

Epidemiological analysis of viral infections as risk factors for multiple sclerosis

Cecilia Ahlgren

**Department of Clinical Neuroscience and Rehabilitation
Institute of Neuroscience and Physiology**

**The Sahlgrenska Academy at
University of Gothenburg**



UNIVERSITY OF GOTHENBURG

2009

Till Charlotte och Jessika

Abstract

Viral childhood diseases have been implicated in the pathogenesis of multiple sclerosis (MS). Swedish mass vaccination programmes resulted in radical changes in the panorama of the targeted infections. Vaccines against measles, mumps and rubella were implemented in the early 1970s. In 1982, Sweden introduced the two-dose measles-mumps-rubella (MMR) vaccine, as the first country in the world. Measles sharply declined in the 1970s. In cohorts born since 1981, measles, mumps and rubella were virtually eliminated. The main aim of this thesis was to investigate whether specific viral childhood diseases, or the vaccinations against them, influence the risk of developing MS. This was accomplished by a study of MS incidence in vaccinated cohorts, by a case-control study and – indirectly – by a study of birth order in sibships.

These studies were based on unique underlying conditions. Individual data on infections and vaccinations were documented in child health and school health records. The proportion of individuals with a history of vaccination-targeted infections was suitable for statistical analyses. Furthermore, there was a long tradition of MS incidence studies in Gothenburg. We updated the Gothenburg MS registry obtaining a study material of 534 incident MS patients born from 1959 to 1990. The incidence of MS was analysed in four population cohorts, each selected to represent a new vaccination programme. Questionnaires on measles, mumps, rubella, or the vaccinations, and two other infections, varicella and infectious mononucleosis were completed by 509 MS patients and 2067 controls, born 1959 to 1986. Data on infections and vaccinations were obtained from the questionnaires and, for a selection of 206 MS patients and 888 controls, also from child health and school health records.

We found no major influence of birth order on MS risk. The observed number of first-born patients did not significantly differ from the expected number, and the proportion of first-borns did not differ from that in a control cohort born during the same period. We found no significant change in the incidence of MS in any of the four population cohorts defined by mass vaccinations. The long-term MS incidence showed a significant gradual age-dependent increase, which was unrelated to the introduction of the vaccination programmes. MS patients and controls reported similar frequencies of measles, mumps, rubella and varicella. The results from the child health and school health records confirmed the results from the questionnaires. Infectious mononucleosis was associated with 2-fold higher MS risk. In the light of this positive finding, the negative findings for the other studied infections are more convincing. Simply being vaccinated against measles, mumps or rubella did not change the risk of MS. Similarly, MMR vaccinated individuals were not at higher or lower MS risk than MMR unvaccinated individuals. Among MMR vaccinated individuals, the MS risk was increased in those vaccinated before age 10 only. Those vaccinated both before and after age 10 had intermediate MS risk.

The specific viral childhood diseases measles, mumps, rubella and varicella do not influence the risk of MS, and may be dismissed as risk factors for MS. Infectious mononucleosis is a moderate risk factor for MS. Vaccination against Epstein-Barr virus infection should be considered. The risk of MS in MMR vaccinated does not differ from that in MMR unvaccinated individuals. The finding that MMR vaccination at a low age only may be related to MS risk needs to be confirmed in other study material.

Original articles

This thesis is based on the following papers, which will be referred to in the text by their roman numerals

- I** Ahlgren C, Taranger J, Johansson L, Andersen O. Elimination of childhood diseases: possible influence on the incidence of multiple sclerosis soon detectable. *Neuroepidemiology*. 2002;21(6):306-9.
- II** Ahlgren C, Andersen O. No major birth order effect on the risk of multiple sclerosis. *Neuroepidemiology*. 2005;24(1-2):38-41.
- III** Ahlgren C, Oden A, Toren K, Andersen O. Multiple sclerosis incidence in the era of measles-mumps-rubella mass vaccinations. *Acta Neurol Scand*. 2009;119(5):313-20.
- IV** Ahlgren C, Oden A, Toren K, Andersen O. A population-based case-control study on viral infections and vaccinations and subsequent MS risk. Manuscript – Submitted

Abbreviations

CI	Confidence interval
CIS	Clinically isolated syndrome
CNS	Central nervous system
CSF	Cerebrospinal fluid
EBV	Epstein-Barr virus
HR	Hazard ratio
IgG	Immunoglobulin G
MMR	Measles - mumps – rubella
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
OB	Oligoclonal band
ON	Optic neuritis
OR	Odds ratio
RA	Rheumatoid arthritis
RR	Risk ratio
SLE	Systemic lupus erythematosus
SSPE	Subacute sclerosing panencephalitis

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INTRODUCTION

A historical retrospective provides the following examples: Jean-Martin Charcot identified MS in 1868 and outlined the classic triad of symptoms: intention tremor, nystagmus and scanning speech [1]. He described transient symptoms, such as diplopia, and progressive symptoms such as paraparesis and ataxia [2]. Charcot's direct experience of patients with MS was limited. He only saw around 30 cases of MS during his career and assumed the disease to be rare [1]. The diagnosis was uncommon until the 1930s. Then, as the number of trained neurologists increased, MS diagnoses increased, emerging out of other disease categories. The most common neurological diagnosis in women throughout the 19th century was hysteria. At the end of the 19th century Charcot's pupil Joseph Babinski described a sign to differentiate weakness due to lesions in the central nervous system (CNS) from weakness caused by hysteria, namely the 1896 "Babinski sign" of extensor toes with plantar stimulation [3, 4]. This sign proved to be of great significance in distinguishing between hysteria and CNS disease. In the first half of the 20th century the diagnosis of hysteria declined and many of these patients were later interpreted as having MS. In the 1920s, males were thought to be more affected than females. By the early 1950s, MS was considered to be one of the most common CNS diseases. As the ascertainment of MS cases improved, the frequency of MS increased and in the 1960s MS incidence was suggested as being slightly higher in women than in men [4]. The first epidemiological reports on MS in the late 19th century, by Sir Byron Bramwell, described a geographical pattern of higher MS prevalence at higher latitude [5]. At the present time, increasing trends in MS incidence are associated with an attenuation of the classic north-south gradient and an increase in the female-to-male ratio of MS over time [6]. A case in point is the large Canadian study in 2006 which revealed a remarkable increase in the female-to-male ratio for at least 50 years, while there was no indication that MS in men had decreased [7]. Changes in MS incidence may offer clues to the aetiology of MS. The role of viral infections, sunlight, and vitamin D are now among the most currently discussed issues [8].

In Sweden, the earliest nationwide MS survey was conducted in 1942. At that time MS prevalence in Gothenburg was estimated at 23 per 100,000 [9]. Dramatically increased MS prevalence to 96 per 100, 000 in Gothenburg in 1988 probably resulted from improved case ascertainment methods [10].

INDICES OF A VIRAL AETIOLOGY IN MS

The uneven world-wide distribution of MS

It is now generally believed that MS is an autoimmune disease in genetically disposed individuals. The epidemiology of MS indicates that it is triggered or facilitated by viral infections. Genetic influence on MS risk have been suggested by twin studies as the recurrence risk in families of people with MS was 30.8% in monozygotic twins, and

4.7% in dizygotic twins, versus 0.1% in the general population of northern Europe [11, 12].

Geographically, MS is distributed throughout the world within three zones, of high, medium, and low frequency. A traditional statement is that high prevalence areas include Northern Europe, the Northern USA and Canada, Southern Australia and New Zealand. Medium prevalence areas include Southern Europe, the Southern USA, and Northern Australia. Low prevalence areas include Asia and South America [13]. Ethnic differences in susceptibility are shown by the white populations of predominantly Caucasian ancestry in high- and medium-risk areas world-wide, and lower risk in black populations, even in high prevalence areas. The risk in black individuals in the United States was half that associated with whites [14]. Variations in MS risk within small geographical areas reflect the significance of ethnicity. In areas where MS is common some groups are at lower risk, including Samis, Turkmen, North and South Amerindians, Canadian Hutterites, Africans and New Zealand Maoris [15]. In the studies on U.S. Army veterans during the World War II, the geographical distribution of residence at enter into active service showed a generally increasing risk for MS with latitude [14]. There was a highly significant positive correlation of Scandinavian ancestry, especially Swedish and MS risk in the US [16]. However, the effect of latitude had more weight than that of ethnicity, when ethnicity was based on surnames [17]. In Australia, a marked variation of prevalence with latitude has been reported [18]. In Western Europe the prevalence of MS was previously regarded as being related to a north-south gradient, but this theory has been refuted [19]. The Nurses' Health Study (NHS) showed a north-south gradient in the U.S. for MS risk in women born 1921 to 1946. However, in the NHS II, including women born 1947 to 1964, the risk of MS in the north was not greater than in the south. These findings suggest that the north-south gradient diminished over time [20].

While the ethnical differences in susceptibility support the idea of genetically based susceptibility for developing MS, migration studies emphasise the importance of environmental factors. Migration studies raised the interest of MS as a disease with an infectious aetiology since the risk of MS changed when moving between high- and low-risk areas before adult age [21]. The most important findings from these studies on migration are that the risk of MS in the migrant changes to that intermediate between the country of birth and the country of immigration. This was shown in the study on US veterans who moved between their birth place and their residence at service entry [22]. Furthermore, the age at migration from high- to low-risk area influenced the risk of developing subsequent MS [23]. However, environmental factors may operate over a period of many years and not only in childhood and adolescence [24].

An epidemiological model used for a general test of an infectious aetiology is cluster analysis. A space-time cluster analysis in Hordaland, Norway, showed that MS patients within the same birth cohort had lived significantly closer to each other than expected during the ages of 13-20 years, with peak clustering at the age of 18 years [25, 26]. Another model has been used in studying possible "MS epidemics". Ingenious studies were performed on MS in relation to a proposed unknown agent,

introduced by the British troops on the Faroe Islands during World War II. It was proposed that MS cases first appeared after 1940 on the Faroe Islands [27]. The suggested association between exposure to British troops and MS epidemics has been questioned, mainly due to the small samples and the weak evidence that MS did not exist in the Faroe Islands before 1940 [28, 29].

Interestingly, a higher percentage of the population in different regions of the world showed positive titres to several infections at an early age in areas with low MS frequency than in areas where MS was common. Alter et al. concluded that if infections are significant risk factors for MS, early infection is associated with low MS risk and later infection with higher MS risk [30].

The hygiene hypothesis

The migration studies had shown increasing MS prevalence with increasing latitude [14]. Leibowitz et al. [31] noted that sanitation increases with latitude. Concerning poliomyelitis, the risk of paralytic poliomyelitis was higher in regions with a high level of sanitation. The similarities between the epidemiological pattern of MS and that of poliomyelitis raised the hypothesis that MS might be correlated with the level of sanitation. Significantly higher sanitary level in childhood for MS patients than controls was found in a population-based case-control study including 85% of all prevalent Israeli MS patients in 1960 and randomly selected and matched controls. Leibowitz et al. also found that the difference between MS patients and controls regarding sanitary level became less marked as the hygiene standard improved over time [31]. The concept of a “hygiene hypothesis” was first explicitly formulated by Strachan in 1989, who found striking associations between family size and position in the household and hay fever in a large follow-up of 17,414 British children for 23 years [32]. Since siblings influence the pattern of exposure to infections, birth order may be a marker of infectious risk factors. Several studies investigated possible associations between the risk of MS and being a first-born or only child [33-41]. The results showed no convincing consistency. However, association with birth order has been found in other autoimmune diseases, as for example inflammatory bowel disease [42].

