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The Relation of Multiple Sclerosis to Early Life and Adolescence Determinants: Insights from Observational Studies

Dissertation

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PREFACE

This cumulative dissertation entitled „The Relation of Multiple Sclerosis to Early Life and Adolescence Determinants: Insights from Observational Studies“ was submitted to the Medical Faculty at the University of Hamburg within the Non-Medical PhD Programme at the University Medical Center Hamburg-Eppendorf.

I was supervised by Prof. Dr. Heiko Becher (University Medical Center Hamburg-Eppendorf until September 2022 / University of Heidelberg since September 2022), Prof. Dr. Christoph Heesen (University Medical Center Hamburg-Eppendorf) and Prof. Dr. Antonia Zapf (University Medical Center Hamburg-Eppendorf).

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My dissertation comprises three thesis articles which will be summarized in this synopsis:

Thesis article 1:	Holz, A., Riefflin, M., Heesen, C., Riemann-Lorenz, K., Obi, N. & Becher, H. (2022). **Breastfeeding and Risk of Multiple Sclerosis: A Systematic Review and Meta-Analysis of Observational Studies.** *Neuroepidemiology.* 56(6):391-401. doi: 10.1159/000526895.
Thesis article 2:	Holz, A., Obi, N., Ahrens, W., Berger, K., Bohn, B., Brenner, H., Fischer, B., Fricke, J., Führer, A., Gastell, S., Greiser, K.H., Harth, V., Heise, J-K., Holleczek, B., Keil, T., Klett-Tammen, C.J., Leitzmann, M., Lieb, W., Meinke-Franze, C., Michels, K.B., Mikolajczyk, R., Nimptsch, K., Peters, A., Pischon, T., Riedel, O., Schikowski, T., Schipf, S., Schmidt, B., Schulze, M.B., Stang, A., Hellwig, K., Riemann-Lorenz, K., Heesen, C., Becher, H. (2024). **Childhood and adolescence factors and multiple sclerosis: results from the German National Cohort (NAKO).** *BMC Neurol.* 24(1), 123. doi: 10.1186/s12883-024-03620-4.
Thesis article 3:	Holz, A., Obi, N., Pischon, T., Schulze, M.B., Ahrens, W., Berger, K., Bohn, B., Brenner, H., Emmel, C., Fischer, B., Greiser, K.H., Harth, V., Holleczek, B., Kaaks, R., Karch, A., Katzke, V., Keil, T., Krist, L., Leitzmann, M., Meinke-Franze, C., Michels, K.B.; Nimptsch, K., Peters, A., Riedel, O., Schikowski, T., Schipf, S., Schmidt, B.,

Thierry, S., Hellwig, K., Riemann-Lorenz, K., Heesen, C., Becher, H. (2025). **The Relation of Multiple Sclerosis to Family History, Lifestyle, and Health Factors in Childhood and Adolescence: Findings of a Case-Control Study Nested Within the German National Cohort (NAKO) study.** *Dtsch Arztebl Int.* 122: online first. doi: 10.3238/arztebl.m2025.0069.

In the following, the personal pronoun „we“ refers to the group of researchers with whom I have jointly published these three thesis articles and whose individual contributions I would like to acknowledge herewith.

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3. LIST OF ABBREVIATIONS

BL	Baseline
BMBF	Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung)
BMI	Body-Mass-Index
C-C	Case-control study
CI	Confidence interval
CTS	Childhood Trauma Screener
DMT	Disease-modifying therapy
EBV	Epstein-Barr-Virus
EnvIMS	Environmental Risk Factors in MS Study
FU	Follow-Up
HR	Hazard ratio
ICD	International Statistical Classification of Diseases and Related Health Problems
IQR	Interquartile range
ISCED	International Standard Classification of Education
MeSH	Medical Subject Headings
MS	Multiple sclerosis
NAKO	German National Cohort (NAKO Gesundheitsstudie)
NOS	Newcastle-Ottawa Scale
OR	Odds ratio
PA	Physical activity
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-Analyses
PRISMA-P	Preferred Reporting Items for Systematic reviews and Meta-Analysis Protocols
RRMS	Relapsing-remitting multiple sclerosis
SD	Standard deviation
SR-MA	Systematic review with meta-analysis
StERKE	Study on risk factors for the occurrence and progression of Multiple Sclerosis (Studie zum Einfluss von Risikofaktoren auf den Krankheitsverlauf und die Entstehung der Multiplen Sklerose)
T1D	Type 1 diabetes

T2DM	Type 2 diabetes mellitus
WHO	World Health Organisation

4. SYNOPSIS

4.1. Background

Multiple sclerosis (MS) is the predominant inflammatory neurodegenerative disease causing disability in young adulthood (1). The course of MS can be seen as a continuum of simultaneous pathophysiological processes that vary greatly from individual to individual and over time (2). During its early phases, MS is predominantly characterized by disseminated lesions in the central nervous system, marked by a multitude of signs and symptoms, often manifested as relapses, i.e. fully or partially reversible episodes of neurological symptoms that subside after a duration of ≥ 24 hours. Typical neurological signs and symptoms include but are not limited to, optic neuritis, gait ataxia, sensory loss, cognitive dysfunction, loss of bladder control (1,3) but also fatigue and depression (4). In later phases, a partial transition from localized lesions to more widespread inflammatory reactions and neurodegeneration occurs in combination with the absence of compensatory processes such as neuroplasticity and remyelination (2). These processes have a considerable impact on the quality of life of those affected (4), and at the same time evoke a high economic burden (5).

In 2020, approximately 2.8 million people worldwide had MS (35.9/100,000 population), corresponding to an increase of 30 % since 2013 (6). As with the prevalence, the global incidence of MS has also increased in recent years (7). The worldwide incidence was calculated to be 2.1 per 100,000 person/years, with Europe (6.8/100,000 person/years) and the Americas (4.8/100,000 person/years) showing the highest rates (6). An increase in prevalence was also observed in Germany from 2012 to 2019, with an average annual increase of 3.4 % from 271 to 337 per 100,000 population (8). The age-adjusted (European Standard Population) incidence in Germany, based on health claims data, in 2012 was estimated at 6.6 to 21.8 per 100,000 person-years depending on the case definition used (9).

Since the first disease-modifying drug was approved by the U.S. Food and Drug Administration in 1993 (10), 20 disease-modifying therapies (DMT) have been approved for the treatment of MS as of 2022 (11). These are mainly aimed at suppressing the inflammatory component of the disease, but the long-term effects and their effect on disease progression are still inconclusive (3). This emphasizes the need for preventive measures, even if the only partially known aetiology (12) and the lack of knowledge about potential risk factors make the development of such measures considerably more difficult (13).

While genetic epidemiological studies have shown the influence of genetic risk factors on the development of MS with degree of kinship, parent-of-origin effects, and sex influencing the magnitude of the risk (13), MS is not solely attributable to genetic factors. Instead, it has been hypothesized that the manifestation of the disease results from an interaction of environmental and lifestyle factors in genetically susceptible individuals (12,14).

So far, an infection with the Epstein-Barr-Virus (EBV) (15,16), increased levels of early life, childhood and adulthood Body-Mass-Index (BMI), and vitamin D deficiency (17) are the few environmental and lifestyle factors that have been identified as risk factors for MS. Although numerous observational studies observed an association between smoking and an increased risk of MS (18), Mendelian randomization studies, summarized by Fazia et al. (17), could not confirm a causal relationship. Regular moderate to vigorous physical activity (PA), on the other hand, has been identified as a protective factor in the development of MS (17).

Other factors that might have either a detrimental or protective influence on the development of MS could be those that occur (pre)natally or during (early) childhood, and adolescence, and thus at a time when the immature immune system is particularly susceptible (19). This has already been shown for other autoimmune diseases such as asthma (20) or type 1 diabetes (T1D) (21).

Among prenatal factors are maternal age and maternal parity before the birth of the offspring developing MS later in life. Haynes and colleagues (22) were able to show an association between higher maternal age and increased incidence of T1D, whereas no association has yet been shown for MS. This was also the case for maternal parity and MS. However, the results were based on a relatively small study (23). In this context, it might be worthwhile to investigate the interaction of these two factors, which to our knowledge has not yet been done.

As an association between active smoking and MS has been observed in multiple observational studies (18), and the harmful effects of tobacco smoke on lung tissue are well known (24), the exposure to passive smoking due to parental smoking, starting as early as during pregnancy and lasting through childhood and adolescence, might be of relevance.

The hygiene hypothesis postulates that people without or with insufficient exposure to, e.g., infections that trigger an immune response develop lower regulatory immunocompetence and are therefore more susceptible to immune-mediated diseases (25). Hence, the exposure to common childhood infections, but also the number of siblings, daycare attendance and contact with pets and/or farm animals in childhood might have an impact on the development of MS. Apart from an EVB infection (15,16), studies yielded conflicting results regarding the association between the occurrence of common childhood infections (measles, chickenpox,

pertussis, rubella, mumps) and the age at infection and MS risk (26,27). Previous studies have also obtained divergent results for the association between the aforementioned hygiene hypothesis-related surrogate factors and MS risk, necessitating further research on these factors (28–33).

In recent years, the influence of the microbiome and thus the interplay between nutrition, the immune system and the gut-associated immune system on the development of immune-mediated and chronic inflammatory diseases has been widely discussed, which ultimately led to an extended version of the hygiene hypothesis. "Extended" in this context means insufficient stimulation of the mucosal immune system to develop a tolerogenic immune milieu (34). Preterm birth and especially the mode of delivery, i.e. vaginal birth or birth by caesarean section, and breastfeeding might play a role in the development of MS since they are closely linked to the development of the (gut-associated) immune system (34,35). However, research on the association between preterm birth (23,33,36–39), mode of delivery (40–42), and breastfeeding (36,43) and the development of MS later in life yielded conflicting results.

Since increased levels of early life, childhood and adolescence BMI have already been identified as risk factors for MS (17) and obesity triggers chronic low-grade inflammation (44), investigating the association between birth weight, weight at the age of ten years as well as BMI at the age of 18 years is induced. In particular, when considering conflicting results of studies taking sex into account (23,33,37,38,45–50).

Another factor that is closely linked to weight in childhood and adolescence is PA during this stage of life. PA in adulthood has already been shown to be a protective factor for the development of MS (17) as well as in many other chronic diseases such as cardiovascular disease, diabetes, obesity and premature death (51). Wesnes and colleagues observed an inverse association between PA during adolescence (13 to 19 years) and MS risk in the Environmental Risk Factors in MS Study (EnvIMS), a population-based multinational case-control study (52). Insufficient time spent outdoors during childhood and adolescence, often linked with low serum vitamin D levels (53), might pose an additional potential risk factor for MS as was investigated by Magalhaes and colleagues once more in the EnvIMS study. They observed a 1.76-fold (95 % confidence interval (CI) 1.27 to 2.46) increased MS risk in persons with low sun exposure during summer and winter and high sun protection compared to persons with high sun exposure and low sun protection (54).

In addition to molecular-biological processes and environmental and lifestyle factors, psychological factors are increasingly being discussed as potential risk factors for MS (55). In particular, since persons with MS often associate stress with disease exacerbation (56). A

recently published systematic review with meta-analysis (SR-MA) showed a weak to moderate effect of psychological stressors on the risk of MS (57).

In short, in addition to the risk factors already associated with MS (EBV infection, increased levels of early life, childhood/adolescence and adulthood BMI, and vitamin D deficiency), there are a variety of environmental and lifestyle as well as psychological factors that might play a role in the aetiology of MS. Especially if considering the time of exposure from the prenatal period to late adolescence, a time of an immature and highly susceptible immune system.
Therefore, we aimed to investigate the association between the multitude of above-described, potential risk factors and the risk of developing MS later in life.

4.2. Methods

4.2.1. Study design and population

To achieve the above-stated research aim, we utilized different approaches comprising different study designs and populations which will be described in more detail below: (i) a systematic review with meta-analysis (**SR-MA**) comprising observational studies investigating the association between having been breastfed and MS risk, (ii) the German National Cohort (NAKO Gesundheitsstudie, **NAKO**) investigating causes for widespread diseases such as cancer, cardiovascular diseases or type 2 diabetes mellitus (T2DM) in the German population, and (iii) the nested case-control study „Study on risk factors for the occurrence and progression of Multiple Sclerosis (**StERKE**)“ investigating risk factors for the occurrence and progression of MS.

Systematic Review with Meta-Analysis (SR-MA)

Since environmental and lifestyle factors might play a decisive role in the development of MS, we conducted a SR-MA focussing on breastfeeding for two reasons: (i) Breastfeeding takes place at a very early stage in infancy and might impact the development of the gut microbiome which in turn influences the development of the immune system (34,35). ii) Breastfeeding as a potential protective or harmful factor was not considered in another SR-MA of natal factors published at the same time (40). Our **SR-MA** therefore aimed to address this research gap. It was performed in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2020 statement (58). Before the actual conduct of the systematic review, a study protocol was developed in accordance with the Preferred Reporting Items for

Systematic reviews and Meta-Analysis Protocols (PRISMA-P) 2015 statement (59). Protocol amendments were documented along with the date of each amendment, a description of the changes and the rationale to do so. The search strategy was developed based on the PICO (population, intervention, comparison, outcome) framework. Search strings were combined with the Boolean operators AND and OR and adapted to the respective databases. The specific search terms used and the search syntax can be found in Supplement Table 1 of the corresponding publication (60) and are therefore not repeated here. The databases MEDLINE, Web of Science and Cochrane Library were first searched on 12 October 2021, supplemented by a search in Google Scholar considering the 100 most relevant results. The database search was repeated on 18 May 2022, shortly before submission to the journal Neuroepidemiology using the same search strategy. We included observational studies, i.e. cohort, case-control, and cross-sectional studies, which reported on the influence of having been breastfed on MS risk. The relevant breastfeeding period was defined from birth to the age of two years in line with the World Health Organisation's (WHO) recommendations on breastfeeding (61). MS had to be diagnosed according to established diagnostic criteria by Schuhmacher, Poser or McDonald (62) although studies based on self-reported MS diagnoses were also included but with a corresponding downgrading in the quality assessment. Studies reporting on disease activity of MS, e.g., relapse rates, were excluded. Only peer-reviewed studies published in English or German were included, without restrictions regarding study setting or publication year. Duplicates were removed before the title and abstract screening. Both the title and abstract screening and the full-text screening were performed by two independent reviewers blinded to each other's decisions. In case of disagreement, a third reviewer was consulted. If there was no full-text access or data was missing, the corresponding author of the respective study was contacted. The quality of the included studies was assessed with the Newcastle-Ottawa Scale (NOS) for non-randomized studies, ranging from 0 points (equals low quality) to 9 points (equals high quality). The domains selection of cases and controls / exposed and non-exposed, the groups' comparability, and the ascertainment of exposure and outcomes were assessed for case-control studies and cohort studies, respectively (63). For the planned sensitivity analysis, described further below, we classified studies as high vs. low quality with a cut-off of ≥ 6 points (high quality) and < 6 points (low quality).

NAKO

The **NAKO** is a population-based prospective cohort study conducted in 18 study regions throughout Germany. Approximately 205,000 participants were recruited by means of age- and

sex-stratified samples randomly drawn from the corresponding local registries of residents. Ethical approval was granted by the responsible ethics committees of the German Federal States in which the respective study centres were located (Bayerische Landesärztekammer (protocol code 13023; approval date: 27 March 2013 and 14 February 2014)) and written informed consent was obtained from all participants. In the course of the standardized data collection, participants underwent extensive biomedical examinations, participated in a face-to-face interview conducted by trained study staff, and completed a self-administered touchscreen questionnaire. The overall aim of the study was to identify causes and risk factors for common diseases such as cancer, T2DM, infections, and cardiovascular diseases. In addition, the study aimed to derive potential preventive measures against the above-mentioned diseases (64,65). Information provided throughout the face-to-face interview on rare diseases, including MS, was the basis for the selection of study participants for the **StERKE** study.

StERKE

The **StERKE** study is a case-control study nested within the NAKO and was conducted between November 2021 and March 2022 in 16 of the 18 NAKO study centres. Ethical approval was granted by the ethics committee of the Hamburg Chamber of Physicians (PV7292). We set up the study to investigate the influence of risk factors on the occurrence and progression of MS as well as to describe disease patterns of affected individuals. Cases were defined as persons who reported a physician-based MS diagnosis either at the NAKO baseline (BL) interview, the questionnaire-based interim survey on average two years after BL, or at the first follow-up (FU) examination until recruitment for StERKE. Controls were randomly selected NAKO participants without MS and were individually matched to each MS case based on sex, birth year, and study centre in a ratio of 2:1. Participants were contacted either by mail or e-mail, depending on whether they had provided an e-mail address during the NAKO BL survey. Written informed consent was obtained from all participants. In the course of the study, participants received up to three reminders either via mail or e-mail. Data was collected by use of an online- or paper-based questionnaire with questions on medical history, family history, stressful life events, PA, time spent outdoors and nutrition. Cases additionally received MS-specific questions including questions on disease manifestation, medication and physical as well as mental impairments.

4.2.2. Multiple sclerosis risk factors

Since the NAKO aimed to identify causes and risk factors for widespread diseases, potential risk factors collected were rather unspecific for MS. By nesting the StERKE study within the NAKO, potential as well as established MS-specific risk factors, previously identified by an extensive literature search and discussions within the interdisciplinary study team, were to be collected in addition to the more general potential risk factors provided by the NAKO. Hence, the covariates and exposure variables collected differed between the two studies, as depicted in Table 1. For reasons of completeness, the variables included in the SR-MA are also listed.

Table 1 Overview of covariates and risk factors included in the analyses

	SR-MA	**NAKO**	**StERKE**
COVARIATES			
Sex	X	X	X
Birth year		X	X
Education		X	X
Migration background		X	
FAMILY HISTORY & (PRE)NATAL FACTORS			
1st or 2nd degree family member with MS			X
Maternal parity until the birth of the participant			X
Maternal age at participant's birth			X
Parental smoking during pregnancy			X
Premature birth (> 4 weeks before due date)		X	
Caesarean section		X	
Birth weight		X	
FACTORS OCCURING DURING INFANCY/CHILDHOOD, ADOLESCENCE AND ADULTHOOD			
Number of siblings		X	
Breastfeeding	X	X	
Common childhood infections (including chickenpox, mumps, rubella, pertussis, measles, and EBV)			X
Daycare attendance / Age at 1st daycare attendance		X	
Parental smoking during the participant's childhood/adolescence (0-18 years)			X
Time spent outdoors during childhood/adolescence (0-18 years)			X
Contact with pets and/or livestock during childhood		X	
Physical activity during teenage years (13-19 years)			X
Weight at the age of 10 years		X	
BMI at the age of 18 years (kg/m²)		X	X

	SR-MA	NAKO	StERKE
Childhood Trauma		X	
Major stressful life events (including death of the partner, death of a close person (other than partner), serious illness of a close person, own serious illness (other than MS)			X
Smoking		X	X

BMI = Body-Mass-Index; EBV = Epstein-Barr-Virus; MS = Multiple sclerosis; NAKO = German National Cohort; SR-MA = Systematic review with meta-analysis; StERKE = Study on risk factors for the occurrence and progression of Multiple Sclerosis

4.2.3. Statistical Analysis

As the statistical methods used differ substantially, they will be described separately for each respective thesis article in the following section.

SR-MA. Characteristics of included studies were summarized with regard to country, study design, MS diagnostic criteria, number of cases and controls in case-control studies and exposed and non-exposed in cohort studies, matching variables as well as adjustment variables. As we anticipated considerable between-study heterogeneity, we calculated a random-effects model applying the Mantel-Haenszel method (66) for dichotomous outcomes to calculate pooled estimates, both Odds Ratios$_{crude}$ (OR) as well as OR$_{adjusted}$ with corresponding 95 % CIs. In the analysis of the OR$_{adjusted}$, OR$_{crude}$ were used if the adjusted ORs were not reported in the respective studies. Overall and between-study heterogeneity was evaluated by calculation of τ^2 applying the DerSimonian-Liard estimator (67) and of I^2 statistics (68), respectively. Subgroup and sensitivity analyses were calculated to assess the impact of the individual study on the overall robustness of the pooled estimates. The subgroup analyses included analyses stratified by study design as well as time of MS onset, i.e. paediatric- vs. adult-onset MS, while the sensitivity analysis included only studies of high quality identified in the quality assessment using the NOS. Results of the respective analyses are presented by forest plots displaying the number of breastfed and non-breastfed individuals, the OR$_{crude}$ and OR$_{adjusted}$ with corresponding 95% CIs and the corresponding study weight for the overall analysis as well as the analyses stratified by study design. Publication bias was visually assessed with funnel plots.

NAKO. Descriptive analyses with regard to birth year, sex, educational level, and exposure variables as depicted in Table 1 were conducted stratified by MS status. Categorical variables were summarized as absolute and relative frequencies, and continuous data as mean and standard deviation (SD).

We performed a multivariable Cox proportional hazards regression to assess the association between childhood and adolescence factors and MS. We adjusted for the covariates educational level and migration background and stratified for the birth year to account for possible cohort effects. Results are displayed as Hazard Ratios (HR) with corresponding 95 % CIs. The outcome was defined as age at self-reported physician-based MS diagnosis. The observation period began at birth and ended either with age at reported MS diagnosis in case of an event or age at the NAKO BL examination in case of no event, whichever came first. The reference categories for categorical variables were defined as (i) female for sex, (ii) absence of the respective factor (for binary variables) or (iii) the lowest/average/normal exposure category (for variables with > 2 categories).

Since the number of missing values was relatively high in the respective exposure categories and only 75,247 (36.8 %) records were complete, multiple imputation was used to calculate and analyse 40 imputed datasets. Missing values were imputed under fully conditional specification, using the default settings of the R-package „mice 3.0“ (69). HRs were first calculated separately for every imputed dataset and subsequently pooled by applying Rubin's rule (70). For comparison, we performed the Cox proportional hazards regression on the subset of complete records. To analyse sex-specific effects, we additionally performed a subgroup analysis stratified by sex.

The proportional hazards assumption was assessed by graphical inspection of the Schoenfeld residuals.

StERKE. Analogous to the NAKO data, descriptive analyses were conducted for the StERKE data with regard to year of birth, education level and exposure variables by case-control status and sex. MS-specific information such as age at first manifestation, defined as first symptoms, age at diagnosis, MS type, use of MS-specific medication or immunotherapy as well as time of diagnosis were separately displayed for MS cases, stratified by sex. Categorical variables were summarized as absolute and relative frequencies. Continuous variables as mean and SD or, in some cases as median and interquartile range (IQR). This was explicitly indicated in Table 1 and 2 of the respective publication (71). To assess the association between family history, prenatal, childhood, adolescence, and adulthood factors and MS, a conditional logistic regression was performed. The outcome was defined as either a prevalent self-reported, physician-based MS diagnosis during the NAKO BL survey or an incident MS diagnosis during the paper-based interim survey approximately two years after the BL assessment or the first FU survey approximately five years after BL. The reference categories for categorical variables

were defined as (i) female for sex, (ii) absence of the respective factor (for binary variables) or (iii) the lowest/average/normal exposure category (for variables with >2 categories). Since not all invited participants took part in StERKE we conducted a second matching to ensure that each participating case had at least one control. In this process, all cases and controls with the same matching variables, i.e. study centre, sex and year of birth, were combined in a matching set according to the method described in Neuhäuser and Becher (72). If there was no suitable matching set for a case, the age limit was relaxed by ± 2 years. Since the data for the variables BMI at the age of 18 and smoking originated from the NAKO BL, we conducted a separate model for these factors with all MS cases (n=746) and matched controls (n=1,492). We calculated a full model including all factors under consideration and a final model obtained from backward selection with a selection limit of $p = 0.10$. To address a potential survival bias by using prevalent cases, we performed subgroup analyses that included: i) only incident cases, ii) incident cases combined with various sub-samples of prevalent cases selected based on the respective disease duration (the time between the year of MS diagnosis and the NAKO BL examination: 2, 5, 10, and 20 years, respectively). Additional subgroup analyses comprised iii) only MS cases with relapsing-remitting MS (RRMS) who reported the use of MS-specific medication in the NAKO BL survey and/or the use of immunotherapy in the StERKE study as well as iv) an analysis stratified by sex. For all subgroup analyses as well as the analysis stratified by sex, the final model was used. Missing values were dealt with either by introducing an additional category or by imputation of the median. A detailed overview of the handling of missing values can be found in eSupplement Table 2 of the respective publication (71).

The analyses were performed with the software R version 4.1.1 (SR-MA), 4.2.0 (NAKO), 4.3.1 (StERKE) (73) using the packages „meta“ (74), „metafor“ (75), „dmetar“ (76), „survival“ (77), „survminer“ (78), „sjPlot“ (79), „MASS“ (80), „mice“ (69), and „micemd“ (81).

