Vaccines are not associated with autism: An evidence-based meta-analysis of case-control and cohort studies

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A B S T R A C T

There has been enormous debate regarding the possibility of a link between childhood vaccinations and the subsequent development of autism. This has in recent times become a major public health issue with vaccine preventable diseases increasing in the community due to the fear of a ‘link’ between vaccinations and autism. We performed a meta-analysis to summarise available evidence from case-control and cohort studies on this topic (MEDLINE, PubMed, EMBASE, Google Scholar up to April, 2014). Eligible studies assessed the relationship between vaccine administration and the subsequent development of autism or autism spectrum disorders (ASD). Two reviewers extracted data on study characteristics, methods, and outcomes. Disagreement was resolved by consensus with another author. Five cohort studies involving 1,256,407 children, and five case-control studies involving 9,920 children were included in this analysis. The cohort data revealed no relationship between vaccination and autism (OR: 0.99; 95% CI: 0.92 to 1.06) or ASD (OR: 0.91; 95% CI: 0.68 to 1.20), nor was there a relationship between autism and MMR (OR: 0.84; 95% CI: 0.70 to 1.01), or thimerosal (OR: 1.00; 95% CI: 0.77 to 1.31), or mercury (Hg) (OR: 1.00; 95% CI: 0.93 to 1.07). Similarly the case-control data found no evidence for increased risk of developing autism or ASD following MMR, Hg, or thimerosal exposure when grouped by condition (OR: 0.90, 95% CI: 0.83 to 0.98; p = 0.02) or grouped by exposure type (OR: 0.85, 95% CI: 0.76 to 0.95; p = 0.01). Findings of this meta-analysis suggest that vaccinations are not associated with the development of autism or autism spectrum disorder. Furthermore, the components of the vaccines (thimerosal or mercury) or multiple vaccines (MMR) are not associated with the development of autism or autism spectrum disorder.

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1. Introduction

Over the past several years much concern has been raised regarding the potential links of childhood vaccinations with the development of autism and autism spectrum disorders (ASD). The vaccinations that have received the most attention are the measles, mumps, rubella (MMR) vaccine and thimerosal-containing vaccines such as the diphtheria, tetanus, pertussis (DPT or DT) vaccine. A rising awareness of autism incidence, prevalence, and the postulated causation of childhood vaccinations has led to both an increased distrust in the trade-off between vaccine benefit outweighing potential risks and an opportunity for disease resurgence. This is especially concerning given the fact that the CDC reported 17 measles outbreaks in the U.S. in 2011 and NSW, Australia also saw a spike in its measles notifications from late 2011 to mid-July 2012 [1,2]. Vaccine-preventable diseases clearly still hold a presence in modern day society and the decision to opt out of MMR or other childhood vaccination schedules because of concerns regarding the development of autism should be properly evaluated with available evidence. To date there have been no quantitative data analysis pooling cohort and case-control studies that have assessed the relationship between autism, autistic spectrum disorder and childhood vaccinations.

This meta-analysis aims to quantitatively assess the available data from studies undertaken in various countries regarding autism rates and childhood vaccination so that the relationship between these two, whatever its significance, can be adequately substantiated.

2. Methods

2.1. Study protocol

We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to conduct our review and analysis [3,4]. The PRISMA guidelines have been
developed in an attempt to standardise reporting in systematic reviews and include a four-phase flow diagram as well as a checklist of 27 items deemed necessary for transparent reporting of results of meta-analyses. A systematic search of the databases Medline (from 1950), PubMed (from 1946), Embase (from 1949), and Google Scholar (from 1990) through to April 2014, to identify relevant articles was completed. The following combinations or search terms were used to search all databases: vaccine; immunise; immunisation; autism; autistic; Asperger; pervasive developmental disorder and PDD. The search strategy was peer reviewed by two independent experts prior to implementation. The reference lists of relevant articles were also searched for appropriate studies. No language restrictions were used in either the search or study selection. A search for unpublished literature was not performed.