Mechanisms by which a virus may induce an autoimmune disease

Mechanisms by which an infection can lead to an autoimmune disease were postulated by Shoenfeld and co-workers: 1) by molecular mimicry the infecting agent may incorporate an epitope that is structurally similar to that of a self-antigen. 2) Polyclonal activation is a mechanism where an infection of B cells results in B cell proliferation, enhanced antibody production, and the generation of circulating immune complexes which may cause damage to self-tissues. 3) The bystander activation mechanism describes a situation where enhanced cytokine production as part of the immune response to infections induces the expansion of autoreactive T cells against the adjacent tissue whose prior number was insufficient to produce an overt disease [43]. Experimental animal models have contributed to a large body of knowledge on these mechanisms. The most studied autoimmune model of MS is experimental autoimmune

encephalomyelitis (EAE), which may be induced by immunisation with myelin protein antigens. A chronic variant of this model resembles relapsing-remitting MS [44]. There is, however, a crucial difference between EAE and MS in that the antigen in EAE is known, whereas the initial cause of the autoimmunity in MS is unknown [45].

Vaccination with live attenuated virus

In contrast to the infection induced by wild virus, vaccination induces a considerably milder infection. However, according to Molina and Shoenfeld, the same mechanisms that act in infectious invasion of the host apply equally to the host's response to vaccination. One possible mechanism by which a vaccine may induce autoimmunity is possible persistence of the viruses in the target tissue, a second is the potential for induction of autoantibodies production, and a third is abnormal cytokine production [46]. Based on these principles a killed vaccine would be less likely than a live attenuated vaccine to activate the innate immune response or cause tissue disruption [47], and the degree of response achieved by vaccination will be much smaller than that induced by the wild strain [48]. Live attenuated viral vaccines may very rarely lead to acute encephalitis [49]. Acute encephalitis occurs after measles vaccination in about 1 in a million recipients, while the incidence of acute encephalitis after natural measles infection is 1 per 1000 [50].

Intrathecal synthesis of antibodies in the cerebrospinal fluid of MS patients

Cerebrospinal fluid (CSF) enriched oligoclonal bands (OBs) are used as an indicator of local production of immunoglobulin G (IgG), and indicate that there is a localised inflammatory process within CNS [51]. Abnormal production of oligoclonal IgG within the CNS, detected as OBs, occurs in more than 95% of clinically definite MS patients [52]. OBs are also found in other CNS disorders, such as neurosyphilis, tuberculous, fungal meningitis, some acute viral CNS infections, in SSPE [53] and in autoimmune diseases other than MS, such as systemic lupus erythematosus (SLE), sarcoidosis, and neuroborreliosis [54, 55]. Since an oligoclonal response in viral encephalitis represents a specific immunological response to the causal antigen or set of antigens, OBs in MS have been investigated to find a specific viral agent. About 70% of MS patients carry intrathecally synthesised CSF enriched antibodies to measles, about 40% against rubella, about 35% against varicella, and about 90% of MS patients carry antibodies to one or more of the measles, rubella and varicella zoster viruses (MRZ reaction) [56, 55]. Approximately one third of MS patients carry CSF antibodies to Epstein-Barr virus (EBV) [57]. While the major oligoclonal response in infectious diseases of the nervous system is directed against the agent that causes the disease, only a small fraction of about 2% of the total intrathecally synthesized oligoclonal IgG in MS, is directed against measles, rubella and varicella viral antigens [56]. It is unclear, however, whether the oligoclonal response in MS is partly directed against causative agents or whether the entire oligoclonal response is a result of polyclonal stimulation of B cells as an unspecific part of the autoimmune process [58].

Common viral childhood infections and CNS involvement

Wild-type measles, mumps and rubella viruses are all known to infect the CNS [44]. Measles infection is extremely contagious. Infection with one strain of wild measles virus provides life-long protection from disease caused by all strains. Secondary infections pneumonia or diarrhoea, often as a consequence of a measles virus induced immunosuppression, are responsible for the greater part of the death rates in acute measles infection. It is generally believed that measles virus is eliminated from infected individuals after recovery from acute measles. However, brain tissue obtained at autopsy in unrelated diseases revealed measles virus mRNA in 20% of subjects [59]. It is possible that all morbilliviruses transiently infect the CNS in their natural hosts, but development of disease is dependent on the efficiency of the immune response [60]. Postinfectious encephalitis occurs in 1/1000 cases with measles, in 3/1000 cases with parotitis, and in 0.2/1000 cases with rubella [61]. Rarely, a subacute form of encephalitis occurs, which is consistent with slow virus involvement. Subacute sclerosing panencephalitis (SSPE) complicates 1 in a million cases of measles infection. It occurs 5-10 years after acute infection. The risk is age-dependent, being at highest if the initial measles infection occurs below the age of 2 years. The rare CNS disease measles inclusion body encephalitis can affect immunocompromised patients weeks to months after both wild-type infection and vaccination [62].

COMMON VIRAL CHILDHOOD INFECTIONS INVESTIGATED FOR AN ASSOCIATION WITH MS

The viruses of the common infections measles, mumps and rubella, for which vaccines are available, have all been implicated in MS. The following review is restricted to some of the population-based or randomised case-control studies, which included at least 100 MS patients. Compston et al. [63] studied viral infections and vaccinations in 177 patients attending London hospitals and 164 HLA-DR matched controls. While reported frequency of mumps was higher in patients than in controls, serological tests showed no significant differences in the frequency of measles, mumps, rubella or varicella seropositivity between patients and controls. However, almost all individuals in the study were seropositive for measles, a situation that involves difficulties in detecting significant differences. Patients had measles and rubella at a significantly later age than controls. However, the risk of MS was significantly increased in association with age at infection only in HLA-DR2 positive individuals. The Italian MS Study Group [64] conducted a hospital-based case-control study including 318 definite or probable MS patients and 1,975 “neurological” or “non-neurological” control patients. Based on questionnaires, the reported frequencies of measles, mumps, rubella and varicella were found to be equal between the MS and the control group. However, infectious mononucleosis (MS patients, $N = 4$ (1.3%), controls, $N = 9$ (1.8%)) was also equally reported by patients and controls, a result which was not in agreement with two other studies [37, 65]. While there were no significant differences in the frequencies of infections, the age at infection was associated with higher MS risk. The risk of MS was increased in individuals who reported one or more infections (measles, mumps, rubella or varicella) after the age of 6 years. However, no

explanation was provided for the choice of age 6 as a cut-off. Grønning et al. [66] compared the mean age at infection in a patient group with that in a matched control group in a Norwegian study with a very high questionnaire response rate. The difference was close to statistical significance for infection with measles, the mean age being 6.6 years in the patient group versus 5.5 years in the control group. The proportion reporting measles, mumps, rubella and varicella was similar between groups. Casetta et al. [67] chose 5 years of age as a cut-off and found a relationship between younger age at infection with measles and rubella and 2-fold increased MS risk, obviously in disagreement with the studies cited here. However, the authors provided no explanation for the choice of age 5. One working hypothesis was that environmental factors before puberty may play a role in MS aetiology, according to the authors. Gusev et al. [68] performed an MS case-control study on environmental risk factors in Russia. In order to analyse the importance of age, events were classified after occurrence before or after puberty (age 15 years). The choice of age was based on the hypothesis that environmental factors operate until age 15. However, few participants reported measles, mumps, rubella or varicella after 15 years of age, and the frequencies of these infections were similar between cases and controls. The authors commented that a lower threshold age was used in earlier investigations in which an influence of a higher age at childhood infections was reported. Bachmann et al. [69] found that MS patients had measles, mumps, rubella, varicella and infectious mononucleosis at a significantly later age in comparison with statistical data on the Swiss population. A questionnaire was published in a newsletter of the Swiss Multiple Sclerosis Society and reached about 3,800 MS patients. More than 600 MS patients responded to the questionnaire. However, the response rate was apparently only 16%. While the patient material in this study was either population-based or random, the “control” material was epidemiological statistic data for the normal Swiss population. The authors claimed that the patients had no knowledge of the hypothesis of age or about the age at infection in the population. Although a patient selection bias is difficult to exclude, the collected data seems to be relatively reliable. Only those patients with childhood infections who were examined by a physician were recorded. The population figures for age at infection were based on data from Swiss health and medical registries. The age intervals were similar to those used in the health and medical registries, and thus not arbitrarily chosen. The results from this large study confirmed those reported by Compston et al. and Grønning et al. [63, 66]. Hernán et al. [41] performed a large nested case-control study within the Nurses’ Health studies. They found increased risk after infectious mononucleosis, and after late infection with measles or mumps. The results were based on questionnaire data. The risk of recall bias was, however, minimised by the exclusion of women who had received a diagnosis of MS before they responded to the baseline questionnaire. Bager et al. [70] took a step forward by using objective registry data for 455 MS patients and 1,801 controls. School health records covered childhood diseases during school years while information on diseases during preschool age was obtained from parents at school entry. Possible recall inaccuracy, however, is likely to have been equal between the patient and the control group. The frequencies of measles (~80%), mumps (~45%) and rubella (~40%) in the study material of Bager et al. were not so high as to hamper detection of significant differences between groups. However, neither prevalence nor age at infection was associated with MS risk. The authors concluded that measles,

mumps, rubella and varicella, even if acquired late in childhood, are not associated with increased risk of MS later in life. A significant association with MS risk would have been difficult to detect if the occurrence of measles in the population was extremely high. For example, Compston et al. found 98% of the MS patients and 99% of the controls being seropositive for measles virus [63]. Pekmezovic et al. [71] found significantly higher MS risk associated with measles, rubella and varicella before or at age 7 than after that age. It is not clear if this specific age was an arbitrary choice. Vaccinations against tuberculosis, poliomyelitis, tetanus and mumps did not change the risk of MS. The results were based on data reported in questionnaires. Zaadstra et al. [72] analysed data on common childhood infections based on questionnaire responses from 2,877 MS patients and 2,673 controls in the Netherlands. They found significantly increased MS risk associated with history of measles, mumps, rubella and varicella. The estimated ORs were between 1.14-1.42 and similar to those of the probe variables for 'broken arm', 'concussion', and 'tonsils removed', which were included to estimate a possible recall bias. Unlike these weak associations, history of infectious mononucleosis doubled the risk of MS. The authors therefore concluded that the association between MS and infectious mononucleosis is selective.