4.3. Results

Since the main purpose of this synopsis is to provide an overall interpretation of the main results of the individual publications that contributed to this project, we will refrain from reporting once more from the description of the main characteristics of the studies included in the SR-MA as well as the description of the NAKO and StERKE participants and would like to refer to the respective publications at this point (60,71,82). Nevertheless, a summary of the most important characteristics is provided at the beginning of each section, followed by a presentation of the results of the main analyses and the subgroup and sensitivity analyses.

4.3.1. Association between having been breastfed and MS risk – systematic review with meta-analysis

Figure 1 and Table 1 in the original publication (60) depict the study selection process as well as the characteristics of the included studies. The initial search in the databases MEDLINE, Web of Science and Cochrane Library supplemented by the search in Google Scholar yielded 425 results in total. After duplicate eradication, title, abstract and full-text screening with regard to the pre-defined in- and exclusion criteria, 15 studies (13 case-control studies, two cohort studies) describing 17 distinct study samples, were included in the systematic review and meta-analysis. 13 of 17 studies were conducted in Europe (n=8) or the Americas (n=5). 15 of 17 studies included participants who were diagnosed with regard to established diagnostic criteria such as Poser criteria, McDonald criteria, International Pediatric Multiple Sclerosis Study Group criteria, or whose diagnoses was confirmed by the International Statistical Classification of Diseases and Related Health Problems (ICD) codes. In the included case-control studies the matching was mainly performed based on sex, age and region. 12 of 15 studies were rated as „high quality studies“ in the risk of bias assessment applying the NOS criteria. The results of the ratings for the respective studies can be found in Supplement Table 2 of the publication (60).

Main results. Table 2 summarizes the results of the meta-analyses on the association between having been breastfed compared to not having been breastfed and MS risk of the crude and adjusted ORs, including analyses stratified by study design. The analysis of the crude ORs showed a protective effect of having been breastfed (OR 0.82, 95 % CI 0.70 to 0.96) with substantial heterogeneity I^2 (68.2 %). The analyses by study design yielded similar results (case-control studies: OR 0.83 (95 % CI 0.70 to 0.98); cohort studies: OR 0.73 (95 % CI 0.49 to 1.09)) with low heterogeneity between the results when comparing case-control and cohort studies. The analysis of the adjusted ORs, if available otherwise using the crude OR, also showed a protective effect of having been breastfed compared to not having been breastfed with regard to MS risk (OR 0.86, 95 % CI 0.75 to 0.99, I^2 = 48.9 %), with subgroup analyses by study design no longer yielding significant results. The forest plots (Figures 2 and 3 in the original publication (60)) display the summary statistics number of events, sample size, crude OR and corresponding 95 % CI for the analysis of the crude OR; adjusted OR and corresponding 95 % CI for the analysis of the adjusted OR of the individual studies contributing to the respective

analyses. Visual inspection of the funnel plot (Figure 4 in the original publication (60)) did not reveal an indication of a relevant publication bias.

Table 2 Crude and adjusted odds ratios, 95 % confidence intervals and heterogeneity for having been breastfed compared to not having been breastfed on MS risk.

Included Studies		Pooled OR (95 % CI), random effects model	Heterogeneity
C-C and cohort studies (n=17[a])	Crude	0.82 (0.70–0.96)	I^2 = 68.2 %
C-C studies (n=15)	Crude	0.83 (0.70–0.98)	I^2 = 71.8 %
Cohort studies (n=2)	Crude	0.73 (0.49–1.09)	I^2 = 0.0 %
C-C and cohort studies (n=17[a])	Adjusted[b]	0.86 (0.75–0.99)	I^2 = 48.9 %
C-C studies (n=15)	Adjusted[b]	0.87 (0.75–1.02)	I^2 = 53.9 %
Cohort studies (n=2)	Adjusted[b]	0.73 (0.49–1.09)	I^2 = 0.0 %

C-C = Case-control study; OR = Odds Ratio

[a]Studies by Hedström et al. (43) and Ragnedda et al. (83) reported results of two distinct study samples and are therefore included in the meta-analysis as two distinct studies.

[b]If adjusted OR were not available, crude OR were used instead.

Subgroup and sensitivity analysis. Overall, subgroup analyses, i.e. by study design and time of MS onset, as well as the sensitivity analysis, i.e. low vs. high quality studies, showed consistent findings with the primary analysis, indicating a protective effect of having been breastfed compared to not having been breastfed on MS risk. Results are displayed in the publication as well as in the corresponding Supplement Table 3 (60).

4.3.2. Association between family history, (pre)natal, childhood, adolescence and adulthood factors and MS risk – NAKO and StERKE study

Figure 1 displays the selection of participants for the respective analysis – NAKO or StERKE. The NAKO cohort comprised a total of 204,899 participants, 860 people with a prevalent MS diagnosis at BL and 203,415 people without MS. For the remaining 624, no information regarding an MS diagnosis was available. These and two other study participants who did not provide information on age at MS diagnosis were excluded. Accordingly, the final analysis included data of 204,273 NAKO participants - 858 prevalent cases and 203,415 controls.

During the period between the NAKO BL and the recruitment of StERKE participants, a total of 136 persons were newly diagnosed with MS. Therefore, the study population for the recruitment of StERKE participants consisted of 996 cases, 203,426 controls and 477 participants with unknown disease status. After excluding unavailable study participants and completion of the matching, 2,493 participants were available for the StERKE study, of which a further 252 participants from the Hanover and Halle study centres had to be excluded due to

non-participation of these centres. Of the 2,241 invited study participants, data from 1,471 StERKE participants - 576 cases and 895 controls - were ultimately analysed. This amounts to a response proportion of 77 % among the cases and 60 % among the controls.

The mean age in both samples was 50 years. 71.8 % of the NAKO participants and 73.4 % of the StERKE participants had a medium level of education based on the International Standard Classification of Education (ISCED)-97. More than 80 % of the NAKO participants had no migration background. NAKO participants with MS were diagnosed at a median age of 36 years, whereas StERKE cases were diagnosed at a median age of 38 years. The most common form of MS in the StERKE sample (57.1 %) was RRMS. Approximately half of the StERKE cases had ever received immunotherapy. The information regarding MS type and immunotherapy were StERKE-specific questions and were therefore not collected for the remaining NAKO participants. Table 3 displays the main characteristics of the NAKO and StERKE participants by sex and MS status/case-control status, respectively.

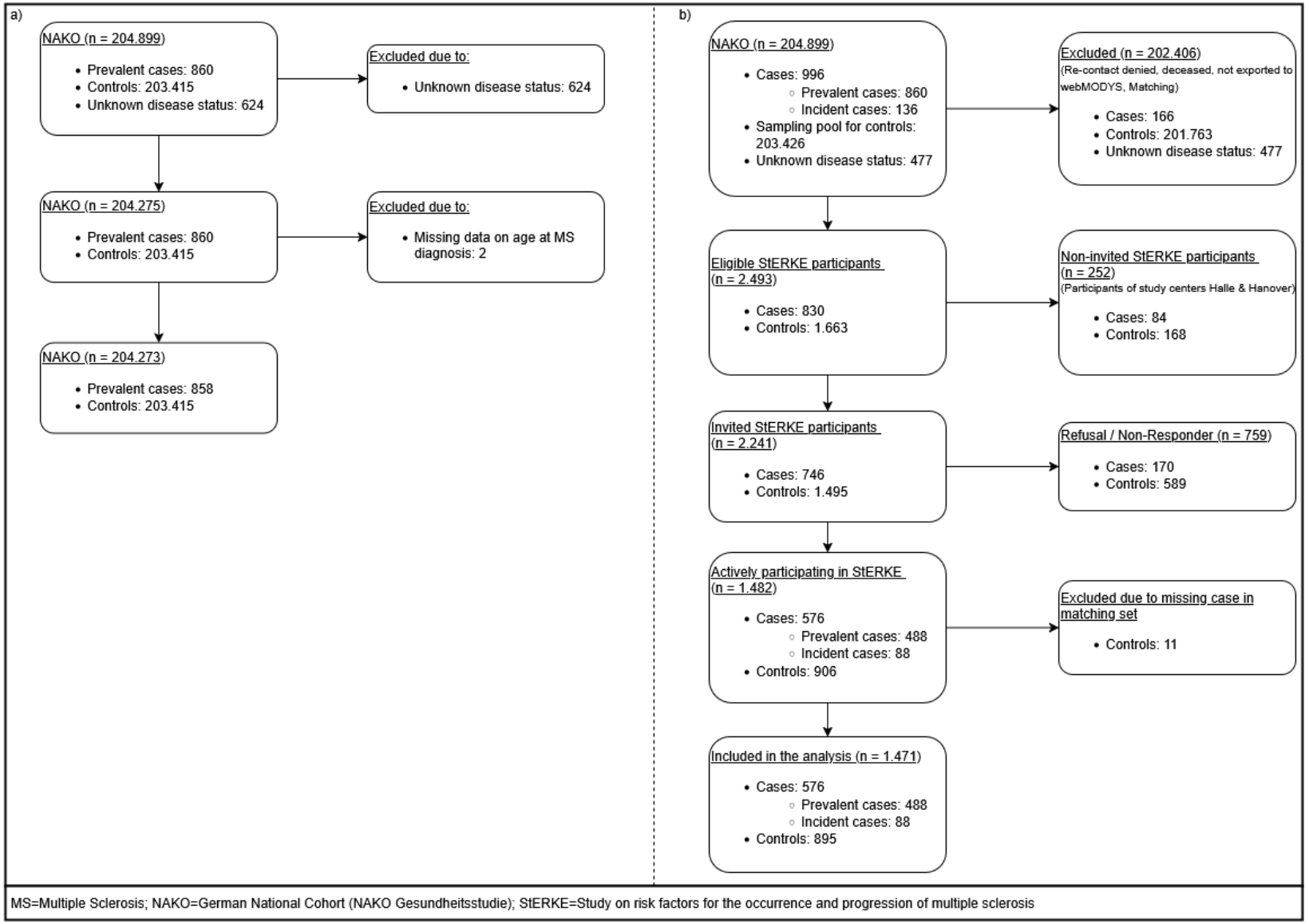

Figure 1 Flow diagram showing the inclusion and exclusion of study participants for a) the NAKO analysis and b) the StERKE analysis

Table 3 Characteristics of the NAKO and StERKE participants by sex and MS status/case-control status

Variable	NAKO				StERKE			
	Women		Men		Women		Men	
	Persons without MS (n=102,494)	Persons with MS (n=579)	Persons without MS (n=100,921)	Persons with MS (n=279)	Controls (n=638)	Cases (n=396)	Controls (n=257)	Cases (n=180)
Age at NAKO baseline (Mean (SD))	50 (12.7)	50 (10.8)	50 (12.8)	49 (10.7)	51 (11.0)	49 (10.9)	52 (11.3)	49 (11.1)
Birth year								
<1955	22,794 (22.2 %)	86 (14.9 %)	22,816 (22.6 %)	39 (14.0 %)	86 (13.5 %)	51 (12.9 %)	44 (17.1 %)	25 (13.9 %)
1955-1964	26,176 (25.5 %)	174 (30.1 %)	24,731 (24.5 %)	82 (29.4 %)	178 (27.9 %)	110 (27.8 %)	90 (35.0 %)	56 (31.1 %)
1965-1974	28,199 (27.5 %)	188 (32.5 %)	27,717 (27.5 %)	90 (32.3 %)	229 (35.9 %)	137 (34.6 %)	68 (26.5 %)	56 (31.1 %)
1975-1984	12,582 (12.3 %)	88 (15.2 %)	12,969 (12.9 %)	48 (17.2 %)	76 (11.9 %)	57 (14.4 %)	33 (12.8 %)	27 (15.0 %)
≥1985	12,743 (12.4 %)	43 (7.4 %)	12,688 (12.6 %)	20 (7.2 %)	69 (10.8 %)	41 (10.4 %)	22 (8.6 %)	16 (8.9 %)
Education[a]								
Low	3,158 (3.4 %)	16 (3.0 %)	1,949 (2.1 %)	4 (1.6 %)	9 (1.5 %)	2 (0.6 %)	2 (0.8 %)	1 (0.6 %)
Medium	70,788 (76.1 %)	406 (75.7 %)	75,328 (82.0 %)	200 (81.0 %)	457 (76.9 %)	286 (79.2 %)	205 (85.4 %)	131 (81.9 %)
High	19,077 (20.5 %)	114 (21.3 %)	14,639 (15.9 %)	43 (17.4 %)	128 (21.5 %)	73 (20.2 %)	33 (13.8 %)	28 (17.5 %)
Migration background[a]								
No	84,988 (82.9 %)	514 (88.8 %)	83,808 (83.1 %)	229 (82.1 %)	--	--	--	--
Yes	17,484 (17.1 %)	65 (11.2 %)	17,092 (16.9 %)	50 (17.9 %)	--	--	--	--
Age at MS diagnosis (Median (IQR))	--	36 (28, 43)	--	36 (28, 45)	--	38 (29, 47)	--	38 (28, 46)
Age at MS manifestation (Median (IQR))[a,b]	--	--	--	--	--	33 (26, 42)	--	35 (27, 41)
Type of MS[a,b]								
Relapsing-remitting	--	--	--	--	--	244 (79.5 %)	--	85 (63.9 %)
Primary progressive	--	--	--	--	--	26 (8.5 %)	--	22 (16.5 %)
Secondary progressive	--	--	--	--	--	37 (12.1 %)	--	26 (19.5 %)
MS-specific medication[a,c]								
No	--	387 (67.9 %)	--	164 (59.2 %)	--	219 (65.6 %)	--	90 (58.4 %)
Yes	--	183 (32.1 %)	--	113 (40.8 %)	--	115 (34.4 %)	--	64 (41.6 %)
No MS at baseline	--	--	--	--	--	62	--	26
Ever immunotherapy[a,b]								
No	--	--	--	--	--	150 (41.7 %)	--	75 (49.7 %)
Yes	--	--	--	--	--	210 (58.3 %)	--	76 (50.3 %)

IQR=Interquartile range; MS=Multiple sclerosis; NAKO=German National Cohort; SD=Standard deviation; StERKE=Study on risk factors for the occurrence and progression of Multiple Sclerosis

[a] Percentages refer exclusively to persons for whom information was available. The data for the categories "missing value" and "unknown" can be found in the respective publications (71,82).

[b] Information only collected in the course of the StERKE study.

[c] Up to 7 days before the NAKO baseline survey

Main analyses. Risk estimates with corresponding 95 % CIs of the multivariate Cox proportional hazards regression, stratified by birth year and adjusted for education and migration background, as well as risk estimates of the conditional logistic regression are presented in Tables 4 and 5, respectively.

Table 4 Multivariable Cox proportional hazards regression on the association between childhood and adolescence factors and multiple sclerosis

	Multivariable Cox Proportional Hazards Model[a]	
Variable	**HR**	**95 % CI**
Sex		
Female	Ref.	
Male	0.48	0.41 – 0.56
Number of siblings		
Only child	Ref.	
1-2 sibling(s)	0.93	0.73 – 1.17
≥3 siblings	0.81	0.61 – 1.09
Premature birth (>4 weeks before due date)		
No	Ref.	
Yes	0.94	0.61 – 1.47
Caesarean section		
No	Ref.	
Yes	1.10	0.80 – 1.52
Birth weight		
Low	0.99	0.74 – 1.33
Average	Ref.	
High	1.14	0.90 – 1.45
Ever breastfed		
No	Ref.	
Yes, ≤4 months	1.02	0.79 – 1.31
Yes, >4 months	0.88	0.69 – 1.11
Contact with pets and/or livestock during childhood		
No	Ref.	
Yes	1.04	0.69 – 1.58
Attended daycare		
No	Ref.	
Yes, 1st attendance at age 3-6 years	0.93	0.77 – 1.12
Yes, 1st attendance at age 1-<3 year(s)	0.77	0.58 – 1.03
Yes, 1st attendance at age <1 year	0.97	0.63 – 1.50
Weight at the age of 10 years compared to peers		
Lower	0.86	0.70 – 1.05
Average	Ref.	
Higher	0.93	0.73 – 1.19
BMI at the age of 18 years (kg/m²)		
Underweight (<18.5)	1.12	0.89 – 1.42
Normal weight (18.5 - <25)	Ref.	
Overweight (25 - <30)	2.03	1.41 – 2.94
Obesity (≥30)	1.89	1.02 – 3.48
Childhood Trauma[b] (per 5 units)	1.08	0.95 – 1.24

BMI = Body-Mass-Index; CI = Confidence Interval; HR = Hazard Ratio; Ref = Reference category

[a]Adjusted for education and migration status, stratified by birth year (categorized as: <1955, 1955-1964, 1965-1974, 1975-1984, ≥1985)

[b]Assessed with the Childhood Trauma Screener (5 – 25 points)

In the analysis comprising all NAKO participants, we observed a reduced risk for men compared to women (HR 0.48, 95 % CI 0.41 to 0.56). Furthermore, the risk of MS was increased 2.03-fold (95 % CI 1.41 to 2.94) for overweight and 1.89-fold (95 % CI 1.02 to 3.48) for obesity at the age of 18 compared to normal weight at this age. For the remaining factors (number of siblings, preterm birth, caesarean section, birth weight, breastfeeding, contact with pets and/or farm animals, age at first daycare attendance, weight at the age of 10 years, and childhood trauma) no association was observed.

The final model of the StERKE analysis showed an association between:

i. the cumulative number of common childhood infections (OR 1.14 per additional infection, 95 % CI 1.03 to 1.25),
ii. the cumulative number of stressful life events (OR 1.25 per additional event, 95 % CI 1.06 to 1.48),
iii. maternal parity until the birth of the participant (OR 0.85, 95 % CI 0.77 to 0.95),
iv. maternal age (per year) at participant's birth (OR 1.03, 95% CI 1.00 to 1.05), and
v. PA during teenage years (OR 0.82 per level increase, 95 % CI 0.71 to 0.95)

and MS. In a combined analysis of maternal parity and maternal age being the firstborn child of a mother ≥ 30 years at delivery was associated with a higher likelihood of MS (OR 2.11, 95 % CI 1.08 to 4.13). Smoking was not strongly associated with MS risk (OR 1.19, 95 % CI 0.99 to 1.43). In addition, known risk factors, i.e. family history, EBV infection, and overweight and obesity in childhood and adolescence were confirmed in our analyses.

For the remaining factors, i.e. passive smoking in childhood and adolescence, time spent outdoors, and own serious illness we did not observe associations with MS.

Table 5 Conditional logistic regression on the association between family history, prenatal, childhood, adolescence and adulthood factors and multiple sclerosis

	Conditional Logistic Regression[a] (576 cases and 895 controls)	
	OR	***95 % CI***
FAMILY HISTORY AND PRENATAL FACTORS		
Family history		
No family member with MS	Ref.	
1st and 2nd degree family member with MS	7.08	3.90 – 12.86
Number of older siblings (per additional sibling)	0.85	0.77 – 0.95
Maternal age (per year) at participant's birth	1.03	1.00 – 1.05

	OR	*95 % CI*
CHILDHOOD INFECTIONS		
Number of common childhood infections (per additional infection) before the age of 18 years[b,c]	1.14	1.03 – 1.25
EBV infection[b]		
No	Ref.	
Yes	3.05	1.80 – 5.16
LIFESTYLE FACTORS IN ADOLESCENCE		
Physical activity (per level increase) during teenage years (13-19 years)	0.82	0.71 – 0.95
BMI at the age of 18 years (kg/m²)[d]		
Underweight (<18.5)	0.86	0.64 – 1.16
Normal weight (18.5 - <25)	Ref.	
Overweight (25 - <30)	1.73	1.22 – 2.44
Obesity (≥30)	2.29	1.18 – 4.46
OTHER FACTORS		
Number of major stressful life events (per additional event)[b,e]	1.25	1.06 – 1.48
Ever smoked[a,b,d]		
No	Ref.	
Yes	1.19	0.99 – 1.43

BMI = Body-Mass-Index; CI = Confidence interval; EBV = Epstein-Barr-Virus; MS = Multiple sclerosis; OR = Odds ratio

[a]Final model as obtained from backward selection with p = 0.10 as selection limit

[b]Cases: Before age at diagnosis; Controls: Before age at diagnosis of the matched case/median age of diagnosis if >1 case per matching set

[c]Including chickenpox, mumps, rubella, pertussis, and measles

[d]The OR estimate for BMI at the age of 18 years and smoking were calculated based on the original matching with all cases (n = 746) and controls (n = 1.492) regardless of their participation in the StERKE study since information on BMI and smoking was available from the NAKO baseline examination.

[e]Including death of the partner, death of a close person (other than partner), serious illness of a close person

Subgroup and sensitivity analyses.

For the NAKO sample, in the analysis stratified by sex, we observed an association between overweight and obesity compared to normal weight and MS risk for women, but not for men. In contrast, there was an association between breastfeeding for ≤ 4 months compared to not having been breastfed and a lower risk of MS for men, but not for women. The risk estimates for the other factors differed only marginally from the estimates of the main analysis. This also applied to the analyses in which only complete cases were included, whereby the CIs were wider than in the main analysis with all approximately 205,000 participants due to the smaller sample.

For the StERKE analyses, the results of the subgroup analyses as well as the analysis stratified by sex differed only marginally from the results of the final model (eSupplement Tables 3, 4, and 5 of the respective publication (71).

Although we acknowledge the importance of subgroup and sensitivity analyses, we would like to refer the interested reader to the respective publications for a detailed presentation of the results of these analyses (71,82).

4.4. Discussion

4.4.1. Summary

In an overarching summary of the studies' individual results, the concept of an association between modifiable environmental and lifestyle factors and MS can be supported. We were able to reproduce results from the literature on previously identified risk factors for MS, i.e. an EBV infection, increased levels of adolescent BMI, and family history of MS. Furthermore, we observed a weak association between smoking and increased MS risk as reported in multiple observational studies. In addition, we attained several novel findings, such as an increase in the odds of the cumulative number of childhood infections, the cumulative number of major stressful live events and being the firstborn to a mother ≥ 30 years at delivery and MS. For PA during teenage years and having been breastfed we observed a protective effect on MS.

4.4.2. Research in Context

Maternal age and **parity.** We observed an association between older maternal age at the birth of the first child and the child's risk of MS, which contradicts the results of an earlier but smaller study (23). However, our results are consistent with studies that have investigated the association between maternal age and the occurrence of T1D (22), another autoimmune disease. Therefore, the underlying pathomechanisms still need to be further elucidated.

Hygiene hypothesis. Among the factors associated with the hygiene hypothesis (common childhood infections, number of siblings, daycare attendance/age at 1st attendance, and contact with pets and/or farm animals) we observed only a positive association between an EBV infection and the cumulative number of childhood infections and the risk of MS. The first finding confirmed an EBV infection as an already known risk factor for MS (15,16), while the latter on the cumulative number of childhood infections contrasts the hygiene hypothesis (25). However, the effect of this finding might rather depend on the age at infection or the underlying pathogen. In addition, it should be taken into account that most NAKO participants were born

and raised before the introduction of standard vaccinations (84). Further studies with younger, presumably vaccinated participants or even birth cohorts might clarify the relevance of these findings.

Our results and the conflicting results of previous studies regarding the remaining factors associated with the hygiene hypothesis (i.e. number of siblings, daycare attendance, contact with pets and/or farm animals) (25,26,28,30–32) highlight the need for further research into these factors and their association with MS risk.

Concerning the "extended" hygiene hypothesis, i.e. the investigation of possible risk factors for the development of MS such as premature birth, birth mode and breastfeeding as contributors to the development of the gut-associated immune system, studies have so far yielded contradictory results (23,33,36–42,60). Both in our SR-MA and in the analysis of the NAKO data, we observed a protective effect of breastfeeding on the risk of MS. No association was observed for preterm birth and mode of delivery. It could be that these factors play only a minor role in the development of MS and that these effects were masked by the large number of factors included in the analyses. Nevertheless, the relevance of birth mode should not be ignored given the increasing number of caesarean sections performed in recent years (85).

Weight. We observed an association between overweight and obesity at the age of 18 years compared to normal weight and MS risk in the NAKO as well as the StERKE analyses, confirming increased levels of adolescent BMI as a risk factor for MS (17). Since overweight and obesity induce a constant minor inflammatory response in the body, one might assume that both increased birth weight and the continuation of increased body weight in childhood compared to normal weight were associated with the risk of MS. However, we did not observe an association between increased birth weight and body weight at the age of ten years with MS risk, which is in line with previous studies (23,33,37,38,49). Thus, the time of onset of overweight/obesity might have an impact on MS risk.

