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Environmental risk factors and biomarkers for autism spectrum disorder: an umbrella review of the evidence

Jong Yeob Kim[1], Min Ji Son[1], Chei Yun Son[2], Joaquim Radua[3, 4, 5, 6], Michael Eisenhut[7], Florence Gressier[8], Ai Koyanagi[9, 10], Andre F. Carvalho[11, 12], Brendon Stubbs[13, 14], Marco Solmi[15, 16, 17], Theodor B. Rais[18], Keum Hwa Lee[19], Andreas Kronbichler[20], Elena Dragioti[21], Jae Il Shin[19] and Paolo Fusar-Poli[17, 22, 23]

1. Yonsei University College of Medicine, Seoul, Republic of Korea.
2. Department of Psychological & Brain Sciences, Washington University in St. Louis, MO, USA.
3. Early Psychosis: Interventions and Clinical-detection (EPIC) Lab, Department of Psychosis Studies, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, United Kingdom.
4. FIDMAG Germanes Hospitalaries, CIBERSAM, Barcelona, Spain.
5. Centre for Psychiatry Research, Department of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden.
6. Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain.
7. Department of Pediatrics, Luton & Dunstable University Hospital NHS Foundation Trust, Luton, United Kingdom.
8. CESP, Inserm UMR1178, Department of Psychiatry, Assistance Publique-Hôpitaux de Paris, Bicêtre University Hospital, Le Kremlin Bicêtre, France.
9. Research and Development Unit, Parc Sanitari Sant Joan de Déu, Universitat de Barcelona, Fundació Sant Joan de Déu, Barcelona, Spain.
10. Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Salud Mental, CIBERSAM, Madrid, Spain.
11. Centre for Addiction & Mental Health, Toronto, Ontario, Canada.
12. Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada.

13. Physiotherapy Department, South London and Maudsley NHS Foundation Trust, London, United Kingdom.
14. Department of Psychological Medicine, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom.
15. Department of Neurosciences, University of Padua, Padua, Italy.
16. Neurosciences Center, University of Padua, Padua, Italy.
17. Early Psychosis: Interventions and Clinical-detection (EPIC) lab, Department of Psychosis Studies, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, United Kingdom.
18. Department of Psychiatry, University of Toledo Medical Center, Toledo, Ohio, USA.
19. Department of Pediatrics, Yonsei University College of Medicine, Seoul, Republic of Korea.
20. Department of Internal Medicine IV, Medical University Innsbruck, Anichstraße 35, 6020, Innsbruck, Austria.
21. Pain and Rehabilitation center and Department of Medicine and Health Sciences (IMH), Faculty of Health Sciences University of Linköping, SE- 581 85 Linköping, Sweden.
22. OASIS service, South London and Maudsley NHS Foundation Trust, London, United Kingdom.
23. Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy.

*Corresponding Author:
Dr. Paolo Fusar-Poli, MD.
Address: Department of Psychosis Studies, Institute of Psychiatry, Psychology & Neuroscience, London SE5 8AF, UK
Tel: +44 (0) 20 7848 0900; fax: +44 (0) 20 7848 0976; E-mail: paolo.fusar-poli@kcl.ac.uk
Prof. Jae Il Shin, MD.
Address: Yonsei-ro 50, Seodaemun-gu, C.P.O. Box 8044, Department of Pediatrics, Yonsei University College of Medicine, Seoul 120-752, Korea
Tel.: +82-2-2228-2050; Fax: +82-2-393-9118; E-mail: shinji@yuhs.ac

* Jong Yeob Kim, Min Ji Son, and Chei Yun Son contributed equally to this article as co-first authors and Jae Il Shin and Paolo Fusar-Poli are joint co-corresponding authors.

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ABSTRACT

Background

Numerous studies have identified the potential risk factors and biomarkers for autism spectrum disorder (ASD). We aim to study the strength and validity of the suggested environmental risk factors or biomarkers of ASD.

Methods

We conducted an umbrella review and systematically appraised the relevant meta-analyses of observational studies (PROSPERO registration: CRD42018091704). We searched PubMed, Embase, and Cochrane Database of Systematic Reviews from inception to 10/17/2018 and screened the reference list of relevant articles. We obtained the summary effect, 95% confidence interval (CI), heterogeneity, and 95% prediction intervals. We examined small study effects and excess significance. We performed analyses under credibility ceilings.