VACCINATIONS AGAINST MEASLES, MUMPS, RUBELLA AND POSSIBLE ASSOCIATION WITH MS

Few studies have been carried out to discover if the introduction of routine mass vaccinations against measles, mumps and rubella have had any effect on the incidence of MS. Bansil et al. [73] interviewed all 27 MS patients in an MS clinic about history of measles infection and measles vaccination. Immunisation records were obtained for 15 of the 27 patients. There was no control group. Only two patients had received two vaccinations. However, there was one important finding. MS did occur in measles vaccinated individuals, even after two vaccinations. The authors concluded that measles vaccination does not prevent MS. They also considered it to be premature to carry out a large-scale population-based study of measles and possible association with MS incidence. Currier [74] performed a type of cohort study. The incidence of measles had markedly decreased in the US after the introduction of the measles vaccine in 1963. He studied a material of 152 consecutive MS patients at a multiple sclerosis clinic. This material was divided into two 12-year birth-year periods, before and after the introduction of the measles vaccine programme, and the numbers of patients were compared. About 80% of the patients born in 1959 or earlier recalled having had measles. All 22 patients born in 1960 or later stated they had had measles vaccination and two possibly measles. Currier concluded that there was no evidence of a beneficial effect of measles immunisation on the occurrence of MS. DeStefano et al. [75] collected MS patients and controls from three health maintenance organisations (HMO) that participated in the Centers for Disease Control and Prevention's Vaccine Safety Datalink project in a large population-based case-control study. Information on vaccinations was ascertained from computerised vaccination records, medical records and interviews. The main results showed similar vaccination histories in MS patients and controls. Vaccination against hepatitis B, influenza, measles or rubella was not associated with risk of MS or optic neuritis (ON), while tetanus vaccination was

associated with decreased risk. Analyses restricted to HMOs' records provided similar results. Age at vaccination was not analysed, and the focus was not on childhood vaccinations. Information was collected on vaccinations received from 10 years of age and later.

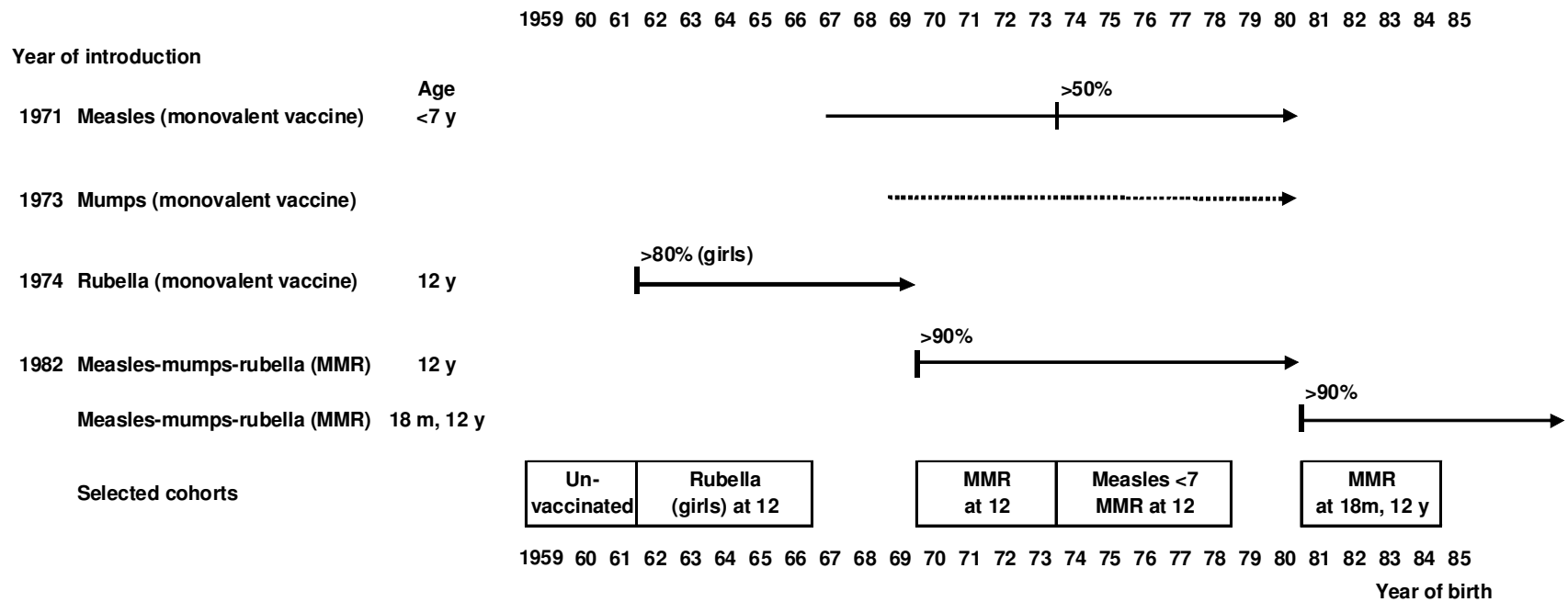
A meta-analysis was performed by Garanieri and Casetta [76], who reviewed 24 case-control studies on common childhood and adolescent infections and MS. Their analysis showed that many studies found no significant differences in frequency of infectious diseases, while later age when acquiring at least one of the childhood infections was the most consistent result.

A NEW INFECTION SPECTRUM IN SWEDEN FOLLOWING NATIONWIDE MASS VACCINATIONS

The Swedish vaccination programmes

The Swedish vaccination programmes against measles, mumps and rubella were implemented nationwide, yet sharply separated in time with several year's intervals. The target for the vaccinations was children born in certain years who had reached certain ages. In the prevaccine era Swedish children were unvaccinated against measles, mumps and rubella, but routinely vaccinated against smallpox, tuberculosis, diphtheria, tetanus, pertussis and polio. More than 80% of 12-year-old children had a history of measles, and about half of them mumps and rubella. Among adults, more than 90% had a history of measles, about 65% mumps [77], about 90% rubella [78], and at least 70% had a history of varicella [79]. In 1971 a monovalent vaccine against measles was introduced and given to children older than 18 months, mainly preschool children [80]. In the later part of the 1970s, the incidence of measles was radically reduced to 12.5% among preschool children born in 1975, and to 5.5% among children born in 1978 (annual data from the child health centres, the Swedish Institute for Infectious Disease Control, personal communication). A monovalent mumps vaccine followed in 1973 [81], given preferentially to a minor group of children with sensorineural deafness. There was, however, never general vaccination against mumps until it was included in the combined measles-mumps-rubella (MMR) vaccine in 1982 [82]. In 1974, 12-year-old girls were vaccinated against rubella. This programme continued until the rubella vaccine was included in the combined MMR vaccine [80]. Sweden was the first country to introduce a two-dose regime of the combined MMR vaccine [83]. This programme was successfully accomplished and reached more than 90% coverage in both age groups of 18-month and 12-year olds as early as 1983, one year after its introduction. The incidence of measles, mumps and rubella was reduced to about 1% in cohorts born in 1981 and later (annual data from the Child Health Centres, the Swedish Institute for Infectious Disease Control, personal communication) (Fig. 1).

Fig. 1 The vaccination programmes and the selected population cohorts



ADVANTAGES AND DISADVANTAGES OF EPIDEMIOLOGICAL APPROACHES

Observations of differing MS risk in different regions of the world led to studies on migration [23, 22, 24]. Migration provides the opportunity to study a “natural experiment” with the advantage of elucidating environmental and genetic contributions to MS aetiology. Studying the effect of changing environment during a short period of time has provided valuable insights into disease causation.

Furthermore, age at migration provides information on a possible susceptibility age for acquiring MS. According to Gale and Martyn [84] who systematically reviewed all relevant migrant studies, the main conclusions drawn from migration studies were: migrants who move from an area where the disease is common to an area where it is rarer show a decrease in MS risk, while those who move in the opposite direction tend to retain the low risk of their country of origin, and the risk is largely established during the first two decades of life. However, the interpretation of results from studying migration entails certain problems. One is the question whether migrants are representative of the population of their country of origin. Migrants tend to be drawn from younger age groups, and are often of higher socioeconomic status and better educated than non-migrants, although this may vary according to the type of migration. Different incidence rates between the country of origin and the country of destination may be due to different levels of awareness of the disease, of availability of health care, or of different levels of case ascertainment efficacy between the countries. Another problem may be small numbers of migrants and low power. Few authors carry out tests of statistical significance of the differences in rates between migrant populations and those of their countries of origin and destination, nor are confidence intervals (CI) given [84]. The large study on US veterans of the World War II [22] constitutes an exception to this by providing highly significant MS risk differences, estimated as risk ratios (RR) and CIs, in migrants depending on the risk in the US state of origin and that of the destination [84].

Birth order has been used as a marker of exogenous risk factors in childhood. Siblings influence the pattern of exposure to infections by determining the age at transmission, the frequency of infections, and the severity of infections [85-87]. A method of investigating possible associations between birth order and MS risk is to calculate the expected birth order number among the siblings of MS patients in comparison with the observed birth order number in a matrix of birth order and sibship size. Alternative techniques are comparing the number of older as opposed to younger siblings, or comparing birth order of a MS patient group with that of a control group. However, sibship studies entail an important problem due to possible changes in sibship size and family structure over time, as explained below in the ‘Discussion’ section.

The two main types of epidemiological study are the *cohort* study and the *case-control* study. A cohort is any designated group of individuals who share a common characteristic and are followed or traced over a period of time. Disease frequency in cohorts of different regions and periods of time may be measured by calculating the prevalence or incidence [88]. *Prevalence* may be defined as the proportion of population that has a disease at a specific point in time. Prevalence is estimated by

dividing the number of existing cases with the total population at a given point in time. The prevalence is, however, influenced by the duration of disease, since higher mortality from the disease reduces prevalence. Therefore, comparing prevalence between different regions or periods of time requires consideration of possible differences in treatment strategies which may influence life expectancy. In aetiological research MS prevalence is seldom of interest. The *incidence* rate is the number of new cases divided by the person-time where the person-time is the summation of time that occurs simultaneously for all members in the cohort. The incidence study may provide aetiological clues, especially when used in combination with an ecological approach. For example published health statistics may be used for an average estimate of the exposure to a certain risk factor in the cohort [88]. The MS incidence may then be calculated in population cohorts which differ regarding a specific risk factor. Regions with an MS registry or other tools to identify all MS patients are well suited for incidence surveys. However, meaningful comparisons between incidence rates require uniformity in diagnosis criteria and methods of case ascertainment. Furthermore, discrepancies in awareness of the MS disease, accessibility of magnetic resonance imaging (MRI), and novel therapies, or availability of health care in general, may create artefacts.

In order to study a possible disease outcome as an effect of an exposure, a *prospective* cohort, defined by specific criteria, is followed over a period of time. The cohort members make up the population at risk. They may share certain characteristics, such as residence and age during a defined period of time. The information on exposure should be available for all members. The relative risk of disease in association with exposure can be determined as the ratio of the incidence rate among exposed individuals to that of non-exposed individuals. The cohort may be closed or open. The ideal study design would be a prospective closed cohort, which adds no new members for example through immigration, and loses members only to death [88]. Members who leave the area of the study remain members of the closed cohort. In this case onset of the disease of interest may occur outside the study area. If the cohort is closed, the population at risk will decrease over time due to mortality. Thus, in order to estimate the disease rate in a closed population cohort, the population at risk has to be estimated in relation to the rate of mortality in the cohort. In contrast to a closed cohort, an open cohort, for example a birth cohort, adds new members through immigration and loses members through migration and mortality. Usually the number of members of an open cohort remains stable. An important advantage of the cohort study, open or closed, is the possibility of calculating both incidence rate and relative risk. However, the prospective cohort is not suited for the study of rare diseases [88].

