

# Association between peripheral manganese levels and attention-deficit/hyperactivity disorder: a preliminary meta-analysis

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**Abstract:** Evidence has suggested that dysregulation of the dopaminergic system may play a significant role in the pathogenesis of attention-deficit/hyperactivity disorder (ADHD) in children. Manganese, a neurotoxicant, has been reported to exert its neurotoxicity by affecting the dopaminergic system. However, the association between peripheral manganese levels and ADHD has not been comprehensively reviewed. This study aimed to investigate the association between peripheral manganese levels and ADHD in children. An electronic search was performed on databases including PubMed, ProQuest, ClinicalKey, Cochrane Library, [ClinicalTrials.gov](http://ClinicalTrials.gov), Embase, Web of Science, and ScienceDirect with last search on March 25th, 2018. As per the inclusion criteria, human observational studies investigating peripheral manganese levels in children with ADHD and controls were included. The meta-analysis was performed using a random-effects model, and possible confounders were examined by subgroup analysis. In total, four articles with 175 ADHD children and 999 controls were recruited. The manganese levels were significantly higher in ADHD children than in controls ( $p=0.033$ ), when studies investigating blood levels and those investigating hair levels were included. However, when only studies investigating blood levels were included, there was no significant difference between ADHD children and controls ( $p=0.076$ ). Our results support higher peripheral manganese levels in children diagnosed with ADHD than those in controls. Further primary studies are needed to clarify this association.

**Keywords:** meta-analysis, manganese, ADHD, pediatric psychiatry, review

## Introduction

Attention-deficit/hyperactivity disorder (ADHD) is defined as “a persistent pattern of inattention and/or hyperactivity-impulsivity that interferes with functioning or development”.<sup>1</sup> ADHD can be categorized into three subtypes: predominantly inattentive, predominantly hyperactive-impulsive, and combined.<sup>2</sup> The prevalence of ADHD is estimated to be 5.29% in children worldwide.<sup>3</sup> Inattention refers to wandering off task, lacking persistence, having difficulty sustaining focus, and being disorganized and is not due to defiance or lack of comprehension. Impulsivity refers to excessive motor activity when it is not appropriate, or excessive fidgeting, tapping, or talkativeness.<sup>1</sup> Further, it may cause impairments in social communication and functional limitations of effective communication, social participation, or academic achievement.<sup>1</sup>

Although the exact pathogenesis of ADHD remains unknown, it has been found that people with ADHD have smaller brain volumes of specific structures, such as the prefrontal cortex and basal ganglia,<sup>2</sup> or have higher exposure to environmental pollution, such as lead<sup>4</sup> or polybrominated diphenyl ethers.<sup>5</sup> One of the most widely

postulated pathogeneses of ADHD is the “dopamine hypothesis”,<sup>2</sup> attributing ADHD to a dysregulation of the dopaminergic system. A review article has shown that ADHD patients have decreased availability of dopamine receptor isoforms and increased dopamine transporter (DAT) binding compared with controls.<sup>6</sup> The most widely used medication for ADHD, methylphenidate with its derivatives, is postulated to have its effects by blocking DAT and promoting dopamine release.<sup>6</sup>

In previous studies, manganese (elemental symbol: Mn), an environmental heavy metal element, has been found to cause neurologic toxicity to humans.<sup>7,8</sup> It can be present in contaminated water, pesticides, batteries, and glass, among others, and then be ingested by humans.<sup>9–11</sup> High concentration of manganese has been found in the basal ganglia of manganese-intoxicated rats.<sup>12</sup> After exposure to manganese, decreased long-term dopamine efflux in rats<sup>13</sup> and attenuated dopaminergic neurotransmission in humans<sup>14</sup> have also been reported. In epidemiological studies, higher levels of manganese in the hair and blood samples from children with ADHD have been found.<sup>15,16</sup> Another study has shown decreased intelligence quotient (IQ) in children with increased hair concentration of manganese.<sup>17</sup> There have been no studies investigating the postmortem levels of in vivo manganese in ADHD patients found in the literature, by using the same search strategy as the current meta-analysis.

Therefore, as a neurotoxin affecting dopaminergic neurotransmission, manganese may play a role in the pathogenesis of ADHD in those exposed to the metal. Among the four datasets in the literature studying “blood” manganese levels, two<sup>16,18</sup> showed a significantly positive association between blood manganese level and ADHD, while the other two<sup>18,19</sup> revealed insignificant results. Three of the four datasets used case–control design, while the other dataset used cross-sectional design. Another case–control study showed a trend of positive association between “hair” manganese level and ADHD without statistical significance.<sup>20</sup> Difference in study design (case–control vs cross-sectional), source of manganese level (ie, blood vs hair), and population (described in the “Discussion” section) may contribute to the heterogeneity of the study results. Although there are two previous meta-analyses<sup>17,21</sup> mentioning the possible relationship between manganese exposure and ADHD in children, they did not directly calculate the association. Therefore, the aim of the current study is to summarize the literature for the possible association between peripheral manganese levels and ADHD in children via a thorough meta-analysis.

