Vaccine 30 (2012) 4292–4298

Contents lists available at SciVerse ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

The combined measles, mumps, and rubella vaccines and the total number of vaccines are not associated with development of autism spectrum disorder: The first case–control study in Asia

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ARTICLE INFO

Article history:
Received 9 September 2011
Received in revised form 6 January 2012
Accepted 7 January 2012
Available online 20 April 2012

Keywords:
Autism spectrum disorder
Vaccination
Measles–mumps–rubella Vaccine
Risk factors
Medical record
Case–control studies

ABSTRACT

Objective: The aim of this study was to investigate the relationship between autism spectrum disorder (ASD) and general vaccinations, including measles–mumps–rubella (MMR) vaccine, in Japanese subjects, a population with high genetic homogeneity.
Patients and methods: A case–control study was performed. Cases ($n = 189$) were diagnosed with ASD, while controls ($n = 224$) were volunteers from general schools, matched by sex and birth year to cases. Vaccination history and prenatal, perinatal, and neonatal factors from the Maternal and Child Health handbook, which was part of each subject's file, were examined. To determine the relationship between potential risk factors and ASD, crude odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated, and the differences in mean values of the quantitative variables between cases and controls were analyzed using an unpaired *t*-test. Moreover, MMR vaccination and the effect of the number of vaccine injections were investigated using a conditional multiple regression model.
Results: For MMR vaccination, the OR was 1.04 (95% CI, 0.65–1.68), and no significant differences were found for the other vaccines. For all of the prenatal, perinatal and neonatal factors, there were no significant differences between cases and controls. Furthermore, regarding the presence of ASD, MMR vaccination and the number of vaccine injections had ORs of 1.10 (95% CI, 0.64–1.90) and 1.10 (95% CI, 0.95–1.26), respectively, in the conditional multiple regression model; no significant differences were found.
Conclusions: In this study, there were not any convincing evidences that MMR vaccination and increasing the number of vaccine injections were associated with an increased risk of ASD in a genetically homogeneous population. Therefore, these findings indicate that there is no basis for avoiding vaccination out of concern for ASD.

1. Introduction

Autism is a life-long neurodevelopmental disorder. Its prevalence was long considered to be approximately 4 in 10,000 [1]. Due to broadening of the nosological categorization and more widespread recognition, however, in recent years the prevalence of autism spectrum disorder (ASD) [2,3], which includes autism, Asperger syndrome, and Pervasive developmental disorder not otherwise specified, has been reported at approximately 1% worldwide [4–6]. Although the pathogenesis of ASD has not yet been elucidated, genetic risk factors are strongly implicated, because the relative risk (λ_s) among siblings is greater than 20, and heritability is estimated to be as high as 38–90% [7–9]. In contrast, because the concordance rate of identical twins is not 100%, one can infer that environmental factors are also involved, and the recent increase in prevalence also indicates the involvement of various types of "novel environmental exposure". A debate has arisen over the contribution of vaccination as one environmental trigger of ASD.

The view that vaccination and ASD onset are related dates back to 1998 when the Lancet article by Wakefield et al. appeared [10] (the paper was retracted in 2010 because of ethical and methodological problems [11]). Thereafter, other published reports suggested a link between the measles–mumps–rubella vaccine

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0264-410X/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.vaccine.2012.01.093

(MMR) and ASD [12–14], and concerns emerged that thimerosal, which is included in other vaccines as a preservative, and vaccination with combined vaccines might be risks for ASD onset [15–17]. Other studies, however, that examined retrospective data and rejected any such link were published in rapid succession [18–26]. For example, some reported an increase in ASD prevalence despite a decline in the MMR vaccination rate [27,28]. In Japan, only two reports have been based on a time-series design, and the results suggested no relationship between MMR and ASD [29,30]. The most prominent articles in the past have focused mainly on the results of ecologic studies, and we will discuss the few existing case–control studies [31–33]. Each study demonstrated no differences between ASD cases and controls, failing to support a conclusion that immunization using MMR increases the risk of ASD onset.