A *historical* cohort design takes advantage of individual information on exposure to potential risk factors already collected for other purposes [89]. Although data on exposure in the historical cohort study are collected retrospectively, the methodological principles of prospective cohort studies and historical cohort studies are the same. There might be a problem in that the cohort design requires knowledge of all cases in the population. A disadvantage is the large number of subjects required if the disease is rare. However, linking registries is a feasible study approach as the number of national registries is increasing. In comparison with the case-control

method, the most important advantage is that the risk of several types of biases is reduced. Recall bias is not present and selection bias may be reduced [89].

A *cluster*, defined as an excess number of cases within a small geographic area or during a short period of time, provides the possibility of testing aetiological hypotheses. However, establishing an exogenous risk candidate for MS, using the cluster cohort method, may be difficult. One problem is that genetic factors may be important. Another problem is low statistical power. The most serious problem, however, is the highly variable latency period from exposure to clinical onset in MS [26].

Ecological or *aggregate* studies focus on the comparison of groups, rather than individuals. They often use published statistics on disease occurrence and exposure in population [90]. MS is particularly well suited for this approach. MS incidence may be compared between different geographical areas or periods of time. An example of an ecological study is the use of reports on seropositivity for childhood infections from different areas in the world in the study by Alter et al. [30]. Although caution has to be exercised in interpreting of results which are based on population averages, ecological studies are useful for detecting associations of exposure distributions with disease occurrence [91, 92].

The *case-control* study design is effective for relatively rare diseases, such as MS. This design is also effective when there is a long latency between exposure to a risk factor of interest and clinical onset of disease, as in MS. While the proportion of individuals exposed to certain risk factors is seldom known in a population cohort, the relative risk of disease can be approximated as the odds ratio (OR) in a case-control study. In a population cohort, the proportion of subjects not diagnosed with MS is much higher than the proportion diagnosed with MS. One of the advantages of the population cohort-based case-control design is that all ascertained cases can be included, while only an appropriate selection of the controls is needed [93]. This is the method of a “nested case-control” study, exemplified by the study on MS and age at infection, nested in the prospective Nurses’ Health Studies [41]. Less restrictive selection methods are commonly used, but almost any case-control study can be thought of as nested within some source population. When the control subjects are not selected from the same source as the cases, or the response rate is low, there is potential for selection bias. In an appropriate selection, control persons should have been identified as cases if they had developed MS. Patients with a chronic disease are known to answer questions on medical history and previous events more accurately than healthy individuals. Patients also tend to over-report exposures and events. Such discrepancy between cases and control leads to recall bias [93]. A few probe variables for events not related to the disease may be included to estimate a possible recall bias [72]. If possible, questionnaire-based data would be validated against another data source. Since the time of the initial subclinical MS onset is unknown, life-style factors, or events close to MS onset may be consequences of the MS disease, rather than causal risk factors. This indistinctness is avoided in practice in research on the common viral childhood diseases, since they are most often experienced many years before the ages at which MS onset commonly occurs.

A particular problem in many studies on aetiological risk factors for MS is the unknown length of the preclinical phase, which may span decades [94].

THE CONDITIONS ON WHICH THIS THESIS IS BASED

These studies were based on unique underlying conditions. Firstly, the Swedish vaccination programmes were successfully performed, and the vaccination programme progress was followed up by the Swedish Institute for infectious Disease Control, especially with regard to vaccine coverage, efficacy of different vaccines and reported adverse events. A child health card in its present form has been in use in Sweden since 1959 which together with the school health record contributed to a valuable source of data on the occurrence of childhood diseases and the vaccinations against them. Secondly, it is easier to draw statistical inferences concerning characteristics which are not extremely common in the population. During the period of the implementation of mass vaccination programmes, the proportion of infected individuals in the population declined, and consequently the ratio between infected and uninfected became more suitable for statistical analyses. Third, there was a long tradition of MS incidence studies in Gothenburg, starting in the 1950s with intensive case ascertainment. Repeat MS incidence studies in Gothenburg [95, 96, 10, 97] provided a register which was updated in the studies included in the present thesis.

We do not contend that measles or any of the other diseases under study causes MS. The question is whether viruses, such as those of measles, mumps, rubella, or varicella contribute to the development of MS by triggering an autoimmune process in genetically disposed individuals.

AIMS OF THIS THESIS

The general aim of the study was to investigate whether common viral childhood diseases influence the risk of developing MS later in life. The specific aims were to:

- Investigate whether the pattern of exposure to infections early in life influences the risk of MS
- Investigate whether the introduction of measles, mumps, and rubella vaccination programmes have influenced the incidence of MS
- Investigate whether measles, mumps, rubella, varicella and infectious mononucleosis influence the risk of MS
- Investigate whether vaccination against measles, mumps and rubella influence the risk of MS

MATERIALS AND METHODS

The study area and population - The study area was Gothenburg on the Swedish west coast close to the 58th latitude. The initial study (Paper I), was restricted to the population of the city of Gothenburg, which was 431,273 in 1980. In the following surveys (Papers III, IV) the study area was extended to the Greater Gothenburg region with a land area of 2314 square kilometres and 731,592 residents in 2000. The 10-digit personal identification code, obligatory in Sweden since 1947, provided an effective tool for the use of multiple sources, to link registries, and trace patients. Population data were obtained from the Swedish Total Population Register, Statistics Sweden (www.scb.se). The number of immigrants to Sweden increased during the study period. The proportion of residents born abroad increased from 4.0% in 1960 to 12% in 2004.

Sources - We had the advantage of the long tradition of MS incidence studies at Sahlgrenska University Hospital in Gothenburg. The Gothenburg MS register was established with intensive case ascertainment from the 1950s and repeatedly updated over the following years [95, 96, 10, 97]. From 1951, the Gothenburg population was exclusively served by the neurology service at Sahlgrenska University Hospital. After 1970, four additional minor associated neurological clinics were established. There was a strong tendency to refer all neurological or suspected neurological problems to the specialists at Sahlgrenska University Hospital, resulting in a “spider” type of case ascertainment [98].

Present patient material – The Gothenburg MS register was updated from multiple sources, including the administrative diagnosis registries of the Departments of Neurology, Neuro-ophthalmology and the Neuropediatric Unit at Sahlgrenska University Hospital, the patient register of the National Board of Health and Welfare, the local MS Society, and by personal visits to the four outpatient neurological clinics in the Greater Gothenburg region. We reviewed all records with the following MS related diagnoses, according to the International Classification of Diseases (ICD) 10, 9, and 8:

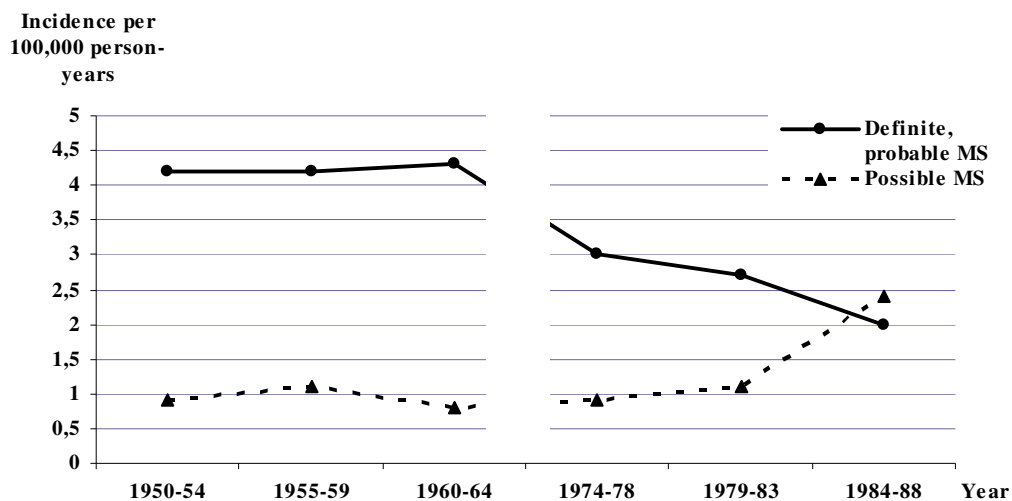
G369; 341X Acute disseminated encephalomyelitis
G359; 340; 340.99 Multiple sclerosis
G368; G378; G379; 341W; 341.09 Demyelinating disorders in the central nervous system
G360; 341A; 341.01 Neuromyelitis optica
G373 Acute transverse myelitis
H46; 377D; 367.02 Optic neuritis
H48,1; 367.03 Retrobulbar neuritis

Diagnostic re-classification – Two of the authors (C.A., O.A.) reviewed the medical records and systematically re-assessed the year of onset, the results of diagnostic lumbar puncture and MRI examinations, the disease course, and the year of onset of secondary progression. The diagnoses of the MS patients in the study of MS incidence in unvaccinated and vaccinated cohorts (Paper I) were re-classified by the authors

according to the Poser criteria [99] as definite, probable, or the additional category of possible MS [10]. In the study on birth order and MS risk (Paper II) the MS patients were diagnosed with definite, probable or possible MS according to the Poser criteria [99] in the preceding Gothenburg MS studies [10, 100]. For the study on mass vaccinations and MS incidence (Paper III) and the case-control study (Paper IV), the authors re-classified the diagnoses according to the criteria of Poser [99], as well as the criteria of McDonald [101]. We included primary progressive MS as a disease compatible with MS presenting as insidious progress from onset for at least one year without remission and with subsequent development of additional foci. We added the category of “clinically isolated syndromes” (CIS) to Poser’s four probability categories. In the study on mass vaccinations and MS incidence (Paper III), we also included possible primary progressive MS (2%), defined as progressive course from onset for at least 1 year without evidence of multifocality provided that diagnostic workup was adequate. Those patients were not, however, included in the case-control study (Paper IV).

We included patients diagnosed with ‘possible MS’ (Paper I) or CIS [102] (Papers III, IV) for the following reasons: i) the reliability of these diagnoses at experienced centres is relatively high ii) the diagnoses were re-assessed by the authors, iii) a possible increase in MS incidence is expected to appear first in these diagnosis categories, and iv) there is a risk of an artefact of erroneously higher ratio of CIS (or possible) cases to definite MS cases at the end of a study. This artefact appears because of the delay from the first isolated syndrome until the second, diagnostic relapse [10]. The consequence is that a rise in incidence may not be detected at an early stage (Fig. 2).

Fig. 2 Artefact at the end of a study



Data from Svenningsson et al., 1990 [10]

The vaccines – 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. *The monovalent measles vaccine* contained the Schwarz live, attenuated measles vaccine strain. *The combined measles-mumps-rubella (MMR) vaccine* contained the Enders Edmonston (measles), Jeryl Lynn (mumps), RA 27/3 (rubella) live, attenuated virus strains [103]. According to a Finnish study, the seropositivity rate was 82% for measles at the 20-year follow-up in individuals who were seronegative prior to vaccination. The corresponding figures for mumps were 74% at the 15-year follow-up, and for rubella 100% at the 20-year follow-up. Fifteen years after the second MMR vaccination, the mean antibody levels were roughly one third of the levels measured right after the second dose [104].