2.2. Eligibility criteria

This review included retrospective and prospective cohort studies and case-control studies published in any language looking at the relationship between vaccination and disorders on the autistic spectrum. No limits were placed on publication date, publication status, or participant characteristics. Studies were included that looked at either MMR vaccination, cumulative mercury (Hg) or cumulative thimerosal dosage from vaccinations to ensure all proposed causes of ASD or regression were investigated. Outcome measures included development of any condition on the autistic spectrum as well as those specifically looking at regressive phenotype. Papers that recruited their cohort of participants solely from the Vaccine Adverse Event Reporting System (VAERS) in the United States were not included due to its many limitations and high risk of bias including unverified reports, underreporting, inconsistent data quality, absence of an unvaccinated control group and many reports being filed in connection with litigation [5,6]. We excluded studies that did not meet the inclusion criteria.

2.3. Study selection

Two authors (LT, AS) independently reviewed the abstracts and methods of returned results to assess for eligibility for inclusion. Disagreements between reviewers were resolved by consensus with the third author (GE).

2.4. Data collection process

Data was extracted manually by one author (LT) which was subsequently reviewed by another author (GE). Where data on multiple endpoints was available, the longest duration between exposure and measurement of outcome was used. Where data on multiple doses of mercury were available, the data used was that when the largest dose was given. Where data was provided adjusted for confounding variables, the result that was adjusted for the most variables was included. Duplicate publications were determined and excluded by juxtaposing authors’ names, sample sizes of treatment and control groups, and subsequent odds and risk ratios.

2.5. Data items

Information was extracted from each paper on (1) study design; (2) country of study; (3) sample sizes (including total number of participants, and number of participants in each treatment arm); (4) intervention (including type, dose and timing of vaccination); (5) outcome measure (including development of autistic disorder, other autism spectrum disorder, or autistic disorder with regression); (6) and measures of effect (including calculated odds and risk ratios and the confounding variables for which they were adjusted).

2.6. Risk of bias in individual studies

Risk of bias was assessed independently by two authors (LT, AS) using the appropriate Newcastle-Ottawa scale (NOS) [7] with disagreements resolved by consensus with the other author (GE). The NOS scale has three components assessing studies on participant selection, comparability, and outcome/exposure assessment. A study is awarded stars for items within each category for a maximum of nine stars. We decided to rate studies as low risk of bias if they received nine stars, moderate risk of bias if they received seven or eight stars, and high risk of bias if they received less.

2.7. Statistical analysis

Pooled odds ratios and 95% confidence intervals were calculated for the effect of vaccinations on the development of autism using a random effects model [8]. For both case-control and cohort studies, an overall pooled odds ratio was calculated. Subsequently we divided the data and performed subgroup analyses to investigate risk of developing either autism alone or ASD alone after MMR, Hg, or thimerosal exposure. In addition we performed subgroup analyses by exposure type investigating the individual likelihood of developing autism or ASD depending on whether the participants

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**Fig. 1.** Flowchart of search strategy.
had received the MMR vaccine, the measles vaccine alone, or had exposure to thimerosal or Hg.

We tested heterogeneity with Cochran’s Q statistic, with \( p < 0.10 \) indicating heterogeneity, and quantified the degree of heterogeneity using the \( I^2 \) statistic, which represents the percentage of the total variability across studies which is due to heterogeneity. \( I^2 \) values of 25, 50 and 75% corresponded to low, moderate and high degrees of heterogeneity, respectively [9]. We quantified publication bias using the Egger’s regression model where effect estimates are graphed against sample size and symmetry of the resultant funnel plot is assessed. This approach assumes that larger studies will produce results nearer the average and smaller studies will be spread on both sides of the average, which is useful to detect bias in meta-analyses that are later contradicted by large trials [10]. In addition, Rosenthal’s fail-safe number was calculated to assess publication bias, which calculates the number of additional ‘positive’ or ‘negative’ studies that would be required to change the outcome of the meta-analysis [11]. All analyses were performed with Comprehensive Meta-Analysis (version 2.0), Biostat, Englewood, NJ (2005).