Worldwide, reports on studies of immunization with vaccines other than MMR are rare. Moreover, parents or legal guardians remain apprehensive about the perceived risk of ASD posed by vaccination [34–37]. Therefore, the purpose of this study was to investigate Japanese subjects, a genetically homogeneous population, regarding links between ASD and immunization with various vaccines, including MMR, as well as the association between ASD and the number of vaccine injections [38]. This is the first case–control study in Asia investigating links between vaccination and ASD onset. These links were examined in ASD cases and controls matched for sex and year of birth based on data found in the Maternal and Child Health (MCH) handbook. This handbook, provided to all mothers by the relevant Japanese health system institution, is a highly reliable record of early development, health, and immunization, and health professionals (e.g. public health nurses, obstetricians, and pediatricians) keep record of most of the data listed in it [39,40]. In this study, therefore, data from the MCH handbook in terms of vaccination history, as well as potential prenatal, perinatal, and neonatal risk factors, were examined.

2. Patients and methods

2.1. Study population

2.1.1. Cases (Fig. 1)

The study analyzed case data from patients of the Yokohama Psycho-Developmental Clinic (YPDC), Kanto area, Japan, which accepts only patients with suspected developmental disorders. Of the patients who initially consulted the YPDC from April 1997 (opening of the clinic) until March 2011, the cases consisted of patients who: (1) were diagnosed with ASD, and (2) had been born between April 1, 1984 and April 30, 1992, the possible time period for MMR vaccination. Subjects whose records in the MCH handbook were missing or illegible and those with a history of vaccination in another country were excluded.

2.1.1.1. Diagnosis of ASD. Patients were diagnosed based on the classifications of pervasive developmental disorders in the Diagnostic and Statistical Manual 4th edition (DSM-IV) and standardized criteria using the Diagnostic Interview for Social and Communication Disorder (DISCO) [41,42]. The DISCO is recognized as one of the best ways to obtain a reliable and valid diagnosis of ASD [43].

One of several child psychiatrists on the team met the patient's parents and used the DISCO to take the patient's developmental history. Another child psychiatrist or clinical psychologist conducted intellectual or developmental tests, such as the Psycho-Educational Profile-Revised and Wechsler Intelligence Scale for Children-Third Edition. After the interview and testing, the diagnosis was made by the team according to the DSM-IV criteria.

2.1.1.2. Period of birth. MMR vaccination in Japan was conducted under specific circumstances. It was introduced in April 1989, and only one vaccination using MMR was included in the immunization schedule. The monovalent mumps and rubella vaccines remained the optimal choice of vaccine for those who did not participate in the MMR program. However, soon after the immunization program commenced, there were several cases of aseptic meningitis, which may have been caused by the mumps vaccine [44]. As a result, in April 1993, the Japanese government ceased extensive inoculation with MMR. Therefore, children born from April 1984 to April 1992 could receive the MMR vaccination, and those children were included in the present study.

2.1.2. Controls (Fig. 1)

One to two controls were selected for each case, matched by sex and year of birth and recruited as volunteers from general schools in the Kanto area, the same area where YPDC patients reside. Consent for participation in the present study was obtained from the parents (or legal guardians) of the students. Students who had previously been recognized as having developmental problems and were already receiving care were excluded, as were those whose records in the MCH handbook were missing or illegible and those with a history of vaccination in another country.

2.2. Source of data

The vaccination history and potential prenatal, perinatal, and neonatal risk factors collected based on the MCH handbook, which was routinely attached to each patient's file, were examined. The targeted vaccines were the MMR, generally used for infants, and the individual vaccines of the same type: the diphtheria–pertussis–tetanus vaccine (DPT); the polio vaccine; the B-encephalitis vaccine; and the Bacillus of Calmette and Guerin vaccine (BCG). For DPT, Polio, and B-encephalitis, there were many subjects who received these vaccines more than once. Therefore, the times of exposure to these vaccines were counted within the period of the first three years, when ASD features first appeared. Maternal hypertension (systolic blood pressure $\geq$140 mmHg or diastolic blood pressure $\geq$90 mmHg), albuminuria or edema, and anemia were examined as prenatal factors. The birth weight, head and chest circumference, duration of labor, delivery method (normal delivery, cesarean section, and obstetrical vacuum extraction or forceps delivery), and Apgar score were examined as perinatal and neonatal factors. Hypertension, albuminuria or edema, and anemia were recorded using a two-category scale (yes/no), and the Apgar score was recorded as an ordinal variable. Duration of labor, birth weight, and head and chest circumference were handled as continuous variables. The delivery method was recorded using a two-category scale (performed/not performed) for each delivery technique.