In this context, **PA** should be considered as it both contributes to the prevention of overweight and obesity and at the same time has an anti-inflammatory effect (86). While children are usually still very physically active, PA declines during puberty (87). We were able to observe an inverse association between PA and MS risk, supporting this assumption as well as corroborating the results of a large multi-regional study also reporting an inverse association between PA levels during adolescence and MS risk (52).

Factors such as maternal weight before and during pregnancy or the occurrence of gestational diabetes, which presumably lead to a dysregulation of maternal and foetal glucose, insulin, lipid

and amino acid metabolism and thus might have an influence on the birth weight of the child (88), were not taken into account in our analyses. Future studies might include these factors to shed light on their influence on the MS risk.

Childhood trauma and **major stressful life events.** Stress is considered a risk factor for other chronic, non-infectious diseases and is associated with the development of both mental and somatic diseases (89). This can also be transferred to the MS context as a recently published SR-MA concluded (57). Childhood trauma, especially physical and sexual abuse, might also play a decisive role in the development of MS, as the systematic review by Polick and colleagues (55) has shown. Consistent with this, our analyses showed an association between the cumulative number of stressful life events and the risk of MS. For childhood trauma, which we analysed based on the sum score of the Childhood Trauma Screener (CTS), no association was found. A more differentiated analysis of the individual subscales of the CTS (emotional, physical, and sexual abuse and emotional and physical neglect scales), might have yielded different results. Nevertheless, the specific biological mechanisms underlying these associations remain to be explored.

Smoking. We were able to observe an association between smoking and increased MS risk, although our effect was not as strong as in the meta-analysis by Degelman and Herman (18). With regard to passive smoking, a distinction should be made between parental smoking during pregnancy and during the participant's childhood and adolescence. Goldacre and colleagues observed an association between maternal smoking during pregnancy and an increased risk of MS for their children, but this was no longer significant after adjustment for year of birth and maternal age (23). Wang and colleagues observed not only an association between passive smoking during childhood and adolescence with an increased risk of MS but also a dose-response relationship (90). We did not see an association with MS for either parental smoking during pregnancy or passive smoking during childhood and adolescence, which could be due to recall bias or social desirability. Furthermore, the NAKO participants grew up at a time when smoking was considered a common habit, so the memory of smoking as a specific exposure may have been reduced.

Time spent outdoors. In contrast to Magalhaes and colleagues (54), we did not observe an association between time spent outdoors and MS risk. This could be due to recall bias on the one hand and the use of a broad time span, which ranged from never to some hours per months

to some hours per day, on the other. Conducting studies using serum samples is preferable, but was not possible in the context of this study.

4.4.3. Strengths and Limitations

This work has various strengths and limitations that concern the methods used and - in the regard of the SR-MA - the included studies themselves.

SR-MA. By adhering to the PRISMA (58) and PRISMA-P (59) guidelines and formulating the research question and search terms based on the PICO scheme using free text terms, Medical Subject Headings (MeSH)-Terms as well as Boolean operators, we were able to ensure a systematic approach to the search, the selection of suitable studies and the description and analysis of the results - both qualitatively and quantitatively. However, it should be noted that language bias might have occurred as we only included studies in German or English. Since publishing in English is common practice, we assume that this bias is very small. With regard to the included studies, the following limitations have to be acknowledged:

i) The included studies used different diagnostic criteria for the outcome of interest - MS - which could have resulted in a different detection rate of MS.

ii) Most of the included studies were case-control studies and therefore prone to recall bias. However, we do not assume that there was differential recall between cases and controls and therefore a possible bias would have rather been towards a null effect.

iii) Different definitions of breastfeeding were used, which reduces the comparability of the studies.

iv) This also applies to the variables considered to adjust for in the studies, although it should be noted that most of the studies adjusted for common risk factors for MS, e.g., EBV infection, serum vitamin D levels, overweight and obesity during childhood and adolescence.

v) Last but not least, only a few studies looked at sex-specific influences despite the known sex-specific distribution of MS.

NAKO and **StERKE.** Our analyses are based on the NAKO, the largest population-based prospective cohort study in Germany so far (64,65). Due to the very large sample size, we were able to compare a sufficiently large group of people with MS with people without MS or, in the nested case-control study, randomly selected and matched controls based on birth year, sex and study centre. The NAKO BL survey already provided us with a considerable body of

information on environmental and lifestyle factors potentially associated with MS, which we were able to supplement with information gathered in the course of the StERKE study. In addition, the high quality of the NAKO data collected by trained personnel based on standard operating procedures and the stringent recruitment management in the StERKE study with up to three reminders per participant which resulted in a high response proportion of 77 % for cases and 60 % for controls should be emphasized.

Nevertheless, the NAKO as well as the StERKE analyses have some limitations that need to be acknowledged. Our analyses are based on self-reported, physician-based MS diagnoses as well as self-reported age at diagnoses, which might have led to possible misclassifications. However, we presume the self-report of having been diagnosed by a physician as rather reliable in light of the psychological impact of such a diagnosis. In addition, Claflin and colleagues evaluated the consistency and validity of self-reported year of diagnosis in 2,245 participants of the Australian Multiple Sclerosis Longitudinal Study and found that self-reported year of MS diagnosis was reliable information to be used in analyses (91). Furthermore, the conduction of subgroup analyses based on the assumption that the statements on the type of MS and the intake of MS-specific medication are a clear indication for a valid MS diagnosis led to similar results as the main analyses.

Selection bias might have occurred as primarily health-oriented people tend to participate in scientific studies. With regard to people with MS, the severity of the disease may be associated with the willingness to participate, as the subjects had to visit the respective study centre for the physical examinations and interviews, which could have led to the participation of less severely affected people. On the other hand, people who are particularly severely affected may have an increased interest in participating in a study. Therefore, participants might have a different risk factor pattern than the general population.

Another limitation is the inclusion of predominantly prevalent MS cases which is considered to be prone to survival bias (92). To counteract this, we performed subgroup analyses, initially including only incident cases and subsequently adding subsamples of prevalent cases to the study sample with regard to disease duration (i.e. the time from MS diagnosis to NAKO BL survey of 2, 5, 10, and 20 years). We did not observe a trend in risk estimates. Although this is not a formal proof of the absence of a survival bias, we consider our results to be sufficiently reliable. As survival after diagnosis is rather long in MS in contrast to diseases such as cancer, the possible survival bias may be less severe in our study.

As the factors investigated occurred predominantly in the participants‘ childhood and adolescence, recall bias might have occurred. For potential risk factors assessed in the NAKO,

we assume no differential recall between cases and controls, since the focus of the survey was on causes and risk factors for common diseases such as cancer or cardiovascular diseases rather than on MS. Differential recall is more likely for the factors assessed in the StERKE study, particularly when cognitive impairment associated with MS disease progression is taken into account. However, since our results are largely consistent with those from prospective studies (23,93,94) and only participants with a certain level of physical and cognitive fitness participated in the NAKO and StERKE studies, we assume that the effects of differential recall were rather small.

We had to deal with a large number of missing values in the analysis of the entire NAKO sample, which made it difficult to classify the missingness. However, the analysis including only complete cases, which led to the same results as the analysis with the imputed values, strengthens our confidence in our approach and the results obtained.

4.4.4. Conclusion and implications of all the available evidence

This project was based on i) a SR-MA incorporating 15 distinct studies, 12 of which were of high quality, and ii) two observational studies providing us with the possibility to answer our research questions based on high quality population-based data. We were able to confirm already identified risk factors while simultaneously identifying new factors associated with MS, thus contributing to the knowledge of MS aetiology. As most of them are modifiable risk factors contributing also to the development of other common diseases, preventive measures such as vaccination programs, promoting smoking cessation, breastfeeding, PA during adolescence and healthy eating behaviours to prevent overweight and obesity might also be applicable in the context of MS prevention. Future studies might initially focus on the investigation of incident cases within birth cohorts with validated MS diagnosis to investigate, e.g., a dose-response relationship between the newly found risk factors and MS or the MS prodrome by applying a lag time between exposure and MS diagnosis.

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6. PUBLICATION 1

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Breastfeeding and Risk of Multiple Sclerosis: A Systematic Review and Meta-Analysis of Observational Studies

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Keywords
Multiple sclerosis · Breastfeeding · Risk factors · Epidemiology · Meta-analysis

Abstract

Introduction: The causes of multiple sclerosis (MS) are not fully understood, yet. Genetic predisposition, environmental and lifestyle factors as well as an interplay thereof constitute relevant factors in the development of MS. Especially early-life risk factors such as having been breastfed may also be of relevance. Breast milk provides the newborn not only with essential nutrients and vitamins but also with numerous immune-active molecules, metabolites, oligosaccharides, and microbial components that are important for the development of the immune system. We present a systematic review and meta-analysis on the influence of having been breastfed during infancy on the risk of developing MS. ***Methods:*** The databases MEDLINE, Cochrane Library, and Web of Science were systematically searched for studies on breastfeeding and MS published between database inception and May 18, 2022. Observational studies comparing persons with MS to healthy controls with regard to having been breastfed during the first 2 years of life were eligible for inclusion. A random effects meta-analysis was calculated to estimate pooled effect sizes using the Mantel-Haenszel method for dichotomous outcomes. The Newcastle-Ottawa Scale was used for quality analysis. ***Results:*** 15 studies (13 case-control, 2 cohort) were included of which 12 were rated as high quality. The meta-analysis of crude odds ratios (ORs) yielded a risk estimate of $OR_{crude} = 0.82$ (95% confidence interval [CI]: 0.70–0.96) for MS in breastfed versus non-breastfed individuals with substantial heterogeneity ($I^2 = 68.2\%$). Using adjusted OR, when available, reduced heterogeneity ($I^2 = 48.9\%$) and resulted in an $OR_{adjusted} = 0.86$ (95% CI: 0.75–0.99). Restricting the analysis to studies with high-quality scores (i.e., ≥6/9 points) resulted in a combined OR_{crude} of 0.79 (95% CI: 0.66–0.94) and an $OR_{adjusted} = 0.83$ (95% CI: 0.71–0.98), respectively. ***Discussion/Conclusion:*** The meta-analysis showed a small protective effect of having been breastfed on MS risk. This adds to the knowledge that breastfeeding is beneficial for the immunological health of a child. Future studies on the influence of having been breastfed on MS risk should apply a uniform definition of breastfeeding and investigate possible sex-specific aspects.

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Introduction

Multiple sclerosis (MS) is an inflammatory and degenerative disease of the central nervous system [1, 2]. The degenerative component of the disease is presumed to be an immune-mediated process, potentially caused by a viral infection [3]. MS is characterized by relapses, disseminated lesions in the central nervous system, and progression of neurological disability resulting in multiple neurological symptoms and signs (walking and cognitive impairment, sensory dysfunction, visual impairment but also fatigue and depression), which have negative effects on quality of life and participation [4].

In 2016, approximately 2.2 million people worldwide suffered from MS which is equivalent to 30.1 cases per 100,000 population. This corresponds to an increase of approximately 10.4% or 22.47 cases per 100,000 population in the age-standardized prevalence since 1990 [5]. A more recent study by Walton et al. [6] estimated MS prevalence to amount to 2.8 million people (35.9 cases per 100,000 population) worldwide in 2020 with an increase of 30% since 2013. MS incidence was estimated to be 2.1 per 100,000 persons/year [6].

The causes of MS are not fully understood, yet [7]. Genetic predisposition, environmental and lifestyle factors as well as an interplay thereof constitute relevant factors in the development of MS [8]. Especially early-life risk factors such as having been breastfed may also be of relevance. Breast milk provides the newborn not only with essential nutrients and vitamins but also with numerous immune-active molecules, metabolites, oligosaccharides, and microbial components that are important for the development of the immune system [9]. This, in turn, is involved in the demyelination of the brain and spinal cord typical of MS. Moreover, having been breastfed has already been shown to be associated with a reduced incidence of other immune disorders such as type 1 diabetes (DT1) and asthma [9].

Some studies have been published on the influence of having been breastfed on subsequent risk of developing MS, but to our knowledge, no systematic review including a meta-analysis has yet been conducted to summarize the evidence. Given the reported increasing prevalence [5] and incidence [6] of MS, a better understanding of the underlying causal factors is needed to possibly reduce the risk of disease and influence the course of MS in those already ill. Being aware of the multitude of factors triggering MS, evidence synthesis on single factors is helpful in weighing the impact of that factor. Hence, the objective of this work was to systematically search for studies and summarize their findings on the impact of having been breastfed on subsequent MS risk to evaluate whether having been breastfed during infancy is a protective factor for the development of MS.

Methods

A systematic review of observational studies including a subsequent meta-analysis was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2020 statement [10].

Search Strategy and Study Selection

A systematic search strategy was developed based on the PICO (population, intervention, comparison, outcome) framework applying the following search terms ([*multiple sclerosis*] *AND* [*breastfeeding OR breast milk OR maternal OR perinatal OR neonatal*] *AND* [*case-control studies OR cohort studies OR cross-sectional studies OR prospective studies OR systematic review OR meta-analysis*]) in the form of a free-text search and controlled vocabulary, i.e., MeSH Terms with automatic explosion to search for more specific terms. The search strategy was adapted to the syntax and subject headings of the corresponding database. MEDLINE, Web of Science, and Cochrane Library were searched on October 12, 2021, for publications published by that date. In addition, a search in Google Scholar (https://scholar.google.com) was conducted using the same search terms and the 100 most relevant studies were considered. We updated the database search on May 18, 2022, using the same search strategy. The search strategy for the respective databases can be found in online supplementary Table 1 (see www.karger.com/doi/10.1159/000526895 for all online suppl. material). Reference lists of included studies were searched for additional studies not identified through the initial search. Prior to the abstract and title screening, duplicates were removed. Selected studies were then subject to full-text screening for eligibility. If there was no full-text access, the authors of the respective study were contacted. The search and study selection were performed by two independent researchers (A.H. and M.R.). In case of discordance, a third researcher (H.B.) was consulted.

Eligibility Criteria

Observational studies, i.e., case-control, cohort, and cross-sectional studies, reporting on the influence of having been breastfed on MS risk in children or adults were included if subjects were breastfed in a period from birth to 2 years of age as this is the period recommended by the World Health Organization [11] for breastfeeding. Additionally, MS had to be diagnosed according to established diagnostic criteria by Schuhmacher, Poser, or McDonald criteria [2]. Studies based on self-reported MS diagnoses were also included but were downgraded in the quality assessment accordingly. Studies on disease activity of MS, i.e., relapse rates or progression of neurological symptoms and signs, were excluded. Only peer-reviewed studies published in English or German were included. No restrictions were made regarding the study setting or publication year.

Data Extraction and Statistical Analysis

For the data extraction, an adaption of the data collection form of the Cochrane Developmental, Psychological and Learning

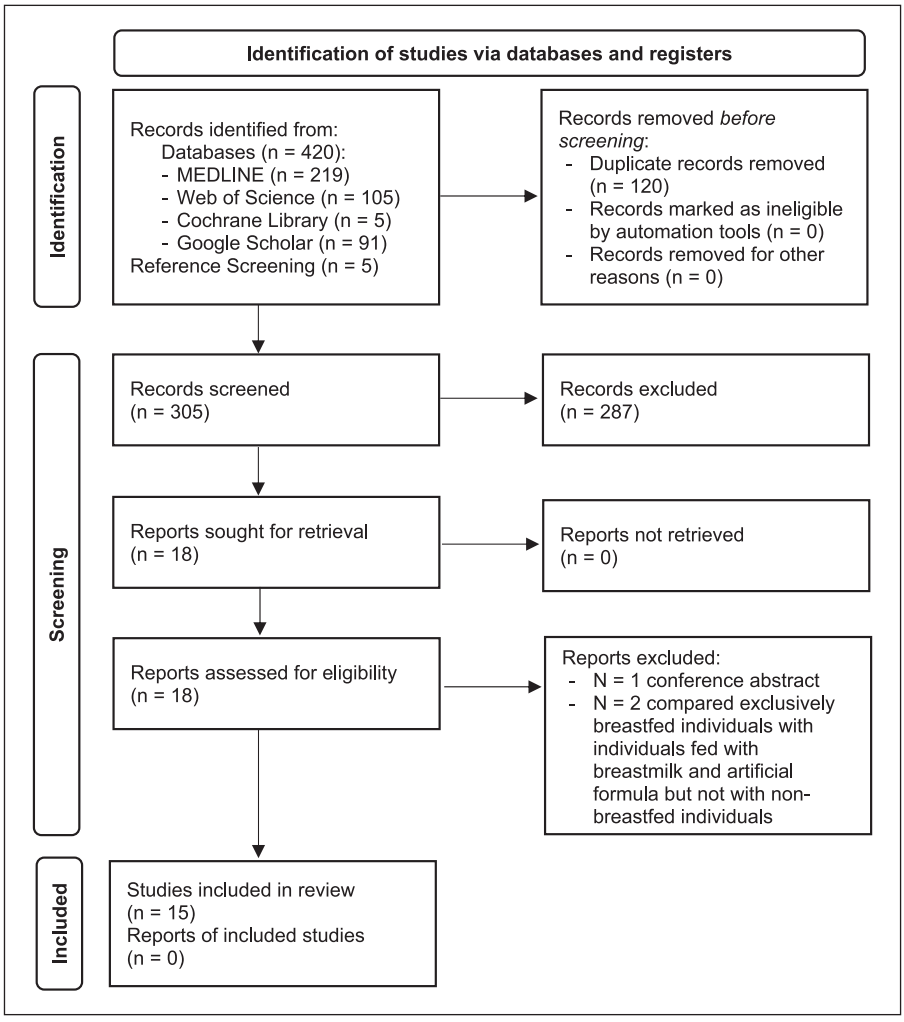

Fig. 1. PRISMA flow diagram: study selection.

Problems Review Group [12] was used. Data were extracted by two independent reviewers (A.H., M.R.) who were blinded to each other's decision. Disagreement was resolved by consultation of a third reviewer (H.B.). Extracted data included author, year, country, diagnostic criteria, number of people per exposure group (breastfed vs. not breastfed), cases (persons with MS [pwMS]) and controls from case-control studies, and number of events (diagnosis of MS) at the end of the study period in cohort studies as well as confounding variables. Crude and adjusted risk estimates, reported below as OR [13], were extracted from the original publications if available. If crude risk estimates were missing, these were calculated by the authors from published data.

Both pooled OR_{crude} and $OR_{adjusted}$ with 95% confidence intervals (CIs) were calculated. Overall and between-study heterogeneity was evaluated by calculation of τ^2 applying the DerSimonian-Liard estimator [14] and of I^2 statistics [15], respectively. As considerable between-study heterogeneity was anticipated, a random-effects model applying the Mantel-Haenszel method [16] for dichotomous outcomes was chosen to calculate pooled effect sizes, for both crude and adjusted risk estimates. Subgroup and sensitivity analyses were performed to identify the influence of each respective study on the robustness of the overall pooled effect estimates. Individual study quality was assessed using the Newcastle-Ottawa Scale (NOS) for non-randomized studies [17], and publication bias was examined visually with funnel plots. All analyses were run in R (version 4.1.1) using the packages meta (version 5.1–1), metafor (version 3.0–2), and dmetar (version 0.0.9000).

Results

Study Selection

The search in the databases MEDLINE, Web of Science, and Cochrane Library supplemented by a search in Google Scholar yielded 425 results. After duplicate eradication, 305 studies were subject to the title and abstract screening of which 287 were excluded since they did not fulfil the predefined eligibility criteria. For two [18, 19] of the remaining 18 studies, full-text screening revealed that

Table 1. Study characteristics of included studies

Author (year)	Country	Study design	Diagnostic criteria	Origin of controls/ non-cases	Breastfed		Not breastfed		Matching variables	Adjustment variables
					cases	controls/ non-cases	cases	controls/ non-cases		
Abbasi et al. (2017) [22]	Iran	C-C	McDonald criteria	P	532	358	46	48	- Age - Sex	- Age - Sex - Education - Ethnicity - Income - Marital status
Al Wutayd et al. (2018) [23]	Saudi Arabia	C-C	NA	A	203	218	46	37	- Age - Sex	- Age - Sex
Alkhawajah et al. (2021) [24]	Saudi Arabia	C-C	McDonald criteria	A	276	557	9	32	- Age - Sex	- Age - Sex - Marital status - Age at first school attendance
Baldin et al. (2021) [25]	Norway	Cohort	ICD codes	R	197	89,463	18	6,213	NA	- Sex - Year of birth - Offspring's year of birth - Offspring birth order - Maternal educational level - Maternal smoking habits - Maternal age at delivery
Brenton et al. (2017) [21]	USA	C-C	McDonald criteria and IPMSSG	P	13	51	23	21	None	IPTW considering: - Gestational age - Sex - Race - Birth weight - Delivery method - Maternal education - Health insurance Sensitivity analysis: - Age - BMI
Conradi et al. (2013) [26]	Germany	C-C	McDonald criteria	P	148	184	67	43	None	- Age - Sex - No. of older siblings - No. of inhabitants for place of domicile between ages 0 and 6 - Daycare attendance between ages 0 and 3
Da Silva et al. (2009) [27]	Brazil	C-C	Poser criteria	A	32	34	42	41	- Age - Sex - Place of birth	None
Goldacre et al. (2017) [28]	UK	Cohort	ICD codes	R	19	115,005	12	49,007	NA	- Year of birth - Maternal age
Graves et al. (2017) [20]	USA	C-C	IPMSSG	P	176	306	89	106	None	- Age - Sex - Race - Ethnicity - Maternal education - Region
Hedström et al. (2020) [19]	Sweden	C-C	McDonald criteria	R	1,126	2,318	641	1,267	- Age - Sex - Residential area	- Age - Residential area
Hedström et al. (2020) [19]	USA	C-C	McDonald criteria	R	121	214	634	598	- Sex - Birth date - Race - Ethnicity - Zip code	- Smoking
Hughes et al. (2013) [29]	Australia	C-C	McDonald criteria	R	186	371	93	168	- Age - Sex - Region	- Education - Smoking history

Table 1 (continued)

Author (year)	Country	Study design	Diagnostic criteria	Origin of controls/ non-cases	Breastfed		Not breastfed		Matching variables	Adjustment variables
					cases	controls/ non-cases	cases	controls/ non-cases		
Pisacane et al. (1994) [30]	Italy	C-C	Poser criteria	P	80	85	13	8	- Age - Sex	- Age group - Sex - Birth weight - Type of delivery - Social class - No. of children in the household
Ragnedda et al. (2015) [18]	Italy	C-C	McDonald criteria	R	242	513	305	506	- Age - Sex	- Sex - Education - Smoking habits - Maternal smoking habits during pregnancy - Other types of milk
Ragnedda et al. (2015) [18]	Norway	C-C	McDonald criteria	R	470	885	267	450	- Age - Sex	- Sex - Education - Smoking habits - Maternal smoking habits during pregnancy
Spencely and Dick (1982) [31]	UK	C-C	NA	S	455	214	101	53	None	None
Tarrats et al. (2002) [32]	Mexico	C-C	Poser criteria	P, M	85	183	9	27	None	No information on specific adjustment variables available

C-C, case-control study; IPMSSG, International Pediatric Multiple Sclerosis Study Group; P, patient; A, acquaintance; R, register; S, spouse; M, medical staff; IPTW, Inverse Proportional Treatment Weighting.

a shortened (<4 months) versus prolonged duration of having been breastfed (≥4 months) was compared. These studies were also included, with the short duration of having been breastfed considered comparable to "not having been breastfed" and the longer duration comparable to "having been breastfed" (see discussion on this point). Of the 18 studies which underwent full-text screening, three studies were excluded as one was a conference abstract and two studies compared having been exclusively breastfed with having been breastfed supplemented with supplementation but not with non-breastfed individuals. The updated search on May 18, 2022 did not reveal any additional studies that met the eligibility criteria. Therefore, 15 studies were ultimately included in the syntheses of results and subsequent meta-analysis. Figure 1 depicts the study selection process.

