 5.0 | 99.20 | 8.10 | 2.80 |
| | | 10 | 107.1 | 1.20 | 1.40 |
| Ba | 0.55 ± 0.060 | 1.0 | 115.7 | 3.70 | 3.90 |
| | | 5.0 | 118.0 | 2.60 | 2.50 |
| | | 10 | 117.6 | 2.70 | 3.20 |
| Pb | 0.12 ± 0.013 | 0.10 | 114.5 | 3.80 | 4.10 |
| | | 1.0 | 111.3 | 1.80 | 2.20 |
| | | 5.0 | 111.3 | 0.900 | 1.00 |

*CV% (1)* intra-assay imprecision, *CV% (2)* inter-assay imprecision

## Determination of 8-OHdG and MDA in urine

8-OHdG was determined by LC/MS/MS coupled with an isotoped internal standard dilution method (Li et al. 2018). MDA was determined by an enzyme-labeled instrument using the TBA method. The LOD of 8-OHdG and MDA were 0.2 μg/L and 0.645 μg/L, respectively.

## Statistical analysis

SPSS (SPSS, version 23.0, Chicago, IL) was used for statistical analysis. 8-OHdG, MDA, and all urinary concentrations of heavy metals were presented as μg/g creatinine (i.e., μg/g Crt.). All the results were achieved by a blind observer method. For the concentrations below LOQ, LOQ/2 was used as the concentration instead of zero. The missing values of body mass index (BMI) were imputed by using the mean value. As data were not normally distributed, Mann-Whitney *U* test was used to examine the significant differences of urinary heavy metal concentrations between different groups. When covariates were not continuous, such as gender and life habits, we use Cochran's chi-squared test to examine the significant differences between groups. All significant levels were set at $p < 0.05$. All children were stratified into quartiles based on their urinary creatinine-adjusted heavy metal concentrations. In binary logistic regression models adjusted by personal information (i.e., age, gender, and BMI), the first quartiles of urinary heavy metal levels in children were used as the reference to examine the odds ratios (ORs) of ADHD correlated with heavy metals exposure. Spearman correlation (two-tailed) was used to test the relationship between ln-transformed heavy metals and 8-OHdG, MDA concentrations.

**Table 3** Concentrations of heavy metal elements in urine for the control and case groups

| | Controls ($n$ = 106) | | Detection frequency (%) | Cases ($n$ = 178) | | Detection frequency (%) | LOD | LOQ | $p$ value |
|---|---|---|---|---|---|---|---|---|---|
| | Median (P25, P75) | Ranges | | Median (P25, P75) | Ranges | | | | |
| Cr | 0.0920 (0.0220, 1.84) | < LOQ–14.7 | 46.2 | 1.48 (0.166, 3.87) | < LOQ–27.4 | 75.3 | 0.02 | 0.06 | 0.0001 |
| Mn | 0.658 (0.0600, 1.66) | < LOQ–11.6 | 68.9 | 1.09 (0.205, 2.35) | < LOQ–44.6 | 74.7 | 0.03 | 0.09 | 0.01 |
| Co | 0.279 (0.167, 0.401) | < LOQ–31.4 | 98.1 | 0.436 (0.296, 0.704) | 0.0120–4.38 | 98.3 | 0.002 | 0.008 | 0.0001 |
| Ni | 2.08 (1.18, 3.25) | < LOQ–15.0 | 96.2 | 4.00 (2.39, 6.68) | < LOQ–26.6 | 94.9 | 0.04 | 0.1 | 0.0001 |
| Cu | 10.9 (8.29, 14.1) | 3.59–126 | 100 | 16.6 (12.9, 24.9) | 2.91–103 | 100 | 0.01 | 0.04 | 0.0001 |
| Mo | 81.9 (60.6, 107) | 13.1–356 | 100 | 93.5 (68.3, 140) | 17.5–440 | 100 | 0.01 | 0.04 | 0.03 |
| Cd | 0.431 (1.18, 3.10) | < LOQ–4.47 | 99.1 | 0.406 (0.220, 0.588) | 0.0140–3.09 | 95.5 | 0.003 | 0.01 | 0.2 |
| Sn | 2.05 (0.623, 1.58) | 0.143–15.8 | 100 | 4.99 (3.03, 8.47) | 0.544–48.8 | 100 | 0.003 | 0.01 | 0.0001 |
| Ba | 2.15 (0.992, 3.43) | < LOQ–577 | 85.9 | 3.40 (1.02, 7.64) | < LOQ–158 | 82.6 | 0.09 | 0.3 | 0.0001 |
| Pb | 0.882 (0.0160, 7.43) | < LOQ–58.8 | 63.2 | 2.64 (0.362, 5.44) | < LOQ–141 | 75.3 | 0.007 | 0.02 | 0.03 |
| 8-OHdG | 4.09 (3.00, 5.61) | 1.16–14.8 | 100 | 5.04 (3.49, 7.17) | 0.440–17.0 | 100 | 0.2 | | 0.003 |
| MDA | 242 (201, 291) | 86.8–535 | 100 | 340 (262, 441) | 52.6–977 | 100 | 0.7 | | 0.9 |

