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Review article

Association between hair lead levels and autism spectrum disorder in children: A systematic review and meta-analysis

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ABSTRACT

A number of studies measured lead levels in hair from children with autism spectrum disorder (ASD) to detect the relationship between cumulated lead exposure and the development of ASD, but results are inconsistent. We aimed to conduct a systematic review and meta-analysis using the published studies to explore the actual association of hair lead levels with ASD in children. We searched PubMed, Embase, PsycINFO, and Cochrane Library databases (up to December 11, 2018). The random-effects model was applied to summarize effect sizes. Subgroup and meta-regression analyses were performed simultaneously. Twenty eligible studies involving 1787 participants (941 autistic children and 846 healthy subjects) were included. Our results of primary analysis showed that there were no statistically significant differences in the levels of hair lead between children with ASD and healthy individuals (Hedges's $g = 0.251$; 95% confidence interval: -0.121, 0.623; $P = 0.187$). We identified 2 sources of between-study heterogeneity: analytical technology and the sample size of patients. Additionally, no publication bias was observed in this meta-analysis. In conclusion, this study does not support the association of hair lead levels with ASD in children, and the involvement of cumulated lead exposure in the occurrence of ASD.

1. Introduction

To date, the exact etiology and pathogenesis of autism spectrum disorder (ASD) remain unclear. However, there is a developing common view that ASD arises from the interaction between genetic susceptibilities and environmental influences (Homberg et al., 2016; Schendel et al., 2014). Among the environmental factors, a great deal of attention has recently been paid to toxic metals (Gorini et al., 2014); thereinto, what especially deserves concern is lead, because lead can bring about particularly detrimental impacts on children's neurodevelopment (Modabbernia et al., 2017; Ye et al., 2017).

Generally, levels of lead exposure in children are assessed through lead content determination in biological specimens (Gorini et al., 2014; Modabbernia et al., 2017; Sealey et al., 2016). As compared to other matrices, hair has more advantages: *a*) the sampling is non-invasive and can be more easily achieved (particularly for pediatric patients) (Appenzeller and Tsatsakis, 2012; Domingues et al., 2016; Skalny et al., 2017a); *b*) this tissue is very stable during storage at room temperature (Appenzeller and Tsatsakis, 2012); *c*) most of all, hair is virtually a external repository of all the elements that enter the body, providing abundant information about the state of long-term exposure of a given substance (Domingues et al., 2016; Dormandy, 1986; Skalny et al., 2017a). Therefore, hair is proposed as a useful analytical material to evaluate lead exposure in children (Bencko, 1995; Domingues et al., 2016; Skalny et al., 2017a). Accordingly, a number of studies applied hair lead levels as biomarker to detect the latent relationship between the status of lead exposure and the genesis of ASD (Modabbernia et al., 2017; Sealey et al., 2016).

Nevertheless, the studies measuring lead levels in hair from autistic children and healthy control (HC) subjects reported inconsistent results. For instance, some studies indicated that children with ASD had significantly elevated levels of hair lead compared with HC individuals (Al-Farsi et al., 2013; Mohamed Fel et al., 2015), whereas others found no correlation between lead contents in hair and childhood autism (Domingues et al., 2016; Skalny et al., 2017a, 2017b). What is more, several articles reported decreased hair lead values in autistic children (Kern et al., 2007; Yasuda et al., 2005). In this context, it is difficult to determine the actual association of hair lead levels with ASD in children.

Thus, in the current study we conducted a systematic review and meta-analysis on this subject, in order to adequately estimate the exact levels of hair lead in children with ASD, and further offer theoretical

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support for clarifying the potential relationship between cumulated lead exposure and the development of ASD.

2. Methods

This systematic review and meta-analysis complied with the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher et al., 2009).

2.1. Search strategy and study selection

Two independent investigators (B.-Q.G. and H.-B.L.) searched for potentially eligible studies using the following databases: PubMed, EMBASE, PsycINFO, and Cochrane Library from inception to December 11, 2018, without language restriction. The databases were searched in [All Fields] (PubMed), [All fields] (EMBASE), or [All Text] (PsycINFO; Cochrane Library) applying the combination of key terms: (lead OR metal* OR element* OR mineral*) AND (autism OR autistic OR pervasive developmental disorder* OR Asperger*). After obtaining all related records, we verified the results and removed duplicates. Then, the titles and abstracts were screened. Inclusion criteria were original human studies of any design that reported necessary data on hair lead levels in children with ASD and HC individuals. Exclusion criteria were *a*) studies with adult subjects, *b*) studies in which ASD was comorbid with other health conditions, *c*) studies with extremely abnormal values, and *d*) studies that overlapped with other one(s). In the last case, only those reporting the largest sample or published as full-text articles in academic journals were included. We also checked the reference sections of retrieved articles to find extra publications. Disagreements were resolved by discussion among all researchers.

2.2. Data extraction

The following information was independently extracted by two trained researchers (B.-Q.G. and Y.-Y.L.): author, year of publication, geographic location, sample sizes, age, gender (percentage of male participants;% male), matching factors, diagnostic method, biomaterial (sampling position), analytical technology, mean lead levels and standard deviations (SDs), unit of measure, and *P*-values (if means and SDs were unavailable). When research did not directly provide the desirable data, the statistics that we wanted were derived from the reported data using methodologies outlined in the Cochrane Handbook (Higgins and Green, 2011). Where data were not available or there was uncertainty with respect to the interpretation of research contents, the study authors were contacted. To maximize the number of studies, when data were only expressed graphically, the numerical values from the graphs were acquired using the method proposed by Sistrom and Mergo (2000). Besides, one included study (Yasuda et al., 2005) provided the means and SDs of log-transformed data, while others reported the results on raw scale. In order to allow the present meta-analysis to be carried out on a common scale, we used the method described by Higgins et al. (2008) to convert data from a logarithmic scale to a original equivalent.

2.3. Quality assessment

The methodological quality of included studies was assessed based on the Newcastle–Ottawa Scale (NOS) (Wells et al., 2009). In the light of the standards proposed in previous publications (Quansah et al., 2015; Viale et al., 2015), we judged a study scored seven or more stars to be of high quality, otherwise it was deemed to be of low quality.