2.3. Selection of case children and matched control children

Among the patients who initially consulted the clinic between April 1997 and March 2011, 1875 cases of ASD were identified. Of these, 89 cases were excluded because the MCH handbook was missing or the vaccination record in the handbook could not be read, and 3 were excluded because they had received MMR vaccination overseas. Of the remaining 1783 cases, 1429 were born before March 1984 or after May 1992, leaving 354 cases (males: n = 286, 80.8%) born between April 1984 and April 1992, the possible time period for MMR vaccination. The ASD group consisted of 280 subjects with Autistic disorder (79.1%), 27 subjects with Asperger disorder (7.6%), and 47 subjects with Pervasive developmental disorder not otherwise specified (13.3%).

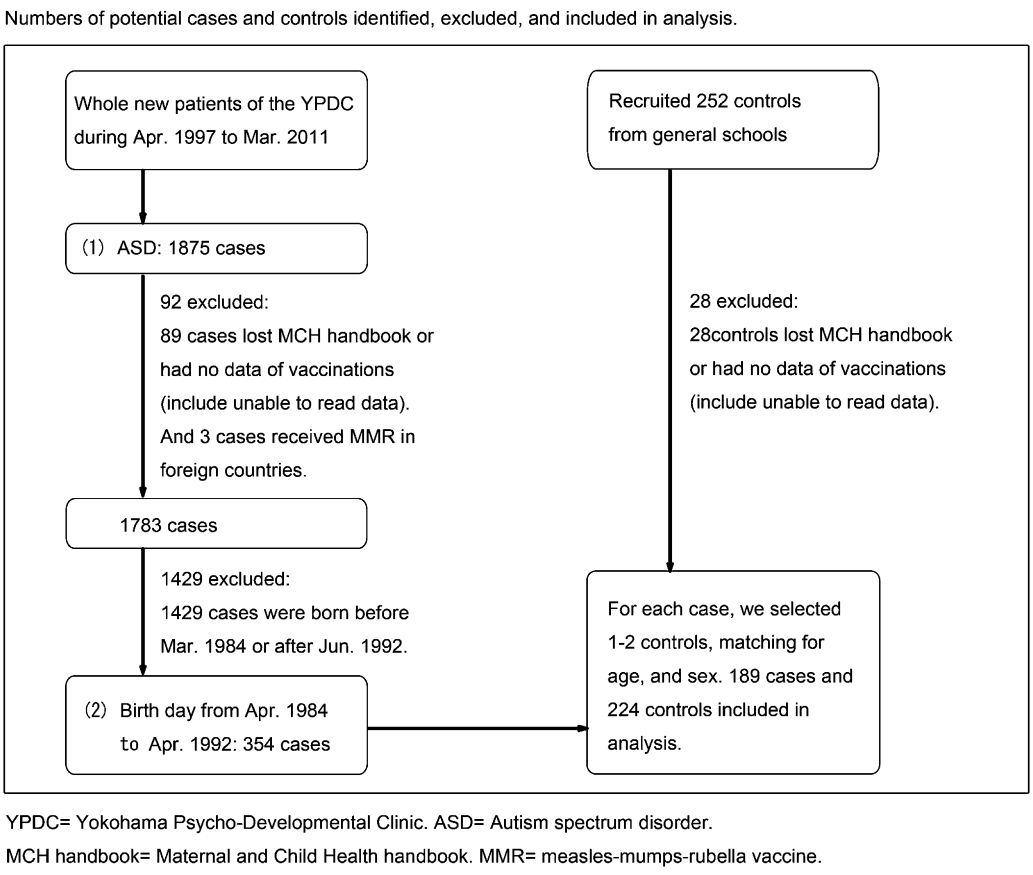

Fig. 1. Numbers of potential cases and controls identified, excluded, and included in analysis. YPDC, Yokohama Psycho-Developmental Clinic; ASD, autism spectrum disorder; MCH handbook, maternal and child health handbook; MMR, measles–mumps–rubella vaccine.

