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

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Lancet Psychiatry 2019; 6: 590–600

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Summary

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

Methods We did an umbrella review and systematically appraised the relevant meta-analyses of observational studies. We searched PubMed, Embase, and the Cochrane Database of Systematic Reviews for papers published between database inception and Oct 17, 2018, and screened the reference list of relevant articles. We obtained the summary effect, 95% CI, heterogeneity, and 95% prediction intervals. We examined small study effects and excess significance. We did analyses under credibility ceilings. This review is registered with PROSPERO, number CRD42018091704.

Findings 46 eligible articles yielded data on 67 environmental risk factors (544 212 cases, 81 708 787 individuals) and 52 biomarkers (15 614 cases, 15 433 controls). Evidence of association was convincing for maternal age of 35 years or over (relative risk [RR] 1·31, 95% CI 1·18–1·45), maternal chronic hypertension (odds ratio [OR] 1·48, 1·29–1·70), maternal gestational hypertension (OR 1·37, 1·21–1·54), maternal overweight before or during pregnancy (RR 1·28, 1·19–1·36), pre-eclampsia (RR 1·32, 1·20–1·45), prepregnancy maternal antidepressant use (RR 1·48, 1·29–1·71), and maternal selective serotonin reuptake inhibitor (SSRI) use during pregnancy (OR 1·84, 1·60–2·11). Only two associations, maternal overweight before or during pregnancy and SSRI use during pregnancy, retained their high level of evidence under subset sensitivity analyses. Evidence from biomarkers was scarce, being supported by p values close to the significance threshold and too few cases.

Interpretation Convincing evidence suggests that maternal factors, such as age and features of metabolic syndrome, are associated with risk of autism spectrum disorder. Although SSRI use during pregnancy was also associated with such risk when exposed and non-exposed groups were compared, this association could be affected by other confounding factors, considering that prepregnancy maternal antidepressant use was also convincingly associated with higher risk of autism spectrum disorder. Findings from previous studies suggest that one possible confounding factor is underlying maternal psychiatric disorders.

Funding None.

Introduction

Autism spectrum disorder is a leading cause of disability in children, and often requires high levels of support, which is costly for society and places a substantial economic, emotional, and physical burden on affected families.[1–4] The prevalence of autism spectrum disorder was estimated to be 2·47% in US children and adolescents in 2014–16[5] and 7·6 per 1000 individuals globally in 2010, when it accounted for 111 disability-adjusted life-years per 100 000 global population.[2]

Given the scarcity of clinical and epidemiological evidence of remission in autism spectrum disorder,[2] numerous investigations are focused on better understanding and advancing risk prediction and prevention of the disorder. The cause of autism spectrum disorder is multifactorial, with various genetic predispositions and environmental risk factors having been associated with an increased risk of autism spectrum disorder.[6–11] Advances have been made in the knowledge of genetic causes of autism spectrum disorder; however, the exact genes have not yet been elucidated. In addition, the results on associations of various kinds of environmental factors for autism spectrum disorder have been inconsistent, hierarchies of evidence have not been determined across different factors, and it is unknown whether these risk factors are prone to bias.

Cohort and case-control studies have reported various types of environmental risk factors or biomarkers of autism spectrum disorder, and these have been meta-analysed by combining the results of multiple studies.[12] However, these analyses are usually restricted to one topic, and assessment of various kinds of bias in the literature is often insufficient. Claimed significant associations are susceptible to bias such as publication bias, reporting bias, and residual confounding bias, resulting in false positives[13] or inflated estimates[14] of the

Research in context

Evidence before this study

We searched PubMed, Embase, and Cochrane Database of Systematic Reviews between database inception and Oct 17, 2018, for meta-analyses of observational studies studying any environmental risk factors and biomarkers of autism spectrum disorder, without language limitations, using the following search terms: "autis*", "Asperge*", and "meta-analysis." Our search showed that numerous risk factors and biomarkers were associated with risk of autism spectrum disorder 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 the literature. One systematic review by Modabbernia and colleagues has comprehensively identified and analysed possible environmental risk factors of autism spectrum disorder and concluded that birth complications accompanied by trauma or ischemia and hypoxia have strong associations with the prevalence of autism spectrum disorder, but overall, quantitative analysis was scarce and bias assessment was incomplete owing to its reliance on previous reports. To overcome these limitations, we did an umbrella review of meta-analyses. We did various tests of bias assessment and applied criteria for determining the level of credibility of the association.

Added value of this study

We identified and analysed 119 unique associations of environmental risk factors or biomarkers with risk of autism spectrum disorder. Among these, only maternal factors, namely advanced age, chronic hypertension, pre-eclampsia, gestational hypertension, and overweight before or during pregnancy, were convincingly associated with an increased risk of autism spectrum disorder. Selective serotonin reuptake inhibitor use during pregnancy was also convincingly associated with an increased risk of autism spectrum disorder, but confounding from underlying maternal psychiatric disorder is possible. Evidence from biomarkers was scarce, supported by few cases and p values close to the significance threshold.

Implications of all the available evidence

Our findings suggest that offspring of mothers who are older, have features of the metabolic syndrome, and might have psychiatric disorders are at an increased risk of developing autism spectrum disorder. Although this finding does not imply that the other environmental risk factors and biomarkers we examined are not meaningful, there is still some uncertainty in their associations that should be resolved. Well-designed prospective cohort studies are needed to draw firmer conclusions.