(P25, P75): 25% quantile, 75% quantile in quartile. The unit of concentration is μg/g Crt.

*LOD* detection limit, μg/L; *LOQ* limit of quantitation, μg/L

## Results

In total, 284 children were enrolled in our study. Among them, 178 children were diagnosed with ADHD by at least two doctors, including 161 boys and 17 girls. Table 1 showed their demographic characteristics. No significant difference in BMI between the case and the control groups ($p$ = 0.128) was observed. However, their dietary habits existed a significant difference ($p$ < 0.001); i.e., children from the case group had higher consumption frequencies of semi-manufactured foods/beverages than those from the control group (Table 1). Materials of daily tableware were also collected (data was not shown in Table 1 because there is no difference between groups).

The medians, quartiles of heavy metals, 8-OHdG, and MDA concentration in urine are presented in Table 3. The detection frequencies of the studied metals in the case group were higher than in the control group. With the exception of urinary Cr in the control group, the detection frequencies of other elements and target compounds were in the range of 80–100%. Except for urinary Cd concentrations, all levels of other heavy metals (i.e., Cr, Mn, Co, Ni, Cu, Mo, Sn, Ba, and Pb) in the case group were significantly higher than those in the control group and most of them in the case group were times higher than those in the control group (e.g., levels of Cr, Mn, Co, Ni, Cu, Mo, Sn, Ba, and Pb in the case group were 16.1, 1.66, 1.56, 1.92, 1.52, 1.14, 2.43, 1.58, and 2.99 times in the control group, respectively). Though urinary Cd in the case group was slightly lower than that in the control group, no significant difference existed between them. Interestingly, the median urinary 8-OHdG and MDA concentrations in the case group were also higher than those in the control group (e.g., 8-OHdG and MDA levels in the case group were 1.23 and 1.40 times in the control group, respectively), which were in consistent with the trends of urinary heavy metal exposure.

As all data were not normally distributed, they were ln-transformation in the unit of μg/g Crt. Levels of Co, Ni, Cu, Mo, and Sn were significantly correlated with 8-OHdG in urine (Fig. 1). Similarly, after ln-transformation, significant correlations between Co, Ni, Cu, Mo, Sn, and MDA concentrations in urine were observed (Fig. 2).

When the control and ADHD children were combined together and the 1st quartile children were used as the reference, only Co, Cu, and Sn exposure correlated well with the OR of ADHD. Moreover, their ORs for the 2nd, 3rd, and 4th quartiles of children gradually increased with the increasing of Co, Cu, and Sn concentrations, suggesting that higher heavy metal exposure lead to higher risks of ADHD occurrence. It is worth stating that after the adjustment of covariates (i.e., age, gender and BMI), the probabilities of having ADHD for children in the fourth quartiles of Sn concentrations increased from 4.360 to 95.61, approximately 95 times of those in the lowest quartile of urinary Sn concentrations (Table 4).