As controls, 252 subjects from the general school population were recruited into the present study. Of these, 28 cases were subsequently excluded because the MCH handbook was missing or the vaccination record could not be read. The goal was to have a matched control for each case. However, since there were not enough controls to match to all cases, 189 subjects were chosen randomly from the ASD group as a case group. The controls were individually matched to cases by age and sex. There were 189 cases, mean age 22.6 years (SD 2.2), and 224 controls, mean age 22.6 years (SD 2.2), with case-to-control ratios ranging from 1:1 to 1:2 (Fig. 1).

2.4. Statistical analysis

2.4.1. Analysis 1

Duration of labor was divided into 2 categories of normal (≤20 h) versus prolonged labor (>20 h). Because an Apgar score of less than 7 has been associated with increased ASD risk [45–48], the Apgar score was divided into 2 categories of normal (≥7 points) and low (<7 points). In order to compare the backgrounds of the cases and controls, the crude odds ratios (ORs) and 95% confidence intervals (CIs) were determined for each outcome. The relationship between ASD onset and the total number of vaccine injections was also investigated. The crude ORs and 95% CI were determined for each. The differences in the mean values of the quantitative variables between cases and controls were examined by an unpaired *t*-test. When necessary, the *t*-test was modified for unequal variances.

2.4.2. Analysis 2

Because this study was only concerned with the theoretical increase in the risk of ASD onset due to the MMR vaccine injection, a conditional logistic model was applied to evaluate the ORs of MMR vaccination after adjusting for other risk factors.

2.4.3. Analysis 3

The OR of the total number of vaccine injections after adjusting for other risk factors was evaluated with the conditional logistic model.

2.4.4. Power analysis

Power analysis was performed in accordance to general power calculation model for chi squared statistics, *t*-test, and a conditional multiple regression model. In brief, power is determined with respect to degree of freedom and predefined alpha level of the study (0.05), number of predictors (in case of a conditional multiple regression model, 4) after assuming effect size (in accordance with Cohen's criteria).

Analysis 1 was performed using SPSS 17.0 for Japan, and Analyses 2 and 3 used the HALBAU 7. ORs were considered significant when the lower 95% CI exceeded 1.0. The *t*-tests were two-sided, and significance was defined as $p < 0.05$. For power calculation G*power v3.1 was used.

2.5. Ethical considerations

This study was approved by the ethics committee at Nagoya University. All data used in this study were clinical data obtained in the course of conventional diagnosis and therapy, and cooperation in the study placed no burden on individual patients. The parents or legal guardians of all of the children in the control group provided their written, informed consent to participate. Personal information regarding subjects in this study and the resulting data

Table 1
The proportions and crude odds ratios (ORs) and 95% confidence intervals (CI) for ASD according to vaccines, prenatal factors, perinatal factors and neonatal factors.

Variable category	*n* (%)		ORs	95% CI	*p*-Value
	Cases (*n* = 189)	Controls (*n* = 224)			
Vaccines					
MMR	47 (24.9)	54 (24.1)	1.04	0.65–1.68	.86
Measles	126 (66.7)	141 (62.9)	1.18	0.77–1.80	.43
Mumps	110 (58.2)	110 (49.1)	1.44	0.96–2.17	.06
Rubella	108 (57.1)	120 (53.6)	1.16	0.77–1.74	.47
DPT	185 (97.9)	219 (97.8)	1.06	0.24–4.75	.94
Polio	184 (97.4)	221 (98.7)	0.5	0.09–2.43	.73
B-encephalitis	167 (88.4)	206 (92.0)	0.66	0.33–1.34	.22
BCG	182 (96.3)	218 (97.3)	0.72	0.21–2.42	.55
Prenatal factors					
Maternal hypertension	6 (3.2)	3 (1.3)	2.42	0.53–12.36	.20
Albuminuria, edema	18 (9.5)	19 (8.5)	1.13	0.55–2.35	.71
Anemia	59 (31.2)	69 (30.8)	1.02	0.66–1.58	.93
Perinatal and neonatal factors					
Prolonged labor (>20 h)[a]	8/169 (4.7)	9/200 (4.5)	1.06	0.36–3.06	.92
Method of delivery					
Cesarean section	21 (11.1)	24 (10.7)	1.04	0.54–2.02	.90
Obstetrical vacuum extraction or forceps delivery[a]	23/168 (13.7)	22/200 (11.0)	1.28	0.66–2.51	.43
Low Apgar score (<7)	6 (3.2)	2 (0.9)	3.64	0.66–26.39	.09

