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Early exposure to the combined measles–mumps–rubella vaccine and thimerosal-containing vaccines and risk of autism spectrum disorder

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ABSTRACT

Objective: This case–control study investigated the relationship between the risk of Autism Spectrum Disorder (ASD) onset, and early exposure to the combined Measles–Mumps–Rubella (MMR) vaccine and thimerosal consumption measured from vaccinations in the highly genetically homogenous Japanese population.
Methods: Vaccination histories at 1, 3, 6, 12, 18, 24, and 36 months from birth were investigated in ASD cases (189 samples), and controls (224 samples) matching age and sex in each case. Crude odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated to determine relationship between MMR vaccination and ASD. The differences in mean values of the thimerosal dosage between cases and controls were analyzed using an unpaired *t*-test. MMR vaccination and thimerosal dosage were also investigated using a conditional multiple-regression model.
Results: There were no significant differences in MMR vaccination and thimerosal dosage between cases and controls at any age. Furthermore, the ORs (95% CIs) of MMR vaccination and thimerosal dosage associated with ASD in the conditional multiple regression model were, respectively, 0.875 (0.345–2.222) and 1.205 (0.862–1.683) at age 18 months, 0.724 (0.421–1.243) and 1.343 (0.997–1.808) at 24 months, and 1.040 (0.648–1.668) and 0.844 (0.632–1.128) at 36 months. Thus, there were no significant differences.
Conclusions: No convincing evidence was found in this study that MMR vaccination and increasing thimerosal dose were associated with an increased risk of ASD onset.

1. Introduction

Autism Spectrum Disorder (ASD) is a type of neurodevelopmental disorder that significantly affects patients' social functions for their lifetime. The etiology and pathology of this disorder are still predominantly unknown. It has been indicated that there is a strong association with genetic factors, and the relative risk among siblings is known to be greater than 20 with heritability estimated to be as high as 50–80% [1–4]. In contrast, the concordance rate of identical twins is not 100%, indicating that environmental factors also play an important role in the onset of ASD [1,4,5]. One of the concepts that has been discussed is whether vaccinations increase the risk of ASD onset.

The view that vaccinations and ASD are related dates back to Wakefield et al.'s article [6]; however, the paper was retracted in 2010 because of ethical and methodological problems [7]. Thereafter, other studies suggested a link between the measles–mumps–rubella vaccine (MMR) and ASD [8,9], and concerns emerged that thimerosal (49.6% ethyl mercury by weight) included in other vaccines as a preservative might increase the ASD risk [10–12]. On the other hand, three case–control studies, which

Abbreviations: ASD, Autism Spectrum Disorder; MMR, measles–mumps–rubella; TCV, thimerosal-containing vaccine; YPDC, Yokohama Psycho-Developmental Clinic; MCH handbook, Maternal and Child Health handbook; DPT, diphtheria–pertussis–tetanus.

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used the Metropolitan Atlanta Developmental Disabilities Surveillance Program [13], the UK General Practice Research Database [14], or the UK Doctors' Independent Network Database [15], were conducted in Western countries and demonstrated no link between MMR vaccination and ASD. In addition, another case–control study that utilized the Centers for Disease Control and Prevention's Vaccine Safety Datalink also did not show significant differences in the amount of exposure to thimerosal between ASD and non-ASD groups at various months following birth [16]. Moreover, according to two meta-analytic articles, published in 2014, there were no associations between exposure to MMR vaccine/thimerosal and ASD onset [17,18]. However, most studies have not considered vaccinations timing and the subject's racial heterogeneity even though genetic factors are known to be strongly involved in ASD onset. As an investigation that specified the race of the study participants, we conducted a case–control study from the Japanese population [19]. Japanese people were proven to be highly genetically homogenous according to the genotyping results of 140,387 single nucleotide polymorphisms [20]. In our previous study, there was not any convincing evidence that MMR vaccination was associated with an increased risk of ASD in Japanese people. However, the effects due to the differences in vaccinations timing and the amount of exposure to thimerosal were not accounted for in this study.

