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A population-based case–control study on viral infections and vaccinations and subsequent multiple sclerosis risk

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Abstract Viral infections are probably involved in the pathogenesis of multiple sclerosis (MS). A recent cohort study in the Gothenburg population revealed no change in MS incidence associated with the introduction of the Swedish measles, mumps and rubella vaccination programmes. The aim of the present study was to clarify whether these infections or vaccinations, and two other infections, varicella and infectious mononucleosis, influence MS risk. We performed a population-based case–control study in Gothenburg that included 509 MS cases and 2,067 controls, born 1959–1986. Data on infections and vaccinations were obtained from questionnaires and from child health and school health records. We found no significant associations between measles, mumps, rubella or varicella and MS risk. These results were consistent between the two source materials. Infectious mononucleosis was associated with significantly higher MS risk (odds ratio 2.03, 95% CI 1.52–2.73). Overall, there was no significant association between measles-mumps-rubella (MMR) vaccination and MS risk, while those MMR vaccinated before age ten only were at significantly higher MS risk (odds ratio 4.92, 95% CI 1.97–12.20). Those MMR vaccinated both before and after age ten had intermediate MS risk. Infection with measles, mumps, rubella and varicella did not influence MS risk in contrast to infectious mononucleosis which conferred doubled MS risk. The association with 'early' MMR vaccination only was an isolated finding, limited by a small number of subjects and multiple testing. Most likely this was a chance finding. Future studies could investigate it on an a priori basis.

Keywords Multiple sclerosis · Viral infections · Vaccinations · Case–control study · Gender

Abbreviations

CI	Confidence interval
CIS	Clinically isolated syndrome
CSF	Cerebrospinal fluid
EBV	Epstein–Barr virus
MMR	Measles-mumps-rubella
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
OR	Odds ratio

Introduction

Multiple sclerosis (MS) is a complex autoimmune disease probably resulting from an interaction between genetic and environmental factors. Epidemiological findings suggesting exogenous causative factors are a decreased risk of MS after migration to a low-risk area before adult age [1–3] and a tendency to increased risk after late occurrence of common childhood infections [4–7].

Serological studies of cerebrospinal fluid (CSF) show that a majority of MS patients carry an intrathecal inflammation

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with immunoreactivity to one or more of the viruses of the childhood or adolescence infections measles, mumps, rubella, varicella zoster or Epstein–Barr virus (EBV) [8–15]. However, viral antibodies may be an unspecific part of the autoimmune process, possibly the result of a polyclonal stimulation of B cells [16]. Case–control studies on serum antibodies generally showed no differences concerning seropositivity to measles, mumps and rubella [5, 17, 18]. Genes related to MS are generally expressed in the immune response, which supports the hypothesis that infections are involved in MS pathogenesis [19]. Experimentally, the risk of development of autoimmune disease differs in non-sterile versus sterile environments [20].

The underlying conditions in Sweden were unique for the study of the possible influence of viral infections on the risk of subsequent MS, as national mass vaccination programmes resulted in increasingly large numbers of people without a history of measles, mumps or rubella. Monovalent vaccines were introduced against measles in 1971, mumps in 1973, and rubella in 1974 [21, 22]. In 1982, a combined measles-mumps-rubella (MMR) vaccine for administration at both 18 months and 12 years of age was introduced in Sweden as the first country in the world [23]. Before the introduction of these vaccination programmes more than 80% of Swedish children had a history of measles [24], and half of them a history of mumps and rubella [25, 26]. The percentage of children with a history of measles declined to 12.5% in children born in 1975 and fell further to 5.5% in children born in 1978 following mass vaccinations. The frequency of these three diseases dropped to about 1% in children born in the 1980s (annual data from Child Health Centres, the Swedish Institute for Infectious Disease Control, personal communication) (Fig. 1).

A recent birth cohort and incidence cohort study was designed to analyse MS incidence during the period of implementation of the Swedish national vaccination programmes. A baseline cohort represented the prevaccine era in inhabitants of Gothenburg born 1959–1961. Four cohorts born between 1962 and 1984 were selected to cover a sharp change into each new vaccination programme. The MS incidence showed an age-related continuous increase throughout the study period, but no stepwise increase from baseline and previous cohorts to any of the cohorts selected to represent a new vaccination programme. Thus, there was no indication that the increasing MS incidence was related to the mass vaccination programmes [27]. However, although the vaccination programmes were coordinated nationwide, our cohorts were still relatively inhomogeneous, and the power was limited. We therefore performed a case–control study which, based on a multiplex data set on each individual, examines whether measles, mumps and rubella infections and vaccinations, and two other infections, varicella and infectious mononucleosis, influence the risk of developing MS.

