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ORIGINAL PAPER

Congenital Cytomegalovirus Infection in Children with Autism Spectrum Disorder: Systematic Review and Meta-Analysis

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Abstract
Association of congenital cytomegalovirus (CMV) infection with autism spectral disorder (ASD) has been suggested since 1980s. Despite the observed association, its role as a risk factor for ASD remains to be defined. In the present review, we systematically evaluated the available evidence associating congenital CMV infection with ASD using PubMed, Web of Science, Cochrane Library, and Embase databases. Any studies on children with CMV infection and ASD were evaluated for eligibility and three observational studies were included in meta-analysis. Although a high prevalence of congenital CMV infection in ASD cases (OR 11.31, 95% CI 3.07–41.66) was indicated, too few events (0–2 events) in all included studies imposed serious limitations. There is urgent need for further studies to clarify this issue.

Keywords Congenital CMV infection · ASD · Risk factor · Prenatal environment · Maternal immune activation · Neurodevelopmental disorder

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that comprises a wide spectrum of functional impairments characterized by deficits in social and communication interaction, and restricted interest and repetitive patterns of behavior. Children with ASD typically manifest developmental deviations by 2–3 years of age, show a wide range of symptoms interfering with daily function, and are expected to diagnose at preschool age for early intervention (Lai et al. 2014). The prevalence of ASD has increased dramatically over time (Elsabbagh et al. 2012), from less than 0.05% in 1966 to approximately 1.2% in 2013 (Lotter 1966; Saemundsen et al. 2013), with wide geographic and ethnic variation (Baio 2014; Idring et al. 2015).

A complex collection of genetic and environmental factors has been associated with the development of ASD, but no single factor has been consistently identified among different studies (Bilder et al. 2009; Ornoy et al. 2015). Recent progress in identifying the thousands of genetic factors has revealed the extraordinary causal diversity and complexity of ASD (de la Torre-Ubieta et al. 2016). Given that ASD seems to result from the interaction of genetic factors and prenatal/postnatal environment, the current challenge in understanding the etiology of ASD is to define the ASD's genetic components and the environmental risk factors in each ASD case.

Cytomegalovirus (CMV) is a neurotropic herpesvirus that can be transmitted to the infant during pregnancy (Alford et al. 1990). Primary CMV infection is usually asymptomatic or self-liming, and remains latent for a lifetime. However, it becomes a serious burden during pregnancy and immunocompromised state. CMV is the most common pathogen

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of intrauterine infection worldwide, the leading non-genetic cause of sensorineural hearing loss (SNHL) in children, and an important cause of neurodevelopmental delay and sequelae. Prevalence of congenital CMV infection has varied between 0.15 and 2% in different countries (Kenneson and Cannon 2007). Infant diagnosis of congenital CMV infection has been based on targeted testing of symptomatic infants within 2–3 weeks of birth, and most asymptomatic infants remain undiagnosed. Approximately 10–15% of infants with congenital CMV infection have clinical signs at birth, and a high population (40–60%) of them will develop CMV-related sequelae. However, another 10–15% of those who are asymptomatic at birth will also present CMV-related sequelae in later development (Dollard et al. 2007).

A link between maternal CMV infection during pregnancy and ASD was already reported in case studies in the early 1980s (Markowitz 1983; Stubbs et al. 1984). Later case studies of children with early manifestation of developmental delay also indicated the association between congenital CMV infection and severe autism (Sweeten et al. 2004; Yamashita et al. 2003). Since retrospective diagnosis of congenital CMV infection after clinical diagnosis of ASD has become feasible by using dried blood spots or preserved dried umbilical cord (Barbi et al. 1996; Tagawa et al. 2009), recent studies have also reported a link between retrospectively diagnosed congenital CMV infection and ASD (Engman et al. 2015; Gentile et al. 2017; Sakamoto et al. 2015).

However, these studies associating congenital CMV infection with ASD are largely based on symptomatic children who suffer from other CMV-related sequelae. It remains elusive whether the observed association is a direct link or an indirect multifactorial phenomenon related to other CMV-related sequelae such as SNHL, vision impairment, cognitive deficit, and cerebral palsy (Ornoy et al. 2015; Smithers-Sheedy et al. 2017). In the present review, we thus compile and analyze the available evidence on whether the prevalence of congenital CMV infection is significantly higher in children with ASD.