The risks related to ASD onset and vaccinations are still debated [21] and many parents and guardians avoid vaccinations due to fear of their children developing ASD [22–27], despite the existence of these studies. Therefore, we conducted a case–control study with Japanese subjects, who are highly genetically homogenous, to further investigate whether exposure to MMR vaccine/thimerosal is related to ASD onset in greater detail. We also accounted for temporal factors from birth to vaccinations. The present study involves the same study population as our previous study [19], and consists of a more in-depth investigation of the vaccine data.

2. Materials and methods

2.1. Study population

2.1.1. Cases (Fig. 1)

Case data from patients of the Yokohama Psycho-Developmental Clinic (YPDC) were used in this study. The YPDC opened in April 1997 and is located in the Kanto area of Japan. It only accepts patients with suspected neurodevelopmental disorders. Of the patients who initially consulted the YPDC from April 1997 until March 2011, eligible case subjects: (1) were diagnosed with ASD, and (2) had been born between April 1, 1986 and April 30, 1992, the possible time period for MMR vaccination.

2.1.2. Diagnosis of ASD

To consider diagnoses, the developmental history and the present illness of patients were obtained through the use of the Diagnostic Interview for Social and Communication Disorder, version 10 (DISCO). This diagnostic interview, the DISCO is a semi-structured interview form for diagnosing ASD. It is recognized as one of the best ways to obtain a reliable and valid diagnosis of ASD [28–30]. Patients were diagnosed based on the application of the ASD algorithm of the Diagnostic and Statistical Manual, 5th edition, using the DISCO data.

2.1.3. Period of birth

MMR vaccination in Japan was conducted under specific circumstances and for only a short period of time. A combined MMR vaccination program commenced from April 1989, and only one vaccination using MMR was included in the immunization schedule. The monovalent mumps, measles, 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 had started, there were several cases of aseptic meningitis, which may have been caused by the mumps vaccine [31]. As a result, in April 1993, the Government ceased extensive inoculation with MMR. Therefore, children who were born from April 1984 to April 1992 could receive MMR vaccination. However, children who were born between April 1984 and March 1986 were able to receive it after the age of three. Therefore, they were excluded from the samples, because autism features always appear before the age of three. As a result, children who were born from April 1986 to April 1992 were included in the present study.

2.1.4. Controls (Fig. 1)

Control subjects were recruited as volunteers from general schools in the Kanto area which is the same area where YPDC patients reside. There were 450 students who were born from April 1986 to April 1992 in these schools. Students who had previously been recognized as having developmental problems and were already receiving care were excluded. We obtained informed consent from 252 students (56.0%).

2.2. MCH handbook and source of data

The vaccination records, such as the type of vaccine, dose, manufacturer, lot number, medical institution, and date of prior vaccinations were obtained from the Maternal and Child Health (MCH) handbook, which is a record provided to all mothers by the relevant Japanese health system institution. It is a highly reliable data record of early development, health, and immunization, and the data are record by health professionals (e.g., public health nurses, obstetricians, and pediatricians) [32,33].

From this handbook, we assessed the type of vaccine, frequency, dose, timing, manufacturer, and lot number of vaccinations that were given by 36 months of age when ASD features become apparent. The targeted vaccines were the MMR and the thimerosal-containing vaccines (TCVs) such as the diphtheria–pertussis–tetanus vaccine (DPT); the polio vaccine; the Japanese B encephalitis vaccine; the flu vaccine; and the hepatitis B vaccine. Case and control subjects whose records in the MCH handbook were missing or illegible and those who were vaccinated outside Japan were excluded.

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 three were excluded because they had received vaccinations outside Japan. Of the remaining 1783 cases, 354 cases (males: $n = 286$, 80.8%) were born between April 1986 and April 1992, the time period when MMR vaccinations were administered to children less than 3 years old.

For the control group, 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 the 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).

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