## Methods

We followed the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines<sup>22</sup> (Figure S1). This preliminary meta-analysis followed an a priori defined but unpublished protocol. The Institutional Review Board of the Tri-Service General Hospital (TSGHIRB: B-105-12) approved the study.

## Eligibility criteria

Inclusion criteria were the following: (a) observational studies, including a cohort or cross-sectional study design comparing peripheral levels of manganese in children with ADHD and controls; and (b) trials in humans. We excluded preclinical studies, review articles, meeting abstracts, and non-human studies; furthermore, to increase the specificity of the effect of manganese on ADHD, we excluded those studies that collected manganese sample sources further away from the diagnosis of ADHD (eg, collecting manganese when ADHD children were just delivered).

## Search strategy and study selection

Two well-trained authors (JH Shih and PT Tseng) performed a systematic literature search from inception until August 30th, 2017 and updated search at March 25th, 2018. The following keywords were used in our search on PubMed, ProQuest, ClinicalKey, Cochrane Library, ScienceDirect, and [ClinicalTrials.gov](http://ClinicalTrials.gov) platforms: “(Manganese OR Mn) AND (ADHD OR attention-deficit/hyperactivity disorder)”. A similar search was conducted in Embase and Web of Science databases. The reference lists of included articles and recent reviews were also hand-searched to identify additional articles.<sup>17,21,23–25</sup>

Two authors (JH Shih and PT Tseng) independently screened the titles and abstracts of all retrieved results for potential eligibility. Both authors reviewed the full text of potentially eligible papers, and a final list of included studies was achieved. Any inconsistencies were resolved through discussion with a third reviewer (MK Wu).

## Data extraction

The primary outcomes were the difference in manganese levels in children with ADHD compared to control groups (calculated as Hedges’ *g* statistic and corresponding 95% CIs and *p*-values). We did not choose difference in mean as the effect sizes (ESs) of our primary outcome due to presumed different units used in each study. The control groups were defined as those without ADHD. In addition,

due to potential confounding effect of specific medication (eg, methylphenidate) on manganese,<sup>18</sup> we did not include the data of manganese level in children with ADHD who had been clearly described as receiving such medication. If data were available, we prefer to choose control groups as healthy (asymptomatic) individuals. When data were not available from the included studies, we contacted the primary authors to request the original data. We contacted the authors via email on two occasions if required (a second email was sent a week later if no response was received following an initial email). If there were no relevant data in the paper regarding manganese levels, wherever possible, we attempted to use another compatible statistical parameter (eg,  $p$ -value and sample sizes) to estimate the ESs according to the protocol in the Comprehensive Meta-Analysis manuals and guidance on the Comprehensive Meta-Analysis website (<https://www.meta-analysis.com/downloads/Meta-analysis%20Converting%20among%20effect%20sizes.pdf>) to convert and pool the ESs into Hedges'  $g$ .

The variables of interest included manganese levels, mean age, gender distribution (in the form of % females), mean body mass index, cognitive performance (in the form of mean IQ), parental tobacco smoking and alcohol consumption, and the type of assay used for detection of manganese levels.

## Methodological quality appraisal

We used the modified Newcastle–Ottawa Scale (NOS) to evaluate the quality of the included studies. In brief, the modified NOS was based on a version previously used in a meta-analysis study published in *British Journal of Psychiatry* in 2013, and score ranges from 0 to 6 (Table S1).<sup>26</sup>

## Meta-analysis procedure

Given the anticipated heterogeneity in the basic population of study, we conducted the meta-analyses with a random-effects model rather than a fixed-effects one.<sup>27</sup> As aforementioned, the primary ES was estimated as Hedges'  $g$  with 95% CI to compare manganese levels in children with ADHD and controls. Subgroup analyses were conducted to determine whether results differed as we grouped them according to the sample sources (eg, blood, hair). We performed subgroup analyses whenever data from at least two independent data-sets were available. In addition, to overcome the limitation of the meta-analysis that we could not adjust the potential confounding effect by clinical variables, we collected the adjusted OR provided by the recruited articles to calculate the pooled adjusted OR.

## Sensitivity test, heterogeneity, publication bias, and meta-regression

We performed sensitivity test with “one-study removal” test to evaluate if the results of the meta-analysis were obtained from any outliers within the recruited studies or not.<sup>28</sup> Heterogeneity was assessed by the Cochran  $Q$  test and its corresponding  $p$ -value.<sup>29</sup> The  $I^2$  statistic was interpreted as the proportion of heterogeneity a study estimates that is due to heterogeneity.<sup>30</sup> We performed meta-regression analyses with unrestricted maximum likelihood random effects when data on each potential moderator were provided by at least five different studies. We assessed publication bias via the inspection of funnel plots<sup>31</sup> and with the Egger's regression test.<sup>32</sup> When evidence of publication bias was found, the Duval and Tweedie's trim-and-fill procedure, which is a validated model to estimate an ES, was employed.<sup>33</sup> The current meta-analysis was conducted using the Comprehensive Meta-Analysis software, version 3 (BioStat, Frederick, MD, USA). The threshold for statistical significance was set at a two-tailed  $p$ -value  $<0.05$ .