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association. Such problems have resulted in an excess of significant associations (p<0·05) in psychological science and other medical fields.[15,16] One systematic overview[7] has comprehensively identified and analysed possible environmental risk factors of autism spectrum disorder. Although this review was informative, the quantitative assessment of bias was incomplete because it relied on reports from the original studies, and definite criteria for determining the credibility of the associations were absent. To overcome these limitations, we did an umbrella review of the relevant meta-analyses. We aimed to generate a hierarchy of evidence and examine the true association of the suggested environmental risk factors and biomarkers with autism spectrum disorder.

Methods

Literature search strategy and eligibility criteria

Three investigators (JYK, MJS, and CYS) searched PubMed, Embase, and the Cochrane Database of Systematic Reviews for papers published between database inception and Oct 17, 2018, using the search terms (Asperge* [All Fields] OR autis* [All Fields]) AND meta [All Fields]. We chose eligible articles by consecutively examining the titles, abstracts, and then the full-text (figure 1). We manually searched the references of the relevant articles and attempted to identify and include eligible studies. Disagreements were resolved via discussion between JYK, MJS, CYS, and JIS.

We included meta-analyses of observational studies examining associations between autism spectrum disorder and potential environmental risk factors or biomarkers. The definition of autism spectrum disorder followed that of the original meta-analysis, whereas risk factors and biomarkers were defined according to WHO.[17,18] Biomarkers were defined as any substance, structure, or process that can be measured in the body or its products that can influence or predict the incidence of outcome or disease.[17] Risk factors were defined as any attribute, characteristic, or exposure of an individual that increases the likelihood of developing a disease or injury.[18]

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.

This review is registered with PROSPERO, number CRD42018091704, and the protocol is available online.[19]

Data extraction

From each meta-analysis, we extracted the first author, publication year, risk factor or biomarker of interest, number of autism spectrum disorder cases and all participants, maximally adjusted individual study estimates and corresponding 95% CI, metrics used for analyses—such as mean difference, Hedges' *g*, Cohen's *d*,

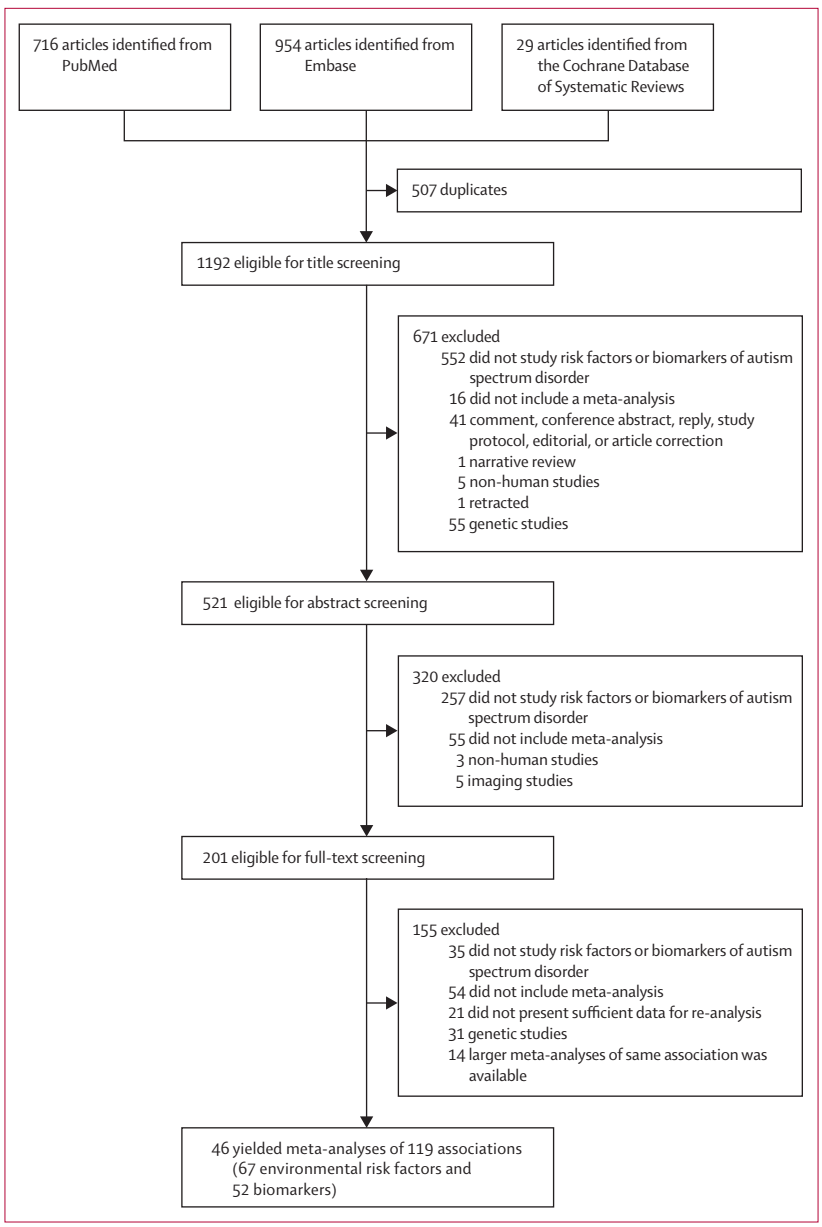

Figure 1: **Literature searches**

See Online for appendix

odds ratio (OR), or relative risk (RR)—and individual study designs (ie, cohort design or case-control design).