2.4. Statistical analysis

All statistical analyses were performed by use of the Comprehensive Meta-Analysis software (version 3.0; Biostat Inc). The majority of effect sizes were generated by sample sizes, mean lead levels, and SDs, the remainder were yielded by sample sizes and *P*-values (i.e., when mean lead levels and SDs were unavailable) (Qin et al., 2016, 2017). When *P*-value was presented as inequality instead of exact value, the *P*-value was rounded down to the closest numerical value to allow compatibility with the meta-analysis programs package (Qin et al., 2016, 2017). The effect sizes were calculated as standardized mean differences in hair lead levels between ASD patients and HC subjects, and converted to Hedges's *g*, which offers not only more conservative estimates of the effect sizes but also a correction factor that is helpful to adjust for the influences of small sample sizes (Munkholm et al., 2016; Qin et al., 2017). The 95% confidence interval (CI) was simultaneously computed to examine the statistical discrepancy of the pooled effect sizes. All analyses were done using the random-effects model as we assume that the true effect sizes vary from study to study. Under this model, the study weight is assigned to each study in accordance with the inverse of total variance, which is equivalent to within-study variance plus between-study variance (τ^2; the actual amount of variance in true effect sizes).

We performed subgroup analyses based on pre-specified factors: the sampling position of hair, the analytical technology, the detection limit of analytical instrument, matching, continent or region, country (all other countries versus the United States). Subgroup analyses were carried out when at least 10 studies were available and at least 2 studies were in each comparator subgroup, by usage of common among-study variance components across subgroups (Mata et al., 2015; Munkholm et al., 2016). Subsequently, meta-regression analyses were implemented with mean age (patients, HC, and all participants), gender (% male; patients, HC, and all participants), sample size (patients, HC, and all participants), total NOS score, and year of publication to further explore the potential sources of heterogeneity. Meta-regressions were conducted using the Method of Moments and *Z*-distribution under random-effects model only if the number of included studies corresponding to a specific covariate was 10 or more (Munkholm et al., 2016). The difference in the summary effect sizes between subgroups was assessed by the Q-statistic and the proportion of between-study variance explained by subgroup membership or covariates was computed as R^2 analog (Munkholm et al., 2016). Sensitivity analysis was conducted by serially removing one study at a time to verify whether a particular study affected the results of the meta-analysis. Finally, a cumulative meta-analysis was undertaken to appraise the effects of accumulated studies over time on the pooled effect sizes.

Heterogeneity between studies was quantified using the I^2 statistic, and its significance was evaluated according to the accompanying Cochran's Q test (Higgins and Green, 2011; Higgins et al., 2003). I^2 values of 25%, 50%, and 75% indicate low, moderate, and high levels of heterogeneity respectively, and $P < 0.1$ was deemed statistically significant because of the low power of the Q test to detect heterogeneity (Higgins and Green, 2011; Higgins et al., 2003). The τ^2 values coming from the random-effects model were also applied to quantify heterogeneity (Mata et al., 2015; Peeters et al., 2015). A τ^2 close to 0 would be considerable homogeneity, and greater than 1 suggests the presence of substantial heterogeneity (Peeters et al., 2015).

For all analyses, a *P*-value of less than 0.05 (two sided) was considered to be statistically significant in this study unless otherwise noted.

2.5. Publication bias

Publication bias was first evaluated by visual inspection of funnel plots, followed by using the Egger's test (Egger et al., 1997). In addition, the classic Fail-safe N test (Soeken and Sripusanapan, 2003) was employed to compute the number of possible omitted studies (with mean effect of zero) that would be required to be appended to the meta-analysis to produce a statistically nonsignificant overall effect ($P > 0.05$).

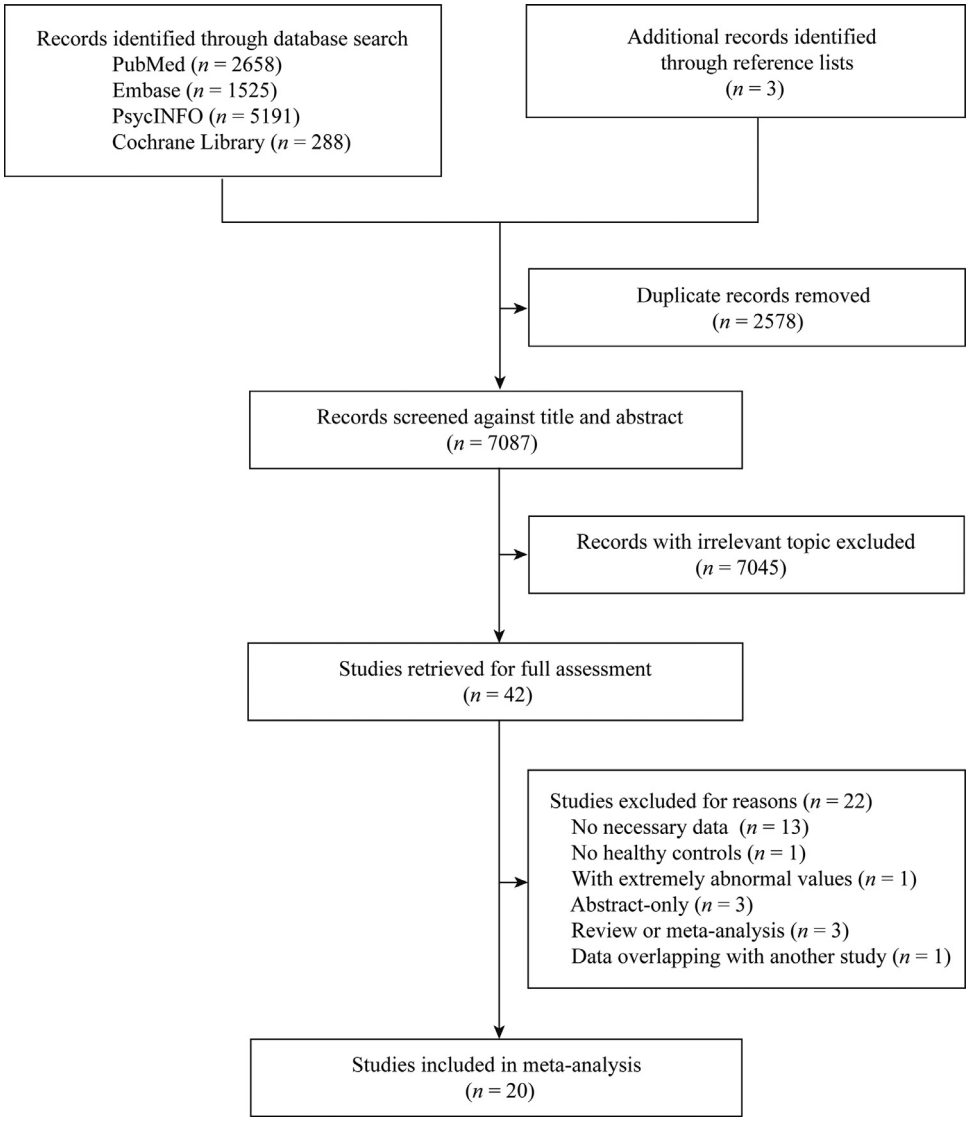

Fig. 1. PRISMA flow diagram of the literature search and study inclusion. Abbreviation: *n*, number of studies.