ORs, odds ratios; CI, confidence intervals; ASD, autism spectrum disorder; MMR, measles–mumps–rubella vaccines; DPT, diphtheria–pertussis–tetanus vaccines; BCG, Bacillus of Calmette and Guerin vaccine.

[a] There were 20 cases and 24 controls who did a cesarean section, and 1 case who did a cesarean section because of prolonged labor. Thus, They were excluded from population of prolonged labor and obstetrical vacuum extraction or forceps delivery.

were rendered anonymous, and analyses were performed using only quantitative data that could not be linked to any particular subject.

3. Results

3.1. Vaccination rate and time of exposure to vaccines

The vaccination rates in cases and controls were as follows: MMR, 24.9% of cases and 24.1% of controls; Measles, 66.7% and 62.9%; Mumps, 58.2% and 49.1%; Rubella, 57.1% and 53.6%; DPT, 97.9% and 97.8%; Polio, 97.4% and 98.7%; B-encephalitis, 88.4% and 92.0%, and BCG 96.3% and 97.3% (Table 1). The mean times of each vaccine injection in cases and controls were as follows: DPT, 3.8 times of cases and 3.7 times of controls; Polio, 1.9 times and 2.0 times; B-encephalitis, 1.7 times and 1.8 times (Table 2).

3.2. Analysis

3.2.1. Analysis 1

For each vaccination, the ORs of cases versus controls were as follows (no significant differences were found): MMR, 1.04 (95% CI, 0.65–1.68); Measles, 1.18 (95% CI, 0.77–1.80); Mumps, 1.44 (95% CI, 0.96–2.17); Rubella, 1.16 (95% CI, 0.77–1.74); DPT, 1.06 (95% CI, 0.24–4.75); Polio, 0.50 (95% CI, 0.09–2.43); B-encephalitis, 0.66 (95% CI, 0.33–1.34); and BCG, 0.72 (95% CI, 0.21–2.42). Maternal hypertension as a prenatal factor had an OR of 2.42 (95% CI, 0.53–12.36), but no significant difference was found between cases and controls. For the other factors as well, cases did not have significantly higher ORs than controls. As a perinatal and neonatal factor, low Apgar score and obstetrical vacuum extraction or forceps delivery had an OR of 3.64 (95% CI, 0.66–26.39) and 1.28 (95% CI, 0.66–2.51), respectively, but no significant difference was found between cases and controls. No other perinatal and neonatal factors showed significant differences between cases and controls (Table 1).

A *t*-test was performed on the mean values of the times of exposure to DPT, Polio, and B-encephalitis, birth weight and head and chest circumference between cases and controls, and no significant differences were found ($p > 0.05$ for all). The minimum number of vaccine injections was 3, and the maximum was 13. The mean (standard deviation) number of vaccine injections of cases and controls was 11.4 (1.7) and 11.4 (1.7), respectively, and there was no significant difference between cases and controls ($t = 0.07$, $p = 0.94$) (Tables 2 and 3).

3.2.2. Analysis 2

Maternal hypertension, low Apgar score, and obstetrical vacuum extraction or forceps delivery, which had higher ORs in the results of Analysis 1, were investigated as confounding factors using a conditional multiple regression model. With regard to the presence of ASD, MMR had an OR of 1.10 (95% CI, 0.64–1.90), and maternal hypertension, low Apgar score, and obstetrical vacuum extraction or forceps delivery had ORs of 4.19 (95% CI, 0.46–38.57), 2.06 (95% CI, 0.18–22.12) and 0.98 (95% CI, 0.50–1.92), respectively. There were no significant differences (Table 4).

Table 2
The comparison of the times of vaccine injection between cases and controls.