The child health and school health records – Childhood illnesses and vaccinations have been documented in a uniform way in Sweden since the present form of the child health and school health record was introduced in 1959 [105]. 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 disease frequencies and vaccination coverage during the accomplishment of the mass vaccination programmes against measles, mumps and rubella have been closely followed by national surveys, based upon annual reports from the child health and school health care systems [106]. Scrutiny of child health and school health records at the City Archives in Gothenburg, by one of the authors (C. A.), confirmed that the national vaccination programmes were accurately accomplished as scheduled in certain age groups and years of birth in Gothenburg. The majority of child health and school health records were available at the City Archives in Gothenburg. In the preliminary cohort study, we used information from national data as well as individual information from child health and school records (Paper I). In the following cohort study of MS incidence, national data was used to define the cohorts, which were selected to represent each of the four vaccination programmes (Paper III). Child health and school health data was used in the analyses of infections and vaccinations as risk factors for MS in the case-control study (Paper IV).

MS incidence in a cohort of 12-year-old residents of Gothenburg, born 1964-1978 (Paper I) – This preliminary study on MS incidence in unvaccinated and vaccinated cohorts (Paper I) was based on a “closed cohort”. We defined the MS patients as having been residents of Gothenburg at age 12, since the vaccination programme was completed at that age. The successive years of birth were selected for the study of MS incidence in relation to measles vaccination coverage at different stages in the population. We updated the Gothenburg MS register from the administrative diagnosis registries of the Departments of Neurology. Since the cohort was “closed”, MS patients who were residents of Gothenburg at age 12 still remained in the cohort, irrespective of residence at MS onset. These patients were found by linking the Gothenburg MS register, the Patient Register of the National Board of Health and Welfare and the general population register of 12-year-old residents of Gothenburg. In order to analyse possible effects of mass vaccinations on MS incidence, we divided the population cohort 1964-1978 into three successive birth cohorts: one unvaccinated against measles (1964-1968), and two following cohorts which were vaccinated against measles with increasing frequency (1969-1973; 1974-1978). The incidence of MS was calculated in 5-year periods. It was possible to follow the first cohort to age

30 years, the second to 25 years, and the third to 20 years. Information on measles vaccination coverage and the frequency of measles in the population was obtained from statistical national data. In addition, we obtained individual data on history of measles and vaccinations from child health and school health records. We used the general population register in Gothenburg to calculate the population at risk in each 5-year cohort and age group. The population at risk was adjusted in such a manner that the annual number of persons who migrated into the area was not added and the annual number of deaths was subtracted. The mortality rate in the age groups studied was, however, negligible. The annual number of persons who emigrated from the area was counted as still belonging to the population at risk of the cohort. The age specific MS incidence estimates with 95% CIs were calculated assuming the MS incidence to be Poisson distributed. The advantage of the use of the so called “closed cohort” was that in addition to exposure to a certain risk factor, both the identified patients and the population at risk had a shared environment during childhood. More common characteristics and “background” exposures should reduce the risk of confounding. A disadvantage was the complex calculations of the population at risk over time. Another disadvantage was the potential risk that patients who emigrated from the cohort and had MS onset after they had moved were lost to follow-up.

MS risk and birth order (Paper II) – The MS incidence from 1950-1964 in Gothenburg was analysed previously [10]. We used the existing database of this population-based incidence cohort of 308 MS patients and their 915 siblings [100], and the additional data obtained from a published Swedish cohort study from Uppsala [107] as a control material. Two methods were applied to analyse MS risk in association with birth order. *Observed versus expected values* – This analysis was performed by using a matrix of birth order and sibship size. As an example, the number of first-born MS patients in a sibship of two children was entered in the column for first born siblings and the row for a sibship consisting of two siblings (Table 1 A, B). MS patients who were only children were not included ($N = 50$). The expected birth order number among the siblings of MS patients, given the number of siblings (n) was calculated under the assumption of equal probability ($1/n$) of each number. The standard deviation corresponding to the uniform distribution over the number 1, ... n was also calculated. A prerequisite for this method is that the family structure in the population is stable over time, as described in the ‘Discussion’ section. *Comparison of two birth cohorts* – We avoided the potential risk of an artefact due to long-term changes in family structure in the population by performing a case-control study including subjects born during a defined period of time. We used a published study on a Swedish birth cohort with information on birth order as control material, originally designed to analyse mortality in association with birth order. The cohort consisted of all 14,200 children born at Uppsala Academic hospital 1915-1929. We selected a subgroup of MS patients from our Gothenburg material born in the same years as the Uppsala cohort ($N = 158/308$), and compared this subgroup with the Uppsala cohort for birth order and the proportion of first-born siblings.

MS incidence in a birth cohort 1959-1984 (Paper III) – This cohort was designed to encompass the entire period of implementation of the Swedish national measles, mumps, rubella vaccination programmes. The study started in the year of birth 1959,

when the present form of a child health and school health record was introduced for routine use in Sweden. MS patients with onset in the Greater Gothenburg region before July 2004 were ascertained. The age segment 10-39 years, the years of birth 1959-1990 and the years of disease onset 1969-2004 constituted the material. We considered the cohort “open”, or “dynamic”, since migration into and out of the study area as well as mortality changed the roster of individuals [88]. The annual average population at risk for each age interval studied was calculated from the general population register. The main purposes were to analyse the gender- and age-specific MS incidence 1) in relation to the introduction of each of four vaccination programmes, and 2) by years of birth as well as years of onset throughout the study period.

Our test hypothesis had two aspects: 1) if infection with measles, mumps, or rubella increases the risk of MS, vaccination against these diseases would decrease this risk, 2) if such mechanisms acting in infection were applicable to the host response to vaccination as well, then vaccinations would increase the risk of MS. We also considered the age at infection with measles, mumps or rubella as well as the age at vaccination against these diseases and hypothetical effects on the incidence of MS.

We combined the incidence study method with an ecological approach by using national population data for the information on vaccination coverage and disease frequencies in the population. Thus, with respect to the measles, mumps and rubella vaccination programmes and the population frequencies of the diseases, we selected a baseline ‘*Unvaccinated birth cohort*’, representing the prevaccine era (1959-1961), and four birth cohorts representing ‘*Rubella vaccination at 12*’ (1962-1966), ‘*MMR vaccination at 12*’ (1970-1973), ‘*Continued MMR vaccination at 12 and measles vaccination at ages 2-6 years*’ (1974-1978), and ‘*MMR vaccination at 18 months and at 12 years*’ (July 1981 to June 1984).

MS incidence in different birth cohorts, each selected to represent the introduction of a new vaccination programme (Paper III) – In order to detect possible stepwise changes in MS incidence in temporal relation to the introduction of any of the new vaccination programmes, the age- and gender-specific MS incidence including CIS, in each of the four selected birth cohorts, was compared to the incidence calculated from the preceding birth cohorts in four independent analyses. Subjects who were born abroad were not included, as they usually had not participated in the Swedish vaccination programmes. In addition, we aimed at relative genetic homogeneity by restricting the inclusion of subjects to those with both parents born in Scandinavia.

MS incidence through years of birth 1959-1990 and years of MS onset 1969-2004 (Paper III) – In order to analyse the longitudinal incidence of MS, we used the total incidence material including 534 probable and definite MS and CIS cases. The risk of MS was estimated 1) by years of birth, and 2) by calendar years of MS onset through the study period. The study-specific age segment was 10-39 years. The main aim of this thesis was to study MS risk in relation to vaccination-targeted childhood infections. We decided to begin the period of observation in the year of birth 1959, the year the present form of the child health and school health record was introduced for

routine use in Sweden [105]. MS patients with disease onset in the Gothenburg region from 1969 were included up to July 2004 when the study was terminated. Consequently, patients were aged 10-39 years at MS onset.

Observed versus expected MS risk values in single years, or sequences of years of birth 1959-1990 and years of MS onset 1969-2004 (Paper III) – In addition to the long-term analysis, we investigated whether the risk of MS in a single year or a sequence of years deviated from that of the preceding year. For this analysis, expected versus observed number of onset events for each year of birth and calendar year was calculated using a hazard function.

A case-control study on MS risk in association with infections and vaccinations (Paper IV) – The study area and the greater part of patient material was the same in the case-control study as in the cohort study in the age group 10-39 years, those born 1959-1990 (Paper III). A smaller part of the proportion of MS patients who had MS onset outside the study area ($N = 93$ MS (18.3%) or who fulfilled the inclusion criteria regarding residence in Gothenburg, but were older than 39 years at onset ($N = 5$ MS (0.9%)) were included in the case-control study. Eight patients who were included in the cohort study were deceased, and 13 patients had emigrated. Twenty-two patients were not contacted for psychological reasons.

Control persons – For the performance of the case-control study (Paper IV), a total of 4,000 control subjects were randomly selected from the general population register in Gothenburg. 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 performed. The number of patients per year of birth declined towards the end of the study, because the maximum risk age for onset of MS was not yet reached by individuals born in the later years. Irrespective of that, the number of controls per year of birth was selected to be equally distributed through years of birth. The purpose was to achieve high power. The female to male ratio in MS was expected to be about 2. The female-to-male ratio in the control material represented that of the population.

The questionnaire – The total number of questionnaires sent to MS patients and controls was 627 and 3,917. MS patients and controls were required to have reached age 18 when receiving the questionnaire (Fig. 2 in Paper IV). Information about infections with measles, mumps, rubella, varicella and infectious mononucleosis, as well as was information about the country of birth of the participant and his or her parents was obtained from the self-administered questionnaire. Swedish origin was defined as being born in Sweden with both parents born in Scandinavia.

The child health and school health records – We selected those study participants who had attended the sixth grade of school within the study area ($N = 1,370$; 273 MS patients and 1,097 controls), since 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. The information about infections with measles, mumps, rubella and varicella

and vaccinations against these diseases was collected from the child health and school health records.

Age at infection – We included all infections in our analyses, irrespective of age at infection and irrespective of whether age information was available. The age at infection was not stated for a considerable number of the reported infections and MS patients recalled age at infection more often than controls. As a consequence, the results would have been influenced if the analyses had been based solely on infections with age information. The risk of MS was significantly increased if the analysis was restricted to those who recalled age at infection with measles (OR 1.34, 95% CI 1.05-1.70), while the risk was decreased in those who recalled having had measles, but not the age (OR 0.70, 95% CI 0.53-0.92). Furthermore, there were few MS patients who contracted one or more of the studied infections after the onset of the MS disease, and the proportion of infections that occurred after the age of 10 years, the lowest age of MS onset in this study, was small. For these reasons, we used “yes” or “no” responses without the additional information on age at infection. Although child health and school health data provided reliable data on age at infection for a selection of 206 MS patients and 888 controls, an age-specific analysis would have required a larger study material.