3. Results

3.1. Identification of studies

The flow chart of study selection is shown in Fig. 1. The initial database searching supplemented by hand search of the reference lists produced a total of 7087 records after removing duplicates. Subsequently, we examined the titles and abstracts, 7045 records were excluded and 42 appropriate citations were identified for full assessment. After scrutinizing the 42 citations, 22 studies were excluded for *a*) no necessary data (Adams et al., 2013; Amin, 2012; Blaurock-Busch et al., 2012; Filon et al., 2017; Gentile et al., 1983; Hodgson et al., 2014; Kim et al., 2016; Lubkowska and Sobiera, 2009; Majewska et al., 2010; Semprun-Hernandez et al., 2012; Skalny et al., 2017c; Yasuda and Tsutsui, 2013; Yasuda et al., 2013), *b*) no HC subjects (Geier et al., 2012), *c*) with extremely abnormal values (Yassa, 2014), *d*) abstract-only (Albizzati et al., 2012; Fido et al., 2002; Tabatadze et al., 2015), *e*) review or meta-analysis (Rimland and Larson, 1983; Rossignol et al., 2014; Saghazadeh and Rezaei, 2017), and *f*) data overlapping with another study (Marlowe and Errera, 1985). The study by Yassa (2014) was excluded for the following reasons: *a*) with extraordinarily large effect size (Hedges's g = 6.898, 95% CI: 5.810, 7.985; Fig. S1), *b*) there has been a significant change in the publication bias before (asymmetric funnel plot; Fig. S2 A) and after (symmetric funnel plot; Fig. S2 B) removing it, which was confirmed by the result of the Egger's test (the *P*-value changed from around 0.05 to 0.415), *c*) the τ^2 value (another index of heterogeneity) declined clearly (from 1.037 to 0.657) after omitting this study. Therefore, 20 full-text articles met all inclusion criteria to be eventually included in the present meta-analysis.

3.2. Study characteristics

The details of 20 eligible studies are displayed in Table 1. Seven studies were conducted in North America (Adams et al., 2006; Kern et al., 2007; Marlowe et al., 1984; Massaro et al., 1983; Obrenovich et al., 2011; Shearer et al., 1982; Wecker et al., 1985), 6 studies in the Middle East (Al-Ayadhi, 2005; Al-Farsi et al., 2013; Blaurock-Busch et al., 2011; Elsheshtawy et al., 2011; Fido and Al-Saad, 2005; Mohamed Fel et al., 2015), 4 studies in Europe (De Palma et al., 2012; Domingues et al., 2016; Skalny et al., 2017a, 2017b), and 3 studies in Asia (Jung et al., 2008; Lakshmi Priya and Geetha, 2011; Yasuda et al., 2005). Year of publication ranged from 1982 (Shearer et al., 1982) to 2017 (Skalny et al., 2017a, 2017b), with all but 4 studies (Marlowe et al., 1984; Massaro et al., 1983; Shearer et al., 1982; Wecker et al., 1985) published after the year 2000. The sample sizes varied broadly, ranging from 12 (Shearer et al., 1982; Wecker et al., 1985) to 200

Table 1
Characteristics of the 20 studies included in the systematic review and meta-analysis.

Study	Country	Sample sizes (ASD/HC)	Age (years) (ASD/HC)	Gender (% male) (ASD/HC)	Matching	Diagnostic method	Biomaterial (sampling position)	Analytical technology	Lead levels (Mean ± SD, μg/g) (ASD/HC)
Adams et al., 2006 [a]	United States	51/40	Mean ± SD 7.1 ± 3.0/7.5 ± 3.0 Range 3–15/3–15	76.5/75.0	Age, gender	By psychiatrist or developmental pediatrician of ASD	Hair (the nape of the neck)	ICP-MS	0.62 ± 0.63/0.81 ± 0.73
Al-Ayadhi 2005 [b,c,d]	Saudi Arabia	67/80	Mean ± SD 9.0 ± 2.40/7.2 ± 6.26	94.0/NA	Age, gender	By a qualified psychologist, psychiatrist or neurologist, according to Rimland's diagnostic criteria E-2	Hair (the nape of the neck)	AAS	3.486 ± 1.763/0.960 ± 1.431
Al-Farsi et al., 2013	Oman	27/27	Mean 5.3/5.5	81.5/74.1	Age, gender, ethnicity	*DSM-IV-TR*/ CARS	Hair (the posterior vertex and posterior temporal regions)	ICP-MS	$P = 0.03$
Blaurock-Busch et al., 2011	Saudi Arabia	25/25	Mean ± SD 5.29 ± 1.9/6.25 ± 2.31	88.0/76.0	Age, gender	*DSM-IV*/CARS/ABC	Hair (the occipital region)	ICP-MS	0.01 ± 0.02/0.01 ± 0.01
De Palma et al. 2012	Italy	44/61	Mean ± SD 9.0 ± 4.0/8.4 ± 3.1 Range 2–17/2–15	84.09/ 40.98	Age, geographical area	*DSM-IV*/CARS	Hair (the nape of the neck)	ICP-MS	$P = 0.052$
Domingues et al., 2016 [b]	Italy	29/36	Mean ± SD 7.3 ± 2.40/8.4 ± 4.22	89.66/ 72.22	Age	*DSM-IV-TR*/ ADOS	Hair (the nape of the neck)	ICP-OES	1.09 ± 0.915/1.05 ± 0.84
Elsheshtawy et al., 2011 [e]	Egypt	32/32	Mean 4.1/4.0 Range 3.8–4.6/3.8–4.6	75.0/75.0	Age, gender	*DSM-IV*/KID-SCID/CARS	Hair (the nape or occipital region)	AAS (flame atomiser)	9.75 ± 1.8/6.8 ± 0.86
Fido and Al-Saad, 2005	Kuwait	40/40	Mean ± SD 4.2 ± 2.2/4.3 ± 2.6 Range 4–7/4–8	100.0/ 100.0	Age, gender	*DSM-IV-R*	Hair (the posterior vertex and posterior temporal regions)	ICP-MS	$P < 0.001$
Jung et al., 2008 [b]	Korea	28/22	Mean ± SD 7.63 ± 7.46/7.77 ± 6.10	67.9/68.2	NA	NA	Hair (NA)	ICP-MS	0.61 ± 1.01/0.19 ± 0.42
Kern et al., 2007	United States	45/45	Mean ± SD 3.0 ± 1.4/3.0 ± 1.4	77.8/77.8	Age, gender, race/ethnicity, vaccination	*DSM-IV*/CARS/M-CHAT	Hair (NA)	ICP-MS	0.19 ± 0.65/0.20 ± 0.37
Lakshmi Priya and Geetha, 2011 [c]	India	45/50	Range 4–12/4–12	80.0/80.0	Age, gender	CHAT/CARS	Hair (the nape of the neck)	AAS (graphite furnace atomizer)	7.75 ± 7.43/1.56 ± 0.18
Marlowe et al., 1984	United States	28/18	Mean ± SD 8.85 ± 4.06/10.83 ± 4.55	85.7/55.6	Race, social class	NSAC	Hair (the nape of the neck)	AAS/HA/ICPT	6.28 ± 2.12/6.66 ± 2.49