3. Results

3.1. Study selection

The search of Medline, PubMed, and Embase returned 519, 718, and 1133 results, respectively. After adjusting for duplicates, 1112 papers in total remained, 953 were excluded immediately on inspection of the abstracts as they clearly did not meet inclusion criteria, leaving 159 papers whose methods sections were analysed in more detail to determine suitability. No unpublished relevant studies were obtained. Five additional papers were found on examination of relevant reference lists. A further 113 were identified as having no possible case-control or cohort data and were excluded, leaving 46 papers to which the inclusion criteria were applied (Fig. 1). A total of five case-control studies and five cohort studies were identified for inclusion in the review.

3.2. Study characteristics

All five cohort studies selected for inclusion were retrospective cohort studies published in English (Table 1). The total sample evaluated among these cohort studies consisted of 1,256,407 children. Two studies [12,13] had data looking specifically at MMR vaccination, two [14,15] had data specifically on cumulative Hg dosage, while one [16] had two data sets looking specifically at thimerosal exposure. All studies looked at the development of autism or other ASD among large populations as the defined outcome, with the exception of one [13] that investigated specifically the development of the regressive phenotype of autism compared to non-regressive autism.

The five case-control studies were published in English and investigated a total sample of 9920 children (Table 2). Four of the five studies had data specifically on MMR vaccination [17–21] and subsequent risk of autism or ASD, two of the five studies had data on the monovalent measles vaccine [18,20], and one study had three data sets investigating cumulative Hg/thimerosal exposure and subsequent risk of developing autism, ASD, or autism with regressive phenotype [22].

3.3. Risk of bias within studies

3.3.1. Cohort studies

Using the NOS, two studies were rated as having low risk of bias [14,16], two as moderate risk [12,15], and one was rated as having a high risk of bias [13]. Specific ratings for each study are included in Table 1. Bias encompassed in the assessment of the study by Uchiyama included selection bias due to recruitment of all participants from a private clinic, poor definition and inadequate description of assessment of regression, and a lack of controlling for comparability between the “MMR Generations” and “pre- and post-MMR Generations”. The study by Madsen also has the potential for bias as a result of investigating MMR vaccination status as opposed to a cumulative dosage of thimerosal or Hg. As the Hg or thimerosal dosage in vaccinations varies, there is a degree of fluctuation in the amount of exposure to the individuals within a population studied. In contrast, when using the binary system of vaccinated versus non-vaccinated in a population with such high immunisation coverage to investigate the risk of ASD, the unvaccinated group is at much higher risk of being non-representative of the larger population for many additional reasons thus creating bias. We have continued to include it in our meta-analysis despite risk of bias as it still provides valuable evidence for the question of the increased risk of autism or ASD in the vaccinated population compared to those unvaccinated, despite bias affecting the implications that can be drawn about the causal nature of the relationship. Follow-up periods for each of the cohort studies varied with time periods of 5 years (at least 3 years of data per individual) [13], 8 years (at least 2 years of data per individual) [15], 8 years [12], 11 years (at least 2 years of data per individual) [14], and individuals followed from 1 to 11 years [16]. The mean length of follow-up of the five cohort studies is 8.6 years, with the range being 5 years to 11 years.

3.3.2. Case-control studies

Using the NOS, one study was assessed as having low risk of bias [19], and four as having moderate risk [17,18,21,22] (Table 2). All case-control studies had good methodology for case and control selection, as well as comparability, however, adequate description of non-response rate was a recurring problem.

3.4. Outcomes

3.4.1. Cohort studies

All five cohort studies included for meta-analysis reported negative findings in their individual investigations of MMR, Hg or thimerosal and autism, other ASD, or autism with regression. Combining the data for a summary odds ratio found no increased risk of developing autism or ASD following MMR, Hg, or thimerosal exposure (OR: 0.98, 95% CI: 0.92 to 1.04; \( I^2 = 0.00, p = 0.45 \)) (Fig. 2). The results of the subgroup analyses investigating the risk of developing either autism alone (OR: 0.99, 95% CI: 0.92 to 1.06; \( I^2 = 0.00, p = 0.80 \)), or ASD alone (OR: 0.91, 95% CI: 0.68 to 1.20; \( I^2 = 55.6, p = 0.10 \)) after exposure to MMR, Hg or thimerosal were not supportive of a causal link (Fig. 3). On dividing the data to investigate each exposure type individually, there was not an increased risk of developing autism or ASD following Hg exposure (OR: 1.00, 95% CI: 0.93 to 1.07; \( I^2 = 0.00, p = 0.89 \)), thimerosal exposure (OR: 1.00, 95% CI: 0.77 to 1.31; \( I^2 = 38.78, p = 0.20 \)), or MMR vaccination (OR: 0.84, 95% CI: 0.70 to 1.01; \( I^2 = 0.00, p = 0.55 \)) alone (Fig. 4).