Materials and methods

Cases

Multiple sclerosis patients included in the study were residents of the Greater Gothenburg region for one or more years, were born in 1959–1986, and had disease onset at age ten or later. The study area and the greater part of the patient material were the same as in the recent cohort study which was restricted to the age group 10–39 years, born 1959–1990 [27]. The percentage of MS patients with disease onset within the study area was 81.9%. A smaller proportion of MS patients, who fulfilled the inclusion criteria regarding residence of Gothenburg, but were older than 39 years at MS onset or had MS onset outside the study area were included in the present case–control study. Eight patients who were included in the cohort study were deceased, and 13 patients had emigrated. We refrained from contacting 22 patients for psychological reasons. Inclusion of cases and controls was restricted to adults aged 18 years or over at the time of receiving the questionnaire (Fig. 2). Briefly, we

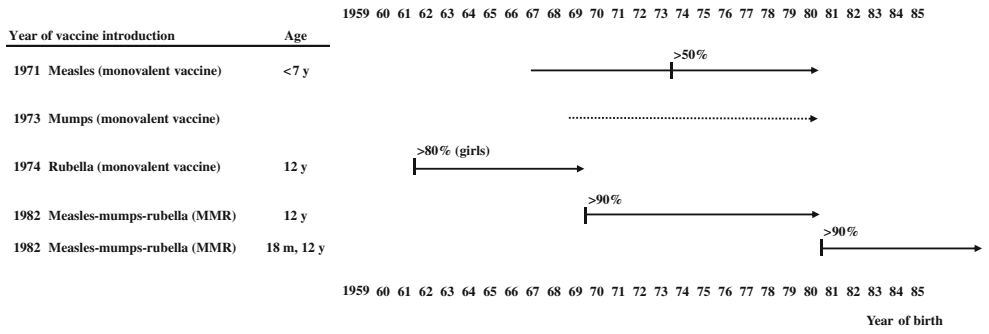

Fig. 1 The Swedish vaccination programmes. The target of the mass vaccinations was children born in certain years who had reached certain ages. *y* years; *m* months

used several sources for obtaining the cases. In February to December 2004, we searched the administrative diagnosis registries at Sahlgrenska University Hospital and the National Patient Register of the National Board of Health and Welfare. Two of the authors (C.A., O.A.) reviewed all records with the diagnoses multiple sclerosis, demyelinating disorders in the central nervous system, acute transverse myelitis, optic neuritis and retrobulbar neuritis, according to the International Classification of Diseases (ICD) 10, 9, and 8. We reclassified all cases according to both the Poser criteria [28] with an added category of "clinically isolated syndromes" (CIS) [29] and the McDonald criteria [30]. According to the modified Poser criteria, 23.2% of the total patient material ($N = 509$) were patients with CIS; according to the McDonald criteria, 21.8% were patients with "possible MS". Table 1 gives details of the proportions of patients in each subgroup of CIS. We included primary progressive MS as a disease compatible with MS presenting as insidious progress from onset for at least 1 year without remission and with subsequent development of additional foci ($N = 19$). Because of the uneven use of magnetic resonance imaging (MRI) examinations through the study period, we used the Poser criteria for our statistical analyses.

Controls

A total of 4,000 control subjects were randomly selected from the general population register in Gothenburg, Sweden. Controls were born in the same years as the patients (1959–1986), and were residents of the study area on May 13, 2004 when the selection was made (Table 2).

Study design

A questionnaire was sent to 627 MS patients and 3,917 controls who fulfilled the inclusion criteria (Fig. 2). Swedish children are regularly examined at child health centres from birth to school entry, and within the school health care system from school entry at age 7 to at least age 12. Childhood diseases and vaccinations are documented in these records. We used this source of child health and school health records in addition to the questionnaires. Participants gave their consent to read their child health and school health records in their completed questionnaires. We selected the study participants who had attended the sixth grade of school within the study area ($N = 1,370$; 273 MS and 1,097 controls), as children received the final vaccination of the Swedish vaccination programme at age 12 years, which usually coincided with the sixth grade of school, and the records of these study participants were relatively easily available (Fig. 2).