(*continued on next page*)

Table 1 (*continued*)

Study	Country	Sample sizes (ASD/HC)	Age (years) (ASD/HC)	Gender (% male) (ASD/HC)	Matching	Diagnostic method	Biomaterial (sampling position)	Analytical technology	Lead levels (Mean ± SD, μg/g) (ASD/HC)
Massaro et al., 1983	United States	23/16	Range 6–12/6–12	NA/NA	Age, geographical area	CARS	Hair (the occipital region)	AAS (graphite furnace atomizer)	3.13 ± 2.35/3.07 ± 2.12
Mohamed Fel et al. 2015	Egypt	100/100	Mean ± SD 6.24 ± 2.43/6.80 ± 3.04	84.0/74.0	Age, gender	*DSM-IV-TR*/ CARS	Hair (the occipital region)	AAS (graphite furnace atomizer)	3.31 ± 3.92/2.06 ± 2.45
Obrenovich et al., 2011 [a,f]	United States	26/39	<6/<6	NA/NA	Age	By pediatric neurologist/*DSM-IV*	Hair (NA)	ICP-MS	0.591 ± 0.611/0.612 ± 0.502
Shearer et al., 1982 [f]	United States	12/12	Mean 8.0/8.4	NA/NA	Social class	NSAC	Hair (NA)	AAS (flame atomiser)	6.332 ± 6.748/7.698 ± 6.337
Skalny et al., 2017a	Russia	74/74	Mean 5.12/5.11	NA/NA	Age, gender	NA	Hair (the occipital region)	ICP-MS	$P = 0.179$
Skalny et al., 2017b	Russia	33/33	Mean 5.0/5.0	100.0/100.0	Age, gender	*DSM-IV-TR*/ *ICD-10*	Hair (the occipital region)	ICP-MS	$P = 0.256$
Wecker et al., 1985 [b,c]	United States	12/40	Range 3–8/3–8 Mean ± SD 5.67 ± 2.39/4.45 ± 2.11	100.0/55.0	Age	*DSM-III*	Hair (the nape of the neck)	AAS	12.6 ± 5.54/10.79 ± 5.33
Yasuda et al., 2005	Japan	200/56	Range 4–9/4–9	100.0/100.0	Age, gender	By mark-seated questionnaire	Hair (NA)	ICP-MS	0.385 ± 0.234/0.887 ± 0.497

Abbreviations: AAS, atomic absorption spectrometry; ABC, Autism Behavior Checklist; ADOS, Autism Diagnostic Observation Schedule; ASD, autism spectrum disorder; CARS, Childhood Autism Rating Scale; CHAT, Checklist for Autism in Toddlers; *DSM-III, Diagnostic and Statistical Manual of Mental Disorders, third edition; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, fourth edition; DSM-IV-R, Diagnostic and Statistical Manual of Mental Disorders, fourth edition, Revised; DSM-IV-TR, Diagnostic and Statistical Manual of Mental Disorders, fourth edition, Text Revision; DSM-5, Diagnostic and Statistical Manual of Mental Disorders, fifth edition;* HA, hydride analysis; HC, healthy controls; *ICD-10, International Classification of Diseases, Revision 10;* ICP-MS, inductively coupled plasma-mass spectrometry; ICP-OES, inductively coupled plasma-optical emission spectrometry; ICPT, induction coupled plasma torch; KID-SCID, Structured Clinical Interview for DSM-IV Childhood Diagnoses; M-CHAT, Modified Checklist for Autism in Toddlers; NA, not available; NSAC, National Society for Autistic Children; SD, the standard deviation; SE, the standard error.

[a] Additional information was provided by the study authors.

[b] Data were reported as mean ± SE instead of the mean ± SD, then the SD was calculated based on the following computational method: the SD equals to the SE multiplied by the square root of the sample size.

[c] Autistic children or HC individuals were divided into several subgroups, data were the means ± SDs of all the subjects in corresponding groups after merging the results of the subgroups.

[d] Children with ASD included 65 cases with autistic disorder and 2 cases with Asperger's syndrome.

[e] The units of hair lead levels in this study were microgram per milligram.

[f] Data were calculated according to the numerical values reading from the corresponding graph in this article.

(Yasuda et al., 2005) ASD patients and from 12 (Shearer et al., 1982) to 100 (Mohamed Fel et al., 2015) HC individuals.

3.3. Quality assessment

The total NOS score across included studies ranged from 5 to 9, with a mean score of 7.2 (Table S1). Overall, we considered that the study qualities of articles incorporated in our meta-analysis are of good quality to make an appropriate evaluation of the association between hair lead levels and children with ASD.

3.4. Association of hair lead levels with ASD in children

We firstly conducted a random-effects meta-analysis on the 20 included studies incorporating a total of 1787 participants (941 autistic children and 846 healthy subjects). The overall pooled result showed that there were no statistically significant differences in the levels of hair lead between autistic children and HC individuals (Hedges's $g = 0.251$; 95% CI: −0.121, 0.623; $P = 0.187$), with the effect sizes in individual studies ranging from −1.615 (95% CI: −1.942, −1.288; $P < 0.001$) (Yasuda et al., 2005) to 2.066 (95% CI: 1.464, 2.668; $P < 0.001$) (Elsheshtawy et al., 2011). Nevertheless, a marked between-study heterogeneity was observed ($I^2 = 92.759\%$; $\tau^2 = 0.657$; $P < 0.001$) (Fig. 2).

3.5. Subgroup analyses

3.5.1. Sampling position of hair

When stratified by sampling position of hair, the hair lead levels in children with ASD were higher than those in HC subjects for the studies collecting hair sample from the nape of the neck (Hedges's $g = 0.461$; 95% CI: 0.022, 0.901; $P = 0.039$), but the same kind of comparisons showed no differences for the studies sampling hair from the occipital region (Hedges's $g = -0.014$; 95% CI: −0.530, 0.503; $P = 0.958$) and the posterior vertex and posterior temporal regions (Hedges's $g = 0.684$; 95% CI: −0.143, 1.511; $P = 0.105$). In addition, no significant differences in the predicted effect sizes were observed among subgroups ($P = 0.253$), suggesting that the sampling position of hair did not explain the heterogeneity in this study (Fig. 3).