3.4.2. Case-control studies

The five case-control studies included in the analysis all individually reported finding no evidence for an association between vaccination and ASD. The overall odds ratio for risk of developing autism or ASD following MMR, Hg, or thimerosal exposure was non-significant when data was grouped by condition (OR: 0.90, 95% CI: 0.83 to 0.98; \( p = 0.02 \)) or grouped by exposure type (OR: 0.85, 95% CI: 0.76 to 0.95; \( p = 0.01 \)). Again the results of the subgroup analyses were similarly negative, with risk of developing autism alone (OR: 0.69, 95% CI: 0.54 to 0.88; \( I^2 = 66.97, p = 0.001 \)) or ASD alone (OR: 0.94, 95% CI: 0.86 to 1.03; \( I^2 = 41.73, p = 0.06 \)) after exposure to MMR, Hg or thimerosal being non-significant. The odds ratios
### Table 1
Characteristics of cohort studies included in the analysis.

<table>
<thead>
<tr>
<th>Study design</th>
<th>Country</th>
<th>Sample size</th>
<th>Participants</th>
<th>Intervention</th>
<th>Outcomes</th>
<th>Risk of bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andrews [14]</td>
<td>Retrospective cohort</td>
<td>109,863</td>
<td>Children born in the United Kingdom from 1988 to 1997 and registered in general practices that contributed to a research database</td>
<td>Cumulative Hg dose from DTP/DT vaccinations</td>
<td>Risk of developing neurodevelopmental disorders (including general developmental disorders, language or speech delay, tics, ADD, autism (ICD-9 code 299.0), unspecified developmental delay, behaviour problems, enuresis, and encopresis)</td>
<td>****</td>
</tr>
<tr>
<td>Hvild [16]</td>
<td>Retrospective cohort</td>
<td>467,450</td>
<td>All children born in Denmark from January 1990 until December 1996</td>
<td>Vaccination with a thimerosal-containing vaccine compared to vaccination with a thimerosal-free formulation of the same vaccine</td>
<td>Risk of developing autistic disorder (ICD-10 code F84.0, DSM-IV code 299.00) or other autistic-spectrum disorders (ICD-10 codes F84.1–F84.9, DSM-IV codes 299.10–299.80)</td>
<td>****</td>
</tr>
<tr>
<td>Madsen [12]</td>
<td>Retrospective cohort</td>
<td>537,303</td>
<td>All children born in Denmark from January 1991 through December 1998</td>
<td>MMR-vaccination at 15 months (vaccine strains: Moraten (measles), Jeryl Lynn (mumps), and Wistar RA 27/3 (rubella))</td>
<td>Odds of developing regressive phenotype of autism as defined by Taylor et al. [36].</td>
<td>**</td>
</tr>
<tr>
<td>Uchiyama [13]</td>
<td>Retrospective cohort</td>
<td>140,887</td>
<td>Infants born at one of three health organisations in USA during 1992 to 1999</td>
<td>MMR vaccination</td>
<td>Risk of developing neurodevelopmental disorders (including autism (ICD-9 code 290.0), other childhood psychosis, stammering, tics, sleep disorders, eating disorders, emotional disturbances, ADD, language delay, speech delay, and coordination disorder)</td>
<td>****</td>
</tr>
<tr>
<td>Verstraeten [15]</td>
<td>Retrospective cohort</td>
<td>1,000,000</td>
<td>Study participated in a controlled trial with a control group</td>
<td>Cumulative Hg exposure from thimerosal-containing vaccinations</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Risk of bias**

- **High risk**
- **Moderate risk**
- **Low risk**
- **Unclear**

- **Selection**
- **Comparability**
- **Outcome**

Based on exposure type did not support a link between measles vaccination (OR: 1.00, 95% CI: 0.59 to 1.67; I² = 30.50, p = 0.23), MMR vaccination (OR: 0.69, 95% CI: 0.53 to 0.90; I² = 66.85, p < 0.001), or thimerosal exposure (OR: 0.89, 95% CI: 0.78 to 1.00; I² = 58.40, p = 0.02) and ASD.