Assessment of exposure

Questionnaires

Information about infections with measles, mumps, rubella, varicella and infectious mononucleosis was obtained from a self-administered questionnaire, together with the country

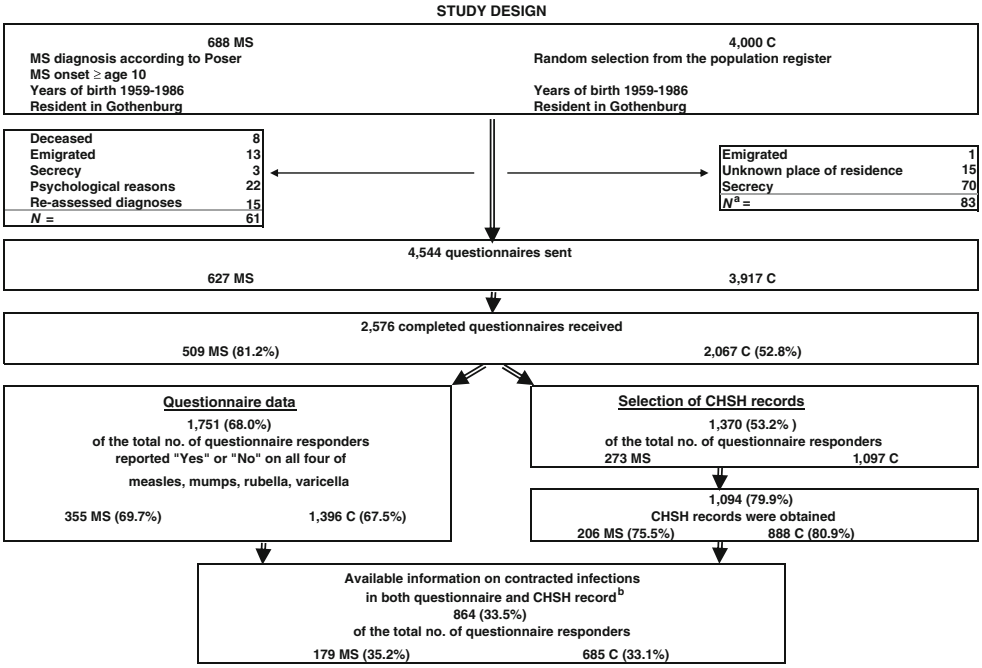

Fig. 2 Study design. Flowchart showing all MS patients and controls eligible for the present study. [a]One person may be included in more than one category. [b]The occurrence but not the absence of specific childhood diseases was registered in the CHSH records. *MS* multiple sclerosis, *C* controls, *CHSH* child health and school health

Table 1 Clinical characteristics of MS patients

Characteristic	Females		Males		Total	
	N	%	*N*	%	*N*	%
Diagnosis						
Probable and definite MS	269	75.4	122	80.3	391	76.8
CIS	88	24.6	30	19.7	118	23.2
CIS category						
ON	27	30.7	8	26.7	35	29.7
BS	12	13.6	9	30.0	21	17.8
M	22	25.0	7	23.3	29	24.6
Long tract or cerebral symptoms	15	17.0	3	10.0	18	15.3
Polyfocal symptoms	12	13.6	3	10.0	15	12.7
Age at onset of MS or CIS (years)						
Mean ± SD	26.5 ± 6.6		26.1 ± 6.9		26.4 ± 6.7	
Range	12–45		10–43		10–45	
MS or CIS onset in the study area	285	79.8	132	86.8	417	81.9
Total number of patients	357	100.0	152	100.0	509	100.0

MS multiple sclerosis, *CIS* clinically isolated syndrome, *ON* optic neuritis, *BS* brain stem syndrome, *M* myelitis, *SD* standard deviation

Table 2 Demographic characteristics of MS patients and controls

Characteristic	MS patients		Controls	
	N	%	*N*	%
Female gender	357	70.1	1,195	57.8
Male gender	152	29.9	872	42.2
Swedish origin[a]	387	76.0	1,492	72.2
Non-Swedish origin[a]	110	21.6	547	26.5
Missing	12	2.4	28	1.3
Total	509	100.0	2,067	100.0

MS multiple sclerosis

[a] Swedish origin was defined as being born in Sweden with both parents born in Scandinavia

of birth of the participant and his or her parents. Swedish origin was defined as being born in Sweden with both parents born in Scandinavia. All infections were included, irrespective of the age at infection and irrespective of whether this additional information was available.

Child health and school health records

Information about infections with measles, mumps, rubella and varicella and vaccinations against these diseases was collected from child health and school health records.