3.5.2. Analytical technology

Children with ASD had increased hair lead levels compared with HC individuals in studies using atomic absorption spectrometry (AAS) as the analytical method (Hedges's $g = 0.794$; 95% CI: 0.228, 1.360; $P = 0.006$). In contrast, the same kind of comparison found no differences in studies employing ICP-MS (Hedges's $g = -0.029$; 95% CI: −0.471, 0.414; $P = 0.899$). Importantly, there was statistical difference in the summary effect sizes between subgroups ($P = 0.025$), indicating that the analytical technology was a important contributor to

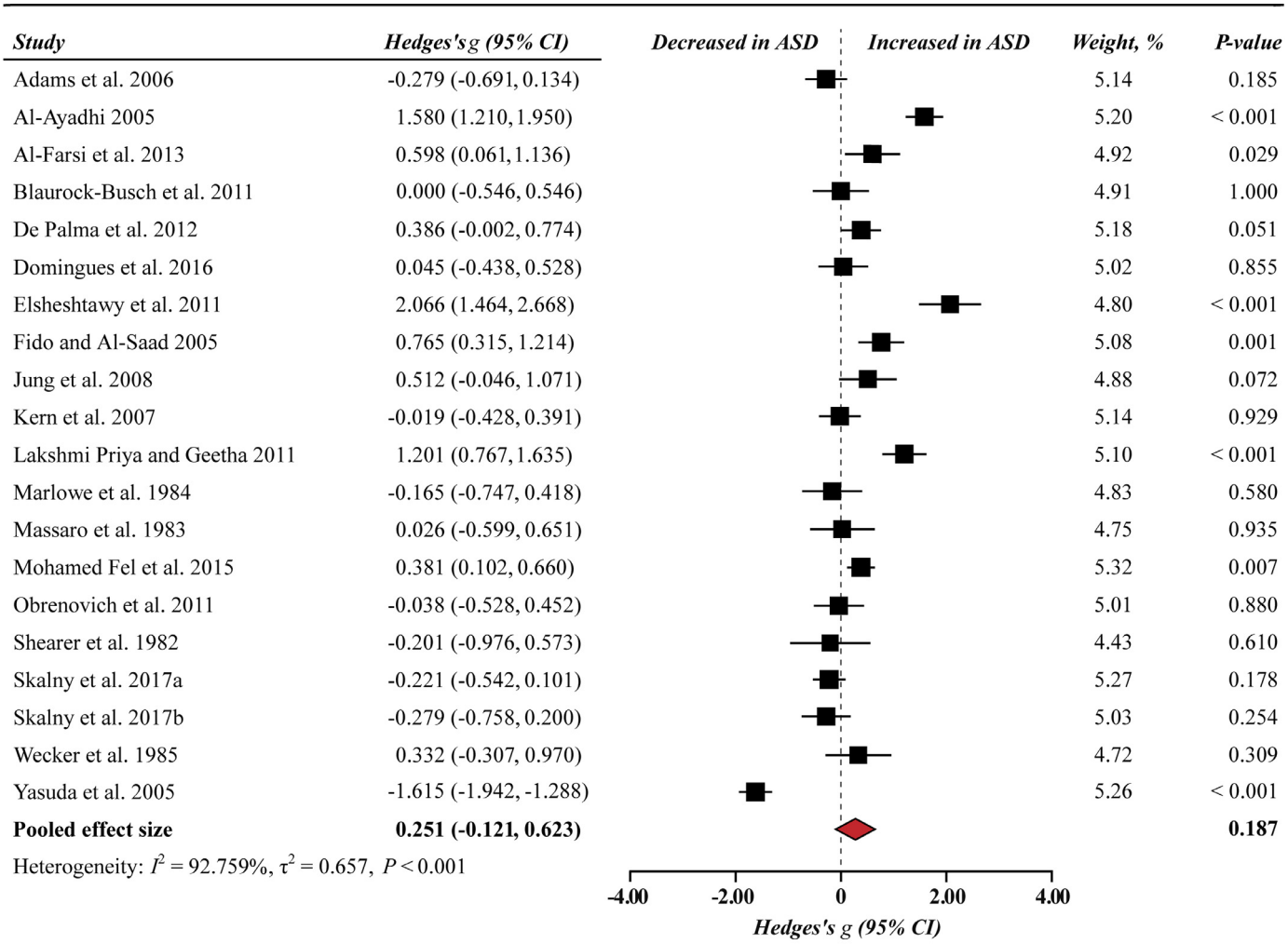

Fig. 2. Forest plot for random-effects meta-analysis. Differences in hair lead levels between children with ASD and HC subjects are shown. The size of each square is proportional to the study weight. Diamond symbol indicates the overall pooled effect size for the full set of studies included in this meta-analysis. Abbreviations: ASD, autism spectrum disorder; CI, confidence interval; HC, healthy controls.

Grouping factor and subgroup	No. of studies	No. of subjects ASD	No. of subjects HC	Hedges's g (95% CI)	P-value	P-value for difference
Sampling position of hair						
The nape of the neck	7	276	325	0.461 (0.022, 0.901)	**0.039**	
I^2 = 90.768%, τ^2 = 0.525, P < 0.001						
The occipital region	5	255	248	-0.014 (-0.530, 0.503)	0.958	0.253
I^2 = 59.968%, τ^2 = 0.066, P = 0.041						
The posterior vertex and posterior temporal regions	2	67	67	0.684 (-0.143, 1.511)	0.105	
I^2 = 0.000%, τ^2 = 0.000, P = 0.642						
Analytical technology						
AAS	7	291	330	0.794 (0.228, 1.360)	**0.006**	
I^2 = 90.042%, τ^2 = 0.520, P < 0.001						**0.025**
ICP-MS	11	593	462	-0.029 (-0.471, 0.414)	0.899	
I^2 = 91.294%, τ^2 = 0.500, P < 0.001						
Detection limit of analytical instrument						
AAS (flame atomizer)	2	44	44	0.987 (-0.125, 2.098)	0.082	
I^2 = 95.126%, τ^2 = 2.445, P < 0.001						
AAS (graphite furnace atomizer)	3	168	166	0.548 (-0.310, 1.405)	0.211	0.169
I^2 = 84.299%, τ^2 = 0.253, P = 0.002						
ICP-MS	11	593	462	-0.028 (-0.475, 0.419)	0.901	
I^2 = 91.294%, τ^2 = 0.500, P < 0.001						
Matching						
Age	3	67	115	0.110 (-1.020, 1.240)	0.849	
I^2 = 0.000%, τ^2 = 0.000, P = 0.658						
Age and gender	10	667	530	0.349 (-0.261, 0.960)	0.262	0.930
I^2 = 96.442%, τ^2 = 1.098, P < 0.001						
Age and geographical area	2	67	77	0.212 (-1.168, 1.591)	0.764	
I^2 = 0.000%, τ^2 = 0.000, P = 0.338						
Continent or region						
Asia	3	273	128	0.004 (-0.870, 0.878)	0.993	
I^2 = 98.269%, τ^2 = 2.734, P < 0.001						
Europe	4	180	204	-0.017 (-0.770, 0.737)	0.966	
I^2 = 56.437%, τ^2 = 0.056, P = 0.076						0.117
Middle East	6	291	304	0.892 (0.270, 1.513)	**0.005**	
I^2 = 90.301%, τ^2 = 0.452, P < 0.001						
North America	7	197	210	-0.051 (-0.638, 0.537)	0.866	
I^2 = 0.000%, τ^2 = 0.000, P = 0.823						
Country						
All other countries	13	744	636	0.406 (-0.057, 0.869)	0.086	
I^2 = 95.224%, τ^2 = 0.889, P < 0.001						0.260
United States	7	197	210	-0.050 (-0.695, 0.594)	0.878	
I^2 = 0.000%, τ^2 = 0.000, P = 0.823						