## Results

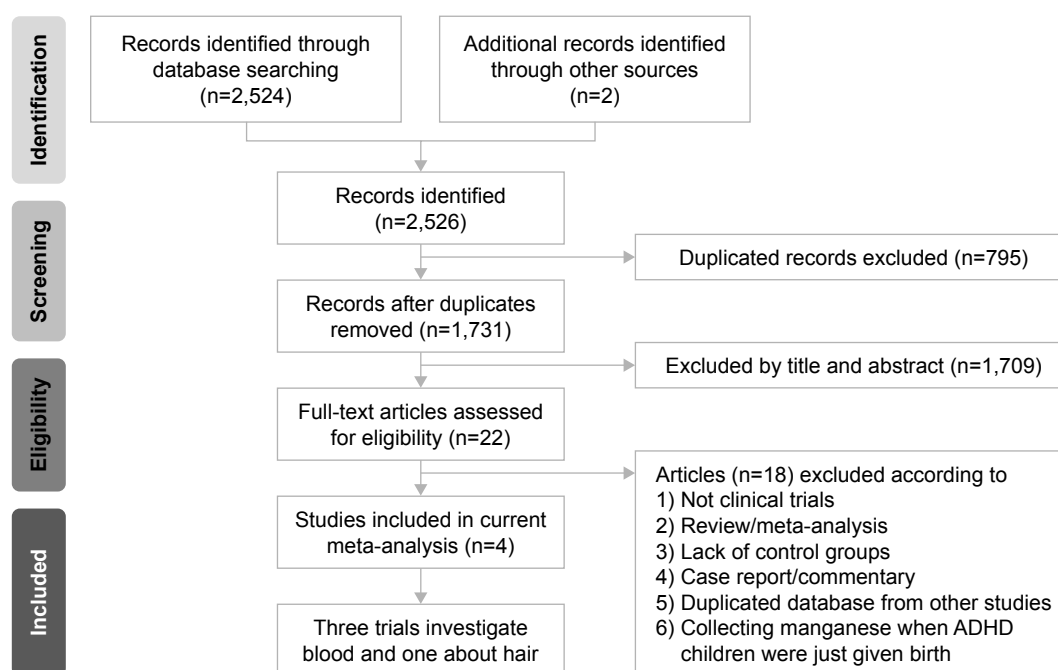
### Study selection

Figure 1 summarizes study selection process. In brief, a total of 22 studies entered the full-text review stage. Eighteen articles were excluded for various reasons (Figure 1). A list of excluded articles is presented in Table S2. In total, four articles met the inclusion criteria, the details of which are summarized in Table 1.<sup>16,18–20</sup>

Among the included studies, four provided comparison of the peripheral manganese levels in children with ADHD and controls (children with ADHD:  $n=175$ , mean age=8.8, mean female proportion=16.0%; controls without ADHD:  $n=999$ , mean age=9.1, mean female proportion=45.9%).<sup>16,18–20</sup> Two of them provided the data of adjusted OR for the association between manganese levels and ADHD.<sup>16,20</sup>

### Characteristics and methodological quality of included studies

Among the recruited studies, the diagnosis of ADHD was made according to widely acceptable criteria, including the *Diagnostic and Statistical Manual of Mental Disorders* (DSM)<sup>16,18,20</sup> or Diagnostic Interview Schedule for Children Version IV.<sup>19</sup> In addition, the sample sources ranged from peripheral blood<sup>16,18,19</sup> to hair sample.<sup>20</sup> The assays used to detect manganese levels included inductively coupled plasma mass spectrometry and graphite furnace atomic absorption spectrometry (Table S1). Regarding the methodological



**Figure 1** The flowchart of meta-analysis.

**Abbreviation:** ADHD, attention-deficit hyperactivity disorder.

quality of included studies, the average NOS score was 15.5 with a SD of 1.0 (Table S1).

## Meta-analysis of overall differences in manganese levels in children with ADHD and controls without ADHD

### Overall

Four articles provided five datasets in this part of meta-analysis.<sup>16,18–20</sup> The meta-analysis suggested that overall manganese levels, either in blood or in hair, in children diagnosed with ADHD were significantly higher than those in controls ( $k=5$ , Hedges'  $g=0.305$ , 95% CI=0.025–0.585,  $p=0.033$ ) (Figure 2A) without significant evidence of heterogeneity ( $Q$  value=8.419,  $df=4$ ,  $I^2=52.486\%$ ,  $p=0.077$ ,  $\tau=0.229$ ), but significant publication bias via inspection of funnel plot and Egger's regression was found ( $t=10.525$ ,  $df=3$ ,  $p=0.002$ ). The significant results of the meta-analysis changed to insignificant after refilling two potential missing data to the left side of means according to Duval and Tweedie's trim-and-fill test (Hedges'  $g=0.145$ , 95% CI=−0.140 to 0.430).