Statistical methods – Paper I: The total number of MS cases fulfilling the inclusion criteria was 58. The incidence of MS was computed in the population of Gothenburg, which was 431,273 in 1980. Census information was obtained from Statistics Sweden and Statistical Yearbooks of Gothenburg, Sweden. Incidence of MS was considered to be Poisson distributed. **Paper II:** The expected birth order number among the siblings of MS patients, given the number of siblings (n) was calculated under the assumption of equal probability ($1/n$) of each number. The standard deviation corresponding to the uniform distribution over the number 1, ... n was also calculated. The comparison of birth order was performed by a test for trend in contingency table, and the proportion of first-borns by Fisher’s exact test. **Paper III:** The age- and gender- specific MS incidence, including CIS, in each of the four selected birth cohorts designed to record possible influences of vaccinations on MS incidence, was compared with the incidence calculated from the preceding birth cohorts selected for this study in four independent analyses (MS patients = 251). The comparisons were performed by the optimal test for comparison of two Poisson distributions, and two times one-tailed P -values were given. The total incidence material including 534 probable and definite MS and CIS was used to analyse the risk of MS by age in successive one-year cohorts in a Poisson regression model. The hazard function of onset of MS, including CIS, was estimated as a function of age and (i) year of birth, (ii) calendar year of onset, and the hazard ratio (HR) for one year versus the preceding one at different ages was calculated from this model (Table 2 in Paper III). **Paper IV:** When we chose the statistical method, we had to consider the following characteristics of the study material. 1) The number of MS patients declined through the years of birth depending on the relationship between year of birth, age at onset, and the date of study termination. If for example, the patients were all required to have reached 39 years of age, the last year of birth to be included in the study would have been 1965. 2) In addition, the population frequency of the vaccination-targeted diseases, especially measles, declined through the years of

birth. At the beginning of the study period, the population frequency of measles was more than 80%, and at the end of the study the frequency of measles had successively declined to 1%. In order to avoid false results due to skewed distributions of patients and childhood disease frequencies over time, we could have selected for example 8 controls matched for sex and year of birth to each MS patient. Stratifying by year of birth in, for example, 1- or 3-year intervals would have been another possibility. We decided, however, to use spline functions in logistic regression whereby we were able to take years of birth into account in a careful way, and to achieve relatively strong power.

Validation – We had access to child health and school health records for a selection of the study participants, as described above. 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%. The percentages of questionnaire responders who did not know whether they had had measles were 14.6% and 18.5%. The percentage of data on infections in the questionnaires that was confirmed by the child health and school health records was 85.8% for measles in the MS group and 85.5% in the control group in this selection of child health and school health records. The corresponding figures were 78.8% and 82.1% for mumps, 69.7 % and 71.2% for rubella and 72.4% and 64.24% for varicella. Sensitivity and specificity of questionnaire data was validated by child health and school health records (Table 4 in Paper IV). Concerning vaccinations, the agreement between data reported in the questionnaires and data registered in the child health and school health records was small. More than one third of MS patients and controls did not recall whether they had received a measles vaccination. Concerning data on vaccinations received only before or at 10 years of age, more than two thirds of the information from the questionnaires was in disagreement with the data provided from the child health and school health records. Therefore, analyses on vaccinations were based exclusively on child health and school health data.

The power to detect significant changes in the incidence of MS related to vaccination programmes (Paper III) – In spite of the relatively large total number of 534 incident MS patients, and the time frame of 25 years, the power was weak, particularly in the youngest cohort. The number of patients in the older birth cohorts, aged 10-34 years (1964-1968), and 10-29 years (1970-1973) was 102 and 62 MS patients, and in the younger birth cohorts aged 10-24 years (1974-1978), and 10-19 years (1981-1984), the number was 37 and 5 MS patients. With the available number of patients, a 33.9% reduction of the incidence in the cohort born 1962–1966, 29.7% in the cohort 1970–1973, and 35.1% in the cohort 1974–1978 compared with that of the preceding cohorts would have been necessary to reach a significant difference. Obviously, the available observation time was increasingly incomplete in the younger cohorts. The birth cohort 1974–1978, during which measles infection declined to 5%, was 24 years, and the birth cohort 1981–1984, in which all three infections measles, mumps, rubella were virtually eliminated, was 19 years old at study termination. In the youngest cohort, a reduction in the MS incidence by about 61.6% would be detected.

We had been able to detect an increased risk of MS in the cohorts born 1962–1966 and 1970–1973 of approximately 37%, and in the cohort born 1974–1978 of 48%.

RESULTS

MS incidence in a cohort of 12-year-old residents of Gothenburg, born 1964-1978 (Paper I) – The cohort was followed up within three sub-cohorts to the ages 15-19 (born 1974-1978), 20-24 (born 1969-1973), and 25-29 years (born 1964-1968). The three age groups represented one unvaccinated cohort and two cohorts with different coverage of measles mass vaccination. A total of 58 MS patients, including 10 possible MS were defined. We obtained information on vaccination status and history of infection with measles from the child health and school health records of 54 MS patients. Few MS patients (1/35) in the oldest age group born 1964-1968 were vaccinated with the monovalent measles vaccine before school entry or with the combined MMR vaccination at age 12 (3/35). The majority of the individuals born 1970 or later had received the combined MMR vaccination at age 12 (88%), according to the national MMR vaccination programme, which was introduced in 1982 and targeted at both 18-months-olds and 12-year-olds. The main result was that no significant change in MS incidence was observed in any of the three age groups. We observed MS in measles vaccinated individuals with no history of measles. Of significance also for the later cohort study (Paper III), we confirmed that the national mass measles vaccination programmes were effectively accomplished in Gothenburg.

MS risk and birth order (Paper II) – Possible associations between birth order and sibship size and the risk of MS was studied using two methods. *Observed versus Expected Values*: We found no association between MS (definite and probable, $N = 211$) and birth order ($P = 0.1411$). The observed number of first-born patients did not differ significantly from the expected number ($P = 0.0871$) (Table 1, A). However, when definite, probable and possible MS ($N = 258$) were included, there was a marginally significant low number of first-borns ($P = 0.0475$) and a significant high birth order compared with the expected values ($P = 0.0381$) (Table 1, B)

Table 1 (Paper II) Distribution by birth order and sibship size. 50 out of 308 patients were the only child and are not included.

A. Definite, probable MS ($N = 211$)

		Birth order										
		1	2	3	4	5	6	7	8	9	10	Total
Sibship size												
2		26	26									52
3		12	13	20								45
4		5	11	4	8							28
5		2	5	6	6	6						25
6		3	4	3	3	5	0					18
7		1	0	1	1	0	3	4				10
8		0	0	0	3	0	3	5	2			13
9		1	1	0	1	1	1	2	1	1		9
10		0	0	2	0	1	1	1	0	1	0	6
11		0	1	1	0	0	0	1	0	0	0	3
12		0	0	0	0	0	2	0	0	0	0	2
Total		50	61	37	22	13	10	13	3	2	0	211

B. Definite, probable and possible MS ($N = 258$)

		Birth order										
		1	2	3	4	5	6	7	8	9	10	Total
Sibship size												
2		32	30									62
3		13	15	23								51
4		7	15	7	11							40
5		3	5	7	9	6						30
6		3	4	3	4	6	2					22
7		1	0	1	1	1	4	4				12
8		0	0	1	3	0	4	7	4			19
9		1	1	0	1	1	1	3	1	1		10
10		0	0	2	1	1	1	1	0	1	0	7
11		0	1	1	0	0	0	1	0	0	0	3
12		0	0	0	0	0	2	0	0	0	0	2
Total		60	71	45	30	15	14	16	5	2	0	258

Population-Controlled Study: No significant association with birth order was found in the subgroup of the MS incidence cohort born between 1915 and 1929 ($N = 158/308$) compared with the Uppsala birth cohort, although there was a possible tendency for a higher birth order number among the MS patients (contingency table, $P = 0.0742$). The proportion of the firstborns in the subgroup of MS sufferers born between 1915 and 1929 in the MS incidence cohort did not differ from the corresponding proportion of first-borns in the Uppsala cohort ($P = 0.220$) either. These results do not suggest a major effect of birth order on the risk of MS.

MS incidence in selected birth cohorts, each representing the introduction of a new vaccination programme (Paper III) – A baseline cohort (born 1959-1961) representing the prevaccine era and four cohorts (born 1962-1966, 1970-1973, 1974-1978, 1981-1984), representing the first years of each new vaccination programme against measles, mumps and rubella, were studied. We had hypothesised that the avoidance of measles, mumps and rubella infections would result in a decrease in the MS incidence. However, there were no significant changes in the incidence of MS correlated with the introduction of the mass vaccination programmes including early monovalent measles (the cohort born 1974-1978, $P = 0.373$ in females, $P = 0.712$ in males) or the combined MMR vaccination with both an early vaccination at 18 months of age and a late vaccination at 12 years (1981-1984, $P = 0.788$ in females, $P = 0.755$ in males) in comparison with the MS incidence estimated from the preceding selected cohorts. We had also raised the question whether a late vaccination would increase the risk of MS in analogy with possible increased MS risk after late infection. However, no increase in the risk of MS was observed in the cohort born 1962-1966, the females of which received rubella vaccination at age 12 ($P = 0.477$ in females, $P = 0.256$ in males), or in the cohort born 1970-1973 who had received a late MMR vaccination at age 12 ($P = 0.541$ in females, $P = 0.546$ in males).

MS incidence through years of birth 1959-1990 and years of MS onset 1969-2004 (Paper III) – The incidence of MS changed significantly during the study period within the age group under study (10-39 years). First, we estimated the risk of MS as a function of age. In females the risk increased by year of birth with a tendency to an age maximum in the twenties (HR = 1.05; 95% CI: 1.02-1.07). The HR 1.05 corresponded to an increase in the risk of MS by 5% per year of birth. However, this change was part of a complex pattern with the increase in the middle age range and a decrease among the youngest and oldest women (HR = 0.91 (0.83-0.98) and 0.86 (0.78-0.94)). There was no significant change in the MS incidence by calendar year of onset. In males there was a gradual risk increase with age by year of birth as well as by calendar year of onset (increasing HR with age to HR maxima 1.09 (1.01-1.17) and 1.08 (1.01-1.16)). We then estimated the probability of MS as a function of year of birth. There was a significant increase in the probability of MS onset for men ($P = 0.0030$) but not for women ($P = 0.1378$). However, in the age interval 20-30 years there was also a significant increase for women ($P = 0.0018$) (Fig. 1 in Paper III). Finally, we estimated the MS one-year risk through the study period of years of birth and calendar years of onset. The observed number of new MS patients in any single year of birth or calendar

year of onset did not significantly deviate from the expected number, as calculated by use of a hazard function.

A case-control study on MS risk in association with infections and vaccinations

(Paper IV) – 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 in Paper IV). ***Origin*** – The risk of MS was significantly higher in individuals of Swedish origin than in individuals of foreign origin (OR 1.32, 95% CI: 1.04-1.68, $P = 0.0224$). However, the risk of MS associated with history of measles, mumps, rubella or infectious mononucleosis did not depend on origin. The 95% CIs of the estimated ORs for MS associated with the different infections in individuals of Swedish or foreign origin overlapped each other. ***Infections*** – There were no significant differences in the frequency 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 males (OR 1.55, 95% CI: 0.88-2.71) (Table 5 in Paper IV). Restricting the analysis 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 in Paper IV). ***Vaccinations*** – We used data based exclusively on child health and school health records for the analyses on 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’ (≤ 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 with none or early vaccination only. ‘Late vaccination’ and ‘Early vaccination only’ were the two extreme groups. Those who had received no vaccination, and those who had received both an early and a late vaccination, had an intermediate risk (Tables 3, 8 in Paper IV). In order to study whether the OR of 2.46 for early vaccination was an effect of 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) of 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 *MMR vaccinations* which constituted more than half of the number of 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 other MMR vaccinations was associated with higher risk of MS (OR 4.92, 95% CI: 1.97-12.20, $P = 0.0006$). '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 in Paper IV).