Vaccines	Cases Mean (±SD)	Controls Mean (±SD)	*p*-Value
DPT	3.8 (±0.8)	3.7 (±0.7)	.78[a]
Polio	1.9 (±0.3)	2.0 (±0.3)	.34[b]
B-encephalitis	1.7 (±0.7)	1.8 (±0.6)	.06[b]

DPT, diphtheria–pertussis–tetanus vaccines.

[a] Student's *t*-test.

[b] Welch's *t*-test.

Table 3
The comparison of quantitative variables between cases and controls.

Variables	Cases Mean (±SD)	Controls Mean (±SD)	*p*-Value
Birth weight (g)	3085.7 (±454.1)	3109.4 (±479.0)	.62[a]
Head circumference (cm)	33.5 (±2.3)	33.6 (±3.0)	.88[b]
Chest circumference (cm)	32.3 (±2.2)	32.3 (±2.7)	.90[a]
The number of vaccine injections (shots)	11.4 (±1.7)	11.4 (±1.7)	.94[a]

[a] Student's *t*-test.

[b] Welch's *t*-test.

Table 4
Odds ratios and 95% confidence intervals of MMR vaccination injection analyzed with a conditional logistic model.

Factor		ORs (95% CI)	*p*-Value
MMR vaccination injection	(−)	1	
	(+)	1.10 (0.64–1.90)	.72
Maternal hypertension	(−)	1	
	(+)	4.19 (0.46–38.57)	.21
Low Apgar score	(−)	1	
	(+)	2.06 (0.18–22.12)	.57
Obstetrical vacuum extraction or forceps delivery	(−)	1	
	(+)	0.98 (0.50–1.92)	.96

ASD, autism spectrum disorder; MMR, measles–mumps–rubella vaccines; ORs, odds ratios; 95% CI, 95% confidence intervals.

3.2.3. Analysis 3

The number of vaccine injections had an OR of 1.10 (95% CI, 0.95–1.26) in a conditional multiple regression model using the same confounding factors as for Analysis 2, maternal hypertension (OR = 3.63, 95% CI, 0.40–33.19), low Apgar score (OR = 2.14, 95% CI, 0.19–23.78), and obstetrical vacuum extraction or forceps delivery (OR = 1.02, 95% CI, 0.52–1.99), and there was no significant difference between cases and controls (Table 5).

3.2.4. Power analysis

Regarding power analysis for chi square statistics and *t*-test, when effect size is set to medium (in accordance to Cohen's criteria), both samples that are characterized in our research had more than 80% power for detecting association, respectively. However, in case, size effect is set to small, calculated power were 52% at chi square statistics and 53% at *t*-test. Similarly, regarding a conditional multiple regression model, our sample had more than 80% of power for detecting association in case of medium effect size. However in case, size effect is set to small, calculated power was 56%.

4. Discussion

The three previous case–control studies focused on the relationship between ASD and MMR. Specifically, the investigation of DeStefano et al. was based on the Metropolitan Atlanta Developmental Disabilities Surveillance Program [31]; Smeeth et al. used data from the UK General Practice Research Database [32]; and DeWilde et al. examined the association using the UK Doctors' Independent Network Database [33]. The aforementioned studies provided no epidemiological evidence for a causal association. The present study is the first case–control study in Asia investigating the relationship between a variety of vaccines including MMR and the risk of ASD onset.

Table 5
Odds ratios of one measure and 95% confidence intervals of the number of vaccine injections analyzed with a conditional logistic model.

Factor		ORs (95% CI)	*p*-Value
The number of vaccine injections	(−)	1	
	(+)	1.10[a] (0.95–1.26)	.19
Maternal hypertension	(−)	1	
	(+)	3.63 (0.40–33.19)	.25
Low Apgar score	(−)	1	
	(+)	2.14 (0.19–23.78)	.54
Obstetrical vacuum extraction or forceps delivery	(−)	1	
	(+)	1.02 (0.52–1.99)	.96

ASD, autism spectrum disorder; ORs, odds ratios; 95% CI, 95% confidence intervals.
[a] OR of the number of vaccine injections means OR of increasing one injection of vaccine.