Findings

A total of 46 eligible articles yielded data on 67 environmental risk factors (cases=544212, population=81708787) and 52 biomarkers (cases=15614, controls=15417). Evidence of association was convincing for greater maternal age (RR=1·31, 95% CI=1·18 to 1·45), maternal chronic hypertension (OR=1·48, 95% CI=1·29 to 1·70), maternal gestational hypertension (OR=1·37, 95% CI=1·21 to 1·54), maternal overweight (RR=1·28, 95% CI=1·19 to 1·36), preeclampsia (RR=1·32, 95% CI=1·20 to 1·45), pre-pregnancy maternal antidepressant exposure (RR=1·48, 95% CI=1·29 to 1·71), and selective serotonin reuptake inhibitor (SSRI) exposure during pregnancy (OR=1·84, 95% CI=1·60 to 2·11). Only two associations, maternal overweight and SSRI during pregnancy, retained high level of evidence under subset sensitivity analyses. Evidence from biomarkers was limited.

Interpretation

Convincing evidence suggests that maternal factors such as age and features of metabolic syndrome are associated with risk of ASD. SSRI use during pregnancy was also convincingly associated with risk of ASD when exposed and non-exposed groups were compared. However, there is a possibility that the association is affected by other confounding factors, considering that pre-pregnancy maternal antidepressant exposure was also convincingly associated with higher risk of ASD. Findings from prior studies suggest that one possible confounding factor is underlying maternal psychiatric disorders.

Funding

None.

Key words: autism; risk factors; environment; epidemiology; meta-analysis; umbrella review

INTRODUCTION

Autism spectrum disorder (ASD) is a leading cause of disability in children, and often requires high levels of support, which impose a high cost on society and a substantial economic, emotional, and physical burden on affected families.[1-4] The prevalence of ASD was estimated to be 2·47% in US children and adolescents,[5] and 7·6 per 1000 persons globally, accounting for 111 disability-adjusted life years per 100000 global population.[2]

Given limited clinical and epidemiological evidence of remission in ASD,[2] numerous investigations focused to better understand and advance risk prediction and prevention of ASD. The etiology of ASD is believed to be multifactorial, with various genetic predispositions and environmental (non-genetic) risk factors having shown to be associated with an increased risk of ASD.[6-11] There have been remarkable advances in the knowledge of genetic causes of autism by the efforts made in the field of genetics, yet the exact genes are not clear. In addition, the results on associations of various kinds of environmental factors for ASD have been inconsistent, and hierarchies of evidence have not been determined across different factors, while it is unclear if these risk factors are prone to biases.

There have been numerous cohort and case-control studies reporting various kinds of significant environmental risk factors or biomarkers of ASD, and these have also been meta-analyzed by combining the results of multiple studies.[12] However, these analyses are usually limited to one specific topic, and assessment of various kinds of biases in literature is often insufficient. Claimed statistically significant associations are susceptible to biases such as publication bias, reporting bias, and residual confounding bias, resulting in false positives[13] or inflated estimates[14] of the association. Such problems have resulted in an excess amount of statistically significant associations ($p<0·05$) in psychological science and other medical fields.[15,16] Recently, one systematic overview has comprehensively identified and analyzed

possible environmental risk factors of ASD.[7] While this overview was informative, quantitative assessment of bias was also incomplete because it relied on reports from the original studies, and definite criteria for determining the credibility of the associations were lacking. To overcome these limitations, we conducted an umbrella review of the relevant meta-analyses. We aimed to generate a hierarchy of evidence and examine true noteworthiness of the suggested environmental risk factors and biomarkers for ASD.

METHODS

Literature search strategy and eligibility criteria

We followed the pre-specified protocol registered in PROSPERO (registration: CRD42018091704).[17] Three investigators (JYK, MJS, and CYS) searched PubMed, Embase, and Cochrane Database of Systematic Reviews from inception to 10/17/2018. We used the following search algorithm: (Asperge* [All Fields] OR autis* [All Fields]) AND meta [All Fields]. We obtained the eligible articles by consecutively examining the titles, the abstracts, and then the full-text (figure 1). We further manually searched the references of the relevant articles and attempted to identify and include eligible studies. Disagreements were resolved by discussion by JYK, MJS, CYS, and JIS.