Methods

Literature Search

This review was conducted in accordance with Cochrane Handbook for Systematic Reviews of Interventions and MOOSE guidelines (Higgins and Green 2017; Stroup et al. 2000). MOOSE checklist was shown in Appendix Table 2. A systematic search of PubMed, Web of Science, Cochrane Library, and Embase was completed on October 8, 2017. To identify relevant studies regarding the association between congenital CMV infection and ASD, synonyms and related terms were combined using thesaurus search terms. The search terms included "congenital" "pregnancy" "prenatal" "perinatal" "maternal" "cytomegalovirus" "infection" "autism" "autistic disorder" "autism spectrum disorder" (Appendix Table 3). There was no date, language or types of publication restriction imposed on the search. Non-English studies were retrieved but then excluded. EndNote version X7 software (Thomson Reuters, New York, NY, USA) was used to store and manage the retrieved citations for screening.

Inclusion and Exclusion Criteria

Inclusion and exclusion criteria for this review were set in compliance with the standard Cochrane review protocols (Higgins and Green 2017). The inclusion criteria were: (1) comparison of the prevalence of congenital CMV infection between control and ASD cases, (2) use of either Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR) (First and Pincus 2000) or DSM-V (Kupper and Regier 2013) for ASD diagnosis, and (3) use of either blood/urine/saliva samples collected within the first three weeks of life, blood/urine spots on filter paper, saliva swab, or dried umbilical cord for congenital CMV infection diagnosis by isolation/molecular detection of CMV. The exclusion criteria were: (1) studies of the limited ASD subgroup (e.g., ASD with or without intellectual disability), (2) studies of the limited congenital CMV infection subgroup (e.g., congenital CMV infection with SNHL or cerebral palsy), and (3) studies of animal models, case reports, and reviews not providing relevant data for this review.

Study Selection

Four authors (KM, KT, HN, and NN) independently screened all titles and abstracts of publications identified by the literature search in order to assess their eligibility. Studies that did not meet the criteria were excluded from this initial stage. Four authors then obtained the full-text articles of the identified eligible studies for independent assessment to decide which studies fulfilled the inclusion criteria. When studies referred to related, previously published protocols or studies, the referenced studies were assessed for criteria eligibility as well. Any disagreement was resolved though discussion until consensus was reached.

Quality Assessment

The Newcastle Ottawa Scale (NOS) was used to assess the quality of included studies (Lo et al. 2014; Ruiz-Goikoetxea et al. 2017; Wells et al. 2014). The NOS consisted of three domains (selection, comparability, and exposure/outcome) and assigned a maximum of 4 points for selection, 2 points

for comparability, and 3 points for exposure/outcome. A total score ranged from 0 to 9 points, with higher points indicating the higher quality.

Statistical Analysis

Studies with similar characteristics were combined for meta-analysis. The relative effect measures were calculated by using odds ratio (OR) statistics based on the reporting from the studies, and the relative effect estimate was assigned with a 95% confidence interval (CI) and a p-value cut-off point of 0.05. Since clinical symptoms of ASD cases were highly dependent on their cultural backgrounds and the true effect would be most likely varied among studies from different countries, a random-effects model was used to summarize the quantification of the pool effect across the studies. Due to the very small number of included studies, a fixed-effects model was also used to test the trend of the estimated effect as well. The *I*-squared and *Q*-statistic were used to determine heterogeneity among studies. *I*-squared > 50% or $P < 0.10$ for *Q*-statistic indicated considerable heterogeneity among studies. Statistical analyses were performed with EZR version 1.35 http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html; Saitama Medical Centre, Jichi Medical University, Saitama, Japan) (Kanda 2013).

Results

Study Selection

The literature search identified 111 records from all databases. Following the elimination of duplicates and non-English records, 71 records were screened by the titles and abstracts and 10 records met the eligibility criteria. The main reasons for exclusion were that articles did not provide relevant data for ASD and/or CMV. Full texts of the resulting 10 studies were further screened for the eligibility criteria. The application of inclusion/exclusion criteria resulted in 7 excluded studies and 3 included studies for meta-analysis. The reasons for exclusion were the lack of relative risk of congenital CMV infection for 4 studies and the inadequate diagnosis of congenital CMV infection for 3 studies (Appendix Table 4). Flowchart of study selection was shown in Fig. 1.