DISCUSSION

INFECTIONS

We found no convincing influence of common viral childhood diseases, such as measles, mumps, rubella or varicella on MS risk. Efficient methodological approaches provided negative results. We studied birth order to analyse a possible impact of exposure to childhood infections in general on the risk of MS. We analyzed the incidence of MS in relation to mass vaccinations against measles, mumps and rubella in a cohort study, and we analysed these infections, and varicella and infectious mononucleosis as risk factors for MS in a case-control study. Only infectious mononucleosis was found to influence the MS risk.

Siblings and exposure to infections during childhood – We found no major impact of birth order on MS risk. The method of using birth order and sibship characteristics as measures for exposure to infections during childhood is indirect. Most population-based studies using a matrix of birth order and sibship size or the case-control technique found no correlation between birth order and MS risk [38, 39, 41] or found low as well as high birth order correlated with MS risk [36]. We used data from the previously reported Gothenburg MS incidence cohort 1950 to 1964 [100] for the study of birth order and MS risk (Paper II). Our analysis of observed versus expected values of birth order and sibship size in probable, definite or possible MS patients showed a trend towards a higher birth order in MS patients. This was probably the result of an artefact, due to diminishing sibship sizes in the population during the period of time the MS patients had their disease onset. Our negative result from the comparison between the Gothenburg MS incidence cohort and the Uppsala birth cohort should be free of this artefact, since the cases and the control persons were born during the same years (Paper II). Nevertheless, the absence of a relationship between MS risk and the indirect variable birth order does not exclude a protective effect of exposure to infections early in life. Later population-based studies on large materials were also negative [108, 109]. However, Ponsonby et al. found that higher exposure to infant siblings in the first 6 years of life was associated with reduced risk of MS.

Furthermore, greater infant contact was associated with reduced risk of infectious mononucleosis and elevated EBV antibodies in controls. It was concluded that the results give support to the theory that siblings alter childhood infection pattern and

related immune responses and reduce the risk of MS [110]. The sample size of the study was not large ($N = 136$ MS patients, 272 controls), but the questionnaire material probably unbiased. The authors selected the age limit at age 6 with respect to strong intrahousehold effects before school age and possible immunomodulation at this stage. The exposure to infants below age 2 was selected because primary infection with MS related viruses such as herpes simplex and enteroviruses commonly occur in the first 2 years, according to the authors.

Mass vaccination programmes and disease frequency in the population – We did not observe any effect of the introduction of measles, mumps, rubella vaccination programmes on the incidence of MS. The cohort born 1974-1978 was especially useful in detecting a possible benefit from the vaccinations. In addition to the combined MMR vaccination at age 12, which almost all children received, half of the children were also vaccinated with the monovalent measles vaccine before school entry. No more than about 10% of the children had a history of wild measles. However, with the power to detect a 35% reduction, we did not observe a decrease in the MS incidence in this cohort in comparison to the incidence calculated from the preceding selected cohorts. Primarily, we considered the absence of infection to be the most important effect of vaccinations. However, certain counteracting age factors may have appeared. The vaccination-induced reduction in disease frequency in the population entails a shift to higher age at infection [111]. Such an effect might have diminished a possible benefit of a low frequency of measles in the cohort 1974-1978. The overall impression of the results from the analyses of MS incidence in the different selected cohorts is that the three vaccination targeted infections do not have a significant role in the risk of MS. Vaccinations in different age groups targeted by the vaccination programmes (Table 1 in Paper III) are discussed below.

History of viral childhood infection – We found no association between history of measles, mumps or rubella and MS risk using the case-control method (Paper IV). These results confirmed the negative results from the analyses of the relationship between MS incidence and mass vaccination programmes (Paper III). A history of varicella, which was not included in the Swedish vaccination programmes, was found to be uncorrelated with MS risk in the case-control analysis (Paper IV).

Several large population-based studies which found no significant differences in the frequency of childhood infections between MS patients and controls, referred to the prevaccine era, i.e. the era before the measles, mumps, rubella vaccination programmes. However, during the prevaccine era almost all individuals of the population probably had a history of measles, mumps and rubella. For example, Compston et al. [63] found that 98% of the MS patients and 99% of the controls were seropositive for measles virus, and 92% of the MS patients and 89% of the controls for rubella. We took advantage of the vaccination-induced decline of the population frequencies of infections which resulted in proportions more suitable for statistical processing. In the present material (Paper IV), approximately 30-40% of the controls had a history of measles, mumps or rubella, and 85% of varicella. In this respect, and with regard to the sample size of our study material, we had the conditions required to detect significant differences in disease frequency between MS patients and controls.

However, no such differences were present. If there is a small effect on MS of these infections, it is not likely to be higher than 30% for measles, 50% for mumps and 20% for varicella, as shown by the upper limits of the corresponding 95% CIs (Table 5 in Paper IV).

Age at infection –We did not perform analyses of age at infection (Paper IV). If we had used the information on age from the questionnaire data, the results would have become erroneous, since a bias had been introduced due to the patients recalling age at infection better than the controls. Had the study material of child health and school health records, been several times larger, the results from age-specific analyses based on these records would have been more reliable.

Age at infection rather than infection per se has been the focus in a considerable number of studies on a possible relationship between childhood diseases and MS. Granieri et al. reviewed 24 case-control studies. The authors found that later age when acquiring at least one of the childhood infections was a consistent result, while there were no significant differences in frequency of infectious diseases in many of these studies [76]. The majority of the subjects included in the studies which found positive correlation between age at infection and MS were children during the first half of the 20th century [63, 69, 41], while most of the subjects included in the studies which found no correlation were children during the second half of the 20th century [109, 72]. In the present thesis (Papers I, III, IV), all subjects were born after 1958. Recall bias due to the patient's ability to recall the age at infection better than controls, as was found in the present thesis, and a tendency to report later age at infection than controls, cannot explain the results in all studies [69]. The question is whether the negative results in more recent studies are due to improved methods only. It has often been stated that the standard of hygiene generally improved during the first four decades of the 20th century. Higher sanitary standard during childhood was assumed to delay infection with polio virus, resulting in higher risk of paralytic polio [112]. The interest for the effect of delayed infection was adapted by the field of MS research. Higher sanitary standard during childhood was found to be associated with higher MS risk in Israelis born from the early to mid-20th century. However, the youngest MS patients at the end of the study differed least from the controls regarding sanitary level [31]. Socioeconomic and sanitary status in childhood was found to significantly differ between MS patients and controls only at extremely low degree in a later Israeli study. The subjects of the latter study were probably born between 1915 and 1961[113]. The observed later age at infection in MS patients may be explained by any factor related to the first half of the 20th century. However, one explanation for the finding in earlier study materials may be that age at infection was a marker for hygiene. Since the middle of the 20th century, discrepancies in sanitary levels in the western world have essentially vanished. According to the hygiene hypothesis, which is supported by findings in type 1 diabetes and EAE, there is a skewed immune responsiveness and increased propensity to develop autoimmune diseases in individuals with delayed or reduced exposure to childhood infections [114]. Infectious mononucleosis is a significant example of a delayed infection which is associated with improved hygiene [115] and increased risk of MS [116]. However, incongruent with the hygiene hypothesis, early infection with EBV has no beneficial effect on the risk of MS, while

retained seronegativity for EBV seems to protect from MS, as described by Ascherio et al. (“The Epstein-Barr paradox”) [117].

History of infectious mononucleosis – We estimated the risk of MS to be increased 2-fold after infectious mononucleosis, which is in agreement with the results of other authors [116, 118]. We had no genetic information available in the present thesis. Interestingly, Nielsen et al. found that the risk of MS was 7.0-fold higher related to interaction between infectious mononucleosis and HLA-DR15 [119]. The EB virus itself is a stronger risk factor for MS than infectious mononucleosis, if this delayed infection is compared to early EBV infection as reference. Seropositivity for EBV is associated with a 10-fold higher MS risk [120]. Other autoimmune diseases also appear to be influenced by the EB virus. Adult patients with MS, systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA) are nearly 100% seropositive for EBV compared with between 84% and 95% for controls [121]. The OR for SLE in association with EBV seropositivity was reported to be about 9 [122]. The OR for RA in association with EBV seropositivity was about 5 and in association with the interaction between EBV seropositivity and HLA-DR4 the OR for RA was 40 [123].

THE LONG-TERM INCIDENCE OF MS

Long-term changes in MS incidence – We observed a continuous risk increase in MS during the study period although without any temporal correlation with the vaccination programmes (Paper III). The interpretation of the MS increase as real was supported by the nonsignificant difference in frequency of MRI examinations between the age groups where MS had significantly increased and other age groups. It was not possible to compare the present estimated MS incidence values with that of the earlier Gothenburg cohort 1950-1964, since the present patient material was based on year of birth, while the earlier Gothenburg cohort was based on MS onset years. Furthermore, the age segment for MS onset was limited to 10-39 years in the present patient material. Thus, we were able to study the MS incidence in the most significant, but not all age groups. However, increasing MS incidence, interpreted as real, was also reported from several other areas, such as Sardinia [124, 125]. A possible age-dependent modifying influence of infections on the developing system has been discussed in Type I diabetes. According to the hygiene hypothesis, normal “education” of the immune system depends on a certain load of infections during the first years of life to protect from autoimmune diseases later in life [126]. If the general exposure to infections early in life decreased over the relatively long period of 3 decades in the present study, increasing incidence of MS during this period would be in agreement with the hygiene hypothesis.

VACCINATIONS

We found no overall difference in MS risk between individuals who were vaccinated against measles, mumps and rubella and the group of individuals who were unvaccinated against these diseases. A similar result was found for the subgroup vaccinated with the combined MMR vaccine. However, the age at vaccination seemed

to be related to MS risk when vaccinations were grouped with respect to age of less than or more than 10 years at administration.

Mass vaccination programmes – As a first approach, we considered the prevention from infection to be the most important effect of vaccinations. Thus, we hypothesised that if the measles, mumps and rubella infections influence MS, vaccination early in life would reduce the risk of MS. We also questioned whether late vaccination, by analogy with late infection [76], would increase the risk of MS. The analyses of MS incidence in population cohorts, as described, revealed no temporal correlation between the incidence of MS and the introduction of any of the vaccination programmes.

Two cohorts were especially useful for the study of a possible effect from late vaccination. The girls in the cohort born 1962-1966 were rubella vaccinated at 12, and children of the cohort born 1970-1973 were MMR vaccinated at age 12. No increase in the MS incidence was observed in any of these cohorts, while we had been able to detect an increase of about 37% with the numbers available. We were not able to observe any beneficial effect on the incidence of MS from the reduced measles frequency in the cohort born 1974-1978. If there had been a harmful effect from late vaccination, such an effect might have attenuated a possible beneficial effect from early vaccination in this cohort, since half of the cohort was vaccinated both early and late. However, we did not detect any influence on MS incidence of vaccinations in any of these cohorts.