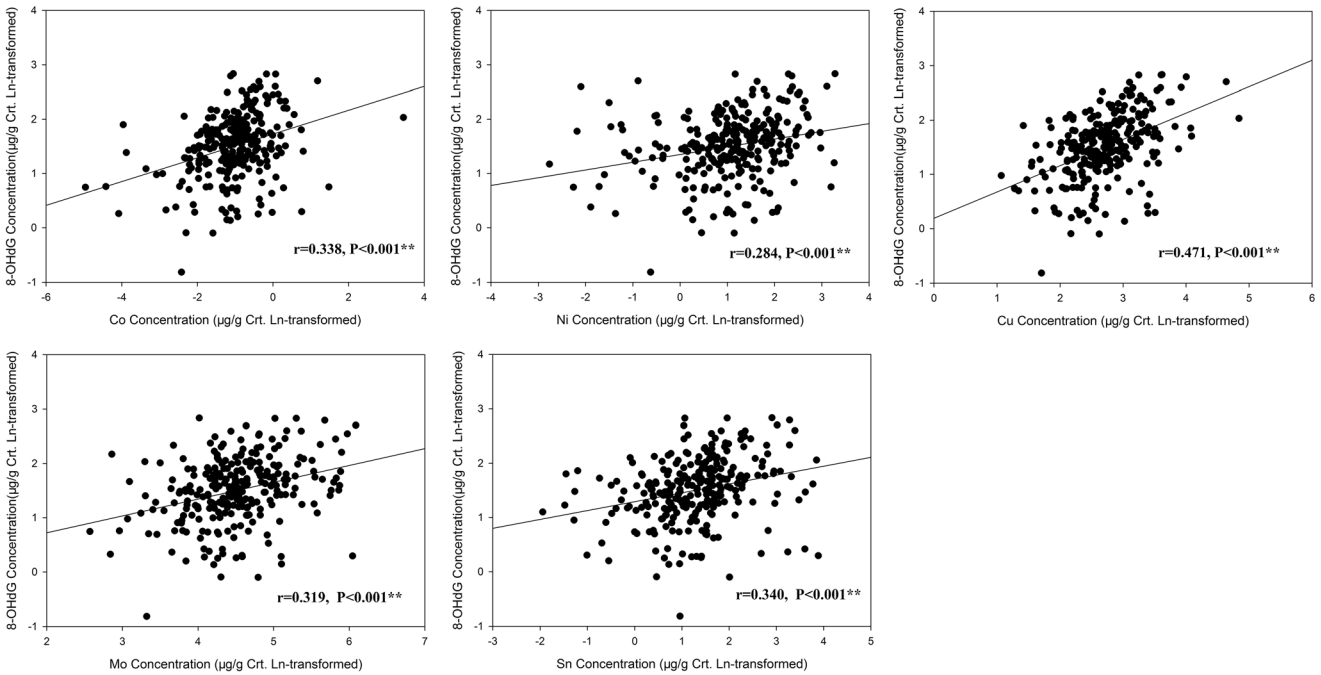


**Fig. 1** Correlations of 5 heavy metal (i.e., Co, Ni, Cu, Mo, Sn) elements with 8-OHdG in children urine samples (all data in the unit of μg/g Crt. were Ln-transformed)

## Discussion

The result showed that the median concentrations of 9 heavy metals (i.e., Cr, Mn, Co, Ni, Cu, Mo, Sn, Ba, and Pb) in urine for the case group were higher than those for the control group (Table 3), suggesting that children with ADHD are exposed to higher levels of heavy metals than those without ADHD.