Study Characteristics

Relevant characteristics of included studies are displayed in Table 1. A total of two cohort studies and 13 case-control studies, of which two studies [18, 19] reported results for two distinct samples, were included. Two of the included studies investigated paediatric-onset MS [20, 21], whereas the remaining studies investigated adult-onset MS [18, 19, 22–32]. Studies were conducted in Europe ($n = 8$), the Americas ($n = 5$), the Middle East ($n = 3$), and Australia ($n = 1$) and published between 1982 and 2021, with most studies ($n = 11$) having been published in the last 10 years. Most of the included studies applied one of the following diagnostic criteria to establish a diagnosis of MS: McDonald criteria [18, 19, 21, 22, 24, 26, 29], Poser criteria [27, 30, 32], or International Pediatric Multiple Sclerosis Study Group (IPMSSG) criteria [20, 21]. In each of the cohort studies by Baldin et al. [25] and Goldacre et al. [28], ICD codes were used to confirm a diagnosis of MS. Al Wutayd et al. [23] and Spenceley and Dick [31] stated that only individuals with a clinically confirmed MS diagnosis were included as cases, without further specifying which diagnostic criteria were used. In case-control studies controls (non-pwMS) were enrolled through registries [18, 19, 29], were acquaintances/spouses of the case [23, 24, 27, 31], health-care workers [32], or were non-pwMS from the same or another hospital ward [20–22, 30, 32] or general practitioner's practice [26]. Sex, age, and region were frequently used as matching variables for pwMS and controls or included as potential confounders in the statistical models. Otherwise,

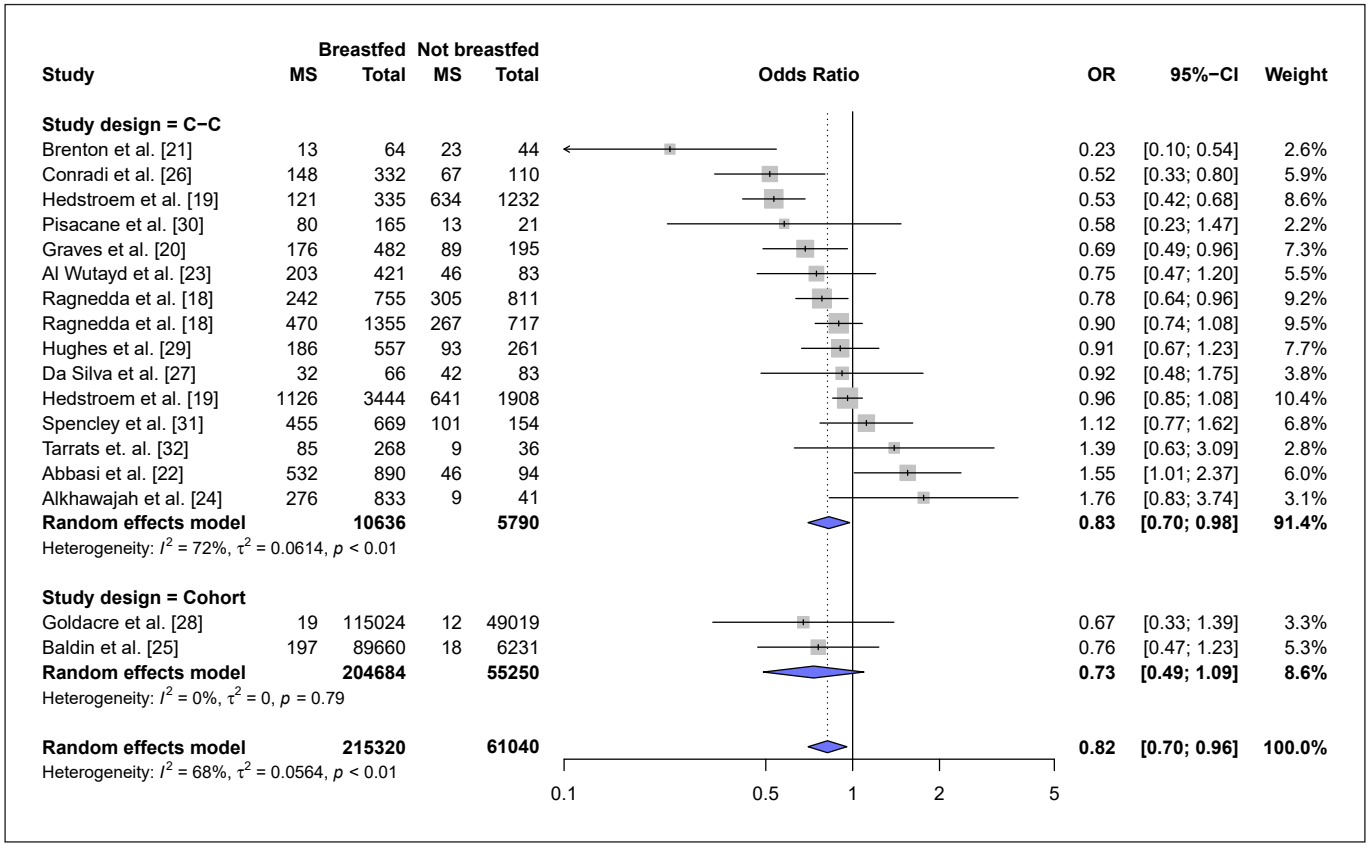

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Fig. 2. Crude OR estimates comparing breastfed with non-breastfed individuals for risk of multiple sclerosis.

Table 2. Crude and adjusted OR, 95% CIs, and heterogeneity for having been breastfed compared to not having been breastfed on MS risk

Included studies		Pooled OR (95% CI), random-effects model	Heterogeneity
C-C and cohort studies (n = 17[a])	Crude	0.82 (0.70–0.96)	I^2 = 68.2%
C-C studies (n = 15)	Crude	0.83 (0.70–0.98)	I^2 = 71.8%
Cohort studies (n = 2)	Crude	0.73 (0.49–1.09)	I^2 = 0.0%
C-C and Cohort studies (n = 17[a])	Adjusted[b]	0.86 (0.75–0.99)	I^2 = 48.9%
C-C studies (n = 15)	Adjusted[b]	0.87 (0.75–1.02)	I^2 = 53.9%
Cohort studies (n = 2)	Adjusted[b]	0.73 (0.49–1.09)	I^2 = 0.0%

[a] Studies by Hedström et al. [19] and Ragnedda et al. [18] reported results of two distinct study samples and are therefore included in the meta-analysis as two distinct studies.
[b] If adjusted OR was not available, crude OR was used instead.

maternal age, education, and smoking habits were most often considered as confounding variables.

Study Synthesis (Meta-Analysis)

Overall, 15 studies comprising 17 distinct study samples investigated the association of having been breastfed compared to not having been breastfed on MS risk and were included in the pooled analysis. For the assessment of risk of bias, the NOS was categorized as follows: ≥6 points equals low risk of bias and ≤5 points equals high risk of bias. The assessment of the respective NOS domains for each study is depicted in online supplementary Table 2. Risk of bias was low in twelve [18–22, 24–30] and high in three studies [23, 31, 32]. Table 2 provides an

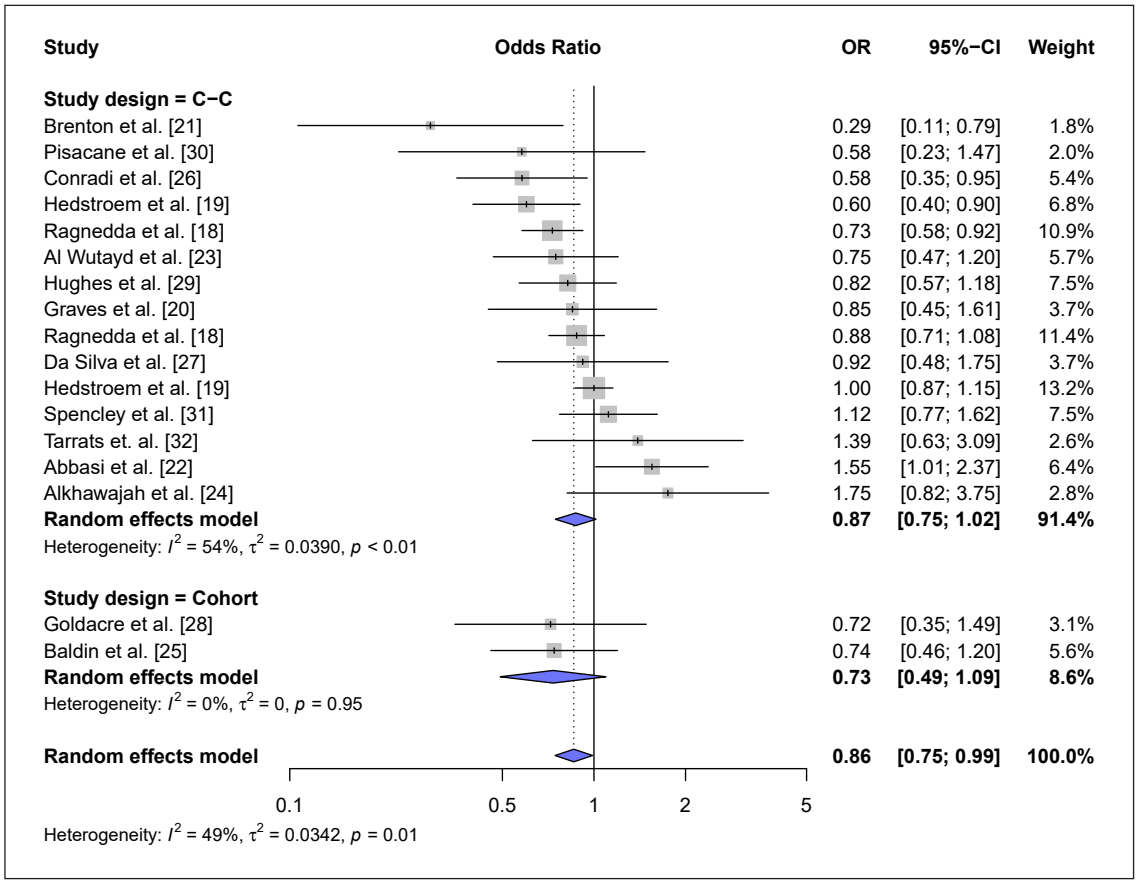

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Fig. 3. Adjusted OR estimates comparing breastfed with non-breastfed individuals for risk of multiple sclerosis.

overview of the results of the meta-analysis of the crude and adjusted ORs including analyses stratified by study design. Unless otherwise specified, all data are from the primary reference of each included study.

The combined crude OR estimates based on a random-effects model showed a protective effect of having been breastfed on MS risk (OR_{crude} = 0.82 [95% CI: 0.70–0.96]) with substantial heterogeneity (I^2 = 68.2%). Subgroup analysis stratified by study design yielded an OR_{crude} of 0.83 (95% CI: 0.70–0.98) and an OR_{crude} of 0.73 (95% CI: 0.49–1.09) for case-control studies and cohort studies, respectively. There is little heterogeneity when comparing the results based on case-control studies with those based on cohort studies (shown in Table 2). Figure 2 displays for each study included in the meta-analysis the summary statistics (number of events and the sample size) for the breastfed and not breastfed groups, the crude OR and corresponding 95% CI for the dichotomous outcome, MS.

The analysis using adjusted OR, when available otherwise using crude OR, also showed a protective effect of having been breastfed compared to not having been breastfed on MS risk with moderate heterogeneity ($OR_{adjusted}$ = 0.86 [95% CI: 0.75–0.99], I^2 = 48.9%). Subgroup analysis stratified by study design did not yield significant risk estimates ($OR_{adjusted}$ for case-control studies = 0.87 [95% CI: 0.75–1.02]; $OR_{adjusted}$ for cohort studies = 0.73 [95% CI: 0.49–1.09]). As in the analysis of the crude ORs, there is little heterogeneity between case-control studies and cohort studies (shown in Table 2). Figure 3 displays for each study the adjusted OR and corresponding 95% CI for the risk of MS. Restricting analyses to studies with low risk of bias [18–22, 24–30], i.e., including only studies with ≥6 points on the NOS, yielded an OR_{crude} of 0.79 (95% CI: 0.66–0.94) and $OR_{adjusted}$ of 0.83 (95% CI: 0.71–0.98). Overall, sensitivity analyses showed consistent results with the primary meta-analysis. Subgroup analyses by time of MS onset, i.e., paediatric versus adult-

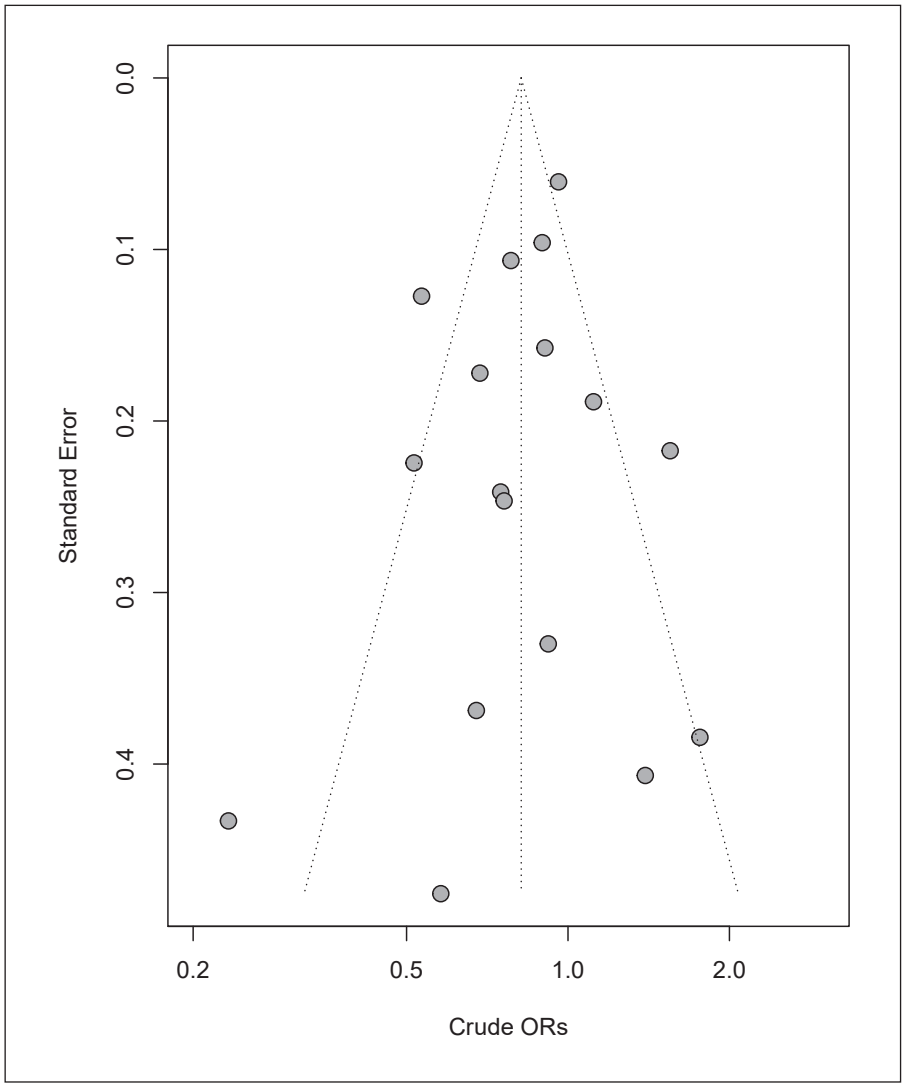

Fig. 4. Funnel plot of crude OR of breastfed versus non-breastfed individuals in relation to risk of multiple sclerosis.

onset MS, also showed a protective effect of having been breastfed on MS risk both in paediatric- and adult-onset MS, although heterogeneity was substantial in the analysis of paediatric-onset MS (shown in online suppl. Table 3).

Publication Bias

Visual inspection of the funnel plot revealed no indication of a relevant publication bias (shown in Fig. 4).

Discussion/Conclusion

A systematic review and meta-analysis were conducted including 15 studies comprising 17 distinct study samples on the influence of having been breastfed on the risk of developing MS later in life. The meta-analysis included two cohort studies with 246 cases and a total cohort size of 259,934 individuals and 15 case-control studies comprising 6,530 cases and 9,896 controls. To our knowledge, this is the first meta-analysis summarizing the current study evidence on the influence of having been breastfed during infancy on the development of MS later in life.

We found a small protective effect of having been breastfed on subsequent MS risk compared to not having been breastfed in analyses of combined OR_{crude} as well as combined $OR_{adjusted}$. When comparing the results of case-control studies with those of cohort studies, there is little heterogeneity, which adds to the judgement of validity of the overall results. Most included studies were of high (≥6/9 points) quality as assessed with the NOS. Although

results need to be interpreted with caution due to moderate to substantial between-study heterogeneity in the case-control studies, findings go in line with the qualitative review by Vieira Borba et al. [9] which concludes that having been breastfed might play a protective role in the aetiology of MS due to the immune-modulatory and toxin-protective effects of breast milk. The protective effect of breast milk has already been shown for allergic and autoimmune diseases other than MS, for instance DT1 [33, 34], and asthma [9, 35, 36]. In their meta-analysis including 43 studies (9,874 patients with DT1), Cardwell et al. [33] found a protective effect of having been breastfed on DT1 risk comparing ever versus never breastfed individuals, irrespective of the mode of breastfeeding, i.e., exclusive or non-exclusive breastfeeding. Restricting the analysis to exclusively breastfed individuals (n = 33 studies, 7,621 individuals with DT1), the protective effect remained with a 26% reduction in DT1 risk. However, it should be taken into account that the between-study heterogeneity was high in these analyses [33]. For asthma, the meta-analysis of three cohort and ten cross-sectional studies by Lodge et al. [35] found a protective effect of having been breastfed on subsequent development of asthma (OR_{pooled} = 0.88 [95% CI: 0.82–0.95]). These results are supported by the meta-analysis of 15 observational studies comprising 7,406 individuals by Harvey et al. [36] who reported a 32% reduction in the odds of wheezing in the first year of life for ever versus never breastfed children.

Not only the influence of breast milk alone, but also the type and duration of breastfeeding, in particular, could play a role in the development of autoimmune diseases such as MS. The included studies by Ragnedda et al. [18] and Hedström et al. [19] used a different dichotomization for breastfeeding and found a protective effect for longer duration (≥4 months vs. <4 months or no breastfeeding). Note that for other autoimmune diseases such as ulcerative colitis and celiac disease, breastfeeding for up to 6 months is recommended [37, 38]. Moreover, for celiac disease [38] and allergy prevention [39], there is indication that continuing breastfeeding while introducing solid foods is beneficial [38, 39], because this might support the tolerance development of the immune system which is crucial for the prevention of autoimmune disorders as well as allergies.

Since our meta-analysis is based on studies of the general population and MS-specific evidence regarding the influence of duration, mode of breastfeeding and optimal time point for introducing solid foods is insufficient, recommendations should be based on general [40] and national recommendations [41] for breastfeeding. Consistent with recommendations for other autoimmune diseases, the newborn should be exclusively breastfed for 4–6 months and solid foods should be introduced while continuing breastfeeding. By implementing these recommendations, women who wish to have children could additionally accrue the well-known benefits of breastfeeding for both mother and child in relation to various diseases (e.g., reduced risk of obesity and diabetes in breastfed children; reduced risk of breast and ovarian cancer in breastfeeding women [40, 42, 43]). Our study has some limitations concerning both the included studies and the conduct of the meta-analysis itself. The included studies used different diagnostic criteria to assess the outcome of interest, which may have led to differences in the detection of MS; however, it was not possible to perform subgroup analyses by diagnostic criteria used.

The majority of studies considered are case-control studies [18–24, 26, 27, 29, 29–32] which are prone to recall bias [44, 45]. However, we do not expect a differential recall in cases and controls, and therefore a possible bias is rather towards a null effect. Breastfeeding was defined differently in terms of duration of breastfeeding, exclusive breastfeeding, and complementary feeding making it difficult to determine the sole effect of having been breastfed on MS risk.

Furthermore, the included studies differed, first, in the set of variables for which they performed adjustment including generally accepted risk factors for MS, e.g., EBV-infection [3], obesity in childhood and adolescence [46–48], smoking [49], or low blood vitamin D concentrations [50]; and second, other potential confounders were not considered. This is partially explained by the different settings and locations of the studies, however, makes it more challenging to combine the estimates. Following the recommendations made above on breastfeeding for MS prevention, future studies should assess breastfeeding in more detail. Given the known female-male ratio in MS prevalence, possible sex-specific aspects should be investigated, as was only the case with the study by Hedström et al. [19], who reported that the protective effect for MS appeared to be higher in males (OR = 0.5 [95% CI: 0.4–0.7]) than in females (OR = 0.9 [95% CI: 0.8–1.1]).

Moreover, only previously published studies in German or English were included, which may have led to studies being overlooked. However, we assume that the number of unidentified studies is relatively small and would not have changed the results of the meta-analysis, as publishing in English-language journals is common practice.

In summary, we found a small protective effect of breastfeeding on MS risk. This adds to the knowledge that breastfeeding is beneficial for the immunological health of a child. Future studies on the influence of having been breastfed on MS risk should apply a uniform definition of breastfeeding and investigate possible sex-specific aspects.

Statement of Ethics

An ethics statement is not applicable because this study is based exclusively on published literature.

Conflict of Interest Statement

CH has received speaker honoraria and research funding from Novartis, Merck, Roche. The other authors have no known conflicts of interest to declare.

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Author Contributions

Concept and design: A.H., H.B.; acquisition, analysis, or interpretation of data: A.H., M.R., N.O., H.B.; drafting of the manuscript: A.H.; critical revision of the manuscript: all authors; statistical analysis: A.H., H.B.; supervision: C.H., N.O., H.B.; all authors read and approved the final manuscript and gave their approval of it to be published.

Data Availability Statement

All data generated or analysed during this study are included in this article and its supplementary material files. Further inquiries can be directed to the corresponding author.

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Supplement Table 1: Search strategy applied in the databases MEDLINE, Web of Science, and Cochrane Library as well as Google Scholar.

Database		Search strategy
MEDLINE	#1	("multiple sclerosis"[All Fields]) OR ("multiple sclerosis"[MeSH Terms])
	#2	("breastfeeding"[All Fields]) OR ("breast feeding"[All Fields]) OR ("breast milk"[All Fields]) OR ("maternal"[All Fields]) OR ("perinatal"[All Fields]) OR ("neonatal"[All Fields]) OR ("breast feeding"[MeSH Terms]) OR ("milk, human"[MeSH Terms])
	#3	("case-control studies"[MeSH Terms] OR "cohort studies"[MeSH Terms] OR "cross-sectional studies"[MeSH Terms] OR "prospective studies"[MeSH Terms] OR "systematic reviews as topic"[MeSH Terms] OR "meta-analysis as topic"[MeSH Terms] OR "case control study"[All Fields] OR "cohort study"[All Fields] OR "cross sectional study"[All Fields] OR "prospective study"[All Fields] OR "systematic review"[All Fields] OR "meta analysis"[All Fields]) OR ("case-control studies"[MeSH Terms] OR "cohort studies"[MeSH Terms] OR "cross-sectional studies"[MeSH Terms] OR "prospective studies"[MeSH Terms] OR "systematic reviews as topic"[MeSH Terms] OR "meta-analysis as topic"[MeSH Terms] OR "case control study"[All Fields] OR "cohort study"[All Fields] OR "cross sectional study"[All Fields] OR "prospective study"[All Fields] OR "systematic review"[All Fields] OR "meta analysis"[All Fields])
	#4	((("multiple sclerosis"[All Fields]) OR ("multiple sclerosis"[MeSH Terms])) AND (("breastfeeding"[All Fields]) OR ("breast feeding"[All Fields]) OR ("breast milk"[All Fields]) OR ("maternal"[All Fields]) OR ("perinatal"[All Fields]) OR ("neonatal"[All Fields]) OR ("breast feeding"[MeSH Terms]) OR ("milk, human"[MeSH Terms]))) AND (("case-control studies"[MeSH Terms] OR "cohort studies"[MeSH Terms] OR "cross-sectional studies"[MeSH Terms] OR "prospective studies"[MeSH Terms] OR "systematic reviews as topic"[MeSH Terms] OR "meta-analysis as topic"[MeSH Terms] OR "case control study"[All Fields] OR "cohort study"[All Fields] OR "cross sectional study"[All Fields] OR "prospective study"[All Fields] OR "systematic review"[All Fields] OR "meta analysis"[All Fields]) OR ("case-control studies"[MeSH Terms] OR "cohort studies"[MeSH Terms] OR "cross-sectional studies"[MeSH Terms] OR "prospective studies"[MeSH Terms] OR "systematic reviews as topic"[MeSH Terms] OR "meta-analysis as topic"[MeSH Terms] OR "case control study"[All Fields] OR "cohort study"[All Fields] OR "cross sectional study"[All Fields] OR "prospective study"[All Fields] OR "systematic review"[All Fields] OR "meta analysis"[All Fields]))
Web of Science	#1	(ALL=”multiple sclerosis”)
	#2	(ALL=("breastfeeding") OR ALL=("breast feeding") OR ALL=("breast milk") OR ALL=(maternal) OR ALL=(perinatal) OR ALL=(neonatal))
	#3	((ALL="case-control study") OR (ALL="cohort study) OR (ALL="cross-sectional study") OR (ALL=”prospective study”) OR (ALL="systematic review") OR (ALL=meta-analysis"))
	#4	#1 AND #2 AND #3
Cochrane Library	#1	MeSH descriptor: [Multiple Sclerosis] explode all trees
	#2	"multiple sclerosis”
	#3	#1 OR #2
	#4	MeSH descriptor: [Breast Feeding] explode all trees
	#5	MeSH descriptor: [Milk, Human] explode all trees
	#6	Breastfeeding

	#7	"breast feeding"
	#8	"breast milk"
	#9	maternal
	#10	Neonatal
	#11	Perinatal
	#12	#4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11
	#13	MeSH descriptor: [Case-Control Studies] explode all trees
	#14	MeSH descriptor: [Cohort Studies] explode all trees
	#15	MeSH descriptor: [Cross-Sectional Studies] explode all trees
	#16	MeSH descriptor: [Prospective Studies] explode all trees
	#17	MeSH descriptor: [Systematic Reviews as Topic] explode all trees
	#18	MeSH descriptor: [Meta-Analysis as Topic] explode all trees
	#19	"case-control study"
	#20	"cohort study"
	#21	"cross-sectional study"
	#22	"prospective study"
	#23	"systematic review"
	#24	"meta-analysis"
	#25	#13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23 OR #24
	#26	#3 AND #12 AND #25
Google Scholar	#1	(“multiple sclerosis”) AND (“breastfeeding” OR “breast feeding” OR “breast milk” OR “human milk” OR “maternal” OR “perinatal” OR “neonatal”) AND (“case-control study” OR “cohort study” OR “cross-sectional study” OR “prospective study” OR “systematic review” OR “meta-analysis”)

Supplement Table 2: Risk of bias assessment for included studies applying the Newcastle-Ottawa Scale.