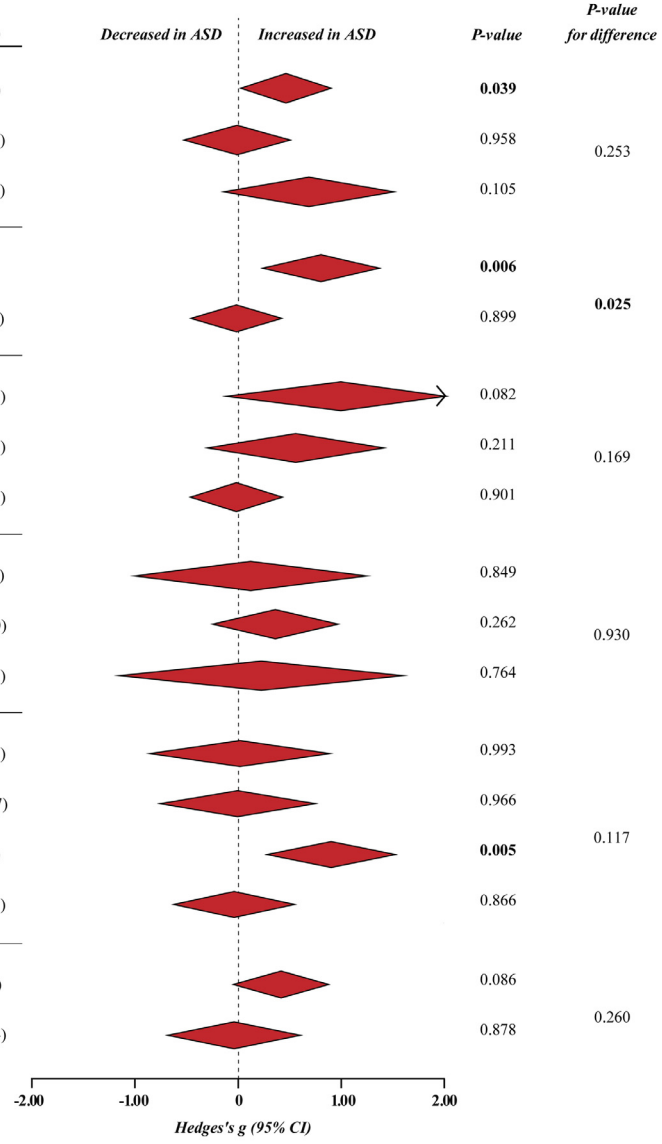

Fig. 3. Subgroup analyses stratified by study-level characteristics. Diamond symbol indicates the summary effect size for each corresponding subgroup. Abbreviations: AAS, atomic absorption spectrometry; ASD, autism spectrum disorder; CI, confidence interval; HC, healthy controls; ICP-MS, inductively coupled plasma-mass spectrometry; No., number.

the heterogeneity in this meta-analysis (R^2 analog = 0.289) (Fig. 3).

3.5.3. Detection limit of analytical instrument

When grouping by detection limit of analytical instrument, there were no differences in the levels of hair lead between autistic children and healthy subjects for studies applying AAS with flame atomiser (Hedges's g = 0.987; 95% CI: −0.125, 2.098; P = 0.082), employing AAS with graphite furnace atomiser (Hedges's g = 0.548; 95% CI: −0.310, 1.405; P = 0.211), and using ICP-MS (Hedges's g = −0.028; 95% CI: −0.475, 0.419; P = 0.901). Besides, no statistically significant differences in the mean effect sizes were shown among subgroups (P = 0.169), suggesting that this grouping factor was not the source of the heterogeneity in the present review (Fig. 3).

3.5.4. Matching

Our analysis indicated that autistic children were not accompanied by altered levels of hair lead as compared to HC subjects in studies employing age (Hedges's g = 0.110; 95% CI: −1.020, 1.240; P = 0.849), age and gender (Hedges's g = 0.349; 95% CI: −0.261, 0.960; P = 0.262), or age and geographical area (Hedges's g = 0.212; 95% CI: −1.168, 1.591; P = 0.764) as matching factor(s). The predicted effect sizes in three subgroups did not significantly differ from each other (P = 0.930), indicating that the subgroup membership was not responsible for the heterogeneity in this study (Fig. 3).

3.5.5. Continent or region

Studies were further stratified by continent or region. As shown in Fig. 3, the hair lead levels in children with ASD were elevated compared with the corresponding values in HC individuals in studies conducted in the Middle East (Hedges's g = 0.892; 95% CI: 0.270, 1.513; P = 0.005), but the same kind of comparisons showed no significant differences in studies from Asia (Hedges's g = 0.004; 95% CI: −0.870, 0.878; P = 0.993), Europe (Hedges's g = −0.017; 95% CI: −0.770, 0.737; P = 0.966), and North America (Hedges's g = −0.051; 95% CI: −0.638, 0.537; P = 0.866). There were no statistically significant differences in the summary effect sizes among subgroups (P = 0.117), suggesting that the heterogeneity in this meta-analysis could not be explained by continent or region.

Table 2
Statistics on meta-regression analyses (results of univariate analyses).