We included meta-analyses of observational studies examining associations between ASD and potential environmental risk factors or biomarkers. The definition of ASD followed that of the original meta-analysis, while the definition of risk factors and biomarkers followed that of the World Health Organization.[18,19] Biomarkers were defined as any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease.[18] Risk factors were defined as any attribute, characteristic or exposure of an individual that increases the likelihood of developing a disease or injury.[19]

We screened for articles without language restriction. We only included meta-analyses that reported either effect estimates of individual study estimates or the data necessary to calculate these. When two or more meta-analyses existed for an association, we included the most recent meta-analysis with the largest number of studies.

Data extraction

From each meta-analysis, we extracted the first author, publication year, risk factor or biomarker of interest, number of ASD cases and total participants, maximally adjusted individual study estimates and corresponding 95% confidence intervals (CI), metrics used for analyses such as mean difference, Hedges' g, Cohen's d, odds ratio (OR), or risk ratio (RR), and individual study designs (i.e. cohort design, case-control design, etc.).

Data analysis

We adopted a series of statistical tests, developed and reproduced in previous umbrella reviews.[20-25] We performed re-analysis on each eligible meta-analysis with individual study estimates extracted from each meta-analysis. We calculated the summary effect size, 95% CI, and p value of eligible meta-analyses using both fixed and random effects model. Statistical significance was claimed at p value < 0·05. We further assessed p values below thresholds such as 10^{-3} or 10^{-6}.[26,27] Additionally, we checked whether the summary effect of the random effects meta-analysis and the effect of its largest component study (the study with smallest standard error) showed concordance in terms of statistical significance.[20-22,24,25] We also checked whether the standard error of the largest study is below 0·10, which is considered as precise effect size.[23] We performed Cochran's Q test and calculated the I^2 statistic for evaluation of heterogeneity.[28,29] We estimated the 95% prediction interval, which is the range where we expect the effect of the association will lie for 95% of similar studies in the future.[30] We assessed the presence of small study effects, i.e. large studies having significantly more conservative results than smaller studies, with the regression asymmetry test proposed by Egger et al.[31] For statistically significant random effects meta-analysis, we adopted the test for excess significance bias, which evaluates whether the observed number of nominally statistically significant studies (p value < 0·05) is too large compared to their

expected number.[32,33] We applied various credibility ceilings to individual observational studies to account for their potential methodological limitations that might result in spurious significant results for the meta-analyses.[34,35] Details of these analytic methods are further explained in the supplementary material. All statistical tests were two-sided. The software used for the analysis was Comprehensive Meta-analysis ver.3.3.070 (Borenstein, NH, USA), RStudio ver. 1.1.453., and R package "metafor" ver.2.0-0 and "pwr" ver.1.2-2.[36-38]

Determining the credibility of evidence

In accordance with previous umbrella reviews,[20-25,39,40] we categorized the strength of the evidence of biomarkers or environmental risk factors for ASD into five levels: convincing (class I), highly suggestive (class II), suggestive (class III), weak (class IV), and not significant (NS). Criteria for the level of evidence used p values under random effects model, the number of ASD cases, heterogeneity presented as I^2, small study effects, excess significance bias, effect estimate under 10% credibility ceiling, and 95% prediction interval (criteria presented in table 1). For associations classified as convincing or highly suggestive evidence, we performed four kinds of subset analyses to confirm the robustness of the associations: We performed subset analyses restricted to prospective cohort studies, cohort studies, study estimates adjusted for covariates, or component studies that ascertained ASD cases using diagnostic methods in line with DSM-III/IV/V or ICD-8/9/10 (which are considered as robust measures compared to other methods such as self-reports). For any associations based on both cohort study(s) and case-control study(s), we also performed subgroup analyses, and assessed whether heterogeneity between the effect of the two subgroups was significant (p value < 0·1 for Cochran's Q test). We also recorded reports from meta-analyses and individual studies of differences of effect of environmental risk

factor of ASD according to important factors such as sex differences and presence of intellectual disability.

Role of the funding source

There was no funding source for this study. All authors had full access to all the study data and the corresponding authors had final responsibility for the decision to submit for publication.