After the combination of the control and case groups, children were divided into four quantiles according to their exposure levels of heavy metal in urine. The first quantile with the

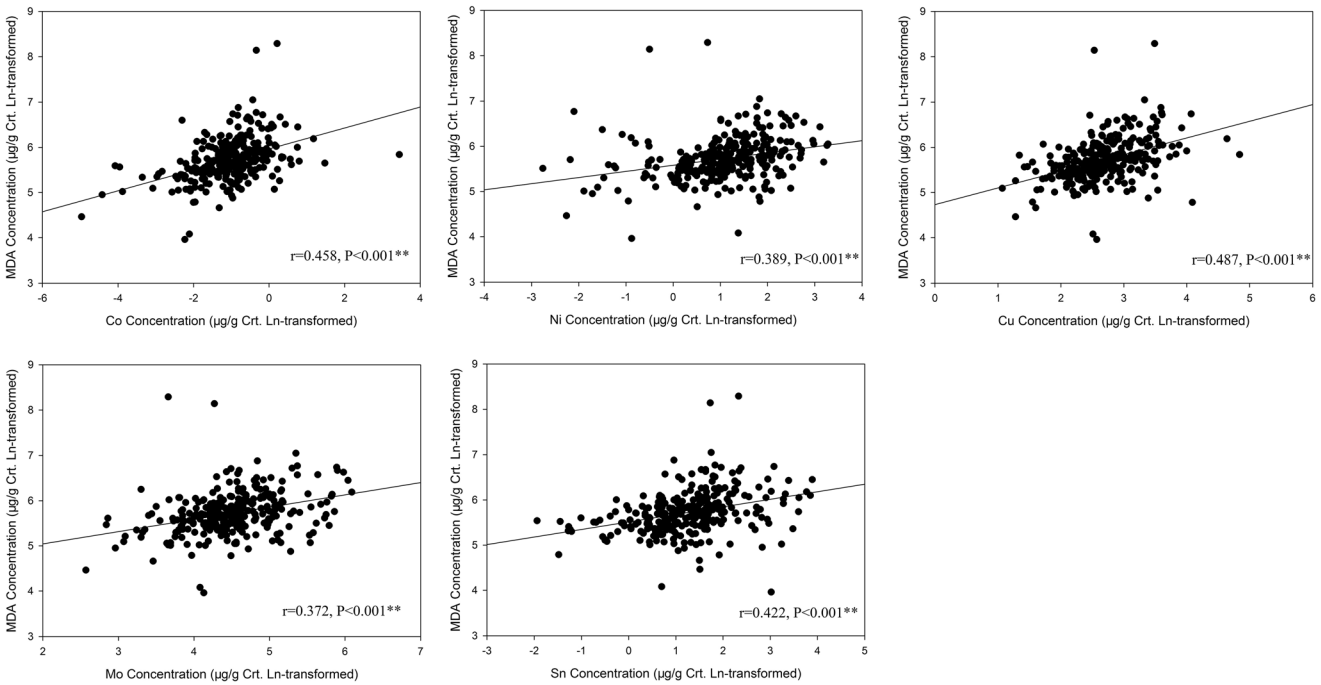


**Fig. 2** Correlations of 5 heavy metal (i.e., Co, Ni, Cu, Mo, Sn) elements with MDA in children urine samples (all data in the unit of μg/g Crt. were Ln-transformed)

**Table 4** Odd ratios (OR) and 95% confidence intervals (CI) of ADHD based on the quartiles of 3 creatinine-adjusted heavy metal concentrations in urine for 284 children from Guangzhou, China