Vaccination categories

Vaccines against measles, mumps, and rubella were administered as vaccinations containing monovalent vaccine strains or as vaccinations with the trivalent MMR vaccine, i.e. the combination of measles, mumps and rubella vaccine strains. Measles, mumps, and rubella vaccinations were either "early", i.e. before or at 10 years, or "late", i.e. after 10 years. The rationale for this division was that the majority of the vaccinations were given around age 12 years (90%), or at ages 1–8 years (54%). Less than 3% of the vaccinations were given at other ages (Table 3). The first analysis was performed on the entire study material of vaccinations with *monovalent or combined measles, mumps and rubella vaccines*. We defined three disjointed categories of vaccinations: 'No vaccination', 'Early vaccination only', and 'Late vaccination'. The group of individuals who received "late" vaccination (>10 years of age) also included those who had received both a late and a prior early vaccination. The group of individuals vaccinated "early only" was restricted to those vaccinated below or at 10 years of age. The major part of the entire material was MMR vaccinations. The second analysis was therefore restricted to the subgroup of the *MMR vaccinations*. Four disjointed vaccination categories: 'No MMR vaccination', 'Early MMR vaccination only', 'Late MMR vaccination only', and 'Both an early and a late MMR vaccination' were defined. Comparisons were made within the group of MMR vaccinations.

Validation

The proportion of MS patients for whom information about their history of measles, mumps, rubella and varicella infections was available from both the questionnaire and from the child health and school health record was 35.2% of the total number of questionnaire responders. The corresponding proportion for controls was 33.1% (Fig. 2). Sensitivity and specificity of questionnaire data was validated by child health and school health records (Table 4).

Table 3 Number of MS patients and controls in four disjointed categories of different vaccinations with respect to age at 'Early' (≤10 years), 'Late' (>10 years), and 'Both early and late' vaccination ($N = 206$ MS patients, 888 controls)

	Vaccination category	No. of MS patients	No. of controls	Total
Monovalent or combined measles, mumps, rubella	No vaccination	75	230	305
	Early vaccination only			
	Age			
	<3	6	36	42
	3–8	9	10	19
	9–10	0	0	0
	Total	15	46	61
	Late vaccination only			
	Age			
	11–13	68	281	349
	>13	3	8	11
	Total	71	289	360
	Both early and late vaccination			
	Age			
	<3 and 11–13	27	265	292
	Other ages	17	57	74
	Total	44	322	366
	Missing information on age	1	1	2
Combined measles, mumps, rubella (MMR)	No vaccination	125	367	492
	Early vaccination only			
	Age			
	<3	5	24	29
	3–8	3	2	5
	9–10	0	0	0
	Total	8	26	34
	Late vaccination only			
	Age			
	11–13	56	294	350
	>13	1	11	12
	Total	57	305	362
	Both early and late vaccination			
	Age			
	<3 and 11–13	12	178	190
	Other ages	3	12	15
	Total	15	190	205
	Missing information on age	1	0	1
Monovalent measles	No vaccination	166	732	898
	Early vaccination only			
	Age			
	<3	19	102	121
	3–8	19	50	69
	9–10	0	0	0
	Total	38	152	190
	Late vaccination only			
	Age			
	11–13	0	0	0
	>13	0	0	0
	Total	0	0	0

Table 3 continued

	Vaccination category	No. of MS patients	No. of controls	Total
	Both early and late vaccination			
	Age			
	<3 and 11–13	0	1	1
	Other ages	0	1	1
	Total	0	2	2
	Missing information on age	2	2	4
Monovalent mumps	No vaccination	202	879	1,081
	Early vaccination only			
	Age			
	<3	0	2	2
	3–8	4	3	7
	9–10	0	1	1
	Total	4	6	10
	Late vaccination only			
	Age			
	11–13	0	2	2
	>13	0	1	1
	Total	0	3	3
	Both early and late vaccination			
	Age			
	<3 and 11–13	0	0	0
	Other ages	0	0	0
	Total	0	0	0
	Missing information on age	0	0	0
Monovalent rubella	No vaccination	163	774	937
	Early vaccination only			
	Age			
	<3	0	1	1
	3–8	0	1	1
	9–10	0	0	0
	Total	0	2	2
	Late vaccination only			
	Age			
	11–13	41	112	153
	>13	2	0	2
	Total	43	112	155
	Both early and late vaccination			
	Age			
	<3 and 11–13	0	0	0
	Other ages	0	0	0
	Total	0	0	0
	Missing information on age	0	0	0

Participation

We performed a test to evaluate the possibility of bias due to uneven frequency of the questionnaire responses from different study periods. The correlation between 4-year periods of birth and participation in the study was tested within the MS group and within the controls by test for trend in contingency table. There was no significant difference in the response rate between different periods (MS patients, $P = 0.1844$, controls, $P = 0.6544$).