RESULTS

A total of 1699 potentially eligible articles were identified by the initial search (figure 1). After screening by title, abstract, and full-text, 46 articles were thought eligible for our umbrella review. Fourteen articles were excluded in the full-text screening because a larger meta-analysis was available (table S1). The eligible 46 articles[12,41-85] yielded 119 associations (67 environmental risk factors and 52 biomarkers). Screening of the reference lists of relevant articles including a previous systematic review[7] did not identify additional eligible articles. A total of 67 associations of environmental risk factors with ASD was based on data of 544212 cases and a total population of 81708787 (table 1-2, S2-S5). Forty-two associations (63%) included both cohort and case-control design studies, eight (12%) used cohort design, six (9%) used case-control designs, and six (9%) included cross-sectional studies. The median number of study estimates in each analysis was 8 (range 2–24). The median number of cases and the total population was 3764 and 502843.

Total of 52 (78%) associations showed statistical significance under the random effects model, of which 33 (49%) had p value < 10^{-3}, and 18 (27%) had p value < 10^{-6}. Fifty-two (78%) associations had more than 1000 ASD cases, of which 16 were supported by p value < 10^{-6}. Out of 52 statistically significant associations, 40 were also supported by the statistically significant result of the largest component study, of which 24 were supported by the statistically significant largest study with standard error < 0·10. Metrics followed that of the original meta-analyses except for one association (extremely low birth weight vs. normal birth weight), where we converted Cohen's d to OR for optimal presentation. Eventually, metrics used were either RR or OR. Effect size was smaller than 2 except for six associations, of which three (congenital cytomegalovirus infection, hearing impairment, visual

impairment) had effect size larger than 10. Only two factors (folic acid supplementation during pregnancy and breastfeeding) were associated with decreased risk of ASD.

Thirty-one associations (46%) showed large heterogeneity (I^2>50%), of which 12 (18%) had very large heterogeneity (I^2>75%). Twenty-four (36%) statistically significant associations had neither small study effects nor excess significance bias. 95% prediction interval excluded the null in only 19 (28%) of the associations. Eleven (16%) associations were retrieved from two individual studies only, and thus small study effects and prediction intervals could not be estimated. Effect sizes of meta-analyses showed a trend toward null value as the standard error of summary estimate decreased (figure 2), and effect sizes of the largest studies were largely similar with the effect sizes of random effects meta-analyses (figure 3). Under the random effects models, while 52 (78%) associations were statistically significant, 41 (61%), 30 (45%), 18 (27%), and 10 (15%) retained statistical significance under respectively 5%, 10%, 15%, and 20% credibility ceilings.

Eventually, seven (10%) associations were graded as convincing evidence (table 1-2). The risk factors with convincing associations were the following: maternal age ≥ 35 years vs. 25 to 29 years, maternal chronic hypertension, maternal gestational hypertension, maternal overweight pre- or during pregnancy, maternal preeclampsia, pre-pregnancy maternal antidepressant exposure vs. unexposed group, and selective serotonin reuptake inhibitor (SSRI) during pregnancy. Effect sizes of these associations ranged from 1·31 to 1·84. Eight (12%) associations were graded as highly suggestive evidence (table 1-2), which are the following: highest maternal age group vs. reference group, maternal age 30 to 34 vs. 25 to 29 years, maternal autoimmune disease exposure, acetaminophen during pregnancy, higher paternal age, per 10-year increase, highest paternal age group vs. reference group, paternal

age>45 years vs. reference group, and paternal age 40-45 years vs. reference group. Eleven (16%) associations were graded as suggestive evidence (table S2), twenty-six (39%) were graded as weak evidence (table S3), and the remaining 15 (22%) did not show statistically significant associations (table S4).

Fifty-two associations of biomarkers comprised a total of 15614 cases and 15417 controls (table 3,S6,S7). Out of 52 meta-analyses of environmental risk factors, 17 (33%) used case-control studies. Two (4%) associations used cross-sectional studies, and study design was not specified in 33 (63%) studies. The median number of study estimates in each analysis was six (range 2–23). The median number of cases and controls was 228 and 215·5.

Out of 52 biomarkers associations, twenty-seven (52%) associations were statistically significant (p value < 0·05), and ten (19%) associations had p value < 10^{-3}. Only three associations, 5-hydroxytryptamine in whole blood, digit ratio (2D:4D ratio), and glutathione disulfide in plasma had p value < 10^{-6}. Moreover, only three associations, namely brain-derived neurotrophic factor in blood, mercury in hair, and mercury in whole blood, were supported by a population with more than 1,000 ASD cases. No associations were based on more than 1,000 cases had p value < 10^{-3}. Thus, no biomarker association was graded as suggestive (class III) or higher level of evidence.