Data analysis

We used a series of statistical tests that were developed and reproduced in previous umbrella reviews.[20–25] We reanalysed 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 models. The level of significance was set at p<0·05. We further assessed p values below two thresholds: 10^{-3} and 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 SE) was concordant in terms of statistical significance.[20–22,24,25] To evaluate heterogeneity, we did a Cochran's Q test and calculated the I^2 statistic.[28,29] We estimated the 95% prediction interval, which is the range in which we expect the effect of the association will lie for 95% of similar studies in the future.[30] Prediction intervals excluding the null value (1 in the case of RRs or ORs) suggest that the association is likely to persist in a future study.[30] We assessed the presence of small study effects (ie, large studies having significantly more conservative results than smaller studies) using the regression asymmetry test proposed by Egger and colleagues.[31] For significant random effects meta-analysis, we used the test for excess significance bias, which evaluates whether the observed number of nominally statistically significant studies (p<0·05) is too large compared with the expected number of such studies.[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 given in the appendix (p 2). All statistical tests were two sided. We used Comprehensive Meta-analysis (version 3.3.070; Borenstein, NH, USA), RStudio (version 1.1.453), and R packages metafor (version 2.0–0) and pwr (version 1.2–2).[36–38]

Determining the credibility of evidence

In accordance with previous umbrella reviews,[20–25,39,40] we categorised the strength of the evidence of biomarkers or environmental risk factors for autism spectrum disorder into five levels: convincing (class I), highly suggestive (class II), suggestive (class III), weak (class IV), and not significant. Criteria for the level of evidence were p values under random effects model, number of autism spectrum disorder cases, the statistical significance of the largest study, heterogeneity presented as I^2, small study effects, excess significance bias, random effects summary estimate under 10% credibility ceiling, and 95% prediction interval. For associations classified as convincing or highly suggestive evidence, we did four types of subset analyses to confirm the robustness of the associations. We did subset analyses restricted to prospective cohort studies, cohort studies, study estimates adjusted for one or more covariates, or component studies that ascertained cases of autism spectrum disorder using diagnostic methods in line with DSM-III/IV/5 or ICD-8/9/10 (which are robust measures compared with other methods, such as self reporting). For any associations based on both cohort studies and case-control studies, we also did subgroup analyses, and assessed whether heterogeneity between the effect of the two subgroups was significant (p<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 autism spectrum

	Grading criteria	Risk factors
Convincing evidence (class I)	$p<1\times10^{-6}$ under random effects; >1000 cases of autism spectrum disorder; p<0·05 of the largest study in meta-analysis; no large heterogeneity; no signs of small study effects; no signs of excess significance bias; retained statistical significance in 10% credibility ceiling; and 95% prediction interval excludes the null value	Maternal age ≥35 years *vs* 25–29 years; maternal chronic hypertension; maternal gestational hypertension; maternal overweight prepregnancy or during pregnancy; maternal pre-eclampsia; prepregnancy maternal antidepressant use *vs* unexposed group; and SSRI use during pregnancy
Highly suggestive evidence (class II)	$p<1\times10^{-6}$ under random effects; >1000 cases of autism spectrum disorder; and p<0·05 of the largest study in meta-analysis	Highest maternal age group *vs* reference group; maternal age 30–34 years *vs* 25–29 years; maternal autoimmune disease; acetaminophen use 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
Suggestive evidence (class III)	$p<1\times10^{-3}$ under random effects and >1000 cases of autism spectrum disorder	Family history of any autoimmune diseases; family history of psoriasis; family history of rheumatoid arthritis; family history of type 1 diabetes; 5-min Apgar score <7; hearing impairment; higher maternal age, per 10-year increase; maternal diabetes (any); maternal infection requiring admission to hospital; paternal age 35–40 years *vs* reference group; and reference group *vs* lowest paternal age group
Weak evidence (class IV)	p<0·05 under random effects	Mercury prenatal or postnatal exposure, by highest dose reported; NO_2 exposure after birth, per 10 ppb increase; O_3 exposure during pregnancy, per 10 ppb increase; O_3 exposure during the third trimester, per 10 ppb increase; PM_{10} prenatal or postnatal exposure, per 10 μg/m³ increase; $PM_{2·5}$ exposure after birth, per 10 μg/m³ increase; $PM_{2·5}$ prenatal or postnatal exposure, per 10 μg/m³ increase; family history of hypothyroidism; congenital cytomegalovirus infection; extremely low birthweight; neonatal jaundice; O_2 treatment; visual impairment; maternal age 25–29 years *vs* <20 years; maternal autoimmune disease developed during pregnancy; maternal infection during pregnancy; maternal obesity during pregnancy; maternal obesity prepregnancy; maternal psychiatric disorder without SSRI use; antidepressant use during pregnancy; use of assisted reproductive technology; birth by caesarean section; paternal age ≤35 years *vs* reference group*; and thimerosal exposure during embryo or early infancy†
Not significant	p>0·05 under random effects	NO_2 exposure during pregnancy, per 10 ppb increase; O_3 exposure after birth, per 10 ppb increase; PM_{10} exposure after birth, per 10 μg/m³ increase; PM_{10} exposure during pregnancy, per 10 μg/m³ increase; $PM_{2·5}$ exposure during pregnancy, per 10 μg/m³ increase; neonatal acidosis; reference group *vs* lowest maternal age group; maternal autoimmune thyroid disease; maternal underweight prepregnancy or during pregnancy; antidepressant use during pregnancy *vs* unexposed group with a history of affective disorder; SSRI discontinuation until 3 months before pregnancy *vs* unexposed group; maternal smoking during pregnancy; mercury exposure by vaccination; measles, mumps, and rubella vaccination; and thimerosal exposure