| | *N* (case, control) | | Model 1[a] OR (95% CI) | *p* value | Model 2[b] OR (95% CI) | *p* value |
|---|---|---|---|---|---|---|
| Co | | | | | | |
| 1st quartile | 26 | 45 | (Reference) | | (Reference) | |
| 2nd quartile | 45 | 26 | 2.996 (1.513, 5.930) | 0.002 | 2.535 (1.053, 6.104) | 0.04 |
| 3rd quartile | 48 | 23 | 3.612 (1.806, 7.223) | < 0.001 | 2.483 (1.066, 5.780) | 0.04 |
| 4th quartile | 59 | 12 | 8.510 (3.876, 18.68) | < 0.001 | 7.249 (2.786, 18.86) | < 0.001 |
| Cu | | | | | | |
| 1st quartile | 24 | 47 | (Reference) | | (Reference) | |
| 2nd quartile | 37 | 34 | 2.131 (1.083, 4.195) | 0.03 | 2.695 (1.138, 6.386) | 0.02 |
| 3rd quartile | 56 | 15 | 7.311 (3.444, 15.52) | < 0.001 | 7.628 (2.870, 20.28) | < 0.001 |
| 4th quartile | 61 | 10 | 11.95 (5.209, 27.40) | < 0.001 | 8.821 (3.162, 24.61) | < 0.001 |
| Sn | | | | | | |
| 1st quartile | 22 | 49 | (Reference) | | (Reference) | |
| 2nd quartile | 38 | 33 | 2.565 (1.292, 5.093) | 0.007 | 4.360 (1.707, 11.14) | 0.002 |
| 3rd quartile | 50 | 21 | 5.303 (2.591, 10.85) | < 0.001 | 11.02 (3.813, 31.82) | < 0.001 |
| 4th quartile | 68 | 3 | 50.49 (14.31, 178.1) | < 0.001 | 95.61 (17.19, 531.7) | < 0.001 |

Model 1 was crude logistic regression model. In model 2, age, gender, and body mass index (BMI) were used as the covariates (unit: μg/g Crt.)

[a] Crude logistic regression model

[b] Adjusted for age, gender, and body mass index

lowest concentration of heavy metal was used as the reference group. The results of logistic regression showed that the ORs of ADHD increased significantly with the increase of urinary Co, Cu, and Sn exposure. In order to further analyze the risk of ADHD caused by heavy metal exposure, and exclude the influence of some confounding factors on the results, ORs of ADHD were adjusted by covariates (i.e., BMI, age, and gender). All ORs showed gradually increased tendencies with the increasing of Co, Cu, and Sn levels, which suggested that these heavy metals may be related to the occurrence of ADHD. Moreover, higher Co, Cu, and Sn exposure in the body will lead to higher occurrence risk of ADHD. The results were consistent with the previous studies (He et al. 2019; Palur et al. 2001; Skalny et al. 2020; Stanton et al. 1991; Tinkov et al. 2019; Yousef et al. 2011). The pathogenesis of ADHD is not clear yet. In addition to the genetic reasons, prenatal, perinatal, and postnatal development environment, family factors, and exposure to high environmental pollution during childhood are all related to the occurrence of ADHD (He et al. 2019; Yousef et al. 2011). Besides Pb, many epidemiological and animal studies have confirmed that high concentrations of heavy metals during the development period will exhibit neurotoxicity and long-term exposure to them will finally inhibit children's learning and memory ability, or result in high incidence of ADHD and autism diseases (Abdullah et al. 2012; Goodlad et al. 2013; Huang et al. 2016; Nigg et al. 2010; Yousef et al. 2011). For example, Cu deposition in thalamus, brainstem, lentiform nucleus, caudate nucleus, dentate nucleus, and brainstem of mice can cause local brain edema, neurodegeneration, demyelination of nerve fibers, and increase of glial cells. With the development of these diseases, necrosis, cystic change, interstitial focus, and cavernous vacuolation may occur in these areas (Chang et al. 1982; Buck 2004); Sally's research indicates that substantial ingestion of Co salts can produce neuro-ocular, cardiac, and thyroid toxicity (Bradberry 2016); Sn contaminants, such as trimethyl Sn (TMT), can selectively damage neurons in a specific area of the central nervous system, especially hippocampal neurons (Balaban et al. 1988; Chang et al. 1982), which play an important role in the altered behavior and the learning and memory abilities (Zhang et al. 2019; Zhou et al. 2017). These results are basically consistent with our study. Among these heavy metals, behavioral alteration or decreased learning focus caused by blood Pb exposure was most reported (Goodlad et al. 2013; Huang et al. 2016; Nigg et al. 2010). Based on continuous researches, people have a comprehensive understanding of the hazards caused by blood Pb exposure and high awareness of vigilance. However, our results showed that high urinary Sn exposure might significantly increase the probabilities of having ADHD for children (4.360 vs 95.61), which suggested that the potential neurotoxicity induced by high Sn exposure should raise our more concerns compared with other heavy metals.