Out of 27 statistically significant associations, only 14 were also supported by a statistically significant result of the largest component study, of which none was supported by a statistically significant result of the largest study of standard error < 0·10. Forty-four associations (85%) had large heterogeneity (I^2>50%), of which 36 (69%) associations had very large heterogeneity. Only eleven (21%) associations retained statistical significance under 10% credibility ceilings, and twelve (23%) statistically significant associations had

neither small study effects nor excess significance bias. 95% prediction intervals excluded the null in only one association (D2:D4 ratio). The detailed results are summarized in table S6-S7. Sensitivity subset analyses were performed on meta-analyses of 15 environmental risk factors graded as convincing (class I) or highly suggestive evidence (class II). Subset analysis restricted to cohort studies (prospective or retrospective) showed that only two associations of class I remained at the same rank (table S8). These were maternal overweight pre- or during pregnancy and maternal preeclampsia. Three associations (SSRI during pregnancy, acetaminophen during pregnancy, paternal age>45 years vs. reference group) remained at highly suggestive evidence. When subset analysis was restricted to only prospective cohort studies, no convincing association was identified, and only two associations (maternal overweight pre- or during pregnancy and SSRI during pregnancy) were still graded as highly suggestive evidence (table S9).

In subset analyses of adjusted study estimates, association of maternal preeclampsia with ASD was downgraded to suggestive evidence, while association of maternal autoimmune disease exposure with ASD was upgraded from highly suggestive evidence to convincing evidence (table S10). In subset analyses limited to component studies that used diagnostic methods in line with DSM III-V or ICD-8/9/10, only two associations, maternal gestational hypertension and maternal autoimmune disease exposure, were downgraded to suggestive evidence (table S11).

A total of 46 associations were eligible for subgroup analyses of cohort studies and case-control studies (table S12). It is worth noting that in all nine class I or II associations that had their level of evidence downgraded in subset analyses of cohort studies (table S8), the effect difference between subgroups of cohort studies and case-control studies was not significant.

Therefore, in these associations, adopting the results and level of evidence of the original meta-analyses of both cohort and case-control studies would also be appropriate. In nine eligible environmental risk factors, at least one individual study reported adjusted study estimates separately by sex or presence of intellectual disability (table S13). Separate meta-analyses by these factors were not feasible due to lack of study estimates (table S13).

DISCUSSION

To the best of our knowledge, the current umbrella review is the first to quantitively appraise the environmental risk factors and biomarkers of ASD. We evaluated associations of ASD with 119 possible risk factors and biomarkers. Our analysis revealed that associations showing convincing evidence (class I) were either maternal factors, such as age and features of metabolic syndrome, or use of antidepressants such as SSRI. Association of ASD with higher paternal age, maternal autoimmune disease exposure, and acetaminophen exposure during pregnancy were graded as highly suggestive evidence (class II), partly because of the presence of small study effects and large heterogeneity. Only two associations, maternal overweight pre- or during pregnancy and SSRI during pregnancy, remained at convincing or highly suggestive evidence. However, the results should be interpreted with caution, because the statistical methods and bias tests applied are not perfect and criteria are based on arbitrary thresholds, although they have been used in recent umbrella reviews of meta-analyses.[20-25]

In our study, components of a maternal metabolic syndrome, that is, chronic hypertension, gestational hypertension, preeclampsia, and overweight were associated with higher risk of ASD in offspring, all graded as convincing evidence. One of the possible underlying mechanism discussed is “fetal programming”, a concept that maternal factors like inflammation and chronic stress can alter the gestational environment and determine long term fetal outcomes.[86,87] Metabolic syndromes are often characterized by chronic low-grade inflammation and insulin resistance. The metabolic and immune system share common signaling pathways.[88] Although the role of an aberrant immune system function in the development of ASD is speculative, there has been evidence of the deleterious role of dysregulation of the maternal immune system on the development of ASD.[89] Several studies

showed that maternal autoantibodies that recognize proteins in the developing fetal brain could cause ASD in offspring of mothers with metabolic syndromes.[90,91] In children with severe ASD, ASD-specific autoantibodies were found to be significantly more prevalent in mothers with diabetes (type 2 or gestational), hypertension, and mothers who were moderately overweight than in healthy mothers.[92] Recently, Jones et al.[90] demonstrated that ASD-specific antigen-induced maternal autoantibodies produced alterations in a constellation of ASD-relevant behaviors in mice. Therefore, one hypothesis is that metabolic syndrome could contribute to the production of ASD-specific maternal autoantibodies through a breakdown of maternal immune tolerance and this can increase the risk for the development of ASD in the offspring.