Table 4 Sensitivity and specificity of questionnaire data validated by child health and school health records

Infection	MS patients ($N = 206$)		Controls ($N = 888$)	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Measles	82.7	92.3	79.4	91.4
Mumps	72.0	88.3	68.0	92.3
Rubella	54.0	85.2	46.9	89.3
Varicella	72.4	80.0	63.8	74.4

Statistical methods

Infections

Logistic regression analyses were applied to study the relationship between infections and MS, age at infection ($\leq$10 or >10 years), and Swedish origin and MS. Independent variables were sex, year of birth, infection and interaction between sex and infection. In order to take year of birth into account in a careful way we used spline functions of year of birth in the logistic regression analyses. The results were expressed as odds ratios (OR). Two-tailed P-values <0.05 were considered to be significant.

Vaccinations

ORs were calculated in order to elucidate the effect on MS risk of vaccinations at different ages before or at 10 years and after 10 years. Logistic models including sex and year of birth (spline functions) were used with MS as the dependent 0–1 variable. The analysis of "Late vaccination versus none, or early only" (see "Results") comprised a 0–1 variable attaining the value 1 if there was a late vaccination and 0 otherwise and another variable attaining the value 1 if there was an early and no late vaccination and 0 otherwise. If β_1 and β_2 are the beta coefficients then $\exp(\beta_2-\beta_1)$ gives the OR of early vaccination only versus late vaccination. The corresponding variance was calculated by use of the covariance between the beta coefficients. The same method was applied for the analysis restricted to the subgroup of MMR vaccinations. All analyses of vaccinations were performed on data obtained from the child health and school health records.

This study was approved by the Regional Ethical Review Board in Gothenburg.

Results

Retrieved data

The questionnaire response rate was 81.2% in the MS group and 52.8% in the control group, providing a study material of 509 patients and 2,067 controls. Child health and school health records of participants who had been in the sixth grade of school within the study area were obtained for 206 MS patients and 888 controls, which corresponds to 75.5% of the MS patients and 80.9% of the controls in this selection (Fig. 2).

General demographic characteristics

The female-to-male ratio in the MS group was 2.35:1. The percentage of study participants of Swedish origin was 76.0% in the MS group and 72.2% in the control group (Table 2).

Clinical characteristics

Table 1 summarises the clinical characteristics of MS ($N = 391$) and CIS ($N = 118$) patients.

Origin

The risk of MS was significantly higher in individuals of Swedish origin than in individuals of foreign origin (OR 1.32, 95% confidence interval (CI) 1.04–1.68, $P = 0.0224$).

Recall of age at infection

Multiple sclerosis patients seemed to recall age at infection better than controls. An analysis of measles infection and MS risk, including only infections which were reported with age information, showed significantly higher frequency of infections in the MS patient group than in the control group (OR 1.34, 95% CI 1.05–1.70). Conversely, when only infections without age information were included, the frequency of infections was significantly lower in the MS patient group than in the control group (OR 0.70, 95% CI 0.53–0.92).

Infections

There were no significant differences in the frequencies of measles, mumps, rubella or varicella reported in the questionnaires between the MS group and the control group, either gender-specific or for both genders combined. The upper limits of the corresponding 95% CIs show that, if there is a small effect, it is not likely to be higher than 30% for measles, 50% for mumps and 20% for varicella. The reported frequency of infectious mononucleosis was significantly higher in the MS group than in the control group for both genders combined (OR 2.03, 95% CI 1.52–2.73) and for females (OR 2.27, 95% CI 1.61–3.22), but not for

Table 5 Odds ratios for MS in individuals with or without a history of each of five infections under study

Infection	No. of MS patients[a]	No. of controls[a]	Females		Males		Total	
			OR	95% CI	OR	95% CI	OR	95% CI
Measles	432	1,683	1.21	0.89–1.63	0.66	0.44–1.01	1.00	0.77–1.30
Mumps	439	1,739	1.31	1.00–1.73	0.92	0.62–1.36	1.17	0.93–1.48
Rubella	410	1,642	0.95	0.72–1.25	0.51	0.33–0.78	0.79	0.62–1.00
Varicella	470	1,906	0.80	0.50–1.27	0.90	0.47–1.73	0.83	0.57–1.21
Infectious mononucleosis	442	1,894	2.27	1.61–3.22	1.55	0.88–2.71	2.03	1.52–2.73