Author	Selection				Comparability	Exposure			Total
Cohort studies	*Representativeness of the exposed cohort*	*Selection of the non-exposed cohort*	*Ascertainment of exposure*	*Outcome of interest was not present at start of the study*	*Comparability of cohorts on the basis of the design or analysis*	*Assessment of outcome*	*Follow-up was long enough for the outcomes to occur*	*Adequacy of follow-up of cohorts*	
Baldin et al. [25]	*	*		*	**	*	*		7/9
Goldacre et al. [28]	*	*	*	*	**	*	*		8/9
Case-control studies	*Adequate case definition*	*Represen-tativeness of the cases*	*Selection of controls*	*Definition of controls*	*Comparability of cohorts on the basis of the design or analysis*	*Ascertainment of exposure*	*Same method of ascertainment for cases and controls*	*Non-response rate*	
Abbasi et al. [22]	*	*		*	**		*		6/9
Al Wutayd et al. [23]		*		*	**		*		5/9
Alkhawajah et al. [24]	*	*		*	**		*		6/9
Brenton et al. [21]	*	*		*	**		*		6/9
Conradi et al. [26]	*	*	*	*	**		*		7/9
Da Silva et al. [27]	*	*	*	*	**		*		7/9
Graves et al. [20]	*	*		*	**		*		6/9

Hedström et al. [19] (Sweden)	*	*	*		**		*	6/9
Hedström et al. [19] (USA)	*	*	*		**		*	6/9
Hughes et al. [29]	*	*	*		**		*	6/9
Pisacane et al. [30]	*	*			**	*	*	6/9
Ragnedda et al. [18] (Italy)	*	*	*		**		*	6/9
Ragnedda et al. [18] (Norway)	*	*	*		**		*	6/9
Spencely & Dick [31]	*	*	*				*	4/9
Tarrats et al. [32]	*	*		*			*	4/9

Supplement Table 3: Subgroup analyses on paediatric- and adult-onset MS - crude and adjusted OR, 95% confidence intervals and heterogeneity for having been breastfed compared to not having been breastfed on MS risk.

Included Studies		Pooled OR (95% CI), random effects model	Heterogeneity
Paediatric-onset MS (n=2[a])	Crude	0.43 (0.15–1.23)	I^2 = 81.4%
Adult-onset MS (n=15[b])	Crude	0.85 (0.74–1.00)	I^2 = 64.5%
Paediatric-onset MS (n=2[a])	Adjusted[c]	0.53 (0.18–1.52)	I^2 = 68.2%
Adult-onset MS (n=15[b])	Adjusted[c]	0.88 (0.76–1.01)	I^2 = 47.1%

[a][20,21]

[b][18-19, 22-32]

[c]If adjusted OR were not available, crude OR were used instead

7. PUBLICATION 2

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RESEARCH

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Childhood and adolescence factors and multiple sclerosis: results from the German National Cohort (NAKO)

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Abstract

Background Multiple Sclerosis (MS) represents the most common inflammatory neurological disease causing disability in early adulthood. Childhood and adolescence factors might be of relevance in the development of MS. We aimed to investigate the association between various factors (e.g., prematurity, breastfeeding, daycare attendance, weight history) and MS risk.

Methods Data from the baseline assessment of the German National Cohort (NAKO) were used to calculate adjusted hazard ratios (HR) and 95% confidence intervals (CI) for the association between childhood and adolescence factors and risk of MS. Analyses stratified by sex were conducted.

Results Among a total of 204,273 participants, 858 reported an MS diagnosis. Male sex was associated with a decreased MS risk (HR 0.48; 95% CI 0.41–0.56), while overweight (HR 2.03; 95% CI 1.41–2.94) and obesity (HR 1.89; 95% CI 1.02–3.48) at 18 years of age compared to normal weight were associated with increased MS risk. Having been breastfed for ≤ 4 months was associated with a decreased MS risk in men (HR 0.59; 95% CI 0.40–0.86) compared to no breastfeeding. No association with MS risk was observed for the remaining factors.

Conclusions Apart from overweight and obesity at the age of 18 years, we did not observe considerable associations with MS risk. The proportion of cases that can be explained by childhood and adolescence factors examined in this study was low. Further investigations of the association between the onset of overweight and obesity in childhood and adolescence and its interaction with physical activity and MS risk seem worthwhile.

Keywords Multiple Sclerosis, Childhood, Adolescence, Epidemiology

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Background

Multiple Sclerosis (MS) is an inflammatory, degenerative disease of the central nervous system (CNS) [1, 2] and the most common inflammatory neurological disease causing disability in early adulthood [3]. MS is characterized by relapses, disseminated lesions in the CNS, and a resulting progression of neurological disability that manifests in various neurological symptoms and signs. It substantially impacts the quality of life of those affected [4] and, in addition to the physical and psychological impairments, MS imposes high direct and indirect costs on the health care system [5]. In Germany, the prevalence was estimated at 337 per 100,000 population in 2019 [6]. Based on claims data, the age-adjusted (European Standard Population) incidence in 2012 was estimated to range from 6.6 to 21.8 per 100,000, depending on the case definition used [7]. In a comprehensive review, Lane et al. reported a wide range of incidence estimates worldwide with predominantly increasing MS incidence [8].

The causes of MS are not fully understood [9]. MS is deemed to be an autoimmune disease [10, 11], which is supported by the observation that the demyelination and subsequent degeneration of nerves within the CNS typical of MS is presumed to be an immune-mediated process, potentially caused by a viral infection [12]. Genetic predisposition, environmental and lifestyle factors, and their interaction constitute relevant risk factors in the development of MS [13]. According to a meta-estimation of hereditary and environmental factors on MS susceptibility, based on twin studies, heritability was estimated to account for 50% of the occurrence of MS. Shared environmental factors such as trans-generational epigenetic modifications or birth month accounted for 21%, and unshared environmental factors, e.g., infections, vitamin D (vit D) deficiency or smoking accounted for 29% of MS liability. The authors highlighted that the investigation of the influence of environmental factors as well as the respective individual lifestyle and infections, e.g., Epstein-Barr virus infection, should be the focus of future research [14]. In particular, early-life exposures that act on the immature immune system might be of relevance in the development of immune-mediated diseases such as MS [15]. The importance of early childhood factors has already been shown for type 1 diabetes [16], asthma [17] as well as for allergies [15, 18]. Of particular relevance in this context is the hygiene hypothesis, which states that individuals who have no or infrequent exposure to, e.g., infections that trigger an immune response, develop a less regulatory immune competence and are more susceptible to immune-mediated diseases [19]. The direct link between the total number of previous infections and the incidence of immune-mediated diseases is difficult to establish in observational studies since study participants remember previous infections only vaguely. Variables such as daycare attendance, number of siblings and birth order, contact with pets or livestock or growing up on a farm, and other social and economic indicators can be considered as surrogates instead [19]. However, these have provided conflicting results with regard to the occurrence of MS [20–26]. Other early childhood factors related to autoimmune diseases include prematurity, the mode of delivery, i.e., vaginal birth or cesarean section, and breastfeeding, all of which affect the development of the infant's immune system [27, 28], but again studies on MS have yielded conflicting results regarding preterm birth [26, 29–33], mode of delivery [34–36] and breastfeeding [37]. As obesity leads to chronic low-grade inflammation [38], weight history during childhood and adolescence might be of relevance. Studies of weight history at the age of 10 [39] and 20 years [40–42], respectively, and also for birth weight [26, 30–32, 43, 44] have so far yielded conflicting results, especially when sex was taken into account. At last, stressful life events in childhood may play a role in the development of MS. Polick and colleagues conducted a systematic review, including twelve studies, most of which demonstrated an association between childhood trauma and subsequent MS. Physical and sexual abuse were the most common traumatic stressors reported in the included studies [45].

Since the causes of MS are not fully understood [9], and a large proportion of the risk might be explained by the interplay of modifiable risk factors [14], it is important to clarify the potential role of these factors. Hence, our study aimed to investigate the association between childhood and adolescence factors and MS risk.

Methods

Study sample

This work was based on data from the baseline assessment of the NAKO, a large population-based cohort study in Germany. The NAKO recruited approximately 205,000 individuals from 18 German study regions based on age and sex-stratified samples randomly drawn from the corresponding local registries of residents. As part of the standardized data collection, subjects underwent several biomedical examinations, participated in a face-to-face interview conducted by trained study assistants, and completed a self-administered touchscreen questionnaire [46]. A more detailed description of the design of the NAKO can be found elsewhere [46, 47]. Our analyses comprised participants who provided information on the presence of a physician-based MS diagnosis as well as on the covariates described below. Participants who answered "Don't know" or "No information" regarding an MS diagnosis were excluded. Furthermore, participants

with MS were excluded if they did not provide information on age at MS diagnosis.

Outcome ascertainment, exposure variables, and covariates

MS diagnosis and age at diagnosis were self-reported by NAKO participants in the rare diseases module of the face-to-face interview administered by trained interviewers.

Exposure variables and covariates were either collected during the face-to-face interview or by completion of a self-administered touchscreen questionnaire. The following two groups of exposures were considered with respect to the time of their occurrence – childhood and adolescence factors. Childhood factors include prematurity, born by cesarean section, birth weight, number of siblings, having had contact with pets and/or livestock during childhood, daycare attendance including age at first attendance, weight history reported as weight at the age of 10 years compared to peers as well as childhood trauma, measured by the Childhood Trauma Screener (CTS) [48]. The CTS is a 5-item brief childhood trauma assessment instrument developed from the original 28-item Childhood Trauma Questionnaire. The five items include the dimensions of emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect [48]. The summary score was used, ranging from 5 to 25 points, with higher scores indicating more severe trauma. Body Mass Index (BMI) at the age of 18 years—calculated from self-reported weight at that age and measured height at baseline—was included as an adolescence factor. We used the following thresholds: <18.5 is equivalent to underweight, 18.5 to <25 to normal weight, 25 to <30 to overweight, and ≥ 30 kg/m^2 to obesity. Data on childhood and adolescence exposures were collected as part of the self-administered questionnaire on touchscreen. Sex, education, migration status as well as birth year, categorized in ten-year birth cohorts (<1955, 1955–1964, 1965–1974, 1975–1984, ≥ 1985), were considered as covariates in the analyses obtained in the face-to-face interview. Education was based on the International Standard Classification of Education 97 (ISCED) [49], with categories summarized as low (ISCED level 0–2), medium (ISCED level 3/4), and high (ISCED level 5/6) education.

Statistical analyses

Descriptive analyses by MS status with respect to birth year, sex, education, migration background, age at onset of MS, and exposure variables were performed. Categorical variables were summarized as absolute and relative frequencies, and continuous variables were summarized as mean and standard deviation (SD). Missing values were displayed.

A multivariable Cox proportional hazards regression was performed to assess the association between childhood and adolescence factors and MS risk. Results are displayed as Hazard Ratios (HR) with corresponding 95% confidence intervals (CI). The outcome was defined as age at self-reported MS diagnosis. The observation period began at birth and ended either at the reported onset of MS in the case of an event or at age at the baseline examination date in the case of no event. The model was stratified by birth year to account for cohort effects, and adjusted for education and migration status. No violation of the proportional hazards assumption was detected by graphically examined Schoenfeld residuals.

The percentage of missing values across the predictor variables ranged from 0% to 43.2% in the group without MS and from 0% to 43.8% in the group with MS (see Table 1). In total, 75,247 records (36.8%) were complete. Multiple imputation was used to create and analyze 40 imputed datasets. Incomplete variables were imputed under fully conditional specification, using the default settings of the mice 3.0 package [50, 51]. HRs were estimated in each imputed dataset separately and subsequently pooled applying Rubin's rules. We analyzed the data separately by sex to investigate sex-specific effects. For comparison, we also performed the analysis on the subset of complete cases.

All statistical analyses were performed with R version 4.2.0 (2022–04-22 ucrt) [52] using the packages "survival" [53], "survminer" [54], "sjPlot" [55], "MASS" [56], "mice" [50] and "micemd" [57].

Results

Of the initial 204,899 NAKO participants, a total of 626 (0.3%) individuals were excluded because they either did not provide information on MS diagnosis ($n=624$) or age at diagnosis ($n=2$). Accordingly, a total of 204,273 subjects were included in the analysis – 858 (579 females, 279 males) with and 203,415 (102,494 females, 100,921 males) without an MS diagnosis.

Table 1 summarizes the characteristics as well as the distribution of childhood and adolescence factors separately for persons with and without MS.

Table 2 summarizes the results of the multivariable Cox regression.

We observed a reduced risk of MS for men compared to women (HR 0.48, 95% CI 0.41 to 0.56).

Compared to normal weight at the age of 18 years, overweight (HR 2.03, 95% CI 1.41 to 2.94) and obesity (HR 1.89, 95% CI 1.02 to 3.48) at the age of 18 years were associated with a higher MS risk. No association with MS risk was observed for the remaining factors including

Table 1 Characteristics of NAKO baseline participants and distribution of childhood and adolescence factors by MS status

Variable	Persons without MS[a] (*n* = 203415)	Persons with MS[a] (*n* = 858)
Birth year		
< 1955	45610 (22.4%)	125 (14.6%)
1955–1964	50907 (25.0%)	256 (29.8%)
1965–1974	55916 (27.5%)	278 (32.4%)
1975–1984	25551 (12.6%)	136 (15.9%)
≥ 1985	25431 (12.5%)	63 (7.3%)
Sex		
Female	102494 (50.4%)	579 (67.5%)
Male	100921 (49.6%)	279 (32.5%)
Age at diagnosis (Mean (SD[a]))	NA	36.3 (11.1)
Education		
Low	5107 (2.8%)	20 (2.6%)
Medium	146116 (79.0%)	606 (77.4%)
High	33716 (18.2%)	157 (20.1%)
Missing	18476	75
Migration background		
No	168796 (83.0%)	743 (86.6%)
Yes	34576 (17.0%)	115 (13.4%)
Missing	43	0
Number of siblings		
Only child	30926 (18.0%)	130 (18.5%)
1-2 sibling(s)	107814 (62.7%)	437 (62.3%)
≥ 3 siblings	33335 (19.4%)	134 (19.1%)
Missing	31340	157
Premature birth (> 4 weeks before due date)		
No	156024 (95.7%)	639 (95.5%)
Yes	7001 (4.3%)	30 (4.5%)
Missing	40390	189
Cesarean section		
No	155208 (94.4%)	629 (94.2%)
Yes	9292 (5.6%)	39 (5.8%)
Missing	38915	190
Birth weight		
Low	17531 (12.6%)	87 (14.8%)
Average	103066 (74.3%)	421 (71.6%)
High	18030 (13.0%)	80 (13.6%)
Missing	64788	270
Ever breastfed		
No	29536 (25.7%)	167 (34.6%)
Yes, ≤ 4 months	45024 (39.1%)	180 (37.3%)
Yes, > 4 months	40532 (35.2%)	135 (28.0%)
Missing	88323	376
Contact with pets and/or livestock during childhood		
No	87293 (50.8%)	348 (49.8%)
Yes	84668 (49.2%)	351 (50.2%)
Missing	31454	159
Attended daycare		
No	47393 (28.6%)	196 (29.2%)
Yes, 1st attendance at age 3-6 years	79977 (48.3%)	336 (50.1%)

Table 1 (continued)

Variable	Persons without MS[a] (*n* = 203415)	Persons with MS[a] (*n* = 858)
Yes, 1st attendance at age 1- < 3 year(s)	28172 (17.0%)	98 (14.6%)
Yes, 1st attendance at age < 1 year	10143 (6.1%)	41 (6.1%)
Missing	37730	187
Weight at the age of 10 years compared to peers		
Lower	43328 (26.6%)	156 (23.5%)
Average	95681 (58.7%)	387 (58.2%)
Higher	24064 (14.8%)	122 (18.3%)
Missing	40342	193
BMI[a] at the age of 18 years (kg/m^2)		
Underweight (< 18.5)	18283 (12.7%)	91 (15.7%)
Normal weight (18.5 - < 25)	108757 (75.8%)	398 (68.7%)
Overweight (25 - < 30)	13558 (9.4%)	68 (11.7%)
Obesity (≥ 30)	2884 (2.0%)	22 (3.8%)
Missing	59933	279
Childhood Trauma[b] (Mean (SD))	7 (2.7)	7 (3.0)
Missing	32174	161

[a] *MS* Multiple Sclerosis, *SD* Standard deviation, *BMI* Body Mass Index

[b] Assessed with the Childhood Trauma Screener (5 – 25 points)

number of siblings, prematurity, cesarean section, birth weight, breastfeeding, contact with pets and/or livestock, age at first daycare attendance, weight at the age of 10 years, and childhood trauma (Table 2).

In separate analyses by sex, we observed an association between overweight at the age of 18 years and an increased MS risk compared to normal weight at the age of 18 years in women (HR 1.55, 95% CI 1.05 to 2.29) but not in men. Furthermore, in contrast to no breastfeeding, breastfeeding duration of ≤ 4 months was related to a reduced risk of MS in men (HR 0.59, 95% CI 0.40 to 0.86) but not in women. Estimates for the remaining variables differed only slightly in women and men compared with the overall group (see Additional file 1).

When we restricted the analysis to complete cases (*n* = 75,247, 36.8% of the total cohort), we obtained similar results in both analyses of the total cohort and stratified by sex. However, due to the reduced sample size, the confidence intervals were considerably larger (see Additional files 2 & 3).

Discussion

The present study was based on data from the baseline assessment of the population-based cohort study NAKO, which included 858 prevalent adult MS cases. We aimed to investigate associations between childhood and adolescence factors and MS risk. We observed associations between overweight and obesity at the age of 18 years and an increased risk of MS compared to normal weight at this age. In analyses stratified by sex, the association between overweight and increased MS risk remained for women but not for men. In contrast to no breastfeeding, a breastfeeding duration of ≤ 4 months was related to a reduced MS risk in men, but not in women. No association with MS risk was observed for the remaining childhood factors including number of siblings, prematurity, cesarean section, birth weight, contact with pets and/or livestock, age at first daycare attendance, weight at the age of 10 years, and childhood trauma.

Our results regarding the association of overweight and obesity with increased MS risk are in line with the meta-analysis of Liu et al. [41] and the study by Gianfrancesco and colleagues [40], both for the overall analysis and for the analyses stratified by sex. Contrary to what might be expected from our results, but in line with previous studies [26, 30–32, 43], we did not observe an association between MS risk and higher birth weight or higher weight at the age of 10 years compared with average weight. Thus, the time of onset of overweight or obesity may have an impact on MS risk. A possible explanation might be the interaction of weight and physical activity throughout childhood. Physical activity contributes to the prevention of overweight and obesity and the resulting anti-inflammatory effect can possibly prevent the development of inflammatory diseases in general [58]. However, physical activity often decreases during puberty [59]. While children still benefit from the positive effects of physical activity in regulating body weight, this may

Table 2 Multivariable Cox proportional hazards regression on the association between childhood and adolescence factors and multiple sclerosis

Variable	Multivariable Cox proportional hazards model[a]	
	HR[b]	95% CI[b]
Sex		
Female	*Ref*	
Male	0.48	0.41 – 0.56
Number of siblings		
Only child	*Ref*	
1-2 sibling(s)	0.93	0.73 – 1.17
≥ 3 siblings	0.81	0.61 – 1.09
Premature birth (> 4 weeks before due date)		
No	*Ref*	
Yes	0.94	0.61 – 1.47
Cesarean section		
No	*Ref*	
Yes	1.10	0.80 – 1.52
Birth weight		
Low	0.99	0.74 – 1.33
Average	*Ref*	
High	1.14	0.90 – 1.45
Ever breastfed		
No	*Ref*	
Yes, ≤ 4 months	1.02	0.79 – 1.31
Yes, > 4 months	0.88	0.69 – 1.11
Contact with pets and/or livestock during childhood		
No	*Ref*	
Yes	1.04	0.69 – 1.58
Attended daycare		
No	*Ref*	
Yes, 1st attendance at age 3-6 years	0.93	0.77 – 1.12
Yes, 1st attendance at age 1- < 3 year(s)	0.77	0.58 – 1.03
Yes, 1st attendance at age < 1 year	0.97	0.63 – 1.50
Weight at the age of 10 years compared to peers		
Lower	0.86	0.70 – 1.05
Average	*Ref*	
Higher	0.93	0.73 – 1.19
BMI[b] at the age of 18 years (kg/m^2)		
Underweight (< 18.5)	1.12	0.89 – 1.42
Normal weight (18.5 - < 25)	*Ref*	
Overweight (25 - < 30)	2.03	1.41 – 2.94
Obesity (≥ 30)	1.89	1.02 – 3.48
Childhood Trauma[c] (per 5 units)	1.08	0.95 – 1.24

[a] Adjusted for education and migration status, stratified by birth year (categorized as: < 1955, 1955–1964, 1965–1974, 1975–1984, ≥ 1985)

[b] *HR* Hazard Ratio, *CI* Confidence Interval, *BMI* Body Mass Index

[c] Assessed with the Childhood Trauma Screener (5 – 25 points)

no longer be the case for adolescents due to lower levels of physical activity. In contrast, in a study based on retrospectively collected data of 1,944 persons with MS and 435,959 persons without MS from the UK Biobank sample, Belbasis and colleagues showed that "plumper than average body size" at the age of 10 years was associated with an increased risk of MS (Odds Ratio (OR) 1.38, 95% CI 1.21 to 1.58). The authors confirmed this result in a subsequent Mendelian Randomization study (OR 1.22, 95% CI 1.05 to 1.41) [39]. This clearly demonstrates the need for future research on the influence of the interaction between body weight and physical activity in childhood and adolescence on the risk of MS. Amidst the hygiene hypothesis [19], we would have expected to observe associations with factors such as a higher number of siblings, younger age at first daycare attendance, contact with pets and/or livestock and decreased MS risk as was reported for other autoimmune disorders [17] and allergies [15, 18]. Regarding the number of siblings, our results are consistent with those of Bager et al. [22] and Banwell et al. [23]. In contrast, one case–control study comprising 245 MS cases and 296 population-based controls reported a decreased risk of MS in participants with ≥ 3 older siblings, but only four participants with MS fell into this category in their study [20]. Another study found that an increasing duration of exposure to a younger sibling aged < 2 years in the first six years of life reduced MS risk [24]. These conflicting results highlight the continuing need for research into MS risk factors. We did not observe an association between daycare attendance and MS risk. In the study by Conradi and colleagues, a protective effect of daycare attendance at the age of 0–3 years on subsequent MS risk was found (OR 0.50, 95% CI 0.31 to 0.80) [20]. Contact with pets and/or farm animals, which has also been addressed in the context of the hygiene hypothesis as a potential factor influencing MS risk [19], was also not related to disease risk in our study, corroborating findings from a systematic review, which investigated the association between pet ownership during childhood and subsequent MS risk [25]. A meta-analysis on pet ownership in infancy and the incidence of the autoimmune diseases asthma and allergic rhinitis at school age also observed no association [60].