Factors listed are associated with increased risk of autism spectrum disorder, except the not significant row. For factors shown as a comparison (ie, *x vs y*), the former (ie, *x*) is associated with increased risk of autism spectrum disorder compared with the latter (ie, *y*). Only two factors, maternal breastfeeding and maternal folic acid supplement during pregnancy, were associated with decreased risk of autism spectrum disorder (not shown in this table). Both were graded as weak evidence (class IV). Heterogeneity was assessed in terms of Cochran's Q test and large heterogeneity was defined as an I^2 statistic of >50%. Small study effects were assessed by Egger's asymmetry test and were claimed at an Egger p value of <0·1 with estimate of the largest component study more conservative than summary estimate under random effects model. Excess significance bias was claimed at p<0·1, with the observed number of significant studies larger than the expected number of significant studies. All statistical tests are two-sided. PM_{10}=particulate matter with a diameter of <10 μm. $PM_{2·5}$=particulate matter with a diameter of <2·5 μm. SSRI=selective serotonin reuptake inhibitor. *The paternal age ≤35 years group has advanced age compared with the reference group. †The meta-analysis supporting the association included studies by Geier and colleagues, for which concerns have been raised about the objective nature of the study population. When study estimates from Geier and colleagues were excluded, the random effects summary estimate of the association changed to 0·98 (0·89–1·07), suggesting no association between thimerosal exposure during embryo and early infancy with autism spectrum disorder.

Table 1: **Summary of level of evidence for associations between environmental factors and risk of autism spectrum disorder**

disorder 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 of the study data and the corresponding authors had the final responsibility for the decision to submit for publication.

Results

1699 potentially eligible articles were identified by our initial search (figure 1). During the screening process, 14 articles were excluded because larger meta-analyses were available (appendix pp 3–4). The eligible 46 articles[12,41–85] yielded 119 associations (67 environmental risk factors and 52 biomarkers). 67 associations of environmental risk factors with autism spectrum disorder were based on data of 544 212 cases and a population of 81 708 787 (tables 1, 2; appendix pp 5–13). 42 (63%) of 67 associations 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 eight (range 2–24). The median number of cases was 3764 and the median total population was 502 843.

52 (78%) of 67 associations were significant under the random effects model, of which 33 (49%) were $p<1\times10^{-3}$ and 18 (27%) were $p<1\times10^{-6}$. 52 (78%) associations had more than 1000 autism spectrum disorder cases, of which 16 were $p<1\times10^{-6}$. Of 52 significant associations, 40 were also supported by the significant result of the largest component study. Metrics were consistent with those of the original meta-analyses except for one association (extremely low birthweight *vs* normal birthweight), where we converted Cohen's *d* to OR; ultimately, we used either RR or OR for all associations. Effect size was smaller than 2 for all but seven associations, of which three (congenital cytomegalovirus

	Author (year)	Number of cases/total population	Number of study estimates	Study design	Effect metrics	Random effects summary estimate (95% CI)	Random effects p value	I^2 (%)	95% prediction interval	Egger p value	Large heterogeneity, small study effect, excess significance bias, or loss of significance under 10% credibility ceiling
Convincing evidence (class I)											
Maternal age ≥35 years vs 25–29 years	Sandin and colleagues (2012)[70]	>1000/NR	11	Cohort, case-control	RR	1·31 (1·18–1·45)	$1·3\times10^{-7}$	20%	1·07–1·6	0·13	None
Maternal chronic hypertension	Xu and colleagues (2018)[81]	22 864/5 994 614	4	Cohort, case-control	OR	1·48 (1·29–1·7)	$2·3\times10^{-8}$	0%	1·1–2·01	0·15	None
Maternal gestational hypertension	Xu and colleagues (2018)[81]	4334/220 713	9	Cohort, case-control	OR	1·37 (1·21–1·54)	$3·2\times10^{-7}$	0%	1·18–1·58	0·67	None
Maternal overweight prepregnancy or during pregnancy	Wang and colleagues (2016)[78]	7872/508 101	5	Cohort	RR	1·28 (1·19–1·36)	$2·2\times10^{-12}$	0%	1·14–1·42	0·14	None
Maternal pre-eclampsia	Dachew and colleagues (2018)[45]	10 699/1 166 307	10	Cohort, case-control	RR	1·32 (1·2–1·45)	$4·9\times10^{-9}$	27%	1·07–1·63	0·80	None
Prepregnancy maternal antidepressant use vs unexposed group	Morales and colleagues (2018)[64]	22 877/2 400 720	7	Cohort, case-control	RR	1·48 (1·29–1·71)	$6·8\times10^{-8}$	24%	1·09–2·02	0·59	None
Selective serotonin reuptake inhibitor use during pregnancy	Andalib and colleagues (2017)[42]	19 670/1 504 264	7	Cohort, case-control	OR	1·84 (1·6–2·11)	$1·2\times10^{-17}$	0%	1·53–2·2	0·78	None
Highly suggestive evidence (class II)											
Highest maternal age group vs reference group	Wu and colleagues (2017)[80]	2254/419 361	19	Cohort, case-control	OR	1·42 (1·29–1·55)	$5·4\times10^{-14}$	59%	1·07–1·86	0·07	Large heterogeneity*
Maternal age 30–34 vs 25–29 years	Sandin and colleagues (2012)[70]	>1000/NR	8	Cohort, case-control	RR	1·14 (1·09–1·18)	$5·5\times10^{-10}$	0%	1·08–1·2	0·41	Loss of significance under 10% credibility ceiling
Maternal autoimmune disease	Chen and colleagues (2016)[44]	9775/961 986	10	Cohort, case-control	OR	1·37 (1·21–1·54)	$6·4\times10^{-7}$	28%	1·05–1·77	0·08	Small study effect
Acetaminophen use during pregnancy	Masarwa and colleagues (2018)[59]	>1000/129 961	5	Cohort	RR	1·2 (1·14–1·26)	$4·3\times10^{-12}$	18%	1·06–1·35	0·05	Small study effect
Higher paternal age, per 10-year increase	Wu and colleagues (2017)[80]	47 373/13 678 773	17	Cohort, case-control	OR	1·21 (1·18–1·24)	$7·8\times10^{-49}$	11%	1·16–1·26	0·07	Small study effect*
Highest paternal age group vs reference group	Wu and colleagues (2017)[80]	2920/539 252	20	Cohort, case-control	OR	1·55 (1·39–1·73)	$8·5\times10^{-15}$	63%	1·09–2·2	0·01	Large heterogeneity and small study effect*
Paternal age >45 years vs reference group	Oldereid and colleagues (2018)[66]	>1000/NR	18	Cohort, case-control	OR	1·43 (1·33–1·53)	$8·0\times10^{-25}$	74%	1·15–1·77	0·01	Large heterogeneity and small study effect
Paternal age 40–45 years vs reference group	Oldereid and colleagues (2018)[66]	>1000/NR	12	Cohort, case-control	OR	1·37 (1·23–1·53)	$1·1\times10^{-8}$	82%	0·99–1·91	0·02	Large heterogeneity and small study effect*