Moderator	No. of comparisions	No. of subjects		Meta-regression				Proportion of variance explained
		ASD	HC	Slope	95% CI (Lower)	95% CI (Upper)	*P*-value	R^2 analog
Mean age of patients	16	647	685	−0.009	−0.185	0.168	0.924	0.000
Mean age of HC	16	647	685	−0.077	−0.240	0.086	0.356	0.000
Mean age of all participants	16	647	685	−0.049	−0.226	0.127	0.584	0.000
Gender (% male) of patients	16	806	705	−0.027	−0.073	0.018	0.238	0.048
Gender (% male) of HC	15	739	625	−0.012	−0.037	0.014	0.380	0.075
Gender (% male) of all participants	15	739	625	−0.024	−0.058	0.009	0.156	0.170
Sample size of patients	20	941	846	−0.008	−0.016	−0.001	**0.037**	**0.293**
Sample size of HC	20	941	846	0.004	−0.014	0.021	0.670	0.000
Sample size of all participants	20	941	846	−0.004	−0.010	0.003	0.247	0.086
Total NOS score	20	941	846	0.057	−0.236	0.350	0.703	0.000
Publication year	20	941	846	0.010	−0.024	0.043	0.579	0.000

Abbreviations: ASD, autism spectrum disorder; CI, confidence interval; HC, healthy controls; No., number; NOS, Newcastle–Ottawa Scale.

3.5.6. Country

When grouping by country, the levels of hair lead in children with ASD were not changed compared with those in healthy subjects in studies from all other countries (Hedges's $g = 0.406$; 95% CI: −0.057, 0.869; $P = 0.086$) or from the United States (Hedges's $g = -0.050$; 95% CI: −0.695, 0.594; $P = 0.878$). Again, there was no significant difference in the predicted effect sizes between subgroups ($P = 0.260$), manifesting that this grouping factor was not a probable cause of the heterogeneity detected in the current study (Fig. 3).

3.6. Meta-regression analyses

We subsequently conducted meta-regression analyses to examine whether certain continuous variables could contribute to significant heterogeneity among studies. Univariate meta-regression analyses revealed that the mean age (patients, HC, and all participants), gender (% male; patients, HC, and all participants), sample size (HC and all participants), total NOS score, and year of publication did not yield moderating effects on the outcome of the meta-analysis ($P > 0.05$ for all analyses) (Table 2). However, a negative correlation was detected between the pooled effect size and the sample size of patients (slope = −0.008; 95% CI: −0.016, −0.001; $P = 0.037$), suggesting that the between-study heterogeneity in this review could also be partially explained by the sample size of autistic children (R^2 analog = 0.293) (Table 2).

3.7. Sensitivity analysis

Sensitivity analysis indicated that the overall pooled estimates were still nonsignificant when each study was systematically omitted, with the exception of one article (Yasuda et al., 2005) where removal rendered the association of hair lead levels and children with ASD significant ($P < 0.05$) (Fig. S3). This is not surprising as the study (Yasuda et al., 2005) had the biggest sample size ($n = 256$) and the smallest effect size (Hedges's $g = -1.615$).

3.8. Cumulative meta-analysis

Lastly, we performed a cumulative meta-analysis to estimate the influences of novel studies on previous pooled outcomes. Our results showed that from 1982 through 2017, there had never been statistically significant differences in hair lead levels between children with ASD and HC individuals (Fig. S4).

3.9. Publication bias

Visual inspection of the funnel plot suggested no publication bias (Fig. 4), which was confirmed by the result of the Egger's test ($t_{18} = 0.834$; $P = 0.415$). Besides, the classic Fail-safe N method revealed that 74 missing studies would need to be added to the meta-analysis to yield a statistically nonsignificant overall effect, further supporting that the outcome of this meta-analysis is unlikely to be caused by publication bias.

4. Discussion

4.1. Overview

Levels of lead exposure in children with ASD have been controversial, and their significance with respect to the etiology of ASD is not elucidated. Here, the primary analysis of this study showed that in comparison with the corresponding values in HC subjects, the lead levels of hair from autistic children were not changed significantly. Therefore, from the perspective of hair lead levels, this study do not support the viewpoint that children with ASD are accompanied by altered levels of hair lead, and there is a potential relationship between cumulated lead exposure and the progression of ASD.

4.2. Interpretation of subgroup and meta-regression analyses

When studies were stratified by the analytical technology, we found that there was statistical difference in the pooled effect sizes between subgroups, indicating that the analytical technology was a important source to the heterogeneity detected in this study (R^2 analog = 28.9%). Additionally, our results indicated that children with ASD had increased hair lead levels in studies using AAS as the analytical method. Presumably, this would more likely to be attributable to the fact that all the three studies (Al-Ayadhi, 2005; Elsheshtawy et al., 2011; Lakshmi Priya and Geetha, 2011), which have three of the biggest effect sizes in all values, applied AAS to measure lead levels in hair. Meanwhile, we also found that there were no differences in hair lead levels between ASD in children and HC subjects in the subgroups of ICP-MS. Among the spectrometric instruments used by included studies, ICP-MS is the most sensitive to determination of toxic metals at extremely low concentrations. It is generally known that the higher the testing capability of analytical technology is, the more accurate and reliable the detection results will be. Consequently, in a certain sense, these results from the subgroups of ICP-MS offers practical support for the reliability of the main outcome that was initially calculated with the full set of studies in this meta-analysis.

Our subgroup analyses also found that although the sampling position of hair did not explain the heterogeneity, the hair lead levels in autistic children were higher in the studies collecting hair sample from the nape of the neck (but not in those sampling hair from other areas). These suggested that the amount of lead secreted to the hair might differ by the growth position of scalp hair. This would be particularly important because previous studies have indicated that hair toxic contents may be in relation to toxic metal excretion rates (Geier et al.,