### Sensitivity test

The significant results of the meta-analysis changed to insignificant after removing any one of the recruited studies, which might be due to smaller sample sizes after removal of one of

the recruited studies. However, the significant results of the meta-analysis changed to more predominantly significant after removing the data from Hong et al (Hedges'  $g=0.425$ , 95% CI=0.191–0.660,  $p<0.001$ ).<sup>19</sup>

### Meta-regression

The meta-regression could be performed only on association between ESs and mean age due to lack of data. To be specific, there was no significant association between ESs and mean age ( $p=0.656$ ).

## Meta-analysis of differences in manganese levels in children with ADHD and controls without ADHD in blood samples

### Blood manganese

Three articles provided four datasets in this part of meta-analysis.<sup>16,18,19</sup> The meta-analysis suggested that the blood manganese levels in children diagnosed with ADHD and controls without ADHD were not significantly different, but a trend of higher blood manganese levels in ADHD children compared to controls was observed ( $k=4$ , Hedges'  $g=0.329$ , 95% CI=−0.035 to 0.693,  $p=0.076$ ) (Figure 2B) with significant evidence of heterogeneity ( $Q$  value=8.394,  $df=3$ ,  $I^2=64.260\%$ ,  $p=0.039$ ,  $\tau=0.295$ ) and significant publication bias via inspection of funnel plot and Egger's regression ( $t=9.335$ ,  $df=2$ ,  $p=0.011$ ). However, the results

Table 1 Characteristics of the recruited studies

Study	Criteria of ADHD	Sample source	Comparison	Subject age	Mean age	Female (%)	Cognitive function		ADHD subtype		Parental history of ADHD	Country
							ADHD (IQ)	Control (IQ)	Inattentive	Hyperactive		
Shin et al <sup>20</sup>	DSM-IV	Hair	ADHD	40	9.4±3.0	15.0	101.8±14.9	112.4±10.4	N/A	N/A	Not mentioned	Republic of Korea
			Control	43	10.3±3.1	16.3						
Hong et al <sup>19</sup>	DISC-IV	Blood	ADHD	43	9.1±0.8	16.3	106.1±13.8	111.0±14.2	N/A	N/A	Not mentioned	Republic of Korea
			Control	847	9.1±0.7	48.5						
Yousef et al <sup>16</sup>	DSM-IV	Blood	ADHD	18	8.3±1.8	N/A	N/A	N/A	38.9%	33.3%	Not mentioned	United Arab Emirates
			Control	74								
Farias et al <sup>18</sup>	DSM-IV	Blood	ADHD-combined	50	8.0±1.2	14.0	102.0±14.0	101.0±17.0	33.0%	0.0%	Not mentioned	Brazil
			ADHD-inattentive	24	9.0±1.4	21.0	93.0±10.0					
			ADHD-combined and Mx	21	9.0±1.8	19.0	98.0±14.0					
			ADHD-inattentive and Mx	11	10.0±1.7	0.0	97.0±10.0					
			Control	35	9.0±1.2	20.0						

**Abbreviations:** ADHD, attention-deficit/hyperactivity disorder; DISC-IV, Diagnostic Interview Schedule for Children Version IV; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; IQ, intelligence quotient; Mx, medication; N/A, not available.

of the meta-analysis did not change after refilling according to Duval and Tweedie's trim-and-fill test.

### Sensitivity test

The insignificant results of the meta-analysis changed to significant after removing the data from Hong et al (Hedges'  $g=0.491$ , 95% CI=0.210–0.771,  $p=0.001$ ).

### Meta-regression

All the meta-regression procedures were not performed because there were less than five datasets.

## Meta-analysis of differences in manganese levels in children with ADHD and controls without ADHD in hair samples

### Hair manganese

Only one article provided one dataset in this part of meta-analysis.<sup>20</sup> Therefore, the meta-analysis was not done due to lack of enough datasets. However, in the research results according to the description in the article by Shin et al,<sup>20</sup> the authors indicated that the proportion of abnormal manganese levels, either higher or lower, in children with ADHD was significantly higher than those in controls ( $p=0.039$ ).

## Meta-analysis of overall adjusted OR for the association between manganese levels and diagnosis of ADHD

Two articles provided two datasets in this part of meta-analysis.<sup>16,20</sup> The meta-analysis of the adjusted ORs indicated significant association between manganese levels and the diagnosis of ADHD ( $k=2$ , adjusted OR=1.849, 95% CI=1.149–2.976,  $p=0.011$ ) (Figure 2C) without significant evidence of heterogeneity ( $Q$  value=0.150,  $df=1$ ,  $P<0.001\%$ ,  $p=0.698$ ,  $\tau=0.001$ ).