Study Characteristics

The three included studies had 9514 participants, of which 180 were described as having ASD and 9334 were controls. One study reported case controls (Gentile et al. 2017),

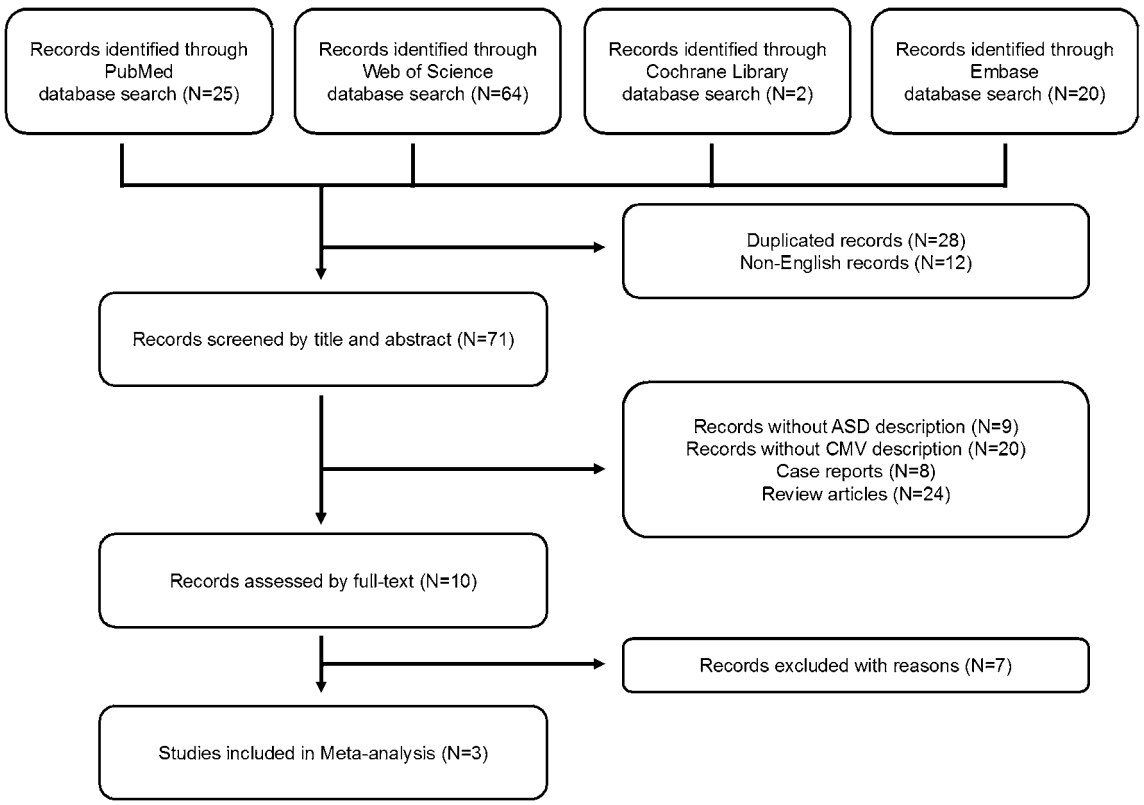

Fig. 1 Flowchart of study selection

whereas two studies used historical controls (Engman et al. 2008, 2015; Koyano et al. 2011; Sakamoto et al. 2015). Sample sizes varied across the included studies. Gentile et al. (2017) had 38 ASD and 44 control cases. Sakamoto et al. (2015) and Engman et al. (2015) had 27 and 115 ASD cases comparing with 3230 and 6060 historical controls, respectively. Events sizes were extremely small in the included studies. Gentile et al. (2017) reported 2 events in ASD cases and no event in control cases. Sakamoto et al. (2015) and Engman et al. (2015) reported 2 and 1 events in ASD cases, respectively. According to the NOS evaluation, the quality of three included studies were assessed as a total score of 4, 7, and 5 points (Wells et al. 2014). The major characteristics of the three studies were summarized in Table 1.