Criteria for convincing evidence (class I) were as follows: $p<1\times10^{-6}$ under random effects, more than 1000 cases of autism spectrum disorder, p<0·05 of the largest study in the meta-analysis, no large heterogeneity, no signs of small study effects, no sign of excess significance bias, retained statistical significance under 10% credibility ceiling, and 95% prediction interval excludes the null value. Criteria for highly suggestive evidence (class II) were as follows: $p<1\times10^{-6}$ under random effects, >1000 cases of autism spectrum disorder, and p<0·05 of the largest study in the meta-analysis. Heterogeneity was assessed in terms of Cochran's Q test and large heterogeneity was defined as an I^2 statistic of >50%. We assessed small study effects via Egger's asymmetry test and such effects were claimed at an Egger p value of <0·1, with the estimate of the largest component study being more conservative than summary estimate under random effects model. Excess significance bias was claimed at p<0·1 with the observed number of statistically significant studies larger than the expected number of significant studies. All statistical tests are two-sided. NR=not reported. OR=odds ratio. RR=relative risk . *Presence of excess significance bias could not be assessed because necessary data were not reported.

Table 2: **Association of environmental factors with risk of autism spectrum disorder graded convincing evidence (class I) or highly suggestive evidence (class II)**

infection, hearing impairment, visual impairment) had an summary estimate greater than 10. Only two factors (folic acid supplementation during pregnancy and breastfeeding) were associated with decreased risk of autism spectrum disorder.

31 (46%) of 67 associations showed large heterogeneity (I^2>50%). 24 (36%) significant associations had neither small study effects nor excess significance bias. 95% prediction intervals excluded the null value in only 19 (28%) of the associations. Effect sizes of meta-analyses showed a trend toward the 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, although 52 (78%) associations were significant, 41 (61%) retained statistical significance under 5% credibility ceilings, compared with 30 (45%) for 10%, 18 (27%) for 15%, and 10 (15%) for 20%.

Seven (10%) of 67 associations were graded as convincing evidence (tables 1, 2). The risk factors with convincing associations were maternal age of 35 years or older versus 25–29 years (RR 1·31, 95% CI 1·18–1·45), maternal chronic hypertension (OR 1·48, 1·29–1·70), maternal gestational hypertension (OR 1·37, 1·21–1·54), maternal overweight before or during pregnancy (RR 1·28, 1·19–1·36), pre-eclampsia (RR 1·32, 1·20–1·45), prepregnancy maternal antidepressant use (RR 1·48, 1·29–1·71), and selective serotonin reuptake inhibitor (SSRI) use during pregnancy (OR 1·84, 1·60–2·11). Eight (12%) associations were graded as highly suggestive evidence (tables 1, 2). 11 (16%) associations were graded as suggestive evidence (appendix p 5), 26 (39%) were graded as weak evidence (appendix pp 6–7), and the remaining 15 (22%) did not show statistically significant associations (appendix pp 8–9).