## Discussion

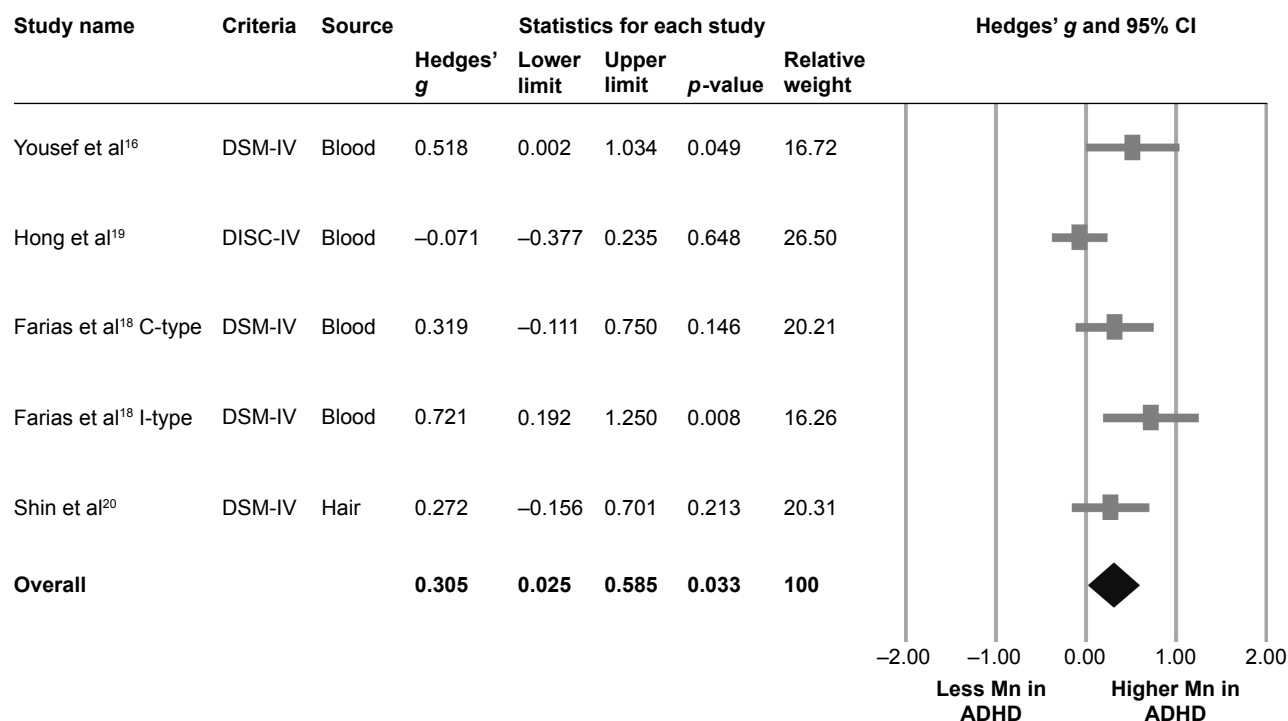
To the best of our knowledge, this is the first meta-analysis to investigate the relationship between peripheral manganese levels and ADHD in children. The results of the current meta-analysis showed significantly higher overall (ie, in blood or hair) peripheral manganese levels in children diagnosed with ADHD than those in controls, with no significant heterogeneity. Among the four included studies with five datasets in total, our results are consistent with two datasets,<sup>16,18</sup> but not with the other three.<sup>18–20</sup> However, among the three studies mentioned,<sup>18–20</sup> two<sup>18,20</sup> also showed a trend of positive association ( $p=0.146$  and  $0.213$ , respectively), though



not significant, which may be due to small number of cases. Sensitivity test showed that the results became insignificant after removing any one of the included studies, which may also be due to small number of cases. Subgroup analysis showed a trend of higher “blood” manganese levels in ADHD

children than in controls, though not significant ( $p=0.076$ ). However, the results changed to significant after removing the data from Hong et al.<sup>19</sup> The change from insignificance to significance may have been due to the difference in the study designs. Among the four studies included in the current