Association Between Congenital CMV Infection and ASD

There were three observational studies providing data for congenital CMV infection in children with ASD (Fig. 2). The pooled estimate from these studies showed that a higher proportion of children in the ASD groups experienced congenital CMV infection compared with the control groups (OR: 11.31, 95% CI 13.07–41.66). The effect estimate showed a statistical significance of $p = 0.0003$. There was no evidence of significant heterogeneity among studies (I-squared = 17%, $P = 0.30$). Publication bias was not assessed because only three studies were identified in the present review. Although the NOS scores of all included studies indicated acceptable quality (a total score ≥ 4 points), too few events (0–2 events) imposed serious limitations on the present review.

Discussion

In the present review, an increased prevalence of congenital CMV infection was found in children with ASD compared to those without. The biological mechanisms underlying an association between congenital CMV infection and ASD were not clear. There were two plausible explanations for this observation. One explanation was a direct link, in which CMV-induced damage to the developing central nervous system causatively contributed to ASD symptoms. Consistent with a direct link, cerebral white matter abnormality by magnetic resonance imaging (MRI) was observed at very high incidence in asymptomatic as well as symptomatic patients with congenital CMV infection (Uematsu et al. 2016; van der Knaap et al. 2004).The other explanation was an indirect multifactorial phenomenon related to congenital CMV infection. Infectious organisms were known to trigger maternal immune activation (MIA) in addition to crossing the placenta and entering the fetal environment (Aronsson et al.

Table 1 Summary of the included studies

Study	Location	Study design	Participants	Age range	Inclusion criteria	Outcome measurements	Study quality (NOS)
Sakamoto et al. (2015)	Japan	Observational study with historical control	N = 3257 ASD: n = 27 Control: n = 3230	ASD: 3–12 years (born 1994–2003) Control: 0 years (born 2008–2010)	ASD: DSM-IV-TR, DSM-V Control: neonatal screening for congenital CMV infection	ASD: qPCR using dried blood spots on filter paper or dried umbilical cord Control: qPCR using urine on filter paper	Total: 4 Selection: 2 Comparability: 1 Outcome/exposure: 1
Engman et al. (2015)	Sweden	Observational study with historical control	N = 6175 ASD: n = 115 Control: n = 6060	ASD: 4–7 years (born 2005–2008) Control: 0 years (born 2003–2004)	ASD: DSM-IV-TR, ADOS-G, DISCO-11 Control: neonatal screening for congenital CMV infection	ASD: qPCR using dried blood spots on filter paper Control: qPCR using dried blood spots on filter paper	Total: 7 Selection: 4 Comparability: 1 Outcome/exposure: 2
Gentile et al. (2017)	Italy	Observational study with case control	N = 82 ASD: n = 38 Control: n = 44	ASD: Mean 4 years (enrolled 2010–2013) Control: Mean 5 years (enrolled 2010–2013)	ASD: DSM-IV-TR, DSM-V, ADOS-G, ADI-R Control: children recruited by general pediatrician	ASD: qPCR using dried blood spots on filter paper Control: qPCR using dried blood spots on filter paper	Total: 5 Selection: 1 Comparability: 2 Outcome/exposure: 2

NOS Newcastle-Ottawa scale

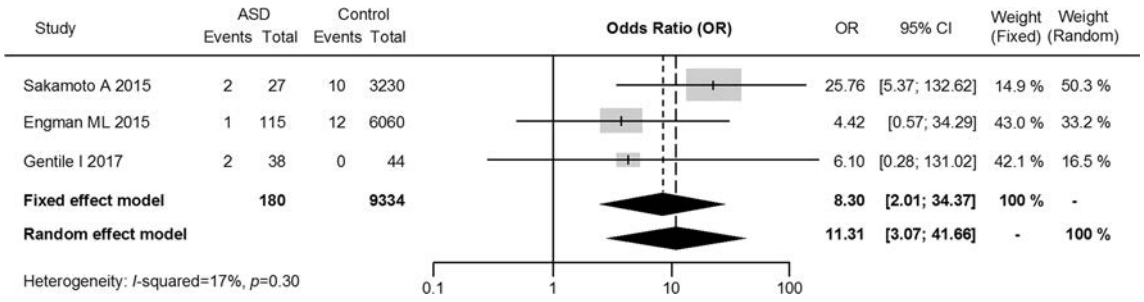

Fig. 2 Relative risk of congenital CMV infection in children with ASD

2002). It had been suggested that MIA disrupted the normal balance of available immune molecules during critical stages of neural development and contributed to an increased risk of neurodevelopmental disorders such as ASD (Hsiao and Patterson 2012; Malkova et al. 2012; Patterson 2011).