52 associations of biomarkers comprised 15 614 cases and 15 433 controls (panel, appendix pp 14–20). Of 52 meta-analyses of biomarkers, 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 was 228 and the median number of controls was 216. 27 (52%) associations were significant, and ten (19%) associations were $p<1\times10^{-3}$. Moreover, only three associations—brain-derived neurotrophic factor in blood, mercury in hair, and mercury in whole blood—were supported by a population with more than 1000 autism spectrum disorder cases. No associations were based on more than 1000 cases and $p<1\times10^{-3}$. Thus, for its level of evidence, no biomarker association was graded as suggestive (class III) or above. Of 27 statistically significant associations, only 14 were also supported by a significant result of the largest component study. 44 associations (85%) had large heterogeneity (I^2>50%), of which 36 (69%) associations had very large heterogeneity (I^2>75%). Only 11 (21%) associations retained statistical significance under 10% credibility ceilings. 95% prediction intervals excluded the null value in only one association (ratio of index finger length to ring finger length [D2:D4 ratio]). Detailed results are shown in the appendix (pp 14–20).

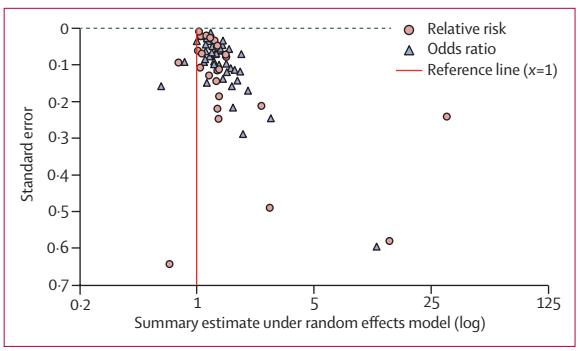

Figure 2: **Summary estimate of random effects meta-analysis of environmental risk factors *vs* SE**
The three outliers with summary estimates of greater than 5 are associations of autism spectrum disorder with congenital cytomegalovirus infection, hearing impairment, and visual impairment.

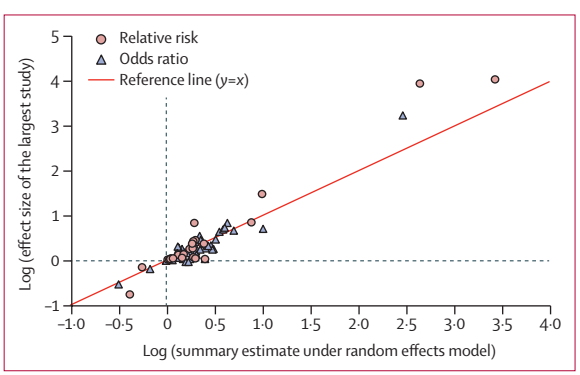

Figure 3: **Effect size of the largest study *vs* summary effect under random effects model for each meta-analysis of environmental risk factors**
The three outliers with a log of the summary estimate that is greater than 2 are associations of autism spectrum disorder with congenital cytomegalovirus infection, hearing impairment, and visual impairment.

We did a sensitivity subset analyses on meta-analyses of 15 environmental risk factors graded as convincing (class I) or highly suggestive (class II) evidence. Subset analysis restricted to cohort studies (prospective or retrospective) showed that only two associations of class I remained at the same rank (appendix p 21). These were maternal overweight before or during pregnancy and maternal pre-eclampsia. Three associations (SSRI use during pregnancy, acetaminophen use during pregnancy, and paternal age >45 years *vs* reference group) remained as highly suggestive evidence. When subset analysis was restricted to only prospective cohort studies, no convincing association was seen, and only two associations (maternal overweight before or during pregnancy and SSRI use during pregnancy) were still graded as highly suggestive evidence (appendix p 22).

In subset analyses of adjusted study estimates, the association of maternal pre-eclampsia with autism

***Panel*: Summary of associations between biomarkers and risk of autism spectrum disorder**

High biomarker level associated with higher risk (class IV)
5-HT in platelet-rich plasma; 5-HT in whole blood; brain-derived neurotrophic factor in blood; lead in erythrocyte; glutamate in blood;* oxidised glutathione in plasma; mercury in brain; mercury in erythrocyte; mercury in red blood cell;* mercury in whole blood; interferon-γ in plasma and serum;* IL-1β in plasma and serum;* IL-6 in plasma and serum; ratio of n-6 LCPUFA to n-3 LCPUFA;* lead in blood and plasma; lead in hair; and antimony in hair.

Low biomarker level associated with higher risk (class IV)
25-OH vitamin D in serum, ng/ml; arachidonic acid in various blood samples; docosahexaenoic acid in various blood samples; ratio of index finger length to ring finger length; eicosapentaenoic acid in children aged younger than 12 years; gamma-aminobutyric acid in brain; oxytocin in saliva; transforming growth factor β1 in plasma and serum;* zinc in plasma; and zinc in plasma, hair, nail, and teeth.

Not significant
5-HT in platelet-poor plasma; ratio of arachidonic acid to docosahexaenoic acid; ratio of arachidonic acid to eicosapentaenoic acid; arsenic in blood;* arsenic in hair; arsenic in urine; cadmium in urine; eicosapentaenoic acid in various blood samples; mercury in hair, mg/g; mercury in hair, ppm; mercury per creatinine in urine, mg/g; homocysteine in plasma; IL-23 in plasma and serum; manganese in blood, plasma, and serum; manganese in hair; nickel in hair; oxytocin in plasma; tumour necrosis factor α in plasma and serum; total n-3 LCPUFA; total n-6 LCPUFA;* vasopressin in plasma; zinc in hair; ratio of zinc to copper in hair;* ratio of zinc to copper in hair, nail, and plasma;* and ratio of zinc to copper in plasma.