Vaccinations – The main result from the analysis of vaccinations showed no overall difference in MS risk between the group of individuals who were vaccinated with any of the monovalent or combined measles, mumps, rubella vaccines, and the group of individuals who were unvaccinated against these diseases. Similarly, an analysis restricted to the subgroup of individuals vaccinated with the combined MMR vaccine showed no difference in MS risk between MMR vaccinated and MMR unvaccinated individuals. The unexpected finding of a relationship between age at vaccination and MS risk may be the result of chance and needs to be confirmed or rejected in another, larger study material. There are no previous studies published on age at vaccination against these childhood diseases and MS risk. There may be a relationship between risk of autoimmunity and the T cell tolerance to myelin, which is age-dependent [127]. Myelination is incomplete at birth, particularly in many tracts in the brain, and is not complete until early adulthood [128]. It is not likely that the viral agents in the vaccines would cause any harmful effects, since the wild viruses themselves did not influence the risk of MS, as demonstrated here.

Vaccination safety – The benefit of the measles, mumps and rubella vaccines clearly outweigh the risk of complications, which are far less common after vaccinations than after the wild virus infections in question. The measles, mumps and rubella vaccine components allow for a mild or inapparent, non-communicable infection. The MMR vaccination induces a protection that appears to persist at least until early adulthood [104]. Systemic adverse events following MMR vaccination are uncommon. Reported adverse reactions are a local reaction, fever or rash. Arthralgias have been reported

after MMR vaccine, thought to be caused by the rubella component. Other reported extremely rare adverse events are parotitis, deafness, encephalitis, and thrombocytopenia [129]. Encephalitis may appear in temporal association with vaccination with measles containing vaccine in 1-2 per million cases, which is far lower than the incidence of encephalitis following wild measles infection (1 per 1000) [130].

A report in 1998 on behavioural and gastrointestinal symptoms after MMR vaccination in 12 children with chronic enterocolitis and regressive developmental disorder raised the debate on MMR vaccine-related autism [131]. However, later studies did not support a causal association between MMR vaccine and autism [132, 133].

A challenge in studying the safety of the MMR vaccination concerning a possible risk of autoimmune disease is the probably long latency from exposure to the development of disease. Furthermore, the latency period from exposure to disease onset may vary in length. For example, the MS risk related to infectious mononucleosis may increase soon after the infection and persist for at least 30 years [118].

In addition to the measles, mumps and rubella vaccinations, other vaccinations have been discussed in connection with MS. A possible association between Hepatitis B vaccination and MS has been discussed since the mid-1990s. The timing of vaccination in relation to MS onset, with respect to the date of the unequivocal first MS related symptoms, is particularly crucial with regard to vaccinations administered after childhood. The relationship with Hepatitis B vaccination has, however, not been confirmed [134-136]. A protective effect on MS risk from tetanus vaccination was reported in several epidemiological studies. The exposure period was defined as after age 10 and more than 5 years before MS onset [75], within 3 years before MS onset [137] or was not specified in several studies [138]. It seems however unclear whether there is a causal relationship between development of MS and tetanus vaccination. The unknown length of the latency period from the exposure to a vaccination to the subclinical onset of MS implies a risk of possible reverse association. It has also been discussed whether adjuvants and preservatives may confer a risk of autoimmune phenomena. Stehr-Green et al. compared the prevalence and incidence of autism in California, Sweden and Denmark with average exposures to thimerosal containing vaccines. In Sweden, few vaccines containing thimerosal as a preservative have been used throughout the history of childhood vaccination programmes. Population based data, representative of Gothenburg showed an increase in autism during a time of decreasing use of thimerosal containing vaccines [139].

Our results, in combination with others [70, 72], suggest that measles, mumps and rubella may now be dismissed as significant risk factors for MS. We confirmed the results of other studies [116, 118], that infectious mononucleosis is a moderate risk factor. To what extent EBV infection, which is a strong risk factor, has a causal relationship to MS is still unknown. Data on DNA, infections, vaccinations, and serology in prospective registries would make it easier to elucidate the role of different risk factors and possible interactions. Awaiting the results from this type of important

but time-consuming research, the current MS epidemiology is mainly focused on the role of EBV, sunlight exposure and vitamin D status. One explanation for sunlight as a protective factor is that exposure to sunlight for most people is the major source of vitamin D, although several confounders related to latitude exist. Furthermore, ultraviolet radiation from sunlight has immunosuppressive effects that could be independent from the synthesis of vitamin D and could thus contribute to MS prevention [8]. Vitamin D promotes the innate immune response to pathogens and also quells an “overzealous” adaptive immune response to pathogens [140].

Concurrently with this development in epidemiology, the idea that the immune system matures by exposure to infections early in life, is a working hypothesis. The observed increase in MS incidence during a period of probably general reduction of childhood infections is in agreement with the hygiene hypothesis. From a public health aspect, it is important to confirm or refute our unexpected finding of higher MS risk associated with age at measles, mumps, rubella vaccination. If confirmed, this finding has implications for the routine childhood vaccination schedules.

CONCLUSIONS

There is no major relationship between birth order and MS risk. However, since birth order is an indirect variable, the absence of such a relationship does not exclude a protective effect of exposure to infections early in life.

The common viral childhood diseases measles, mumps, rubella and varicella do not influence the risk of MS, and can reasonably be dismissed as suspected risk factors for MS.

Infectious mononucleosis is a moderate risk factor for MS.

The search for other strong viral risk factors for MS, besides EBV, should continue. Vaccination against EBV should be considered, particularly in DR15 positive children.

The risk of MS in individuals, vaccinated against measles, mumps, rubella, does not differ from the risk in individuals unvaccinated against these diseases. Similarly, MMR vaccinated individuals are not at higher or lower MS risk than MMR unvaccinated individuals.

The finding that age at MMR vaccination may be related to MS risk needs to be confirmed. If confirmed, childhood vaccination schedules should be revised.

SVENSK SAMMANFATTNING

Infektioner är troligen involverade i sjukdomsutvecklingen av multipel skleros (MS). Speciellt har vanliga barnsjukdomar som mässling, påssjuka och röda hund diskuterats. Svenska vaccinationsprogram resulterade i stora förändringar i förekomsten av dessa infektioner. Under tidigt 1970-tal infördes vaccin mot mässling, påssjuka och röda hund. Sverige var först i världen med att 1982 introducera mässling-påssjuka-röda hund (MPR) vaccination vid både 18 månader och 12 års ålder. Förekomsten av mässling sjönk radikalt under 1970-talet. Nästan inga barn födda efter 1980 fick mässling, påssjuka eller röda hund. Huvudsyftet med denna avhandling var att undersöka om vissa barnsjukdomar, eller vaccinationer mot dem, påverkar risken att få MS. Syftet uppnåddes genom en studie av MS-incidens i vaccinerade kohorter, en fall-kontrollstudie och – indirekt – genom en studie av ordningsföljd i syskonskaran.

Förutsättningarna för våra studier var unika. Genomförandet av vaccinationsprogrammen var mycket effektivt och det övervakades i nationella rapporter. Individuella uppgifter om barnsjukdomar och vaccinationer dokumenterades i barn- och skolhälsovårdsjournaler. Proportionen personer i befolkningen som haft barnsjukdomar minskade så att data blev mer hanterbara för statistiska analyser. Dessutom fanns det en lång tradition av studier av MS-incidens i Göteborg. Vi utnyttjade befintliga data på en kohort av 308 MS patienter med MS-debut i Göteborg för att studera om ordningsföljden i syskonskaran påverkar risken att få MS. Vi använde också publicerade data på en kohort med samma födelseår som jämförelse. Efter uppdatering av det göteborgska MS-registret hade vi ett material bestående av 534 MS patienter födda 1959-1990. Vi undersökte om MS incidensen hade förändrats i någon av de fyra populationskohorter vi valt för att representera introduktionen av vart och ett av vaccinationsprogrammen. I en fall-kontrollstudie inom samma population analyserades mässling, påssjuka, röda hund, vaccinationer mot dessa infektioner, samt vattkoppor och körtelfeber som riskfaktorer för MS. En enkät besvarades av 509 MS-patienter och 2067 kontrollpersoner, födda 1959-1986. Information om barnsjukdomar och vaccinationer inhämtades från enkätsvaren, och för ett urval på 206 MS-patienter och 888 kontrollpersoner också från barn- och skolhälsovårdsjournaler.

Ordningsföljden i syskonskaran ser inte ut att påverka risken för MS. Det observerade antalet förstfödda patienter skiljde sig inte från det förväntade antalet och andelen förstfödda skiljde sig inte från motsvarande andel förstfödda i jämförelsekohorten från samma tid. Vi upptäckte inte någon förändring i MS-incidensen som hade samband med introduktionen av något av de fyra vaccinationsprogrammen. Däremot såg vi att utvecklingen av den långsiktiga incidensen av MS visade en ökande trend. Denna ökning syntes i vissa åldrar och hade inte samband med vaccinationsprogrammen. Fall-kontrollstudien visade att mässling, påssjuka, röda hund och varicella förekom lika ofta hos MS-patienter och kontrollpersoner enligt både enkätsvaren och barn- och skolhälsovårdsjournalerna. Körtelfeber var förenat med ökad MS-risk. Det positiva resultatet för körtelfeber och de negativa resultaten för de övriga studerade

infektionerna gör den samlade bilden av resultaten övertygande. Individer som är vaccinerade mot mässling, påssjuka och röda hund har samma risk att få MS som de som inte är vaccinerade mot dessa infektioner. Det gäller även för MPR-vaccination. Inom den MPR-vaccinerade gruppen hade de som bara vaccinerats före 10 års ålder högre MS-risk än övriga i gruppen. De som var MPR-vaccinerade både före och efter 10 års ålder hade samma MS-risk som de som inte MPR-vaccinerats.

Mässling, påssjuka, röda hund och vattkoppor påverkar inte risken att få MS och kan förkastas som riskfaktorer för MS. Körtelfeber medför en måttligt ökad risk för MS. Vaccination mot Epstein-Barr-virusinfektion, som är orsaken till körtelfeber, kan övervägas. MS-risken är densamma hos de som är MPR-vaccinerade och de som inte är det. Det överraskande fyndet att MS-risken är högre om man bara har MPR-vaccinerats vid låg ålder, måste bekräftas eller förkastas i ett nytt studiematerial.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to all those who have contributed to the completion of this thesis. In particular, I wish to thank:

Professor *Oluf Andersen* my supervisor, for sharing a vast knowledge in MS epidemiology and for all patience and constructive discussions

Professor *Kjell Torén*, my co-supervisor for inspiring discussions and initiatives

Professor *Anders Odén*, my co-author, for statistical help and great discussions

Professor *Lars Frisé*n, for generous neuro-ophthalmological advice and assistance

Senior consultant *Victoria Romanus*, for providing vaccination data from the Swedish Institute for Infectious Disease Control

Malte Nordqvist for excellent technical assistance

All the patients and volunteers who have participated in the studies

My children, *Charlotte* and *Jessika* for love, understanding and encouragement

My friends, for listening and endless support

This thesis was supported by grants from the Research Foundation of the Multiple Sclerosis Society of Gothenburg, the Foundation of the Swedish Association of Persons with Neurological Disabilities, Stockholm, the Edith Jacobsson Foundation and the foundation of Anna-Lisa and Bror Björnsson, Gothenburg, Sweden

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