Our estimate regarding breastfeeding has a wide confidence interval, however, it is in line with our recent meta-analysis on the association between having been breastfed and MS risk (OR 0.86, 95% CI 0.75 to 0.99) [37]. In our meta-analysis, it also became apparent that the effect of breastfeeding on MS risk might differ between men and women, as shown in the included study by Hedström and colleagues, who observed that breastfeeding duration of ≥ 4 months compared to < 4 months was related to a reduced MS risk in men but not in women

(OR 0.5, 95% CI 0.4 to 0.7) [61]. In our present analyses, we observed a HR of 0.59 (95% CI 0.40 to 0.86) for a breastfeeding duration of ≤ 4 months and a HR of 0.70 (95% CI 0.48 to 1.03) for a breastfeeding duration of > 4 months compared to no breastfeeding for men, suggesting that having been breastfed at all is the most relevant factor.

For the remaining childhood factors, i.e., prematurity, born by cesarean section, and childhood trauma no association with MS risk was observed. Regarding the first two factors, our results are consistent with the systematic review and meta-analysis by Badihian and colleagues, in which studies were summarized narratively for preterm birth and meta-analytically for the factor cesarean section, the latter yielding a pooled OR of 0.90 (95% CI 0.52 to 1.56) [34]. Regarding childhood trauma, our results are in contrast to the systematic review by Polick and colleagues [45]. However, the comparability of our results is limited by the fact that we only investigated the sum score of the CTS, but not the individual scales (emotional, physical, and sexual abuse and emotional and physical neglect scales) that comprise the sum score. Such an analysis of MS risk using the subscales of the CTS was beyond the scope of our study and will be incorporated as a main focus in subsequent work.

The primary strengths of this study comprise the large number of participants, its population basis, a comprehensive examination and assessment program that is performed by trained study personnel based on written standard operating procedures as well as the stringent quality assurance of the data collection and usage [46, 47]. The cohort enables analyses with both a sufficiently large group of individuals with MS and a wide range of potential risk factors and covariates to be considered, hence enabling us to contribute to the existing evidence regarding the associations between childhood and adolescence factors and the risk of MS.

Nevertheless, our study also has limitations. It cannot be excluded that the MS status as well as disease severity are related to participation readiness. Participants had to visit the respective study center for the examinations and the interview, therefore a selection of less severely affected MS cases might have occurred. Furthermore, severely affected individuals might have a different risk profile than less severely affected individuals. Hence, selection bias toward less severely affected cases may have resulted in an underestimation of associations in population-based MS cases as a whole. On the other hand, participants with MS and especially more severely affected individuals might be more motivated to participate in a study that investigates disease-related risk factors. Accordingly, future studies should account for the potential disease severity during the course of the disease. Also, as in all population-based cohort studies, there might be a healthy participant bias, implying that participants have a different risk factor pattern than the general population.

Moreover, the analyses are based on self-reported MS diagnoses. To our knowledge, no study to date has examined the sensitivity and specificity of self-reported MS diagnoses and reported HRs might be attenuated toward null. A validation study of the diagnoses, by use of information on treatment, medical records, and/or health insurance data is underway, however not been completed yet. Claflin and colleagues evaluated the consistency and validity of self-reported year of MS diagnosis among 2,245 participants in the Australian Multiple Sclerosis Longitudinal Study. 88% to 92% of respondents were able to recall their year of diagnosis with a deviation of ≤ 1 year. Thus, patient-reported year of diagnosis appears to be reliable information to use in analyses [62].

As early childhood factors were the main focus of our study, which for most study participants occurred on average 40 to 50 years ago, recall bias may have occurred. However, we assume that this is not a differential recall (thus, the bias not being different between MS cases and participants without MS), since the purpose of the data collection in the NAKO did not focus on MS and risk factors specific to MS at the time of recruitment, but rather on the investigation of widespread diseases such as cancer, diabetes mellitus, or cardiovascular diseases (see [47]). The observation that people with MS suffer increasingly from cognitive and memory dysfunction as the disease progresses could be more decisive than the pure temporal component with regard to a recall bias. However, due to the design of the NAKO, only individuals with a certain level of physical and cognitive fitness participated in the study. Therefore, we assume that the risk of a recall bias caused by this is rather low. However, if differential recall had occurred between participants with MS and without MS, the resulting bias was not strong, as our results are largely consistent with prospective studies of incident MS cases which have evaluated childhood and adolescence factors [30, 63, 64]. To some extent, our study might be biased due to a certain degree of misclassification (e.g., breastfeeding duration). Furthermore, some subgroups comprised only a few MS cases, thus the power here is low.

At the time of our analyses, it was not yet possible to determine the number of childhood-acquired infections by determining the viral load in the biosamples collected during the NAKO baseline examination. For this reason, we had to use surrogate variables such as number of siblings, daycare attendance, or pet ownership. As the determination of viral load via biospecimen analyses will be possible in the near future, the investigation of the

interplay between viral load with childhood and adolescence factors is worth considering. Genetic factors may also be considered in these analyses.

Furthermore, we were unable to include other, lesser-known but potentially equally important risk factors such as month of birth, maternal vit D serum levels during pregnancy, or exposure to vaccinations in our analysis. For example, the month of birth, which is linked to the mother's exposure to sunlight during pregnancy and thus directly to the maternal vit D serum level, could be regarded as a precursor to the vit D level of the MS case. The link between vit D deficiency and an increased risk of MS has already been shown [65]. Thus, future studies should take these factors into account.

As shown in Table 1, many variables included in the analyses had a high proportion of missing values (e.g., "Ever breastfed" with 43.8% and 43.2% in persons with and without MS, respectively). It is difficult to clearly classify the type of missingness. However, following the classification by Rubin [66] missing values for most variables seem to be classifiable as missing completely at random. Hence, we used multiple imputation and compared the results with those of the complete case analysis. Both methods showed similar results, supporting the validity of the results using multiple imputation (see Additional file 2 & 3).

Conclusions

In summary, based on this study the proportion of MS cases that can be explained by childhood and adolescence factors considered in this study was low. Nevertheless, we emphasize the observed association between overweight and obesity at the age of 18 years compared with normal weight at the age of 18 years and increased risk of MS. Stratified by sex, the association between overweight and increased MS risk was only found in women but not in men. Furthermore, a breastfeeding duration of ≤ 4 months compared to no breastfeeding was related to a reduced MS risk only in men. Given the large sample size, our study contributed to the existing evidence from previous studies. Our finding of an association between overweight and obesity during adolescence offers potential for MS prevention. In order to reduce the incidence of MS or at least delay its onset, the association between weight gain, onset of overweight and obesity in childhood and adolescence and their interaction with physical activity level should be investigated in longitudinal studies. In particular, sex-specific effects should be taken into account in future studies. Furthermore, the collection of biosamples during the NAKO baseline examination allows the investigation of the interplay of genetic factors, viral load, and childhood and adolescence factors in the future.

Abbreviations

BMBF	German Federal Ministry of Education and Research
BMI	Body Mass Index
CI	Confidence Interval
CNS	Central nervous system
CTS	Childhood Trauma Screener
HR	Hazard Ratio
ISCED	International Standard Classification of Education
MS	Multiple Sclerosis
NAKO	German National Cohort
OR	Odds Ratio
SD	Standard deviation
Vit D	Vitamin D

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12883-024-03620-4.

Additional file 1: Supplementary Table S1. Multivariable Cox proportional hazards regression on the association between childhood and adolescence factors and multiple sclerosis stratified by sex (n_{women}= 103073; n_{men} = 101200).

Additional file 2: Supplementary Table S2. Multivariable Cox proportional hazards regression on the association between childhood and adolescence factors and multiple sclerosis – complete case analysis for the total sample and stratified by sex.

Additional file 3: Supplementary Figure S3. Forest Plot: Multivariable Cox proportional hazards regression on the association between childhood and adolescence factors and multiple sclerosis – Imputation model and complete case analysis.

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Authors' contributions

Conceptualization and design: A.H., H.B. (Heiko Becher), N.O., C.H., K.R.-L.; Methodology: A.H., H.B. (Heiko Becher); Formal analysis and investigation: A.H., H.B. (Heiko Becher); Writing – original draft preparation: A.H.; Writing – review and editing: A.H., N.O., W.A., K.B., B.B., H.B. (Hermann Brenner), B.F., J.F., A.F., S.G., K.H.G., V.H., J.-K.H., B.H., T.K., C.J.K.-T., M.L., W.L., C.M.-F., K.B.M., R.M., K.N., A.P., T.P., O.R., T.S., S.S., B.S., M.B.S., A.S., K.H., K.R.-L., C.H., H.B. (Heiko Becher); Funding acquisition: H.B. (Heiko Becher); Resources: NAKO; Supervision: H.B. (Heiko Becher), N.O. All authors read and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the NAKO e.V. but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of the NAKO e.V.

Declarations

Ethics approval and consent to participate

The German National Cohort (NAKO) was performed in line with the principles of the Declaration of Helsinki. The study was approved by the responsible local ethics committees of the German Federal States where all study centers were located (Bayerische Landesärztekammer (protocol code 13023; Approval date: 27 March 2013 and 14 February 2014)).
Written informed consent was obtained from all participants in the German National Cohort (NAKO).

Consent for publication

Not applicable.

Competing interests

Financial interests: C.H. has received research funding and speaker honoraria from Novartis, Merck, and Roche.
The other authors have no relevant financial or non-financial interests to disclose.

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Publisher's Note

Supplementary Table S1: Multivariable Cox proportional hazards regression on the association between childhood and adolescence factors and multiple sclerosis stratified by sex (n_{women} = 103073; n_{men} = 101200)

	Multivariable Cox proportional Hazards Model[a]			
	Women		Men	
Variable	**HR[b]**	**95% CI[b]**	**HR[b]**	**95% CI[b]**
Number of siblings				
Only child	*Ref.*		*Ref.*	
1-2 sibling(s)	0.94	0.75 – 1.19	1.03	0.72 – 1.47
≥3 siblings	1.00	0.74 – 1.34	0.87	0.55 – 1.37
Premature birth (>4 weeks before due date)				
No	*Ref.*		*Ref.*	
Yes	0.86	0.49 – 1.51	1.12	0.55 – 2.27
Cesarean section				
No	*Ref.*		*Ref.*	
Yes	1.07	0.70 – 1.65	1.14	0.67 – 1.92
Birth weight				
Low	1.04	0.72 – 1.48	1.08	0.60 – 1.93
Average	*Ref.*		*Ref.*	
High	1.02	0.72 – 1.44	1.28	0.89 – 1.83
Ever breastfed				
No	*Ref.*		*Ref.*	
Yes, ≤4 months	1.21	0.96 – 1.51	0.59	0.40 – 0.86
Yes, >4 months	0.85	0.65 – 1.09	0.70	0.48 – 1.03
Contact with pets and/or livestock during childhood				
No	*Ref.*		*Ref.*	
Yes	1.00	0.70 – 1.41	1.23	0.79 – 1.93
Attended daycare				
No	*Ref.*		*Ref.*	
Yes, 1st attendance at age 3-6 years	0.81	0.64 – 1.03	1.17	0.84 – 1.64
Yes, 1st attendance at age 1-<3 year(s)	0.78	0.57 – 1.07	1.00	0.64 – 1.57
Yes, 1st attendance at age <1 year	0.77	0.52 – 1.14	0.49	0.19 – 1.25
Weight at the age of 10 years compared to peers				
Lower	0.85	0.67 – 1.08	1.00	0.69 – 1.43
Average	*Ref.*		*Ref.*	
Higher	0.94	0.70 – 1.25	1.31	0.88 – 1.96
BMI[b] at the age of 18 years (kg/m²)				
Underweight (<18.5)	0.96	0.72 – 1.27	1.06	0.62 – 1.81
Normal weight (18.5 - <25)	*Ref.*		*Ref.*	
Overweight (25 - <30)	1.55	1.05 – 2.29	0.78	0.45 – 1.37
Obesity (≥30)	1.78	0.96 – 3.30	1.45	0.66 – 3.17
Childhood Trauma[c] (per 5 units)	1.11	0.96 – 1.29	1.05	0.81 – 1.37

[a]Adjusted for education and migration status, stratified by birth year (categorized as: <1955, 1955-1964, 1965-1974, 1975-1984, ≥1985)

[b]HR = Hazard Ratio, CI = Confidence Interval, BMI = Body Mass Index

[c]Assessed with the Childhood Trauma Screener (5 – 25 points)

Supplementary Table S2: Multivariable Cox proportional hazards regression on the association between childhood and adolescence factors and multiple sclerosis – complete case analysis for the total sample and stratified by sex

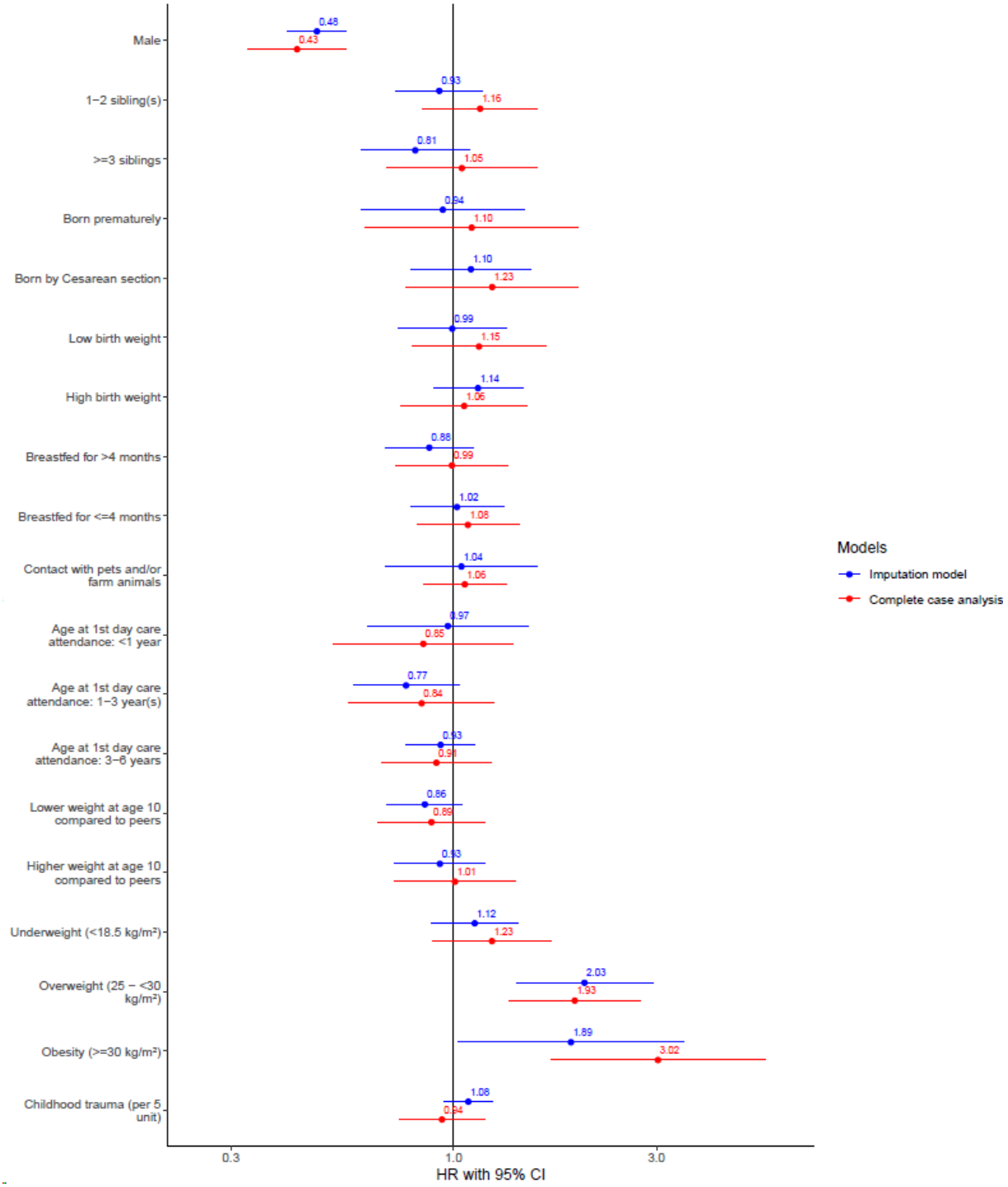
	Multivariable Cox proportional Hazards Model[a] (n = 75247; 310 participants with and 74937 participants without an MS[b] diagnosis**)**		**Multivariable Cox proportional Hazards Model – Women[a] (**n = 41296; 228 women with and 41068 women without an MS[b] diagnosis**)**		**Multivariable Cox proportional Hazards Model – Men[a] (**n = 33951, 82 men with and 33869 men without an MS[b] diagnosis**)**	
Variable	**HR[b]**	**95% CI[b]**	**HR[b]**	**95% CI[b]**	**HR[b]**	**95% CI[b]**
Sex						
Female	*Ref.*					
Male	0.43	0.33 – 0.56				
Number of siblings						
Only child	*Ref.*		*Ref.*		*Ref.*	
1-2 sibling(s)	1.16	0.85 – 1.58	1.07	0.75 – 1.53	1.47	0.75 – 2.89
≥3 siblings	1.05	0.70 – 1.57	1.10	0.69 – 1.73	0.94	0.39 – 2.27
Premature birth (>4 weeks before due date)						
No	*Ref.*		*Ref.*		*Ref.*	
Yes	1.10	0.62 – 1.96	0.95	0.48 – 1.86	1.83	0.58 – 5.80
Cesarean section						
No	*Ref.*		*Ref.*		*Ref.*	
Yes	1.23	0.77 – 1.97	1.27	0.73 – 2.21	1.14	0.48 – 2.68
Birth weight						
Low	1.15	0.80 – 1.65	1.24	0.84 – 1.83	0.78	0.29 – 2.12
Average	*Ref.*		*Ref.*		*Ref.*	
High	1.06	0.75 – 1.49	1.09	0.72 – 1.67	0.99	0.56 – 1.75
Ever breastfed						
No	*Ref.*		*Ref.*		*Ref.*	
Yes, ≤4 months	1.08	0.82 – 1.43	1.25	0.92 – 1.69	0.71	0.38 – 1.30
Yes, >4 months	0.99	0.73 – 1.34	0.90	0.63 – 1.30	1.06	0.58 – 1.91
Contact with pets and/or livestock during childhood						
No	*Ref.*		*Ref.*		*Ref.*	
Yes	1.06	0.85 – 1.33	1.00	0.77 – 1.30	1.30	0.84 – 2.02
Attended daycare						
No	*Ref.*		*Ref.*		*Ref.*	
Yes, 1st attendance at age 3-6 years	0.91	0.68 – 1.22	0.84	0.60 – 1.19	1.13	0.63 – 2.03
Yes, 1st attendance at age 1-<3 year(s)	0.84	0.57 – 1.25	0.89	0.56 – 1.41	0.78	0.36 – 1.68
Yes, 1st attendance at age <1 year	0.85	0.52 – 1.38	0.97	0.58 – 1.65	0.36	0.08 – 1.58
Weight at the age of 10 years compared to peers						
Lower	0.89	0.67 – 1.18	0.80	0.56 – 1.13	1.13	0.67 – 1.92
Average	*Ref.*		*Ref.*		*Ref.*	
Higher	1.01	0.73 – 1.40	0.95	0.65 – 1.38	1.18	0.62 – 2.25
BMI[b] at the age of 18 years (kg/m^2)						
Underweight (<18.5)	1.23	0.89 – 1.70	1.16	0.81 – 1.66	2.09	1.01 – 4.33
Normal weight (18.5 - <25)	*Ref.*		*Ref.*		*Ref.*	
Overweight (25 - <30)	1.93	1.35 – 2.75	2.37	1.57 – 3.58	1.21	0.60 – 2.45
Obesity (≥30)	3.02	1.70 – 5.39	2.91	1.42 – 5.93	3.41	1.26 – 9.24
Childhood Trauma[c] (per 5 units)	0.94	0.75 – 1.18	0.91	0.70 – 1.18	1.05	0.65 – 1.70

[a]Adjusted for education and migration status, stratified by birth year (categorized as: <1955, 1955-1964, 1965-1974, 1975-1984, ≥1985)

[b]MS = Multiple Sclerosis, HR = Hazard Ratio, CI = Confidence Interval, BMI = Body Mass Index

[c]Assessed with the Childhood Trauma Screener (5 – 25 points)

Supplementary Figure S3: Forest Plot: Multivariable Cox proportional hazards regression on the association between childhood and adolescence factors and multiple sclerosis – Imputation model and complete case analysis



8. PUBLICATION 3

Holz, A.**,** Obi, N., Pischon, T., Schulze, M.B., Ahrens, W., Berger, K., Bohn, B., Brenner, H., Emmel, C., Fischer, B., Greiser, K.H., Harth, V., Holleczek, B., Kaaks, R., Karch, A., Katzke, V., Keil, T., Krist, L., Leitzmann, M., Meinke-Franze, C., Michels, K.B.; Nimptsch, K., Peters, A., Riedel, O., Schikowski, T., Schipf, S., Schmidt, B., Thierry, S., Hellwig, K., Riemann-Lorenz, K., Heesen, C., Becher, H. (2025). **The Relation of Multiple Sclerosis to Family History, Lifestyle, and Health Factors in Childhood and Adolescence: Findings of a Case-Control Study Nested Within the German National Cohort (NAKO) study.** Dtsch Arztebl Int. 122: online first. doi: 10.3238/arztebl.m2025.0069.

Originalarbeit

Multiple Sklerose: Familienvorgeschichte, Lebensstil und gesundheitliche Faktoren im Kindes- und Jugendalter

Ergebnisse einer in die NAKO Gesundheitsstudie eingebetteten Fall-Kontroll-Studie

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Zusammenfassung

Hintergrund: Multiple Sklerose (MS) ist eine neuroinflammatorische, mutmaßlich autoimmune Erkrankung. Die Exposition gegenüber Umwelt- und Lebensstilfaktoren bei genetisch suszeptiblen Personen könnte zum Ausbruch einer MS beitragen. Zu den bereits bekannten Risikofaktoren gehören eine genetische Prädisposition, eine Infektion mit dem Epstein-Barr-Virus (EBV), Rauchen sowie ein erhöhter Body-Mass-Index (BMI).

Methode: Zur Untersuchung des Zusammenhangs zwischen potenziellen Risikofaktoren und MS führten wir zwischen 2021 und 2022 eine in die NAKO Gesundheitsstudie eingebettete Fall-Kontroll-Studie durch.

Ergebnisse: Insgesamt nahmen 576 Personen mit MS (Fälle) und 895 Personen ohne MS (Kontrollen) teil. Neben bereits bekannten Risikofaktoren zeigte sich, dass Infektionen in der Kindheit (Odds Ratio [OR] 1,14 pro zusätzlicher Infektion, 95-%-Konfidenzintervall: [1,03; 1,25]), belastende Lebensereignisse (OR 1,25 pro zusätzlichem Ereignis, [1,06; 1,48]), das erstgeborene Kind einer bei Geburt ≥ 30 Jahre alten Mutter zu sein (OR 2,11 [1,08; 4,13]) mit einer erhöhten und körperliche Aktivität in der Jugend (OR 0,82 pro Anstieg des Aktivitätslevels um ein Aktivitätslevel [0,71; 0,95]) mit einer geringeren Wahrscheinlichkeit für MS assoziiert waren.

Schlussfolgerung: Es zeigten sich Assoziationen zwischen einigen neuen Faktoren, zum Beispiel der kumulativen Anzahl von Infektionen im Kindesalter, und MS. Diese Ergebnisse können zum Wissen über die Ätiologie der MS beitragen und sollten in künftigen Studien vertiefend untersucht werden. Andere bereits bekannte Risikofaktoren für MS wurden in dieser Studie bestätigt.

Zitierweise
Holz A, Obi N, Pischon T, Schulze MB, Ahrens W, Berger K, Bohn B, Brenner H, Emmel C, Fischer B, Greiser KH, Harth V, Holleczek B, Kaaks R, Karch A, Katzke V, Keil T, Krist L, Leitzmann M, Meinke-Franze C, Michels KB, Nimptsch K, Peters A, Riedel O, Schikowski T, Schipf S, Schmidt B, Thierry S, Hellwig K, Riemann-Lorenz K, Heesen C, Becher H: The relation of multiple sclerosis to family history, lifestyle, and health factors in childhood and adolescence: Findings of a case–control study nested within the German National Cohort (NAKO) study.
Dtsch Arztebl Int 2025; 122: online first.
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Multiple Sklerose (MS) ist eine neuroinflammatorische, mutmaßlich autoimmune Erkrankung, die zu Beeinträchtigungen im frühen Erwachsenenalter führt (1) und die Lebensqualität der Betroffenen erheblich beeinträchtigt (2).