Biomarkers for autism spectrum disorder were classified as either weak evidence (class IV) or not significant. No biomarker for autism spectrum disorder was graded as suggestive (class III) or higher level of evidence. Criteria for suggestive evidence (class III) were $p<1\times10^{-3}$ under random effects and >1000 cases of autism spectrum disorder. Criteria for weak evidence (class IV) was p<0·05 under random effects. Criteria for not significant evidence was p>0·05 under random effects. All statistical tests are two-sided. 5-HT=5-hydroxytryptamine. IL=interleukin. LCPUFA=long-chain polyunsaturated fatty acids. *Associations where small study effects were claimed.

spectrum disorder was downgraded to suggestive evidence; whereas the association of maternal autoimmune disease with autism spectrum disorder was upgraded from highly suggestive evidence to convincing evidence (appendix p 23). In subset analyses restricted to component studies that used diagnostic methods in line with DSM III–5 or ICD-8/9/10, only two associations—maternal gestational hypertension and maternal autoimmune disease—were downgraded to suggestive evidence (appendix p 24).

46 associations were eligible for subgroup analyses of cohort studies and case-control studies (appendix pp 25–26). Notably, in all nine class I or II associations that had their level of evidence downgraded in subset analyses of cohort studies (appendix p 21), 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 (appendix pp 27–28). Separate meta-analyses by these factors were not feasible because of a scarcity of study estimates.

Discussion

This umbrella review is the first to quantitively appraise the environmental risk factors and biomarkers of autism spectrum disorder. We evaluated associations of autism spectrum disorder with 119 possible risk factors and biomarkers. Our analysis showed that associations with convincing evidence (class I) were either maternal factors, such as age and features of metabolic syndrome, or use of antidepressants, such as SSRIs. The associations of autism spectrum disorder with higher paternal age, maternal autoimmune disease, and acetaminophen use 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 before or during pregnancy and SSRI use during pregnancy—remained as convincing or highly suggestive evidence after several sensitivity subset analyses. However, these results should be interpreted with caution, because the statistical methods and bias tests applied are not infallible and the criteria are based on arbitrary thresholds, although they have been used in umbrella reviews of meta-analyses.[20–25]

In our study, components of a maternal metabolic syndrome—ie, chronic hypertension, gestational hypertension, pre-eclampsia, and overweight—were associated with increased risk of autism spectrum disorder in offspring, which were all graded as having convincing evidence. One possible underlying mechanism is fetal programming, a concept that maternal factors (such as inflammation and chronic stress) can alter the gestational environment and determine long-term fetal outcomes.[86,87] Metabolic syndromes are often characterised by chronic low-grade inflammation and insulin resistance. The metabolic and immune systems share common signalling pathways.[88] Although the role of an aberrant immune system function in the development of autism spectrum disorder is speculative, evidence exists on the deleterious role of maternal immune system dysregulation on the development of autism spectrum disorder.[89] Several studies[90,91] have shown that maternal autoantibodies that recognise proteins in the developing fetal brain could cause autism spectrum disorder in offspring of mothers with metabolic syndromes. Although prevalence of diabetes (type 2 or gestational) and hypertension was higher in mothers whose children had autism spectrum disorder

compared with mothers whose children showed typical development,[92] in children with severe autism spectrum disorder, autism spectrum disorder-specific autoantibodies were significantly more prevalent in mothers with diabetes (type 2 or gestational) and mothers who were moderately overweight compared with mothers without these conditions.[91] Jones and colleagues[90] showed that autism spectrum disorder-specific antigen-induced maternal autoantibodies altered various autism spectrum disorder-relevant behaviours in mice. Therefore, one hypothesis is that metabolic syndrome could contribute to the production of autism spectrum disorder-specific maternal autoantibodies through the breakdown of maternal immune tolerance, which can increase the risk of developing autism spectrum disorder in the offspring.

Convincing evidence showed that maternal age, when the comparison is restricted to age groups of 35 years or older versus 25–29 years, was associated with an increased risk of autism spectrum disorder. Accumulation of mutations, high rate of complications, and increased chance of exposure to medications or pollutions could underlie the increased risk of autism spectrum disorder in the advanced maternal age group.[80] Advanced paternal age was also associated with an increased incidence of autism spectrum disorder. 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—showed sufficiently low p values ($<1\times10^{-6}$) and 95% prediction intervals excluded the null value despite high heterogeneity and presence of small study effects. In two of the comparisons (paternal age >45 years and paternal age 40–45 years *vs* reference group), subset analyses of prospective studies showed p values of less than 1×10^{-3} with no evidence of small study effects, indicating the existence of meaningful associations. An increased rate of de novo mutations[93] and epigenetic alternation[80] could underlie the association.