Immune molecules such as major histocompatibility (MHC) class I proteins, complement proteins, chemokines, and cytokines guided the normal development of neural circuits, and thereby were critical for normal brain development of fetus during pregnancy (Bilbo and Schwarz 2012; Corriveau et al. 1998; Deverman and Patterson 2009; Stevens et al. 2007). Altered cytokine expression early in life had been associated with the risk of severe developmental delay and neurodevelopmental disorders (Ornoy et al. 2015; Yoon et al. 1997a, b, 1996). Indeed, the levels of specific cytokines were elevated in serum and amniotic fluid of pregnant women bearing a child with ASD (Abdallah et al. 2012; Goines et al. 2011). Although these modulating effects of MIA on the development of ASD symptoms were convincing in animal models, they were still inconclusive in human ASD cases.

The present meta-analysis suggested a statistically significant association between congenital CMV infection and ASD. No randomized controlled study was identified in the present literature search, which caused a high possibility of confounding factors, such as genetic status of children, medication status, or comorbidity of other developmental disorders that could not be ruled out. Instead, all identified studies were observational studies, which were prone to unclear risk of bias in the selection of participants and blinding of outcome assessments across studies. In addition to these inherent limitations in observational studies, the most critical limitation in the present meta-analysis was the small sample size with very few events and the inability to infer causal relationships between exposure and outcome variables. Another limitation of the present review was the variable sensitivity of CMV detection in different samples; dried blood spots on filter paper, dried umbilical cord, and urine on filter paper (Boppana 2010; Koyano et al. 2011; Leruez-Ville et al. 2011; Ross et al. 2015). Future studies will need to consider these potential confounders and include more participants with more events to strengthen statistical power.

Conclusions

Although the present meta-analysis of three observational studies revealed a significant association between congenital CMV infection and ASD, too few events (0–2 events) in all included studies imposed serious limitations. There is urgent need for further studies with more events to clarify this issue.

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Author Contributions All authors were involved in the design of the study. KM, KT, HN, and NN performed the literature search, evaluated the relevant records for inclusion in the systematic review and meta-analysis, and conducted the statistical analysis. KM and NN drafted the initial manuscript. MY, YT, TK, MM, SK, ST, MN, NS, MN, MTI, IM, and KI revised the article critically for important points. All authors approved the final manuscript as submitted.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interests.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Appendix

See Appendix Tables 2, 3, and 4.