In den letzten Jahrzehnten haben die weltweite Prävalenz und Inzidenz der MS weiter zugenommen (1, 3), während ihre Ätiologie immer noch nur teilweise geklärt ist (4–6). Vermutlich führt die Exposition gegenüber Umwelt- und Lebensstilfaktoren bei genetisch suszeptiblen Personen zur Manifestation der Erkrankung (7, 8).

Die Odds Ratios (OR) der bereits identifizierten MS-assoziierten Risikofaktoren eines erhöhten Body-Mass-Indexes (BMI), eines Vitamin-D-Mangels (9) und einer Epstein-Barr-Virus-Infektion (EBV) liegen zwischen 1,14 (BMI) (9) und 3,33 (EBV) (10), was auf kleine bis mittlere Effektstärken hindeutet. Moderate bis intensive körperliche Aktivität (KA) im Erwachsenenalter hingegen wurde als Schutzfaktor identifiziert (9). Obwohl die Mehrheit der Beobachtungsstudien einen Zusammenhang zwischen Rauchen und einem erhöhten MS-Risiko zeigten (11), konnte ein kausaler Zusammenhang bislang nicht bestätigt werden (9).

Faktoren, die vor der Geburt oder in der (frühen) Kindheit/Jugend auftreten – also zu einer Zeit, in der das Immunsystem noch sehr suszeptibel ist (12) –, könnten bei der Entwicklung der MS besonders bedeutsam sein (12). Zu ihnen zählen zum Beispiel

- die Anzahl älterer Geschwister
- das Alter der Mutter
- Infektionen in der Kindheit
- Passivrauchen
- körperliche Aktivität (KA)
- im Freien verbrachte Zeit.

Tabelle 1

Charakteristika der StERKE-Teilnehmenden nach Geschlecht und Fall-Kontroll-Status, Deutschland, 2021–2022

	Frauen		Männer	
Variable	**Kontrollen n = 638**	**Fälle n = 396**	**Kontrollen n = 257**	**Fälle n = 180**
Alter (zur NAKO Basiserhebung) (Mittelwert [SD])	51 (11,0)	49 (10,9)	52 (11,3)	49 (11,1)
MS in der Familienvorgeschichte				
kein Familienmitglied mit MS	624 (97,8 %)	358 (90,4 %)	253 (98,4 %)	160 (88,9 %)
Familienmitglied ersten oder zweiten Grades mit MS	14 (2,2 %)	38 (9,6 %)	4 (1,6 %)	20 (11,1 %)
Anzahl älterer Geschwister				
keine	243 (38,1 %)	166 (41,9 %)	88 (34,2 %)	74 (41,1 %)
1 älteres Geschwisterkind	229 (35,9 %)	143 (36,1 %)	92 (35,8 %)	61 (33,9 %)
≥ 2 ältere Geschwisterkinder	166 (26,0 %)	87 (22,0 %)	77 (30,0 %)	45 (25,0 %)
mütterliches Alter bei Geburt des Teilnehmenden (Mittelwert [SD])	27 (5,7)	28 (5,9)	27 (5,6)	27 (5,0)
Elterliches Rauchverhalten während der Schwangerschaft				
Mutter rauchte während der Schwangerschaft*1	50 (8,8 %)	22 (6,2 %)	14 (6,2 %)	13 (8,2 %)
Vater rauchte während der Schwangerschaft*1	191 (38,7 %)	129 (41,0 %)	81 (41,8 %)	62 (42,2 %)
Infektionen in der Kindheit *1,*2				
Windpocken	466 (83,4 %)	291 (83,1 %)	149 (73,4 %)	116 (76,3 %)
Mumps	241 (46,5 %)	165 (50,5 %)	102 (54,0 %)	81 (55,5 %)
Röteln	188 (38,7 %)	135 (44,0 %)	64 (36,6 %)	53 (41,7 %)
Pertussis	105 (21,0 %)	75 (23,7 %)	47 (24,2 %)	31 (23,1 %)
Masern	278 (54,2 %)	190 (57,4 %)	113 (59,2 %)	92 (61,7 %)
EBV-Infektion	22 (4,0 %)	42 (13,1 %)	6 (2,8 %)	8 (5,3 %)
Mutter und/oder Vater rauchten in der Kindheit/Jugend des Teilnehmenden (0–18 Jahre)*1	331 (53,3 %)	213 (54,8 %)	137 (55,2 %)	95 (54,6 %)
im Freien verbrachte Zeit in der Kindheit und Jugend (0–18 Jahre)				
keine bis einige Stunden/Monat	45 (7,1 %)	19 (4,8 %)	16 (6,2 %)	7 (3,9 %)
einige Stunden/Woche	112 (17,6 %)	73 (18,4 %)	27 (10,5 %)	30 (16,7 %)
einige Stunden/Tag	481 (75,4 %)	304 (76,8 %)	214 (83,3 %)	143 (79,4 %)
körperliche Aktivität in der Jugend (13–19 Jahre)				
sehr niedrig	19 (3,0 %)	21 (5,3 %)	2 (0,8 %)	4 (2,2 %)
niedrig	76 (11,9 %)	60 (15,2 %)	17 (6,6 %)	12 (6,7 %)
mittel	223 (35,0 %)	134 (33,8 %)	60 (23,3 %)	56 (31,1 %)
hoch	320 (50,2 %)	181 (45,7 %)	178 (69,3 %)	108 (60,0 %)
BMI im Alter von 18 Jahren (kg/m²)*1, *3				
Untergewicht (< 18,5)	161 (22,9 %)	61 (16,6 %)	24 (6,6 %)	14 (8,9 %)
Normalgewicht (18,5 bis < 25)	485 (68,9 %)	251 (68,2 %)	296 (81,1 %)	113 (71,5 %)
Übergewicht (25 bis < 30)	45 (6,4 %)	44 (12,0 %)	40 (11,0 %)	24 (15,2 %)
Adipositas (≥ 30)	13 (1,8 %)	12 (3,3 %)	5 (1,4 %)	7 (4,4 %)
belastende Lebensereignisse*2				
Tod des Partners	15 (2,3 %)	13 (3,9 %)	2 (0,8 %)	3 (1,7 %)
Tod einer nahestehenden Person (außer Partner)	185 (29,0 %)	144 (36,4 %)	69 (26,8 %)	52 (28,9 %)
schwerwiegende Erkrankung einer nahestehenden Person	126 (19,7 %)	91 (23,0 %)	32 (12,5 %)	34 (18,9 %)
eigene schwere Erkrankung (außer MS)	40 (6,3 %)	34 (8,6 %)	13 (5,1 %)	13 (7,2 %)
jemals geraucht*1, *2, *3	480 (49,2 %)	252 (51,9 %)	259 (55,8 %)	147 (64,2 %)

*1 Prozentangaben beziehen sich ausschließlich auf Personen, für die Informationen vorlagen. Die Daten für die Kategorien „nein" und „unbekannt/fehlender Wert" können der *eTabelle 1* entnommen werden.
*2 Fälle: vor Alter bei Diagnose; Kontrollen: vor Alter bei Diagnose des gematchten Falls/medianes Alter bei Diagnose der gematchten Fälle, wenn > 1 Fall pro Matchingset
*3 Verteilung aus der Gesamtzahl der infrage kommenden 746 Fälle und 1 492 Kontrollen
BMI, Body-Mass-Index; EBV, Epstein-Barr-Virus; MS, multiple Sklerose; NAKO, NAKO Gesundheitsstudie; SD, Standardabweichung

Ein möglicher Zusammenhang mit solchen Faktoren wurde bereits für andere Autoimmunerkrankungen wie Asthma bronchiale (13) oder Typ-1-Diabetes (T1D) gezeigt (14). Neben molekularen und umweltbedingten Faktoren werden zunehmend auch psychische Faktoren als potenzielle Risikofaktoren für MS diskutiert. So zeigte eine kürzlich veröffentlichte Metaanalyse einen schwachen bis moderaten Effekt psychologischer Stressoren auf das MS-Risiko (15).

Da die Anzahl von Studien zu einigen dieser modifizierbaren Risikofaktoren für MS gering ist und zudem widersprüchliche Studienergebnisse und/oder eine große methodische Heterogenität vorliegen, sind weitere Untersuchungen dieser Faktoren notwendig.

Um die der MS zugrunde liegenden Ursachen besser zu verstehen, haben wir eine Fall-Kontroll-Studie durchgeführt. Ziel war es, Assoziationen zwischen potenziellen Risikofaktoren und der Entstehung der MS zu identifizieren. Folgende potenzielle Risikofaktoren wurden hierbei untersucht

- eine familiäre Vorgeschichte
- Infektionserkrankungen in der Kindheit
- Passivrauchen
- die Anzahl älterer Geschwister
- das Alter der Mutter bei der Geburt
- im Freien verbrachte Zeit in der Kindheit und Jugend
- körperliche Aktivität in der Jugend
- belastende Lebensereignisse (BLE)
- BMI im Alter von 18 Jahren und
- Rauchen.

Methoden

Die hier präsentierten Analysen basieren auf Daten der Basiserhebung der NAKO Gesundheitsstudie (NAKO) (16) und der StERKE-Studie (StERKE, Studie zum Einfluss von Risikofaktoren auf den Krankheitsverlauf und die Entstehung der Multiplen Sklerose), einer in die NAKO eingebetteten Fall-Kontroll-Studie. Fälle wurden definiert als Personen mit einer selbstberichteten ärztlich gestellten MS-Diagnose. Kontrollen waren zufällig ausgewählte und den Fällen basierend auf Geburtsjahr, Geschlecht und Studienzentrum individuell zugeordnete (Matching-Verhältnis 2:1) NAKO-Teilnehmende ohne MS. Im Rahmen der NAKO-Basiserhebung wurden die Daten mittels eines Interviews erhoben, wohingegen die Teilnehmenden der StERKE-Studie einen Fragebogen ausfüllten. Die Teilnehmenden wurden in den Analysen nur dann als exponiert betrachtet, wenn die Exposition vor der MS-Diagnose (bei Fällen) oder in der Zeit vor dem Alter bei MS-Diagnose des gematchten Falls (bei Kontrollen) auftrat. Es wurde eine bedingte logistische Regression durchgeführt, um den Zusammenhang zwischen den beobachteten Expositionen und dem Auftreten einer MS zu untersuchen. Die Ergebnisse werden als Odds Ratios (OR) mit 95-%-Konfidenzintervallen berichtet.

Alle statistischen Analysen wurden mit R Version 4.3.1 (2023–06–16) (17) durchgeführt. Die angewendeten Methoden werden im *eMethodenteil* (inklusive *eSuppelement-Tabellen 1–5)* ausführlich beschrieben.

Tabelle 2

Charakteristika der StERKE-Teilnehmenden mit multipler Sklerose nach Geschlecht, Deutschland, 2021–2022

	Frauen	Männer
Variable	**n = 396**	**n = 180**
Alter bei MS-Diagnose (Median [IQR])	38 (29, 47)	38 (28, 46)
Alter bei MS-Manifestation (Median [IQR])	33 (26, 42)	35 (27, 41)
fehlender Wert	43	28
MS-Verlaufsform		
schubförmig remittierend	244 (79,5 %)	85 (63,9 %)
primär progredient	26 (8,5 %)	22 (16,5 %)
sekundär progredient	37 (12,1 %)	26 (19,5 %)
unbekannt	89	47
MS-spezifische Medikation (bis zu 7 Tage vor der NAKO-Basiserhebung)		
nein	219 (65,6 %)	90 (58,4 %)
ja	115 (34,4 %)	64 (41,6 %)
keine MS-Diagnose zum Zeitpunkt der NAKO-Basiserhebung	62	26
jemals eine Immuntherapie erhalten		
nein	150 (41,7 %)	75 (49,7 %)
ja	210 (58,3 %)	76 (50,3 %)
unbekannt	36	29
Zeitpunkt der MS-Diagnose		
inzidenter Fall (Diagnose nach der NAKO-Basiserhebung)	62 (15,7 %)	26 (14,4 %)
Diagnose ≤ 2 Jahre vor der NAKO-Basiserhebung	36 (9,1 %)	16 (8,9 %)
3 bis ≤ 5 Jahre vor der NAKO-Basiserhebung	35 (8,8 %)	19 (10,6 %)
6 bis ≤ 10 Jahre vor der NAKO-Basiserhebung	87 (22,0 %)	31 (17,2 %)
11 bis ≤ 20 Jahre vor der NAKO-Basiserhebung	106 (26,8 %)	51 (28,3 %)
> 20 Jahre vor der NAKO Basiserhebung	70 (17,7 %)	37 (20,6 %)

IQR, Interquartilsabstand; MS, multiple Sklerose; NAKO, NAKO Gesundheitsstudie

Ergebnisse

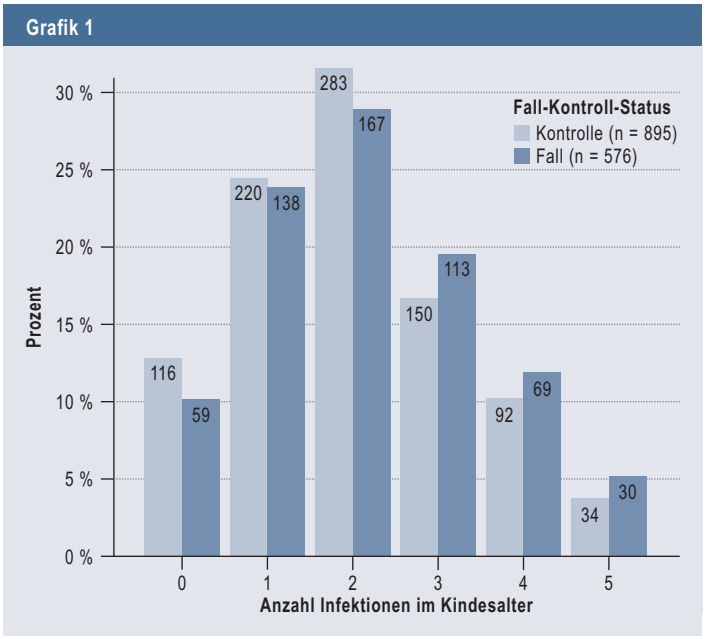
Insgesamt nahmen 576 Personen mit MS (396 Frauen, 180 Männer) (Fälle) und 895 Personen ohne MS (638 Frauen, 257 Männer) (Kontrollen) teil. Dies entspricht einer Antwortquote von 77,2 % bei den Fällen und 60,0 % bei den Kontrollen.

Die Stichprobe umfasste 70 % Frauen und 30 % Männer mit einem Durchschnittsalter von 50 Jahren. In *Tabelle 1* und *eTabelle 1* sind die wichtigsten Charakteristika der StERKE-Teilnehmenden sowie die Verteilung der Expositionsvariablen und Kovariaten nach Geschlecht und Fall-Kontroll-Status getrennt dargestellt.

Das mittlere Alter bei Manifestation der MS betrug 33 und 35 Jahre bei Frauen beziehungsweise Männern. Das mittlere Alter bei Diagnosestellung lag bei beiden Geschlechtern bei 38 Jahren. In 80 % der Fälle wurde eine schubförmig remittierende MS (RRMS) diagnostiziert. 58 % der Fälle hatten jemals eine Immuntherapie erhalten. Unter den StERKE-Teilnehmenden befanden sich 88 (15,3 %) inzidente Fälle *(Tabelle 2)*.

Die *Grafiken 1* und *2* veranschaulichen die Verteilung der kumulativen Anzahl von Infektionserkrankungen im Kindesalter beziehungsweise von BLE vor der MS-Diagnose.

Grafik 1



Verteilung der kumulativen Anzahl von Infektionen im Kindesalter (darunter: Windpocken, Mumps, Röteln, Pertussis, Masern) der StERKE-Teilnehmenden vor Diagnose der multiplen Sklerose nach Fall-Kontroll-Status, Deutschland, 2021–2022

Tabelle 3 zeigt die Ergebnisse der Hauptanalyse. Die Verwandtschaft ersten oder zweiten Grades mit einer an MS erkrankten Person ergab ein OR von 7,08 [3,90; 12,86] verglichen mit keinem an MS erkrankten Familienmitglied. Das mütterliche Alter bei der Geburt des Teilnehmenden war mit MS assoziiert (OR 1,03 pro Jahr [1,00; 1,05), wohingegen für die Anzahl älterer Geschwister ein inverser Zusammenhang beobachtet wurde (OR 0,85, [0,77; 0,95]). Die Kombination beider Faktoren, das heißt das älteste Kind einer bei Geburt 30-jährigen oder älteren Mutter zu sein, war mit einer erhöhten Wahrscheinlichkeit für MS assoziiert (OR 2,11, [1,08; 4,13]). Wir haben einen direkten Zusammenhang zwischen der kumulativen Anzahl von Infektionserkrankungen in der Kindheit (OR 1,14 pro zusätzlicher Infektion [1,03; 1,25]) und MS beobachtet. Für Personen mit fünf Infektionen, das heißt der Maximalanzahl untersuchter Erkrankungen, zeigte sich ein OR von 1,93 ($= 1{,}14^5$) [1,18; 3,06] im Vergleich zu Personen, die keine dieser Erkrankungen berichteten. Teilnehmende, die eine EBV-Infektion hatten, hatten eine 3,05-fach [1,80; 5,16] erhöhte Wahrscheinlichkeit an MS zu erkranken im Vergleich zu Personen, die nicht erkrankt waren. Des Weiteren zeigte sich, dass die Odds für MS umso geringer waren, je höher die körperliche Aktivität in der Jugend (OR 0,82 [0,71; 0,95]) war. Im Vergleich zu Normalgewicht waren Übergewicht und Adipositas im Alter von 18 Jahren mit MS assoziiert (OR 1,73 [1,22; 2,44] beziehungsweise OR 2,29 [1,18; 4,46]). Außerdem war die kumulative Anzahl BLEs mit einem erhöhten OR für MS assoziiert (OR 1,25 pro zusätzlichem Ereignis [1,06; 1,48]). Abschließend beobachteten wir eine schwache Assoziation zwischen dem Rauchen vor der MS-Diagnose und MS (OR 1,19 [0,99; 1,43]).

Die übrigen Faktoren – eigene schwere Erkrankung (außer MS), Passivrauchen, das heißt elterliches Rauchen während der Schwangerschaft sowie in der Kindheit und Jugend des Teilnehmenden, und die im Freien verbrachte Zeit –, waren nicht mit MS assoziiert. Die Schätzer der Subgruppenanalyse sowie der nach Geschlecht stratifizierten Analyse unterschieden sich nur geringfügig von den Ergebnissen der Hauptanalyse *(eSupplement-Tabellen 3, 4* und *5)*.

Diskussion

Diese Analyse basiert auf der StERKE-Studie, einer in die bevölkerungsbezogene Kohortenstudie NAKO eingebetteten Fall-Kontroll-Studie. Unsere Studie hat mehrere neue Erkenntnisse hervorgebracht. So beobachteten wir, dass die kumulative Anzahl von Infektionserkrankungen in der Kindheit sowie BLE mit dem MS-Risiko assoziiert sind. Es ist bekannt, dass ein höheres Alter bei Erstgebärenden mit verschiedenen negativen Folgen für das Kind verbunden sein kann (18), und in dieser Studie fanden wir einen Zusammenhang mit dem MS-Risiko. Jüngste Studien haben gezeigt, dass KA im Erwachsenenalter ein Schutzfaktor für MS ist (9). Beruhend auf unseren Ergebnissen kann diese Erkenntnis auch auf die KA im Jugendalter erweitert werden. Darüber hinaus konnten wir bereits bekannte Risikofaktoren für MS, darunter eine familiäre Vorgeschichte (7), eine EBV-Infektion (10, 19) und einen erhöhten BMI in der Kindheit/Jugend (9), bestätigen.

Bezüglich des höheren Alters Erstgebärender konnte in einer kleineren Studie kein Zusammenhang mit MS festgestellt werden (20). Unsere Ergebnisse stimmen jedoch mit Studien überein, die andere Autoimmunerkrankungen, zum Beispiel Diabetes mellitus Typ 1 (T1D), untersucht haben (21). Die zugrunde liegenden Pathomechanismen sollten allerdings noch weiter untersucht werden.

Frühere Studien resultierten in gegenteiligen Ergebnissen in Bezug auf die kumulative Anzahl an Infektionskrankheiten in der Kindheit sowie des Alters, in dem diese auftraten (22, 23). Wir beobachteten einen Zusammenhang zwischen der kumulativen Anzahl, jedoch nicht mit der jeweiligen Infektion, und dem MS-Risiko. Die Mehrheit der StERKE-Teilnehmenden wurde vor der Einführung der Standardimpfungen gegen die untersuchten Infektionskrankheiten geboren (24) und war dementsprechend den mit einer Infektion verbundenen Risiken ausgesetzt. In Anbetracht der – trotz der Impfbemühungen – steigenden Prävalenz der MS (1) könnten künftige Studien, die jüngere, vorwiegend geimpfte Personen oder sogar Geburtskohorten einschließen, dazu beitragen, die Relevanz der Ergebnisse einzuordnen. Unsere Ergebnisse stehen zusätzlich in Kontrast zur Hygienehypothese (25). Hierbei bleibt zu berücksichtigen, dass die Wirkung von Infektionen im Kindesalter vom Alter bei Infektion oder der Art des Erregers abhängen könnte. Für MS fehlen bislang eindeutige Belege für die Hygienehypothese (26).

Wir beobachteten einen Zusammenhang zwischen der kumulativen Anzahl von belastenden Lebensereignissen (BLE) und einem erhöhten MS-Risiko. Stress ist ein häufiger Risikofaktor für chronische, nichtübertragbare Erkrankungen (NCD) und wird mit der Entwicklung psychischer und somatischer Krankheiten in Verbindung gebracht (27). Es hat sich gezeigt, dass BLE eine direkte Wirkung auf das Immunsystem haben (28). Die spezifischen immunmodulierenden Mechanismen, die diesem Zusammenhang zugrunde liegen, müssen noch erforscht werden.

Unsere Ergebnisse zu KA in der Jugend bestätigen die Resultate einer großen, multi-regionalen Studie, in der ein inverser Zusammenhang zwischen KA in der Jugend

und dem MS-Risiko beobachtet wurde (29). KA trägt zur Regulierung des Körpergewichts bei und hat eine entzündungshemmende Wirkung, die bei der Entwicklung chronischer, inflammatorischer Krankheiten wie MS bedeutsam sein könnte (30). Dies spiegelt sich auch in unseren Ergebnissen zum Zusammenhang zwischen Übergewicht und Adipositas im Alter von 18 Jahren und MS wider, die einen erhöhten BMI in der Jugend als Risikofaktor für MS bestätigen (31).

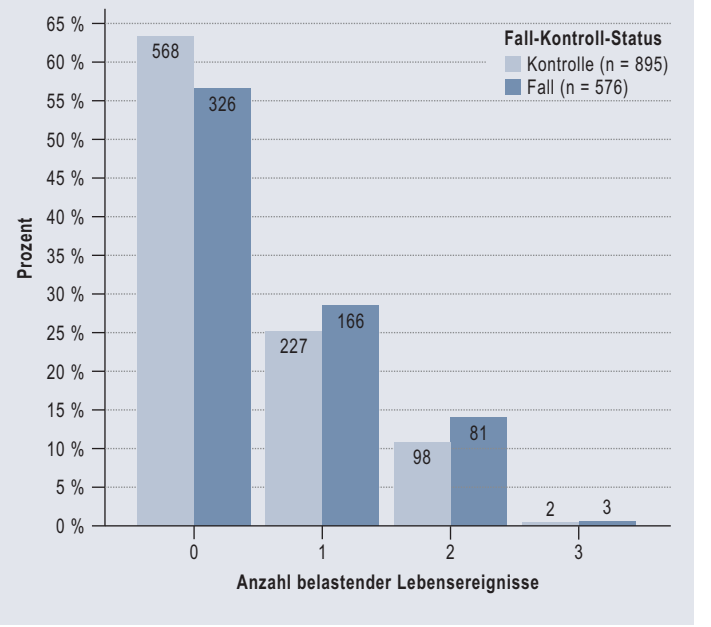
Die Exposition gegenüber Tabakrauch führt zu einer Reizung der Lunge mit erhöhter proinflammatorischer Zellaktivierung und Veränderung von Proteinen hin zu einer autoantigenen Aktivierung, was wiederum mit dem MS-Risiko in Verbindung gebracht werden kann (11, 32). In unserer Studie fanden wir einen schwachen Zusammenhang zwischen Rauchen und MS. Während die Mehrzahl der Beobachtungsstudien eine Assoziation zwischen Rauchen und dem MS-Risiko beobachtete (11), konnte eine kürzlich veröffentlichte Übersichtsarbeit, in der Mendel'sche Randomisierungsstudien zusammengefasst wurden, keine kausale Wirkung zeigen (9).