Convincing evidence showed that maternal SSRI use during pregnancy was associated with a higher risk of autism spectrum disorder than that of unexposed groups. However, this association must be interpreted carefully. In another meta-analysis, when maternal groups with prepregnancy antidepressant exposure were compared with unexposed maternal groups, the association with autism spectrum disorder was also graded as convincing evidence. This finding 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-analyses[53,64] have attempted to discern between the two possible causes of autism spectrum disorder. When maternal groups with a psychiatric disorder but no SSRI use during pregnancy were compared with maternal groups without a psychiatric disorder and also not exposed to SSRIs during pregnancy, an increased incidence of autism spectrum disorder was seen in the former group (OR 1·81, 95% CI 1·44–2·29), supporting the idea that presence of a maternal psychiatric condition is an independent risk factor for autism spectrum disorder.[53] When SSRI-exposed groups were compared with SSRI-unexposed groups with a history of affective disorder (a setting in which the possibility of confounding by psychiatric disorder is minimised), the association with autism spectrum disorder was not significant (RR 1·18, 95% CI 0·91–1·52).[64] Analyses restricted to sibling studies also showed a non-significant association (RR 0·96, 95% CI 0·65–1·42).[64] Notably, when individuals with paternal antidepressant exposure during the maternal pregnancy period were compared with an unexposed group, the former group had an increased risk of autism spectrum disorder (RR 1·29, 95% CI 1·08–1·53).[64] Overall, these findings suggest that maternal psychiatric disorder might act as an independent risk factor for autism spectrum disorder and the association between SSRI use during pregnancy and autism spectrum disorder needs to be further verified in adequately designed future studies.

Maternal autoimmune disease was associated with an increased risk of autism spectrum disorder, graded as having a highly suggestive association, with the 95% prediction interval excluding the null value. In mothers with autoimmune diseases, immune response mediators and autoantibodies might play a role in fetal neurodevelopment, resulting in adverse fetal outcomes such as autism spectrum disorder. Family history of psoriasis, rheumatoid arthritis, type 1 diabetes, or any type of autoimmune disease was also associated with an increased risk of autism spectrum disorder, graded as suggestive evidence. Potential links between the production of autism spectrum disorder-specific brain-reactive maternal autoantibodies and maternal autoimmunity might underlie this association (appendix p 29).[94,95] However, in the identified meta-analyses, small study effects existed across the associations; therefore, more well conducted studies are needed to confirm the association between maternal autoimmune disease and autism spectrum disorder.

We identified and analysed 52 biomarkers for autism spectrum disorder. Identifying robust evidence of biomarkers for autism spectrum disorder can result in early diagnosis and better treatment of the disease.[96] The association of the D2:D4 ratio with a risk of autism spectrum disorder was supported by $p<1\times10^{-6}$ without any signs of bias, meeting the criteria for convincing evidence—except for the number of autism spectrum disorder cases, being supported by 277 cases. However, most of the associations of biomarkers were supported by p values close to the significance threshold ($p>1\times10^{-3}$) and too few cases, implying the possibility of false positive findings. Similar findings were seen in umbrella reviews of biomarkers for other psychiatric disorders.[21,97,98]

Although our review provided more strict and objective criteria for assessing the associations compared with previous reviews (appendix p 29), our study has some

limitations. First, despite using the most rigorous criteria to assess the credibility of the associations developed and reproduced in previous studies,[20–25] we cannot be sure that we excluded all of the biases that are inherent to individual studies. In addition, biases that might have been caused by the respective characteristics of study design, diagnosis of autism spectrum disorder, genetic causes of autism spectrum disorder in the population, or gender effects, were not fully assessed in our study, partly owing 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 have method-related 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 the meta-analysis could be overestimated. When we reanalysed an eligible meta-analysis studying thimerosal exposure during embryo or early infancy[82] after excluding individual studies with a concern about the objective nature of the study population,[99,100] the level of association changed from class IV ($p<0·05$) to not significant ($p>0·05$). Likewise, the results of meta-analyses should always be interpreted with caution. Third, we could not assess environmental risk factors of autism spectrum disorder 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 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 not been studied in meta-analyses could have been excluded from our review. Fifth, because the possibility of genetic or environmental confounding cannot be ruled out in findings of observational studies, it might be hard to establish causations from some associations. What were classified as risk factors might actually act as biomarkers that predict the ascertainment of autism spectrum disorder, rather than being causal factors.

Despite these limitations, this umbrella review mapped the association of autism spectrum disorder with various environmental risk factors and biomarkers. Out of 119 identified associations, only several maternal factors—advanced age (≥35 years), chronic hypertension, pre-eclampsia, gestational hypertension, and overweight before or during pregnancy—were convincingly associated with autism spectrum disorder, without any signs of bias. SSRI use during pregnancy was also convincingly associated with the disorder, but confounding from underlying maternal psychiatric disorders is likely. We cannot state that other associations are not meaningful, but there remains some uncertainty in them that should be resolved. Further well designed studies with accurate assessment of potential biases are needed.

Contributors

JYK, MJS, CYS, and JIS designed the study. JYK, MJS, and CYS did the literature search and screening, extracted, analysed, and interpreted the data, and made the figures and tables; any discrepancies were resolved via discussion between JYK, MJS, CYS, JIS, and PFP. All authors drafted and critically revised the manuscript. All authors approved the final version of the manuscript for publication. JYK, MJS, and CYS contributed equally to the manuscript as joint first authors and JIS and PFP contributed as joint corresponding authors.

Declaration of interests

We declare no competing interests.

Acknowledgments

BS is supported by a clinical lectureship (ICA-CL-2017-03-001) jointly funded by Health Education England and the National Institute for Health Research (NIHR). BS is part funded by the NIHR Biomedical Research Centre at South London and Maudsley NHS Foundation Trust. BS is also supported by the Maudsley Charity, King's College London and the NIHR South London Collaboration for Leadership in Applied Health Research and Care funding. This paper presents independent research. The views expressed in this publication are those of the authors and not necessarily those of the acknowledged institutions.

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