## A



## B

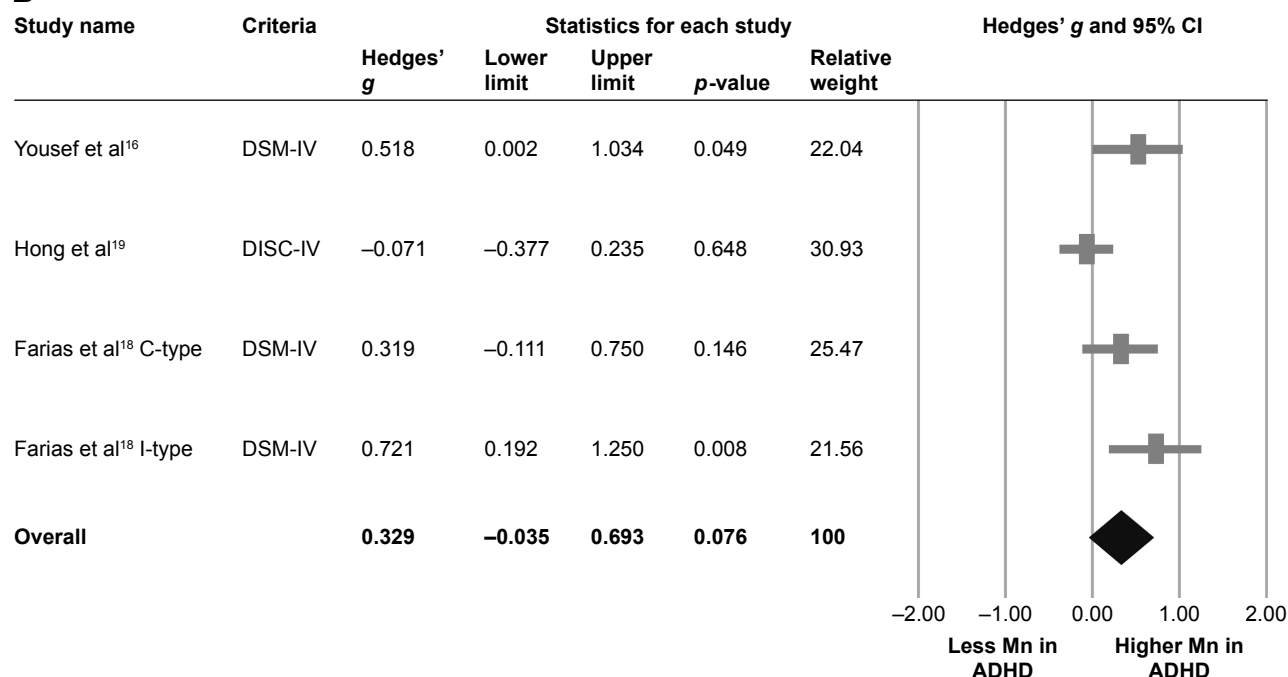
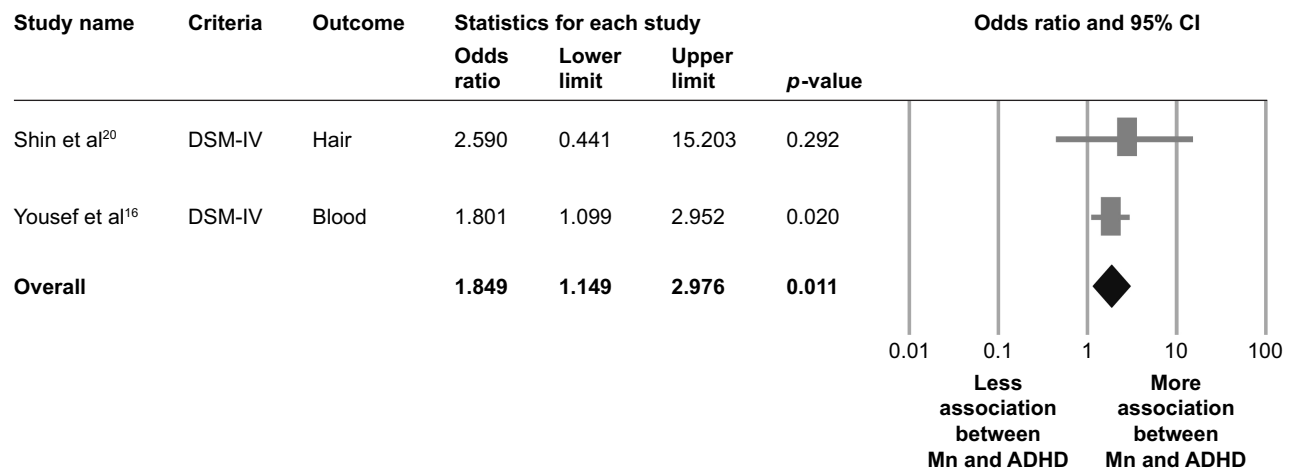


Figure 2 (Continued)

## C



**Figure 2** Meta-analysis of (A) overall manganese levels in ADHD children and controls, (B) blood manganese levels in ADHD children and controls, and (C) pooled adjusted OR for the association between manganese levels and ADHD.

**Notes:** (A) There were significantly higher overall manganese levels in ADHD children than those in controls (Hedges'  $g=0.305$ , 95% CI=0.025–0.585,  $p=0.033$ ). (B) There were no significantly different blood manganese levels in ADHD children and controls (Hedges'  $g=0.329$ , 95% CI=–0.035 to 0.693,  $p=0.076$ ). (C) There was a significant association between manganese levels and diagnosis of ADHD as shown by pooled adjusted OR (OR=1.849, 95% CI=1.149–2.976,  $p=0.011$ ).

**Abbreviations:** ADHD, attention-deficit/hyperactivity disorder; C-type, combined type; DISC-IV, Diagnostic Interview Schedule for Children Version IV; DSM-IV, *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*; I-type, inattention type.

meta-analysis, the study by Hong et al<sup>19</sup> used cross-sectional design, while the other three studies<sup>16,18,20</sup> used case–control design. Moreover, the study by Hong et al<sup>19</sup> used Diagnostic Interview Schedule for Children Version IV, while the other studies used DSM criteria.<sup>16,18,20</sup>

Although the meta-analysis was not done in the “hair” subgroup due to the availability of only one dataset, the result of this article showed that the hair manganese levels were significantly higher in ADHD children than in controls. To overcome the potential confounding effects, we performed a meta-analysis of overall adjusted ORs, which revealed a significantly positive association (adjusted OR=1.849,  $p=0.011$ ) between manganese levels and ADHD in children, with no significant heterogeneity.