These previous studies were conducted using relatively heterogeneous samples in terms of genetic makeup. Conversely, the Japanese population is thought to be highly homogenous on the genetic level (which gives us the opportunity to minimize the effect of population-specific risk factors that might interact with environmental exposures (i.e. immunization)), and almost all Japanese parents have an MCH handbook. The fact that highly reliable information concerning the pregnancy, perinatal, and neonatal periods is collected in the handbook was advantageous for conducting this research.

In this study, we could not find the evidence that MMR vaccination increases the risk of ASD onset. The present results support the findings from the previous case–control studies conducted in Caucasian populations. Furthermore, we could not find any evidences that other types of vaccines or a combined effect of multiple vaccines was associated with ASD onset. Therefore, this study did not support the theory that vaccinations should be avoided to reduce the risk of ASD onset. We should be more concerned about acquiring infectious diseases by avoiding vaccinations.

In the results of this study, the 95% CIs of vaccinations, especially DPT, Polio, and BCG had a wide range because of small power. The sample size was not large enough to absolutely exclude the possibility that DPT, Polio, and BCG vaccinations increased the risk of ASD onset. Additionally, there were no theories about an increase in the risk of ASD onset concerns with any single types of vaccine injection that were included in this study, other than the MMR vaccine. Then a conditional logistic model was applied not to DPT, Polio, and BCG, but to MMR which was more concerned with the risk of ASD onset. This study was limited to data from the MCH handbook, which from the viewpoint of conducting an investigation is a highly reliable vaccination data source. On one hand we believe we have obtained very reliable results. However, on the other hand, the information in this handbook does not include several factors which were known to increase the risk of ASD onset, such as parental age at birth, bleeding, birth order, previous fetal loss, maternal prenatal medication use exclusively for hypertension and maternal toxaemia which were included in this study [49], and coexisting conditions that may influence vaccinations received, for example cardiovascular disease, other physical diseases or anomaly, epilepsy, or allergy. We were not able to investigate such conditions in controls because of the nature of the data collection procedure, which involved community-based sampling. Moreover, the relationship is unclear between the time periods when ASD was diagnosed and when the child was vaccinated. It has been hypothesized that early exposure to thimerosal and immune globulin preparations influence neuropsychological deficits in children include ASD [16,50]. Additionally, it is possible that parents lost motivation regarding vaccination before ASD was diagnosed because of problems such as the child's inability to sit still or frequent tantrums. Even so, both groups showed a high vaccination rate for each of the vaccines, and because the main topic of this study was to investigate whether vaccination increases the risk of ASD onset, we believe these effects can be ignored.

In future studies on vaccination and ASD, investigations with larger sample sizes are expected, and we anticipate examining factors which were known to increase the risk of ASD onset, coexisting conditions that may influence vaccination, such as cardiovascular disease, other physical diseases or anomaly, epilepsy, or allergies, the age at ASD diagnosis and vaccines injection, and reasons why vaccinations were not performed. We also look forward to

prospective studies that include pregnancy, delivery, or even preconception factors that may be associated with ASD.

In this study, we could not find any convincing evidence that MMR vaccination and increasing the number of vaccine injections were associated with an increased risk of ASD in a genetically homogeneous population. If such an association exists, it is so rare that it could not be identified in this large regional sample. Therefore, our findings indicate there is no basis for avoiding vaccination out of concern for ASD. This study investigated the link between vaccination and the risk of ASD, but it does not guarantee the safety or efficacy of the vaccines. Adverse reactions from vaccines other than a link with ASD exist. Such adverse reactions must be studied, and safer and more effective vaccines must be developed. At one time in Japan, mumps vaccine in the MMR vaccine caused several cases of aseptic meningitis. We should continue to investigate the safety and efficacy of vaccines carefully and the biological features of ASD in greater depth to improve outcomes related to long-term function and quality of life.

Acknowledgments

A part of this study is the result of research grants from the Ministry of Health, Labor and Welfare of Japan, and "Integrated research on neuropsychiatric disorders" carried out under the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan. The authors would like to warmly thank all the students who took part in this research and their parents and teachers. They are grateful to Professor Nobuyuki Hamajima and Professor Yutaka Inaba for advice and for checking the statistical analyses. Special thanks are expressed to Toru Yoshikawa, Hiroshi Fujioka, Yuko Yoshida, and Yuriko Hachiya for their assistance.

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