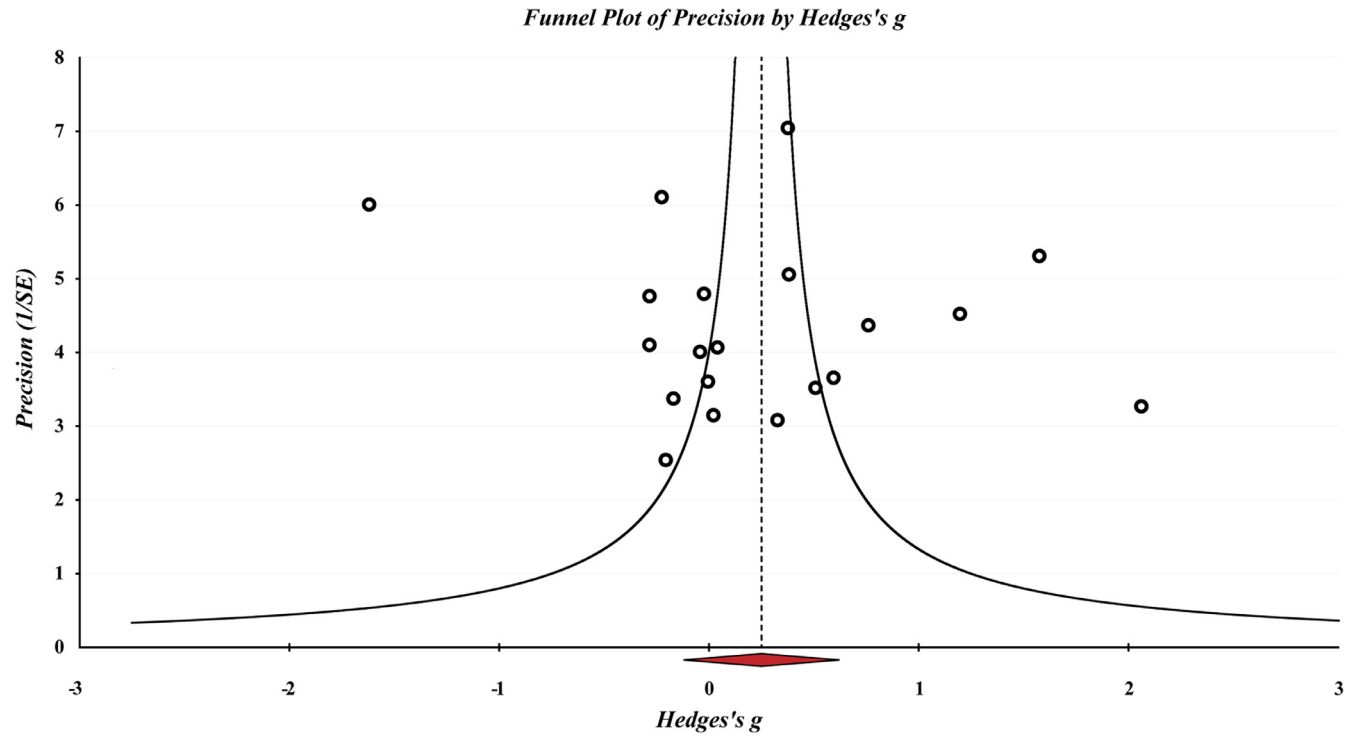

Fig. 4. Funnel plots evaluating publication bias in observed studies comparing hair lead levels between autistic children and healthy subjects. The figure depicts the effect sizes (Hedges's *g*) of studies against their precisions (inverse of SE). Circles represent observed studies. Diamond symbol indicates the overall pooled effect size based on observed studies. Abbreviation: SE, the standard error.

2012; Mohamed Fel et al., 2015). It implies that the difference in the sampling sites might affect the analysis results. Thus, this factor should be taken into account in future investigation examining the hair lead levels in children.

When studies were stratified by continent or region, our results showed that, apart from the studies conducted in the Middle East, the hair lead concentrations in children with ASD did not differ from the corresponding values in HC subjects in the studies from Asia, Europe, and North America. These findings revealed that many variables, including the environmental factors and behavior habits, relevant to the levels of cumulated lead exposure in children with ASD would vary between the Middle East and other geographical locations. However, the specific reasons why the higher levels of lead in hair samples of autistic children were detected exclusively in the Middle East but not elsewhere, are fairly complicated and cannot be ascertained at this stage, which is worthy of further investigation.

Through meta-regression analyses, a meaningful correlation was revealed between the summary effect size and the sample size of patients, suggesting that the between-study heterogeneity in this study could also be partially explained by the number of autistic children (R^2 analog = 29.3%). According to our opinion, it seems reasonable, since the sample sizes of patients in this meta-analysis varied widely, ranging from 12 to 200 autistic children. Actually, of additional concern is the slope in the regression equation, because it is negative value. This means that a larger sample size of patients will bring about a smaller difference in hair lead levels between children with ASD and HC subjects. In other word, it will render the pooled effect size to be closer to zero (more nonsignificant) or even negative. As a general rule, the more participants being surveyed, the smaller the sampling error will be, and the more reliable are the research results. Therefore, our findings of meta-regression analyses provide forceful support for our overall pooled results.

It has been reported that there is a remarkable difference in the prevalence of ASD between the genders, with a ratio of 4–5:1 corresponding to affected males to females (Giarelli et al., 2010; Qin et al., 2016). However, in the current study we haven't found a linkage between the gender (% male; patients, HC, and all participants) and the overall pooled effect size. These indicate that the lead levels in hair sample from autistic children may not be affected by sex. From the view of deep significance, it is also suggestive of no distinction in the extents of cumulated lead exposure between boys and girls who were diagnosed with ASD. These findings are to some extent consistent with the results of one prior study (Kalkbrenner et al., 2010), in which the authors reported correlations between exposure to several hazardous pollutants (including lead) and children with ASD according to sex, but did not find statistically significant differences.

4.3. Strengths and limitations

This study has several strengths. First, we did an expansive and detailed literature search, thereby increasing our possibility to capture all related studies. Second, our work was done by strict application of inclusion and exclusion criteria, well-established methods of data transformation, and robust statistical analyses. Third, we thoroughly identified additional valuable data and the latent sources of between-study heterogeneity by means of a series of subgroup and meta-regression analyses. Fourth, our results revealed that the outcome of this study did not arise from publication bias.

Accompanied by its strengths, this study has a few limitations. First, there is still a certain portion of statistical heterogeneity remained unexplained by the variables examined in this study. Presumably, the primary environmental factors and behavior disorders (such as pica) in relation to the levels of lead exposure may also be potential contributors. However, due to the paucity of reporting in the eligible studies, we were unable to explore the effects of these moderators on the results. Second, although most of the studies applied the Diagnostic and

Statistical Manual of Mental Disorders (DSM) as a diagnostic tool for childhood autism, the versions of DSM were diverse. Moreover, nearly half of these studies employed various auxiliary scales simultaneously. The diagnostic criteria of DSM in distinct versions are different, and the sensitivity and specificity of the auxiliary instruments for diagnosing ASD vary markedly. Therefore, there is no way to analyze the independent effects of diagnostic methods on the outcome of this meta-analysis. Third, almost all of the included studies have introduced the laboratory operating processes for the analysis of hair samples, such as the approaches of washing, digestion, dilution, and quality control. Nonetheless, there were considerable differences in the treatment way of each step among studies. In addition, experimenter reliabilities in different laboratories may also have influences on the testing results, but we are not in a position to know the relevant information from the inclusive articles. Consequently, these confounding factors have not been analyzed in this study.

5. Conclusions

This study does not support the association of hair lead levels with ASD in children, and the involvement of cumulated lead exposure in the occurrence of ASD.

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Conflict of interest

The authors declare that they have no actual or potential conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2019.05.022.

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