Convincing evidence showed that maternal age, when the comparison is restricted to age groups of ≥ 35 years vs. 25 to 29 years, was associated with a higher risk of ASD. Accumulation of mutations, high rate of complications, and increased chance of exposure to medications or pollutions are possible mechanisms that underlie the higher risk of ASD in higher maternal age group.[80] Higher paternal age was also associated with a higher incidence of ASD. Three comparisons (per 10-year increase in paternal age, highest paternal age group vs. reference group, paternal age>45 years vs. reference group, and paternal age 40-45 years vs. reference group) represented risk factor as higher paternal age showed sufficiently low p value ($< 10^{-6}$) and 95% prediction intervals excluded the null despite high heterogeneity and presence of small study effects. In two of the comparisons, subset analyses of prospective studies showed p value $< 10^{-3}$ with no evidence of small study effects, indicating the existence of meaningful associations. Increased rate of *de novo* mutations[93] and epigenetic alternations[80] are proposed potential mechanisms underlying the association.

Convincing evidence showed that maternal exposure to SSRI during pregnancy was associated with a higher risk of ASD when compared with unexposed groups. However, the association must be interpreted carefully. In another meta-analysis, when maternal groups with pre-pregnancy antidepressant exposure were compared with unexposed maternal groups, the association with ASD was also graded as convincing evidence. This raises the question of whether underlying psychiatric conditions of mothers have caused confounding by indication in classical comparisons (SSRI-exposed vs. SSRI-unexposed). Several other meta-analytic attempts have been made to discern between the two possible causes of ASD.[53,64] When maternal groups with psychiatric disorder but with no SSRI exposure during pregnancy were compared with unexposed groups, a higher incidence of ASD was observed in the former group (OR=1·81, 95% CI=1·44 to 2·29), supporting the idea that presence of a maternal psychiatric condition is an independent risk factor for ASD.[53] Meanwhile, when SSRI-exposed groups were compared with unexposed groups with a history of affective disorder (setting in which the possibility of confounding by psychiatric disorder is minimized), the association with ASD was nonsignificant (OR=1·18, 95% CI=0·91 to 1·52).[64] Analyses restricted to sibling studies also showed a non-significant association (RR=0·96, 95% CI 0·65 to 1·42).[64] Interestingly, when group with paternal antidepressant exposure during the maternal pregnancy period were compared with unexposed group, there was higher risk of ASD in the former group (RR=1·29, 95% CI=1·08 to 1·53).[64] Overall, these findings suggest that while maternal psychiatric disorder may act as an independent risk factor for ASD, the association between SSRI exposure during pregnancy and ASD needs to be further verified in adequately designed future studies.

Maternal autoimmune disease exposure was associated with higher risk of ASD, graded as highly suggestive association, with 95% prediction intervals excluding the null. In mothers with autoimmune diseases, immune response mediators and autoantibodies might play a role in fetal neurodevelopment, resulting in adverse fetal outcomes such as ASD. Family history of psoriasis, rheumatoid arthritis, type 1 diabetes, or any type of autoimmune disease was also associated with a higher risk of ASD, graded as suggestive evidence. Several researchers have tested the potential link between the production of ASD-specific brain-reactive maternal autoantibodies and maternal autoimmunity.[94,95] Martin et al.[94] showed that rhesus monkeys exposed prenatally to human IgG collected from mothers of multiple children diagnosed with ASD consistently demonstrated increased whole-body stereotypies and hyperactive behaviors, suggesting a potential autoimmune etiology in a subgroup of patients with ASD. Brimberg et al.[95] reported that mothers of a child with ASD that were positive for anti-brain antibodies were significantly more likely to be positive for anti-nuclear autoantibodies, which are frequently observed in patients with various kinds of autoimmune diseases. They also found that there was a significantly increased incidence of some autoimmune in mothers with circulating ASD-specific anti-brain antibodies than those with negative anti-brain antibodies.[95] Despite such findings, in the identified meta-analyses, small study effects existed across the associations, and no significant association of ASD with maternal autoimmune disease was identified when the analysis was restricted to certain types of autoimmune diseases (e.g., autoimmune thyroid disease).[44] Therefore, more studies are needed to confirm the association between maternal autoimmune disease exposure and ASD.