A comparison of the two previous meta-analyses<sup>17,21</sup> mentioning the association between manganese and ADHD and the current study is summarized in Table 2. In the two studies, the one by Rodríguez-Barranco et al<sup>17</sup> calculated only the association between manganese exposure and IQ, and the other by Scassellati et al<sup>21</sup> did not perform a meta-analysis for manganese exposure.

Since “dopamine hypothesis”<sup>22</sup> is currently the most widely postulated etiologic scheme for ADHD, disrupted dopamine neurotransmission caused by manganese exposure found in several previous studies<sup>13,14</sup> has prompted us to perform this study. Our study results also support the positive association between peripheral manganese levels and ADHD. Although there have been no studies investigating the effect of removing excessive manganese from children with ADHD, the results of our current study suggest that this treatment approach may be promising.

Because manganese levels in children (biological monitoring) are affected by environmental manganese levels, such as in soil, water, and food, it would be difficult to thoroughly investigate. Therefore, we chose blood and hair levels as an indicator of internal dose.

The current study has several limitations. First, although our main results showed higher manganese levels in children diagnosed with ADHD than those in controls, significant publication bias and heterogeneity were found. Further, the results changed to insignificant after refilling two potential missing data by using Duval and Tweedie's trim-and-fill test

**Table 2** Comparison of previous and current meta-analyses

Study	Study designs included	Manganese exposure measure	Observed effect
Scassellati et al <sup>21</sup>	Case–control	N/A	N/A
Rodríguez-Barranco et al <sup>17</sup>	Case–control, cohort, cross-sectional	Blood, cord blood, hair, placenta, tooth, water	Full-scale IQ, performance IQ, verbal IQ
Current study	Case–control, cross-sectional	Blood, hair	ADHD

**Abbreviations:** ADHD, attention-deficit/hyperactivity disorder; IQ, intelligence quotient; N/A, not available.

(Hedges'  $g=0.145$ , 95% CI=-0.140 to 0.430). The publication bias may be overcome when more primary researches are published. The heterogeneity might be due to the difference in study designs. Among the four included studies, the study by Hong et al<sup>19</sup> used cross-sectional design, while the other three studies<sup>16,18,20</sup> used matched case-control design. Another source of heterogeneity may have come from the fact that Farias et al<sup>18</sup> had two datasets of different diagnostic criteria, which were ADHD-combined type (C-type) and ADHD-inattentive type (I-type), respectively. Further, the four included studies investigated different populations: in terms of countries, for example, their subjects were from United Arab Emirates,<sup>16</sup> Brazil,<sup>18</sup> and Republic of Korea.<sup>19,20</sup> Second, due to the small number of eligible studies, we had to combine the results from blood and hair samples, which could be a source of bias. Third, there were only two datasets provided with adjusted ORs, so the potential confounding factors may not have been addressed well enough. Fourth, due to difficulties in experimental designing and ethical concerns, few studies<sup>34,35</sup> provided central nervous system (CNS) concentrations of manganese, and none of them studied the relations between CNS manganese concentrations and ADHD. Although CNS concentrations are likely to provide more accurate information for manganese neurotoxicity, only blood or hair concentrations were available for this meta-analysis. Fifth, although eligible articles studied manganese levels in blood and hair, there have been no well-validated biomarkers for manganese exposure in children.<sup>36</sup> Moreover, hair samples are susceptible to exogenous contamination.<sup>36</sup> Sixth, although we provided evidences of higher manganese levels in ADHD children than in controls, we could not provide further detailed information about "to what extent" the manganese levels were higher than controls. Because the peripheral samples of manganese and method of detection in each included study were different from the others, we could not directly count and pool the difference in mean manganese levels between ADHD children and controls in each study. Seventh, although two of the studies provided information about ADHD subtype,<sup>16,18</sup> there was still limited information about the correlation between peripheral manganese levels and ADHD subtype. Therefore, we could not derive further evidence about the correlation between peripheral manganese levels and ADHD subtype. Finally, because there had been limited articles included in the current meta-analysis, the subjects distribution was as restricted as only from three countries, including United Arab Emirates,<sup>16</sup> Brazil,<sup>18</sup> and Republic of Korea.<sup>19,20</sup> Therefore,

when clinicians apply our results into clinical practice, they should pay more attention about the applicability due to limited number of countries included and regional bias.

## Conclusion

The results of this meta-analysis support higher peripheral manganese levels in children diagnosed with ADHD than those in controls. Further studies are warranted to replicate these findings, especially in other countries and in different ADHD subtypes, and to quantify the degree of the association for it to be useful in clinical settings.

## Disclosure

The authors report no conflicts of interest in this work.