Data from questionnaires ($N = 509$ MS, 2,067 controls)

MS multiple sclerosis, *OR* odds ratio, *CI* confidence interval

[a] Number of MS patients and controls who responded "Yes" or "No"

males (OR 1.55, 95% CI 0.88–2.71) (Table 5). However, there was no significant difference in MS risk associated with infectious mononucleosis between women and men. When comparing the OR for women versus that for men the difference between the two estimates was not significant ($P = 0.2576$). Restricting the analysis of infections to probable and definite MS patients, genders combined, did not substantially alter the result (data not shown). There was no significant difference between MS patients and controls with respect to the frequencies of measles, mumps, rubella or varicella infections registered in the child health and school health records (Table 6).

Infections after the lowest age of MS onset

In the total material of patients and controls, none of the measles, mumps or rubella infections occurred above the age of 10 years in more than 5%, with 10 years being the lowest age of MS onset in this study. Varicella occurred in approximately 13%, and infectious mononucleosis in approximately 10% above the age of 10. None of the MS patients reported measles or rubella after the onset of MS. Seven patients (1.4%) reported mumps or varicella after the onset of MS. One MS patient reported possible infectious mononucleosis in the same year as the onset of MS.

Combinations of infectious mononucleosis with other infections

The OR for MS in individuals with a history of infectious mononucleosis and measles versus those with a history of infectious mononucleosis only was 0.84 (95% CI 0.45–1.58). Similar analyses were performed for ORs in individuals with a history of infectious mononucleosis and mumps, rubella or varicella. No significant changes in MS risk were associated with any of these combinations (Table 7).

Vaccinations

We used data based exclusively on child health and school health records for the analyses of vaccinations and MS risk. The first analysis was performed on the entire study material including *monovalent and combined measles, mumps and rubella vaccines*. Simply having been vaccinated against measles, mumps or rubella did not change the risk of MS. 'Early vaccination only' ($\leq$10 years of age) was associated with increased risk of MS (OR 2.46, 95% CI 1.27–4.76, $P = 0.0075$) in comparison to late, or both early and late vaccination. 'Late vaccination' (>10 years of age) was associated with decreased risk (OR 0.64, 95% CI 0.43–0.94, $P = 0.0238$) in comparison to no vaccination or

Table 6 Odds ratios for MS in individuals with or without a history of each of four infections under study

Infection	OR	95% CI
Measles	1.00	0.68–1.45
Mumps	1.16	0.83–1.62
Rubella	1.16	0.83–1.64
Varicella	1.30	0.93–1.81

Data from child health and school health records ($N = 206$ MS, 888 controls)

MS multiple sclerosis, *OR* odds ratio, *CI* confidence interval

Table 7 Odds ratios for MS in individuals with a history of infectious mononucleosis and any other specific infection, versus individuals with a history of infectious mononucleosis only

Infection	OR	95% CI	*P*-value
Measles	0.84	0.45–1.58	0.5963
Mumps	1.37	0.75–2.49	0.3042
Rubella	0.67	0.36–1.23	0.1935
Varicella	1.08	0.32–3.64	0.9074

Data from questionnaires ($N = 509$ MS, 2,067 controls)

MS multiple sclerosis, *OR* odds ratio, *CI* confidence interval

early vaccination only. 'Late vaccination' and 'Early vaccination only' were the two extreme groups. Those who had received both an early and a late vaccination had an intermediate risk (Tables 3, 8). In order to study whether the OR of 2.46 for early vaccination was an effect from the vaccination per se, or whether it was confounded by infection, we calculated the MS risk in vaccinated individuals with a history of measles versus vaccinated individuals with no history of measles in the corresponding age group. There was no significant influence on MS risk in measles vaccinated individuals depending on whether they had a history of natural measles or not. Rather, the risk of MS associated with natural measles infection before or at 10 years of age tended to be higher in the opposite direction (OR 1.23, 95% CI 0.92–1.64) to that expected if the observed higher MS risk associated with vaccination had been mediated by measles infection. The second analysis was restricted to the subgroup of the *MMR vaccinations*. The MS risk was not significantly different in the MMR vaccinated group from that in the category of 'No MMR vaccination'. 'Early MMR vaccination only' in comparison to late only, or both early and late vaccination was associated with higher risk of MS (OR 4.92, 95% CI 1.97–12.20, $P = 0.0006$). After adjusting for multiple comparisons (the 25 similar comparisons possible to perform) using the Bonferroni method, the *P*-value was 0.015. 'Late MMR vaccination only' in comparison to other MMR vaccinations was associated with decreased risk of MS (OR 0.40, 95% CI 0.17–0.93, $P = 0.0342$). The category of 'Both an early and a late MMR vaccination' was not associated with increased MS risk (Tables 3, 9).