Fifty-two biomarkers for ASD were identified and analyzed. Identifying robust evidence of biomarker for ASD can result in early diagnosis and better treatment of the disease.[96]

Association of D2:D4 ratio with a risk of ASD was supported by sufficiently low p value ($p<10^{-6}$) without signs of biases, meeting the criteria for convincing evidence except for the number of ASD cases, being supported by 277 cases. However, most of the associations of biomarkers were supported by p values close to the significance threshold (p value>10^{-3}) and too few cases, implying a significant possibility of false positive findings. Similar findings were observed in umbrella reviews of biomarkers for other psychiatric disorders.[21,97,98]

Findings from our study differed significantly from that of the previous umbrella review which systematically evaluated environmental risk factors for ASD using different approaches to determine the credibility of the association.[7] The previous review concluded that birth complications accompanied by trauma or ischemia and hypoxia have shown strong links to ASD, while in our review, those markers were graded as class III evidence (5-min Apgar score < 7, class III; O2 treatment, class IV; neonatal acidosis, NS) due to p value close to significance threshold or few cases. These risk factors should be interpreted with caution, because autism is not thought to be a disorder of brain damage, such as hypoxia, but of aberrant brain development. Also, we should also consider the populations that were studied and whether the diagnosis was truly confirmed using objective criteria, because the broadening of the definition of ASD could result in labeling some individuals with differences in socialization as having ASD. In addition, because there are many comorbidities to be considered in evaluating prenatal or perinatal factors, using unadjusted study estimates in a meta-analysis can lead to overestimation of the results. The previous review also asserted that pregnancy-related factors such as maternal obesity, maternal diabetes, and caesarian section have shown weak association with ASD.[7] While our review agreed on the listed associations (maternal obesity, class IV; caesarian section, class IV; maternal diabetes, class

III), we further concluded that other pregnancy-related maternal factors, such as preeclampsia, hypertension, and psychiatric disorders were convincingly associated with ASD. In contrast with the previous review[7], our review quantitatively appraised risk factors in terms of not only small study effects but also other various tests of assessing potential biases. We discerned between the likely and the less likely associations and graded the credibility of the associations using objective criteria utilizing rigorous statistical tests developed and reproduced in prior studies,[20-25,39,40] which is why we believe our review provides the most reliable and objective evidence of associations between environmental risk factors and ASD.

There are some limitations to our study. First, despite the most rigorous criteria we used to assess the credibility of the associations developed and reproduced in prior studies,[20-25] we cannot be sure we ruled out all the biases inherent to individual studies. In addition, biases that might have caused from the respective characteristics of study design, diagnosis of ASD, genetic causes of ASD in the population, or gender effects, were not fully assessed in our study, partly due to insufficient reporting of the original studies. Second, we did not assess the quality of component studies of the meta-analyses as it was beyond the scope of our umbrella review. If component studies are flawed with serious methodologic problems, or if crude unadjusted study estimates which can be more exaggerated than adjusted study estimates are used in the meta-analysis, the effect of meta-analysis could be overestimated. Surely, when we re-analyzed an eligible meta-analysis studying thimerosal exposure during embryo or early infancy[82] after excluding its individual studies known for their flawed methodology,[99,100] the level of association changed from class IV ($p < 0{\cdot}05$) to not significant ($p > 0{\cdot}05$). Likewise, the results of meta-analyses should always be interpreted with caution. Third, we could not assess environmental risk factors of ASD according to important factors

such as sex or presence of intellectual disability, because most individual component studies did not report the adjusted estimates separately by these factors. To overcome this limitation, future observational studies should report adjusted study estimates of risk factors separately by such factors, and if possible, make raw population data open to researchers. Fourth, we studied biomarkers and environmental risk factors reported in published meta-analyses and therefore, associations that have been studied only in large trials could have been missed in our review. Fifth, because the possibility of genetic/environmental confounding cannot be ruled out in findings of observational studies, it may be hard to establish causations from some associations. What were classified as risk factors might actually act as biomarkers that predict the ascertainment of ASD, rather than being causal factors.