It is gratified that people have gradually realized the harm of Sn to neuron development, especially organic Sn. For example, TMT is reported to have strong neurotoxicity as TMT

can produce a complex neurologic syndrome, such as delirium, disorientation, memory defects, stuttering, depression, fatigue, and insomnia (Feldman et al. 1993). In vivo studies also showed that TMT can produce the neurotoxic symptoms in rats, which is described as tail chasing, mutilation of the tail, vocalization, impaired learning, aggression, and tremor (Dyer et al. 1982; Segal 1988). Prenatally TMT-exposed rats showed impaired cognitive performances in both the step-through passive avoidance test and the shuttle box active avoidance test, which suggested that prenatal TMT exposure could be considered as a putative experimental model of ADHD (Tamburella et al. 2012). However, direct clinical evidence or epidemiological studies are still limited. Our study initially demonstrated the evidence of childhood ADHD which might be triggered due to environmental Sn exposure from the clinical and population perspectives.

Sources of heavy metal exposure are wide in daily life. Our questionnaire indicated that though materials of tableware used in case and control groups did not show difference (data not shown here), the frequency of semi-manufactured food/beverage consumption in the case group was higher than in the control group, which is consistent with their exposure to heavy metals in urine. This phenomenon reminds us that foods contaminated by heavy metals during cooking or package might be another source of diet. In recent years, TMT has been used as a substitute of Pb-free plastic stabilizer to be widely used in food containers and packaging (Feldman et al. 1993; Palur et al. 2001; Yanofsky et al. 1991). TMT can contaminate foods due to its volatility (Feldman et al. 1993; Palur et al. 2001; Yanofsky et al. 1991).

Oxidative stress is considered to be the imbalance between oxidation and antioxidation in vivo, which tends to oxidize and produce excessive free radicals. Though pathogenesis of ADHD triggered by pollutants is not elucidated yet, ROS and cell damages induced by pollutants are often the initial steps in vitro and in vivo experiments. For example, the main neurotoxicity of TMT is due to its cytotoxicity and oxidative stress on nerve cells. Cerebellar granule cells exposed to TMT will release more lactate dehydrogenase (LDH, a marker of cytotoxicity) than those non-exposed to TMT, suggesting that TMT has cytotoxicity to cerebellar granule cells as it can induce a variety of oxidative radicals ($(O_2)^-$, $H_2O_2$, NO) in neuronal cells (Segal 1988). Moreover, lipid peroxidation appeared in 10% of mice brains when they were injected with TMT after 24 h, showing a distinctive oxidative stress (Segal 1988).