Table 2 MOOSE checklist

Background	
Problem definition	While a growing body of evidence suggests that congenital CMV infectio is associated with ASD, this association was inconsistent in different studies
Hypothesis statement	Prevalence of congenital CMV infectio are significantly increased in children with ASD
Description of study outcomes	Prevalence of congenital CMV infectio in children with ASD
Type of exposure or intervention used	Congenital CMV infection
Type of study designs used	Observational studies with historical control or case control
Study population	Children with ASD and controls
Search strategy	
Qualifications of searchers (eg, librarians and investigators)	Four investigators (KM, KT, HN, and NN), who are all experienced pediatrician actively engaged in multiple clinical researches, prformed literature search
Search strategy, including time period included in the synthesis and keywords	PubMed, Web of Science, Cochrane Library, and Embase databases up to October 2017 Keywords: "congenital", "pregnancy", "prenatal", "perinatal", "maternal", "cytomegalovirus", "infection", "infections" in combination with "autistic disorder", "autism", "autism spectrum disorder"
Effort to include all available studies, including contact with authors	References of all retrieved articles and recent reviews were reviewed
Databases and registries searched	PubMed, Web of Science, Cochlane Library, and Embase databases
Search software used, name and version, including special features used (eg, explosion)	EndNote X7 (Thomson Reuters, New York, NY)
Use of hand searching (eg, reference lists of obtained articles)	References of all retrieved articles and recent reviews were reviewed
List of citations located and those excluded, including justification	Figure 1 and Table 1, Appendix Table 4
Method of addressing articles published in languages other than English	Non-English articles were retrieved but then excluded
Method of handling abstracts and unpublished studies	The search process was not restricted upon full-text articles, but also conference abstracts and unpublished studies
Description of any contact with authors	Not applicable
Methods	
Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	The inclusion criteria were presented in the section "Inclusion and exclusion criteria"
Rationale for the selection and coding of data (eg, sound clinical principles or convenience)	Study characteristics were extracted independently by four investigators (KM, KT, HN, and NN)
Documentation of how data were classified and coded (eg, multiple raters, blinding, and interrater reliability)	Data were independently extracted and analyzed by four investigators (KM, KT, HN, and NN) and final decision was reached by consensus
Assessment of confounding (eg, comparability of cases and controls in studies where appropriate)	Table 1
Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictiors of study results	Table 1
Assessment of heterogeneity	The *Q*-statistic and *I*-squared were used to explore the heterogeneity among studies
Description of statistical methods (eg, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose–response models, or cumulative meta-analysis) in sufficient detail to be replicated	Description of methods of meta-analysis were descrived in the section "Statistical analysis"
Provision of appropriate tables and graphics	One flowchart, one forest plot, and one table were presented in the main text. Three appendix tables were provided
Results	
Graphic summarizing individual study estimates and overall estimate	Figure 2
Table giving descriptive information for each study included	Table 1
Results of sensitivity testing (eg, subgroup analysis)	Not applicable
Indication of statistical uncertainty of findings	95% confidence intervals were presented with all summary effect estimates
Discussion	
Quantitative assessment of bias (eg, publication bias)	Table 1

Table 2 (continued)

Discussion	
Justification for exclusion (eg, exclusion of non-English-language citations)	Figure 1, Appendix Table 4
Assessment of quality of included studies	Table 1
Conclusions	
Consideration of alternative explanations for observed results	We cannot exclude chance, residual or unmeasured confounding as alternative explanation for the present results
Generalization of the conclusions (ie, appropriate for the data presented and within the domain of the literature review)	We cannot generaleze the present results
Guidelines for future research	More studies were needed to assess the risk of ASD in children with congenital CMV infection
Disclosure of funding source	The authors received no specific funding for this work

Table 3 Search terms

ID	Search terms
#1	Congenital
#2	Pregnancy
#3	Prenatal
#4	Perinatal
#5	Maternal
#6	Cytomegalovirus
#7	Infection
#8	Infections
#9	Autism
#10	Autistic disorder
#11	Autism spectrum disorder
#12	#1 or #2 or #3 or #4 or #5
#13	#7 or #8
#14	#12 and #6 and #13
#15	#9 or #10 or #11
#16	#14 and #15

Table 4 Summary of the excluded studies

Study	Location	Study design	Reason for exclusion
Yamashita et al. (2003)	Japan	Observational study without control	Relative risk of congenital CMV infection is not available This study identified 2 ASD cases in 7 congenital CMV infection cases with symptoms
Yamazaki et al. (2012)	Japan	Observational study without control	Relative risk of congenital CMV infection is not available This study identified 2 ASD cases in 11 congenital CMV infection cases with cochler implantees
Gentile et al. (2014)	Italy	Observational study with control	Laboratory congenital CMV infection diagnosis is not adequate This study identified 18 and 21 anti-CMV antibody-positive cases in 54 ASD and 46 control cases
Inaba et al. (2016)	Japan	Observational study without control	Relative risk of congenital CMV infection is not available This study identified 5 ASD cases in 9 congenital CMV infection cases with sensorineural hearing loss
Mahic et al. (2017)	Norway	Observational study with control	Laboratory congenital CMV infection diagnosis is not adequate This study revealed no association between anti-CMV antibody-positive mothers and ASD children
Glazier et al. (2017)	United States	Observational study without control	Laboratory congenital CMV infection diagnosis is not adequate ASD diagnosis is not adequate This study revealed association between high anti-CMV-positive mothers and high SRS2 score children
Garofoli et al. (2017)	Italy	Observational study without control	Relative risk of congenital CMV infection is not available This study identified 4 ASD cases in 70 congenital CMV infection cases with or without symptoms

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