Discussion

In the present material, infection with measles, mumps, rubella or varicella did not influence subsequent MS risk, opposed to a significant twofold higher MS risk related to a history of infectious mononucleosis. Restricting the analysis to probable and definite MS cases did not substantially

Table 8 Odds ratios for MS associated with three disjointed categories of monovalent or combined measles, mumps, rubella vaccinations: 'No vaccination', 'Early vaccination only', and 'Late vaccination'

Compared groups	OR	95% CI	*P*-value
No vaccination versus vaccination	1.22	0.77–1.92	0.4101
Early vaccination only[a] versus late vaccination[b]	2.46	1.27–4.76	0.0075
Late vaccination[b] versus none, or early only	0.64	0.43–0.94	0.0238
Both an early[a] and a late vaccination[b] versus others	1.02	0.62–1.66	0.9510

Data from child health and school health records ($N = 206$ MS, 888 controls). The number of individuals in different categories is shown in Table 3

MS multiple sclerosis, *OR* odds ratio, *CI* confidence interval

[a] ≤10 years only

[b] >10 years only, or both >10 and ≤10 years

Table 9 Odds ratios for MS associated with four disjointed categories of measles, mumps, rubella (MMR) vaccinations: 'No MMR vaccination', 'Early MMR vaccination only', 'Late MMR vaccination only', and 'Both an early and a late MMR vaccination'

Compared groups	OR	95% CI	*P*-value
No MMR vaccination[a] versus MMR vaccination	1.13	0.62–2.05	0.6849
Early MMR vaccination only[b] versus others[c]	4.92	1.97–12.20	0.0006
Late MMR vaccination only[d] versus others[c]	0.40	0.17–0.93	0.0342
Both an early and a late MMR vaccination versus others[c]	0.85	0.38–1.92	0.7017
Early MMR vaccination only[b] versus both an early and a late MMR vaccination	3.99	1.57–10.53	0.0052
Late MMR vaccination only[d] versus both an early and a late MMR vaccination	0.95	0.41–1.40	0.9077

Data from child health and school health records ($N = 206$ MS, 888 controls). The number of individuals in different categories is shown in Table 3

MS multiple sclerosis, *OR* odds ratio, *CI* confidence interval

[a] MMR = combined measles, mumps, rubella

[b] ≤10 years only

[c] MMR vaccinations given at other ages

[d] >10 years only

alter the findings. Vaccination with measles, mumps, rubella vaccines had no overall influence on the risk of later MS. We found early MMR vaccination to be associated with a higher risk of subsequent MS than late MMR vaccination. However, this result was an unexpected, isolated finding, based on a small number of subjects and therefore, it provides relatively weak evidence.

We have used data not only from questionnaires, but also from a source of more objective data in child health and school health records, where childhood infections were documented from birth to the teen years. The child health and school health records were produced many years in advance of the present study in a uniform social system and MS was not an issue when these records were written. The frequencies of infections may be underestimated in these records, but any such effect would probably be equal for patients and controls. The results for infections with measles, mumps, rubella, varicella and MS risk based on the child health and school health records confirmed the corresponding results based on the questionnaires. There is always a possibility that questionnaire responses are affected by recall bias, and this may have occurred in the present study. The questionnaire response rate in the control group (52.8%) was lower than in the MS group (81.2%). Furthermore, since the permissions to retrieve the child health and school health records were given in the questionnaire responses, the difference in the questionnaire response rate, if biased, may have introduced a corresponding selection bias in the selection of the child health and school health records. However, there was no uneven frequency of questionnaire responses from different study periods. Therefore, the possibility that MS patients and controls decided to participate in the study depending on their experience of childhood infections and vaccinations, and that this affected the results, would be subtle.

We included all infections in our analyses, irrespective of age at infection and irrespective of whether age information was available. The reason for this was that the age at infection was not stated for a considerable number of the reported infections, and the MS patients tended to recall age at infection better than the controls, introducing potential bias in age-specific analyses. We had access to data on age at infection in the child health and school health records, but the sample size did not allow age-specific analyses. Therefore, we used "Yes" or "No" responses without using the additional information on age at infection in the analyses of frequency of infection in MS patients and controls. However, we used the information on age at infection for a simplified analysis on measles before or after age 10, the age limit which was used in the analyses on vaccinations. The data material on age at vaccination was larger, and had a wider distribution than age at infection, and was easily divisible into two age groups.