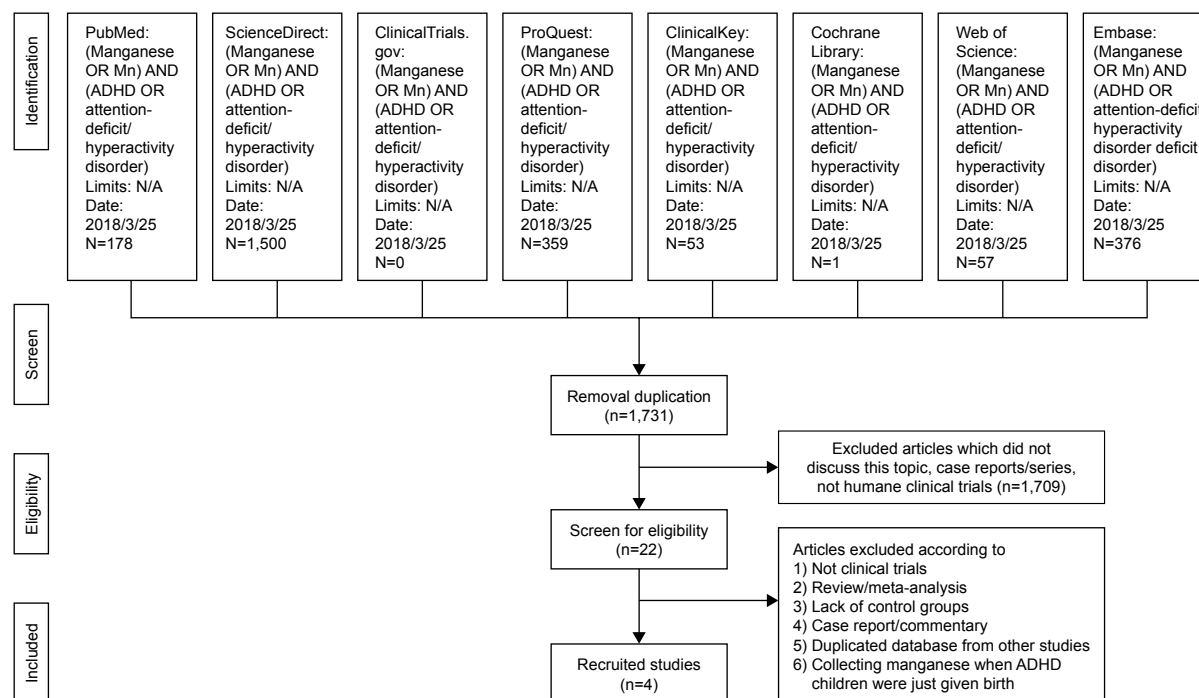
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## Supplementary materials



**Figure S1** Whole flowchart of the meta-analysis.

**Abbreviations:** ADHD, attention-deficit/hyperactivity disorder; N/A, not available.

**Table S1** Results of Newcastle–Ottawa Scale scores of recruited studies

Study	Criteria	Source	Sample method	Newcastle–Ottawa Scale					Country
				Selection	Performance	Detection	Information	Total	
Shin et al <sup>1</sup>	DSM-IV	Hair	Inductively coupled plasma mass spectrometry	1	5	4	5	15	Republic of Korea
Hong et al <sup>2</sup>	DISC-IV	Blood	Graphite furnace atomic absorption spectrometry	3	5	5	4	17	Republic of Korea
Yousef et al <sup>3</sup>	DSM-IV	Blood	N/A	2	5	4	4	15	United Arab Emirates
Farias et al <sup>4</sup>	DSM-IV	Blood	Graphite furnace atomic absorption spectrometry	2	5	4	4	15	Brazil

**Abbreviations:** DSM-IV, *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*; DISC-IV, *Diagnostic Interview Schedule for Children Version IV*; N/A, not available.

**Table S2** Excluded studies and reasons**Not related to manganese and ADHD (n=4)**Konstantopoulos et al<sup>5</sup>Hu et al<sup>6</sup>Konrad et al<sup>7</sup>Han et al<sup>8</sup>**Meta-analysis (n=2)**Rodríguez-Barranco et al<sup>9</sup>Scassellati et al<sup>10</sup>**Review articles (n=3)**Zeng et al<sup>11</sup>Grandjean and Landrigan<sup>12</sup>Lucchini et al<sup>13</sup>**Not definite diagnosis of ADHD (n=5)**Hong et al<sup>14</sup>Bouchard et al<sup>15</sup>Lucchini et al<sup>16</sup>Chan et al<sup>17</sup>Ericson et al<sup>18</sup>**Compare treatment effect of micronutrient but not related to manganese and ADHD (n=1)**Rucklidge et al<sup>19</sup>**Use the same data sample sources of Hong et al<sup>2</sup> (n=1)**Bhang et al<sup>20</sup>**Lack of control (n=1)**Liu et al<sup>21</sup>**Collecting manganese when ADHD children were just given birth (n=1)**Ode A et al<sup>22</sup>**Abbreviation:** ADHD, attention-deficit/hyperactivity disorder.**References**

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