Due to the limitation of human ethics, it is impossible to get human brain tissue samples. Hence, we determined urinary 8-OHdG and MDA. 8-OHdG, a biomarker of DNA damage, is produced by the reaction of hydroxyl radical and superoxide anion with DNA molecules, and is often used to evaluate the degree of oxidative damage and degree of repairing this damage in vivo (Saiki et al. 2011). MDA, a biomarker of lipid damage, is one of the end products of the peroxidation reaction between oxygen free radicals and cell membrane unsaturated fatty acids and can indirectly reflect the content of oxygen free radicals and the degree of oxidative damage (Misra et al. 2009; Moon et al. 2014). So both of them are sensitive indicators of oxidative stress (Chauhan et al. 2004). Compared with other tissues, brain tissue is more sensitive to oxidative stress due to its dense lipid content, high oxygen consumption, relatively low antioxidant level (Simsek et al. 2016). Our results showed that children with ADHD had significantly higher levels of 8-OHdG and MDA than those without ADHD, suggesting that children with ADHD burdened more oxidative stress in body, including brain. This result is consistent with previous studies (Chauhan et al. 2004; Chovanova et al. 2006; Misra et al. 2009; Moon et al. 2014; Simsek et al. 2016). Correlation studies also showed that urinary Co, Ni Cu, Mo, and Sn concentrations were well correlated with 8-OHdG and MDA in urine, suggesting that exposure to these heavy metals could trigger or increase oxidative DNA and lipid damages in the body, which partially confirmed the results of previous animal or cell experiments from the perspective of an epidemiological study.

The mechanism of metal correlation with ADHD is not clear yet. Many metal-enzyme complexes were located in the body, including the nervous system (Toren et al. 1996). Therefore, their imbalance can lead to a subsequent physical reaction. For example, Cu and Zn participate in SOD enzymatic mechanisms that protect against free radicals and therefore serve an important adjunct role in oxidative balance. Hence, low Cu/Zn SOD concentration is related to the higher occurrence of ADHD (Russo 2010). Furthermore, some metals (e.g., Zn) are necessary for the construction and function of the brain (Black 1998). For example, Zn is important for the conversion of dietary pyridoxine to its active form (i.e., pyridoxal phosphate). And pyridoxine is necessary to convert tryptophan into 5-hydroxytryptamine, which is closely related to ADHD (Bilici et al. 2004; Quist and Kennedy 2001). Moreover, Zn is basic for the production and modulation of melatonin, which is considered as a necessary factor in ADHD due to its regulation function to dopamine (Arnold and DiSilvestro 2005).

In previous studies, researchers mainly use the questionnaire based on the DSM criteria to assess and diagnose ADHD instead of clinical diagnosis (Casas et al. 2015; Harley et al. 2013; Tewar et al. 2016). Hence, there may be deviation or wrong judgment in the enrolled subjects. But this phenomenon did not exist in this study as all ADHD children recruited were diagnosed by at least two doctors from the hospital which makes the conclusions more reliable. However, there were still some limitations in our research. Firstly, neurotoxicities are not limited to heavy metals. Many environmental pollutants, such as BPA, pesticides, herbicides, and persistent organic pollutants, are reported with neurotoxicities (Li et al.

2018). Secondly, factors affecting the ADHD are complicated, including genetic (i.e., the child's intelligence quotient (IQ)), environmental, and family factors (Li et al. 2018). It is hard to discriminate against their contribution. Our aim is to investigate the relationship between urinary heavy metal exposure and ADHD. It only involves environmental factors, but it is difficult to exclude the interference of genetic and family factors. Thirdly, family factors, such as the incomes and the education levels of children's parents, were not discussed in this study. Fourthly, the total concentrations of metal were determined in this study, not metal species which often have different toxicities.

## Conclusions

The significant differences of urinary 9 heavy metal (Cr, Mn, Co, Ni, Cu, Mo, Sn, Ba, and Pb) median concentration were observed between children in the case and control groups. Higher Co, Cu, and Sn exposure in urine could increase the odd risks of ADHD, particularly Sn exposure. Moreover, Co, Ni Cu, Mo, and Sn exposure correlates well with urinary 8-OHdG and MDA, suggesting that heavy metal exposure increases oxidative stress. In conclusion, environmental heavy metal exposure (especially Co, Cu, and Sn) is linked to the risk of ADHD clinically.

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### Compliance with ethical standards

This study was approved by the Research Ethics Committee of South China Normal University.

**Conflict of interest** The authors declare that there is no conflict of interest.

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