### 3.5. Publication bias

Egger’s regression analysis suggested that there was no evidence of publication bias for cohort studies (p = 0.12). In addition, Begg and Mazumdar’s rank correlation [23] suggested a symmetrical plot (p = 0.07). For case-control studies, Egger’s regression analysis suggested the presence of publication bias, however, Begg and Mazumdar analysis revealed that the studies were symmetrical on the funnel plot (p = 0.21). Moreover, the fail-safe number was 159 and due to the comprehensive nature of the literature search performed it is unlikely that such a large number of studies would have been missed by the search. In addition, due to the controversial nature of the topic and the high volume of publication on this issue for both sides of the argument it is unlikely that so many papers on one side of the argument (that would have met our inclusion criteria) remain unexplained.

![Fig. 2. Combined estimate for vaccines and autism or ASD.](image-url)
Table 2
Characteristics of case-control studies included in the analysis.

<table>
<thead>
<tr>
<th>Study design</th>
<th>Country</th>
<th>Case participants</th>
<th>Control participants</th>
<th>Intervention</th>
<th>Outcomes</th>
<th>Risk of bias</th>
</tr>
</thead>
</table>
| DeStefano [17]     | Case-control  | All children aged 3 to 10 years in 1996 in the Metropolitan Atlanta Developmental Disabilities Surveillance Program (MADSP) with autism | Children from regular education programs matched to cases based on age, gender and school of attendance | Exposure to MMR vaccine | MMR exposure in cases and control groups | **
| Mrozek-Budzyn [18] | Case-control  | All children aged 6 to 13 from three managed care organisations (MCO) with a diagnosis of autism according to ICD 9 codes 299.0 or 299.8 supplemented with the Autism Diagnostic Interview-Revised administered to mothers | The first 2 children who visited the physician after the time of the autistic child visit matched for birth year, gender and practice | Exposure to monovalent measles vaccine or MMR vaccine | Odds ratio of having autism, based on vaccination status and type of vaccine used | ****
| Price [22]         | Case-control  | All children aged 6 to 13 from three managed care organisations (MCO) with a diagnosis of autism according to ICD 9 codes 299.0 or 299.8 supplemented with the Autism Diagnostic Interview-Revised administered to mothers | Randomly selected from the MCO matched for year of birth, gender, and MCO | Hg exposure from vaccinations since birth | Odds of having autism, ASD, or ASD with regression per μg of Hg per kg of body weight | ****
| Smither [19]       | Case-control  | All children registered in the UK General Practice Research Database (GPRD) who were born in 1973 or later with a first diagnosis of autism between 1987 and 2001 | Children from the GPRD with no diagnosis of PDD matched by year of birth, sex, and general practice | MMR vaccination | Odds of having autism or other PDD based on MMR status | ****
| Uno [21]           | Case-control  | All children from the Yokohama Psycho-Developmental Clinic (YPDC), Kanto area, with a diagnosis of ASD based on DSM-IV and using the Diagnostic Interview for Social and Communication Disorder (DISCO) who were born between April 1, 1984 and April 30, 1992 | Volunteers from general schools in the Kanto area matched by sex and year of birth | MMR monovalent measles vaccine exposure | Odds of having ASD based on vaccination status | **

4. Discussion

This meta-analysis of five case-control and five cohort studies has found no evidence for the link between vaccination and the subsequent risk of developing autism or autistic spectrum disorder. Subgroup analyses looking specifically at MMR vaccinations, cumulative mercury dosage, and thimerosal exposure individually were similarly negative, as were subgroup analyses looking specifically at development of autistic disorder versus other autistic spectrum disorder.

Four of the five cohort studies included in this review investigated very large populations and were of sound methodology, which is of great importance as our review question has implications at the population level, and thus required such data for optimal applicability.