Despite these limitations, this umbrella review covered and mapped the association of ASD with a wide range of environmental risk factors and biomarkers. Out of 119 identified associations, only several maternal factors, which are higher age, chronic hypertension, preeclampsia, gestational hypertension, overweight pre- or during pregnancy, were convincingly associated with ASD, without any signs of biases. SSRI exposure during pregnancy was also convincingly associated but confounding from underlying maternal psychiatric disorders is highly possible. One cannot state that other associations are not meaningful, but there is still some uncertainty in them that should be resolved. Further well-designed studies with accurate assessment of potential biases are needed to confirm the true association.

Contributors

JYK, MJS, CYS, and JIS contributed to the concept and design of the study. JYK, MJS and CYS contributed to the literature search, literature screening, data extraction, data analysis, data interpretation, and construction of figures and tables, and any discrepancies were resolved with discussion by JYK, MJS, CYS, JIS and PFP. All authors drafted and critically revised the manuscript. All authors gave approval to the final version of the manuscript for publication. The corresponding authors (JIS and PFP) attest that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. JYK, MJS, and CYS contributed equally to the manuscript (joint first authors) and JIS and PFP are joint co-corresponding authors.

Declaration of interests

We declare no competing interests.

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Research in context panel

Evidence before this study

Numerous risk factors and biomarkers were shown to have associations with risk of autism spectrum disorder (ASD) in systematic reviews and meta-analyses. However, some results have been inconsistent, and it is unclear if the claimed associations are prone to biases in literature. One systematic review performed by Modabbernia and colleagues has comprehensively identified and analyzed possible environmental risk factors of ASD and concluded that birth complications related birth complications accompanied by trauma or ischemia and hypoxia have shown strong links to ASD, but overall, quantitative analysis was lacking, and bias assessment was incomplete due to its reliance on previous reports. To overcome these limitations, we performed an umbrella review of meta-analyses. We searched PubMed, Embase, Cochrane Database of Systematic Reviews from inception to 10/17/2018 for meta-analyses of observational studies studying any environmental risk factors and biomarkers of ASD, without language limitations. We used the following keywords: "autis*", "Asperge*", and "meta-analysis." We performed various tests of bias assessment and applied criteria for determining the level of credibility of the association.

Added value of this study

A total of 119 unique associations of environmental risk factors or biomarkers with risk of ASD were identified and analyzed. Among these, only maternal factors, namely greater age, chronic hypertension, preeclampsia, gestational hypertension, and overweight pre- or during pregnancy, were convincingly associated with an increased risk of ASD. Selective serotonin reuptake inhibitor (SSRI) exposure during pregnancy was also convincingly associated with increased risk of ASD, but confounding from underlying maternal psychiatric disorder is possible. Evidence from biomarkers was limited, supported by few cases and p value close to significance threshold.

Implications of all the available evidence

Our findings suggest that offspring of mothers who are older, having certain metabolic syndromes, and perhaps under psychiatric disorders are at higher risk of developing ASD. While this does not imply that the other environmental risk factors and biomarkers are not

meaningful, there is still some uncertainty in them that should be resolved**.** Well-designed prospective cohort studies are needed to draw firmer conclusions.

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Figure legends

Fig 1. Flow chart of literature searches

Fig 2. Summary estimate of random effects meta-analysis of environmental risk factors versus standard error. The Y-axis labelled “Standard error” represents the standard error of random effects summary estimate of each meta-analysis. The X-axis labelled “ Summary estimate under random effects model (log scale)” represents the the summary estimate under random effects of each meta-analysis, presented in log scale. The three outliers having summary estimate>5 are associations of autism spectrum disorder with congenital cytomegalovirus infection, hearing impairment, and visual impairment. These studies were not funded by industry nor did the authors declare any conflict of interest.

Fig 3. Log (effect size of the largest study) versus log (summary effect under random effects model) for each meta-analysis of environmental risk factors. The Y-axis labelled “Log (effect size of the largest study)” represents the log of the effect estimate of the largest component study (study with smallest standard deviation) of each meta-analysis. The X-axis labelled “Log (summary estimate under random effects model)” represents the log of the summary effect estimate under random effects of each meta-analysis. The three outliers having log of the summary estimate>2 are associations of autism spectrum disorder with congenital cytomegalovirus infection, hearing impairment, and visual impairment. These studies were not funded by industry nor did the authors declare any conflict of interest.