Several studies were focused on age at childhood infection rather than frequency of these infections in MS patients and controls [4, 6]. Studies based on interviews or questionnaires found no significant differences in frequency of infections [31, 32]. However, the interview or questionnaire technique often entails problems with recall bias. Population-based case–control studies with stronger study designs using more objective and less biased data such as serology [5], pre-symptomatic questionnaire data [7] or prospective registries [33], still found no significant differences between MS patients and controls in the frequencies of preceding common viral childhood infections. The results of a large population-based case–control study in the Netherlands showed significant differences, but these were reported to be eliminated when a correction was applied for an estimated recall bias [34]. Our findings of equal frequency of infections in MS patients and controls, and of a twofold higher MS risk associated with infectious mononucleosis agree with the findings in other studies [35, 36].

We have shown, at first in a cohort analysis [27] and now in a case–control study that infection with measles, mumps or rubella does not significantly influence the individual risk of developing MS. We used data from the child health and school health records here in an analysis of the risk of subsequent MS in individuals exposed to different vaccinations, taking sex and the year of birth into account. The chosen cut-off at 10 years isolates a distinct group of late vaccinations at about 12 years of age (90%) from the group of early vaccinations at age 1–8 years of age (54%). Less than 3% of the vaccinated participants were vaccinated at other ages. From an immunological point of view, however, the age limit is arbitrary. An analysis of the entire study material of vaccinations with monovalent or combined measles, mumps, and rubella vaccines showed no significant difference in the risk of MS between vaccinated and unvaccinated individuals. The risk of MS was higher in individuals vaccinated before or at 10 years only than in those vaccinated after that age, or both before and after. The effect of vaccination seemed to be independent of natural infection, since the risk of MS in the measles vaccinated individuals was not influenced by their history of measles infection. The analysis of the subgroup of MMR vaccinations showed similar results to the analysis of the entire material. There was no overall significant change in MS risk depending on whether or not individuals were MMR vaccinated. Among MMR vaccinated individuals, the risk of MS was higher in those who had received early vaccination only. A late vaccination was associated with lower MS risk. Further analyses of MMR vaccinations revealed that the combination of an early and late vaccination showed approximately the same risk as a late vaccination only, suggesting that a late vaccination

may neutralise the risk of MS in individuals who had received early vaccination only.

A number of questions concerning a possible "susceptibility age" are raised by these results. One possible explanation may be that T cell tolerance to myelin is age dependent [37]. However, our unexpected finding that early MMR vaccination was associated with higher risk of MS than late MMR vaccination may be the result of chance and should be treated with caution. The group of study participants who had received early vaccinations only was small ($N = 61$), less than 10% of the vaccinated individuals with available child health and school health records. Eight MS patients and 26 controls were MMR vaccinated at a young age only. Although significant in the present Gothenburg material, the association with 'early MMR vaccination only' was an isolated finding, which was based on a small number of subjects and only provides relatively weak evidence. There have been no studies published which considered age at measles, mumps, rubella vaccination and the subsequent risk of MS. Thus, the possible importance of age at vaccination needs to be confirmed or refuted in other study material. We confirmed the result obtained by a previous large population-based case–control study showing that ever having been vaccinated against measles, mumps or rubella does not increase the risk of MS [38].

In summary, infectious mononucleosis increased the risk of subsequent MS, while there was no indication that infection with measles, mumps, rubella or varicella, alone or as a group, explains the development of MS. Any effect on the risk of developing MS from these infections must be small. Furthermore, there was no indication that infectious mononucleosis augments the significance of other infections. The possible benefit of including a vaccine against EBV in the vaccination programme should be investigated.

Conclusions

The strength of this study is that it exploited the large differences between the numbers of exposed and unexposed individuals that occurred during the mass vaccination campaigns against measles, mumps and rubella. Furthermore, we used information from two sources, questionnaire data and data from the more objective child health and school health records, and these two sources gave very similar results. In the same patients and with the same methods, we have shown that measles, mumps, rubella and varicella infections do not influence the risk of subsequent MS. We confirmed that the risk of MS is increased by a factor of two following infectious mononucleosis. There was no overall effect of the measles, mumps and rubella vaccinations on MS risk. The most likely explanation for the unexpected finding that age at vaccination is of significance for the risk of MS is that it is a chance finding, but future studies could investigate it on an a priori basis.

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