The current meta-analysis is the only quantitative analysis of pooled data on the topic. In the process of searching the literature 12 systematic reviews were identified and reference lists searched for additional data [24–35]. Eleven of the 12 identified reviews shared the current conclusion that there was no evidence for a link between vaccination and autistic spectrum disorder, advocating continuation of current immunisation practices. The only review to suggest that a link could not be excluded was that by Rataficzak [32] looking into the aetiology of autism and concluded that it is
multifactorial involving genetics and/or inflammation of the brain caused by a wide variety of environmental toxins, one of which may be mercury.

Of specific mention, a 2012 Cochrane review examining five RCTs, one controlled clinical trial, 27 cohort studies, 17 case-control studies, five time-series trials, one cross-over trial, two ecological studies, and six self-controlled case series studies looked at the effectiveness of the MMR vaccination and its associated adverse effects [25]. Congruent with our current study, this review found no qualitative evidence for a link between the MMR vaccination and autism. As every treatment has the possibility of adverse events, those found to be associated to MMR vaccination included aseptic meningitis, febrile seizures and thrombocytopenic purpura in specific populations. Many conditions were found to be unlikely to be associated with vaccination, one of which was autism.

Publication bias was not found in the study, which may be due to the important public health question of the question. While we thought it more important to include only studies that strictly adhered to a case-control or cohort study protocol and drew it’s participants from reliable sources, we recognise that there does exist data from VAERS that reported positive results, however, due to the aforementioned reasons these papers were excluded. It could be considered that duplicate data may be influencing the results as two of the five cohort studies were performed at the population level in Denmark with a crossover of birth cohorts. While the two studies looked at different interventions (one MMR and the other thimerosal-containing vaccines) the outcome data was the same, so while being an interesting comparison to one another, may not provide completely individual results to contribute to this meta-analysis. However, a sensitivity analysis of these studies from Denmark did not change the overall result. An important strength of this meta-analysis is the length of follow-up of the cohort studies, with an average of 8.6 years.

In conclusion, this meta-analysis provides no evidence of a relationship between vaccination and autism or autism spectrum disorders and as such advocate the continuation of immunisation programs according to national guidelines.

As with any treatment or behaviour, one must weigh the benefits and risks to determine their course forward. While at the level of the individual avoidance of immunisation may be seen as conferring lower risk by avoiding possible associated adverse events, the increase in parents deciding to take this course of action has substantially decreased ‘herd immunity’ among populations, subsequently increasing the risk of catching potentially more serious infectious diseases. Thus the risk incurred by not immunising a child is increasing substantially as levels of immunisation coverage fall. In regards specifically to the fear of a child developing autism following immunisation, the data consistently shows the lack of evidence for an association between autism, ASD and vaccination, regardless of whether the intervention was the MMR vaccine itself or one of its components, providing no reason to avoid immunisation on these grounds.

5. Epilogue

As an epidemiologist I believe the data that is presented in this meta-analysis. However, as a parent of three children I have some understanding of the fears associated with reactions and effects of vaccines. My first two children have had febrile seizures after routine vaccinations, one of them a serious event. These events did not stop me from vaccinating my third child, however, I did take some proactive measures to reduce the risk of similar adverse effects. I vaccinated my child in the morning so that we were aware if any early adverse reaction during the day and I also gave my child a dose of paracetamol 30 min before the vaccination was given to reduce any fever that might develop after the injection. As a parent I know my children better than anyone and I equate their seizures to the effects of the vaccination by increasing their body temperature. For parents who do notice a significant change in their child’s cognitive function and behaviour after a vaccination I encourage you to report these events immediately to your family physician and to the ‘Vaccine Adverse Event Reporting System’.

Author contributions

Dr Guy D. Eslick had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Guy D. Eslick; acquisition of data: Luke Taylor, Amy L. Swerdleger; analysis and interpretation of data: Guy D. Eslick; drafting of the manuscript: Luke Taylor, Amy L. Swerdleger; critical revision of the manuscript for important intellectual content: Guy D. Eslick, Luke Taylor, Amy L. Swerdleger; statistical analysis: Guy D. Eslick; study supervision: Guy D. Eslick.

Conflict of interest statement

None.

References