Unsere Studie weist zahlreiche Stärken auf. So konnten wir eine große Stichprobe untersuchen und qualitativ hochwertige Daten verwenden, die im Rahmen der umfangreichen Datenerhebung der NAKO-Basiserhebung gesammelt wurden. Das stringente Rekrutierungsmanagement in der StERKE-Studie mit bis zu drei Erinnerungsschreiben pro teilnehmender Person hat zusätzlich zu einer hohen Rücklaufquote geführt (77 % bei den Fällen, 60 % bei den Kontrollen).

Dennoch weist unsere Studie auch Schwächen auf. Bei der Mehrzahl der Fälle handelt es sich um prävalente Fälle, was gemeinhin als anfällig für einen Survival Bias angesehen wird (33). Um dies zu berücksichtigen, haben wir die Schätzer unserer Hauptanalyse dahingehend geprüft, dass wir zunächst nur inzidente Fälle und deren Kontrollen in unsere Analyse einschlossen, um die Stichprobe anschließend sukzessive um Untergruppen von Matchingsets mit zunehmender Intervalllänge zwischen Diagnose und Rekrutierung zu erweitern. Hierbei konnten wir keinen Trend in den Schätzern beobachten *(eSupplement-Tabelle 3)*. Obwohl dies kein formaler Beweis ist, halten wir unsere Ergebnisse dennoch für ausreichend valide. Zudem ist die Überlebensrate von Personen mit MS nach der Diagnose hoch, im Gegensatz zur Überlebensrate bei anderen Erkrankungen wie zum Beispiel manchen Krebsarten.

Unsere Analysen basieren auf selbstberichteten ärztlich gestellten Diagnosen, wodurch es zu einer möglichen Fehlklassifizierung gekommen sein könnte. Wir gehen jedoch davon aus, dass die Selbstauskunft angesichts der psychologischen Auswirkungen, die mit einer solchen Diagnose einhergehen, zuverlässig ist. Zur Plausibilitätsprüfung führten wir eine Subgruppenanalyse durch, die nur Teilnehmende mit schubförmig remittierender multipler Sklerose (RRMS) umfasste, die die Behandlung mit MS-spezifischer Medikation oder einer Immuntherapie berichteten. Die Ergebnisse waren den Resultaten der Gesamtheit aller Fälle sehr ähnlich *(eSupplement-Tabelle 4)*. Auch wenn wir die Einschränkung der selbstberichteten MS-Diagnose anerkennen, betrachten wir zusätzliche Angaben zur MS-Verlaufsform

Grafik 2



Verteilung der kumulativen Anzahl von schwerwiegenden belastenden Lebensereignissen (darunter: Tod des Partners, Tod einer nahestehenden Person [außer Partner], schwerwiegende Erkrankung einer nahestehenden Person) der StERKE-Teilnehmenden vor Diagnose der multiplen Sklerose nach Fall-Kontroll-Status, Deutschland, 2021–2022

oder zur MS-spezifischen Medikation als klaren Hinweis auf eine valide Diagnose. Darüber hinaus könnten Menschen mit chronischen Krankheiten ein größeres Interesse an der Teilnahme an einer Studie haben, die darauf abzielt, mögliche Krankheitsursachen zu untersuchen. In Anbetracht der häufigen Multimorbidität von Menschen mit MS könnten diese in der NAKO überrepräsentiert sein.

Eine weitere Schwäche unserer Studie stellt die Untersuchung des Zusammenhangs zwischen einer EBV-Infektion und MS durch die Abfrage dar, ob die Teilnehmenden jemals an infektiöser Mononukleose erkrankt waren (34). Eine EBV-Infektion in der Kindheit verläuft meist asymptomatisch, sodass es hier zu einem Underreporting der Häufigkeit einer EBV-Infektion gekommen sein könnte. Dies könnte das vergleichsweise niedrige OR von 3,05 im Vergleich zu anderen Studien erklären (10, 19).

Da die meisten in dieser Studie untersuchten Faktoren Ereignisse betreffen, die durchschnittlich 40–50 Jahre zurückliegen, könnte es zu einem zwischen Fällen und Kontrollen differenziellen Recall Bias gekommen sein, da sich Personen mit MS ihre Erkrankung durch potenzielle Risikofaktoren erklären und sich demnach besser an zurückliegende Ereignisse erinnern. Dies könnte auf die Faktoren Rauchen, im Freien verbrachte Zeit und vor allem Infektionserkankungen in der Kindheit zutreffen. Wir gehen jedoch davon aus, dass die Teilnehmenden aufgrund der Eindeutigkeit der untersuchten Faktoren, zum Beispiel schwere BLE, die meisten Fragen angemessen beantwortet haben (35). Ein objektives Maß für Infektionen in der Kindheit wäre ein Antikörpertest zur Bestätigung einer frühen Infektion. Allerdings ermöglicht auch dieser es nicht, den genauen Zeitpunkt der Infektion zu bestimmen, was für die Beurteilung der Auswirkungen auf das heranreifende Immunsystem bedeutsam sein könnte.

Im Rahmen dieser Analysen haben wir zahlreiche potenzielle Risikofaktoren für MS untersucht, die bislang kaum berücksichtigt

Tabelle 3

Bedingte logistische Regression zur Assoziation zwischen Familienvorgeschichte, pränatalen sowie in der Kindheit, Jugend und im Erwachsenenalter auftretenden Faktoren und multipler Sklerose, Deutschland, 2021–2022

	bedingte logistische Regression*1 (576 Fälle and 895 Kontrollen)	
	OR	[95-%-KI]
Familienvorgeschichte und pränatale Faktoren		
MS in der Familienvorgeschichte		
kein Familienmitglied mit MS	Ref.	
Familienmitglied ersten oder zweiten Grades mit MS	7,08	[3,90; 12,86]
Anzahl älterer Geschwister (pro zusätzlichem Geschwisterkind) (kontinuierlich)	0,85	[0,77; 0,95]
mütterliches Alter (pro Jahr) bei Geburt des Teilnehmenden (kontinuierlich)	1,03	[1,00; 1,05]
Kinderkrankheiten		
Anzahl Infektionen im Kindesalter (pro zusätzlicher Infektion) vor dem 18. Lebensjahr*2,*3 (kontinuierlich)	1,14	[1,03; 1,25]
EBV-Infektion*2		
nein	Ref.	
ja	3,05	[1,80; 5,16]
Lebensstilfaktoren im Jugendalter		
körperliche Aktivität (pro Levelanstieg) im Teenageralter (13–19 Jahre) (kontinuierlich)	0,82	[0,71; 0,95]
BMI im Alter von 18 Jahren (kg/m²)*4		
Untergewicht (<18,5)	0,86	[0,64; 1,16]
Normalgewicht (18,5 bis < 25)	Ref.	
Übergewicht (25 bis < 30)	1,73	[1,22; 2,44]
Adipositas (≥30)	2,29	[1,18; 4,46]
weitere Faktoren		
Anzahl belastender Lebensereignisse (pro zusätzlichem Ereignis)*2,*5 (kontinuierlich)	1,25	[1,06; 1,48]
jemals geraucht*1,*2,*4		
nein	Ref.	
ja	1,19	[0,99; 1,43]

*1 finales Modell nach Backward Selection mit p = 0,10 als Selektionsgrenze; Nagelkerke's R^2 = 0,142
*2 Fälle: vor Alter bei Diagnose; Kontrollen: vor Alter bei Diagnose des gematchten Falls/medianes Alter bei Diagnose der gematchten Fälle, wenn >1 Fall pro Matchingset
*3 darunter: Windpocken, Mumps, Röteln, Pertussis und Masern
*4 Die ORs für BMI im Alter von 18 Jahren und Rauchen wurden auf Grundlage des ursprünglichen Matchings mit allen Fällen (n = 746) und Kontrollen (n = 1492) unabhängig von ihrer Teilnahme an der StERKE-Studie berechnet, da die Daten zum BMI und Rauchen aus der NAKO-Basiserhebung stammen.
*5 darunter: Tod des Partners, Tod einer nahestehenden Person (außer Partner), schwerwiegende Erkrankung einer nahestehenden Person
BMI, Body-Mass-Index; KI, Konfidenzinterval; EBV, Epstein-Barr-Virus; MS, multiple Sklerose; OR, Odds Ratio

wurden. Wir fanden Assoziationen zwischen der kumulativen Anzahl von Infektionen in der Kindheit sowie schweren BLE, einem höheren Alter Erstgebärender, einem geringeren Maß an KA in der Jugend und MS. Darüber hinaus bestätigten wir bekannte Risikofaktoren für MS, darunter eine MS-Familiengeschichte, eine EBV-Infektion, Übergewicht und Adipositas in der Kindheit und Jugend sowie Rauchen. Diese Ergebnisse tragen zur Evidenzbasis bestehender Präventionsmaßnahmen für andere nichtübertragbare Krankheiten (NCDs), wie zum Beispiel Herz-Kreislauf-Erkrankungen oder Infektionskrankheiten bei Kindern, bei und legen den Schluss nahe, dass diese ebenfalls im MS-Kontext nützlich sein könnten. Impfkampagnen, Rauchentwöhnungsprogramme und Initiativen zur Förderung von KA und gesunden Essgewohnheiten zur Vermeidung von Übergewicht und Adipositas könnten vielversprechende Strategien in der MS-Prävention sein. Darüber hinaus können unsere Ergebnisse als Grundlage für weitere Studien dienen, zum Beispiel für die Untersuchung einer Dosis-Wirkungs-Beziehung zwischen den neu identifizierten Risikofaktoren und der Krankheitsschwere der MS sowie des MS-Prodroms durch Berücktigung einer Latenzzeit. Die NAKO Gesundheitstudie bietet viele Möglichkeiten, um

- ausschließlich inzidente MS-Fälle zu untersuchen
- geschlechtsspezifische Analysen durchzuführen
- Sekundärdaten zur Bestätigung der selbstberichteten MS-Diagnose zu nutzen
- Bioproben zu verwenden, zum Beispiel zum Nachweis von Antikörpern gegen Infektionskrankheiten.

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Ethik
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Zusatzmaterial
vollständige Literatur inkl. eLiteratur, eMethodenteil, eTabellen, eSupplement:
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Zusatzmaterial zu dem Beitrag

Multiple Sklerose: Familienvorgeschichte, Lebensstil und gesundheitliche Faktoren im Kindes- und Jugendalter

Ergebnisse einer in die NAKO Gesundheitsstudie eingebetteten Fall-Kontroll-Studie

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eMETHODENTEIL

Studiendesign und Stichprobe

Diese Analysen basieren auf Daten der StERKE-Studie (StERKE, Studie zum Einfluss von Risikofaktoren auf den Krankheitsverlauf und die Entstehung der Multiplen Sklerose) und der NAKO Gesundheitsstudie.

Die NAKO Gesundheitsstudie ist eine große bevölkerungsbasierte Kohortenstudie, die 18 Studienregionen in Deutschland umfasst. Die Basisuntersuchung wurde zwischen 2014 und 2019 mit insgesamt 205 053 Teilnehmenden (103 471 Frauen und 101 582 Männer) im Alter von 19–74 Jahren durchgeführt, wobei der Anteil der 40- bis 74-Jährigen im Vergleich zu den 19- bis 39-Jährigen höher ausfiel. Eine detailliertere Beschreibung des Designs der NAKO ist an anderer Stelle zu finden (16). Die StERKE-Studie ist eine in die NAKO eingebettete Fall-Kontroll-Studie, die zwischen November 2021 und März 2022 in 16 der 18 NAKO-Studienzentren durchgeführt wurde. Personen, die bei der fragebogenbasierten Zwischenerhebung – im Mittel zwei Jahre nach der Basiserhebung – oder bei der ersten Nachuntersuchung bis zur Rekrutierung von StERKE eine inzidente MS-Diagnose oder bei der NAKO-Basisbefragung eine prävalente arztbasierte MS-Diagnose angaben, wurden als Fälle definiert. Bei den Kontrollen handelte es sich um zufällig ausgewählte NAKO-Teilnehmende ohne MS, die basierend auf ihrem Geburtsjahr, ihrem Geschlecht und dem Studienzentrum individuell einem Fall zugeordnet wurden (Matching-Verhältnis 2 : 1).

Im Rahmen der Rekrutierung wurden die ausgewählten und infrage kommenden Personen (746 Fälle, 1 492 Kontrollen) entweder per Post oder per E-Mail kontaktiert, je nachdem, ob sie bei der NAKO-Basiserhebung eine E-Mail-Adresse angegeben hatten. Die schriftliche Einverständniserklärung zur Teilnahme an der StERKE-Studie wurde vor Beginn der Studie von allen Teilnehmenden eingeholt. Die Ethikkommission der Ärztekammer Hamburg erteilte die Genehmigung zur Durchführung der Studie (PV7292). Bei ausbleibender Response wurden die Teilnehmenden im Verlauf der Studie bis zu dreimal an ihre Teilnahme erinnert. Der in der StERKE-Studie verwendete Fragebogen enthielt Fragen

- zur medizinischen Vorgeschichte der Teilnehmenden und zur Familienvorgeschichte
- zu belastenden Lebensereignissen (BLE)
- zum Passivrauchen in der Kindheit und Jugend
- zur Dauer der im Freien verbrachten Zeit und zur körperlichen Aktivität im Teenageralter.

Den Personen der Gruppe „Fälle“ wurden zusätzlich Fragen zu ihrer Krankheit, zu den mit der Krankheit verbundenen Beeinträchtigungen sowie zu Medikamenten und Immuntherapien gestellt.

Outcomes

Die *eSupplement-Tabelle 1* gibt einen Überblick über die Expositionsvariablen und Kovariaten, die in die Analysen einbezogen wurden. Soziodemografische Faktoren, der Raucherstatus und der Body-Mass-Index (BMI) im Alter von 18 Jahren wurden bereits während des durch geschultes Studienpersonal durchgeführten Interviews oder anhand eines Touchscreen-Fragebogens, den die Teilnehmenden ausfüllen konnten, im Rahmen der NAKO-Basiserhebung erfasst. Die übrigen Variablen wurden mit Hilfe des StERKE-Fragebogens erhoben.

Der Bildungsgrad wurde basierend auf der Internationalen Standardklassifikation des Bildungswesens (ISCED 97) in die Kategorien niedriger (ISCED-Stufe 0–2), mittlerer (ISCED-Stufe 3/4) und hoher (ISCED-Stufe 5/6) Bildungsgrad eingeteilt (e1). Der Umfang körperlicher Aktivität im Teenageralter wurde aus zwei Fragen zu leichter und intensiver körperlicher Aktivität abgeleitet, die auf dem Godin Leisure-Time Exercise Questionnaire (e2) basieren, und in folgende Kategorien eingeteilt: sehr geringe, geringe, mittlere und hohe körperliche Aktivität *(eTabelle 2)*. Der BMI im Alter von 18 Jahren wurde anhand des selbst angegebenen Gewichts in diesem Alter und der gemessenen Größe berechnet und in folgende Kategorien eingeteilt: Untergewicht ($< 18{,}5\ kg/m^2$), Normalgewicht ($18{,}5$ bis $< 25\ kg/m^2$), Übergewicht (25 bis $< 30\ kg/m^2$) und Adipositas ($\geq 30\ kg/m^2$). Die Fragen zu schweren BLE basierten auf Fragen aus dem „Gesundheitsfragebogen 18–64 Jahre” (Studie zur Gesundheit Erwachsener in Deutschland – DEGS) des Robert Koch-Instituts (e3). Die Angaben zu durchgemachten Infektionen in der Kindheit waren selbstberichtet. Die Teilnehmenden wurden gebeten, das Jahr der jeweiligen Infektion anzugeben und ob die jeweilige Infektion vor oder nach dem 18. Lebensjahr aufgetreten war. Aus den Einzelangaben wurde die kumulative Anzahl der Infektionen berechnet und als Summenwert in die anschließenden Analysen einbezogen. Die Teilnehmenden wurden in den Analysen nur dann als exponiert betrachtet, wenn die Exposition vor der MS-Diagnose (bei Fällen) oder vor dem Alter bei MS-Diagnose des gematchten Falls (bei Kontrollen) auftrat.

Statistische Analysen

Soziodemografische Faktoren und Expositionsvariablen wurden deskriptiv nach Fall-Kontroll-Status dargestellt. Die Darstellung der MS-spezifischen Informationen wie das Alter bei MS Diagnose, das Alter bei MS-Manifestation – definiert als das Auftreten erster Symptome-, die MS-Verlaufsform und die MS-spezifische Medikation erfolgte stratifiziert nach Geschlecht.

Der Anteil fehlender Werte unter den Prädiktorvariablen reichte von 0–28 % in der Gruppe der Fälle und von 0–27 % in der Gruppe der Kontrollen *(eTabelle 1)*. Informationen zum Umgang mit fehlenden Werten sind in der *eSupplement-Tabelle 2* zu finden.

Wir führten eine bedingte logistische Regression durch, um den Zusammenhang zwischen den oben beschriebenen Expositionsvariablen und dem Auftreten einer MS zu untersuchen. Die Ergebnisse werden als Odds Ratios (OR) mit den entsprechenden 95-%-Konfidenzintervallen dargestellt. Das Outcome wurde definiert als die Selbstangabe einer ärztlich gestellten MS-Diagnose. Teilnehmende der Gruppe „Fälle“ und Teilnehmende der Gruppe „Kontrollen“ mit identischen Werten der Matching-Variablen wurden nach der in Neuhäuser und Becher (e4) beschriebenen Methode zu einem gemeinsamen Matching-Set zusammengefasst. Sofern für einen Fall keine Kontrolle desselben Geschlechts, Studienzentrums und Geburtsjahres verfügbar war, wurde die Altersbedingung auf ±2 Jahre gelockert, was dazu führte, dass jedem Fall mindestens eine Kontrolle zugeordnet werden konnte. Die mittlere absolute Differenz der Geburtsjahre in den Matching-Sets betrug 0,2 Jahre. In 75 % der Matching-Sets wiesen Fälle und Kontrollen das gleiche Geburtsjahr auf. Für Variablen, die bereits in der NAKO-Basisuntersuchung

erhoben wurden, das heißt BMI im Alter von 18 Jahren und Raucherstatus, wurden die ORs auf der Grundlage eines separaten Modells mit allen für die StERKE-Studie infrage kommenden 746 Fällen und 1 492 Kontrollen berechnet, um die Genauigkeit der Schätzungen zu erhöhen. Das Gesamtmodell umfasste alle zuvor beschriebenen Expositionsvariablen und Kovariaten. Das finale Modell wurde durch Backward Selection mit einer Selektionsgrenze von $p = 0{,}10$ ermittelt.

Um einen durch den Einschluss prävalenter Fälle potenziell verursachten Survival Bias zu berücksichtigen, führten wir fünf Subgruppenanalysen durch: Sie umfassten zunächst

- nur inzidente Fälle und anschließend
- Gruppen inzidenter Fälle in Kombination mit verschiedenen Unterstichproben prävalenter Fälle entsprechend der Krankheitsdauer (Zeit zwischen MS-Diagnose und der NAKO-Basisuntersuchung von 2, 5, 10 beziehungsweise 20 Jahren).

In weiteren Analysen untersuchten wir:

- nur Fälle mit schubförmig remittierender MS, die über die Einnahme MS-spezifischer Medikamente berichteten oder in der Vergangenheit eine Immuntherapie erhalten hatten.

Darüber hinaus wurde eine nach Geschlecht stratifizierte Analyse durchgeführt.

Für alle Subgruppenanalysen wurde das finale Modell verwendet.

Alle statistischen Analysen wurden mit R Version 4.3.1 (2023–06–16) (17) unter Verwendung der Pakete sjPlot (e5), survival (e6), tidyverse (e7) durchgeführt.

eTabelle 1

Charakteristika der StERKE-Teilnehmenden nach Geschlecht und Fall-Kontroll-Status, Deutschland, 2021–2022

	Frauen		Männer	
Variable	**Kontrollen n = 638**	**Fälle n = 396**	**Kontrollen n = 257**	**Fälle n = 180**
Geburtsjahr				
< 1955	86 (13,5 %)	51 (12,9 %)	44 (17,1 %)	25 (13,9 %)
1955–1964	178 (27,9 %)	110 (27,8 %)	90 (35,0 %)	56 (31,1 %)
1965–1974	229 (35,9 %)	137 (34,6 %)	68 (26,5 %)	56 (31,1 %)
1975–1984	76 (11,9 %)	57 (14,4 %)	33 (12,8 %)	27 (15,0 %)
≥ 1985	69 (10,8 %)	41 (10,4 %)	22 (8,6 %)	16 (8,9 %)
Alter (zur NAKO-Basiserhebung) (Mittelwert [SD])	51 (11,0)	49 (10,9)	52 (11,3)	49 (11,1)
Bildungsgrad				
niedrig	9 (1,5 %)	2 (0,6 %)	2 (0,8 %)	1 (0,6 %)
mittel	457 (76,9 %)	286 (79,2 %)	205 (85,4 %)	131 (81,9 %)
hoch	128 (21,5 %)	73 (20,2 %)	33 (13,8 %)	28 (17,5 %)
unbekannt/fehlender Wert	44	35	17	20
MS in der Familienvorgeschichte				
kein Familienmitglied mit MS	624 (97,8 %)	358 (90,4 %)	253 (98,4 %)	160 (88,9 %)
Familienmitglied ersten oder zweiten Grades mit MS	14 (2,2 %)	38 (9,6 %)	4 (1,6 %)	20 (11,1 %)
Anzahl älterer Geschwister				
keine	243 (38,1 %)	166 (41,9 %)	88 (34,2 %)	74 (41,1 %)
1 älteres Geschwisterkind	229 (35,9 %)	143 (36,1 %)	92 (35,8 %)	61 (33,9 %)
≥ 2 ältere Geschwisterkinder	166 (26,0 %)	87 (22,0 %)	77 (30,0 %)	45 (25,0 %)
mütterliches Alter bei Geburt des Teilnehmenden (Mittelwert [SD])	27 (5,7)	28 (5,9)	27 (5,6)	27 (5,0)
Mutter rauchte während der Schwangerschaft				
nein	515 (91,2 %)	331 (93,8 %)	212 (93,8 %)	146 (91,8 %)
ja	50 (8,8 %)	22 (6,2 %)	14 (6,2 %)	13 (8,2 %)
unbekannt/fehlender Wert	73	43	31	21
Vater rauchte während der Schwangerschaft der Mutter				
nein	302 (61,3 %)	186 (59,0 %)	113 (58,2 %)	85 (57,8 %)
ja	191 (38,7 %)	129 (41,0 %)	81 (41,8 %)	62 (42,2 %)
unbekannt/fehlender Wert	145	81	63	33
Windpockeninfektion*[1]				
nein	93 (16,6 %)	59 (16,9 %)	54 (26,6 %)	36 (23,7 %)
ja	466 (83,4 %)	291 (83,1 %)	149 (73,4 %)	116 (76,3 %)
unbekannt/fehlender Wert	79	46	54	28
Mumpsinfektion*[1]				
nein	277 (53,5 %)	162 (49,5 %)	87 (46,0 %)	65 (44,5 %)
ja	241 (46,5 %)	165 (50,5 %)	102 (54,0 %)	81 (55,5 %)
unbekannt/fehlender Wert	120	69	68	34
Rötelninfektion*[1]				
nein	298 (61,3 %)	172 (56,0 %)	111 (63,4 %)	74 (58,3 %)
ja	188 (38,7 %)	135 (44,0 %)	64 (36,6 %)	53 (41,7 %)
unbekannt/fehlender Wert	152	89	82	53

Pertussisinfektion*1				
nein	396 (79,0 %)	242 (76,3 %)	147 (75,8 %)	103 (76,9 %)
ja	105 (21,0 %)	75 (23,7 %)	47 (24,2 %)	31 (23,1 %)
unbekannt/fehlender Wert	137	79	63	46
Maserninfektion*1				
nein	235 (45,8 %)	141 (42,6 %)	78 (40,8 %)	57 (38,3 %)
ja	278 (54,2 %)	190 (57,4 %)	113 (59,2 %)	92 (61,7 %)
unbekannt/fehlender Wert	125	65	66	31
EBV-Infektion*1				
nein	530 (96,0 %)	278 (86,9 %)	206 (97,2 %)	142 (94,7 %)
ja	22 (4,0 %)	42 (13,1 %)	6 (2,8 %)	8 (5,3 %)
unbekannt/fehlender Wert	86	76	45	30
Mutter und/oder Vater rauchten in der Kindheit/Jugend des Teilnehmenden (0–18 Jahre)				
nein	290 (46,7 %)	176 (45,2 %)	111 (44,8 %)	79 (45,4 %)
ja	331 (53,3 %)	213 (54,8 %)	137 (55,2 %)	95 (54,6 %)
unbekannt/fehlender Wert	17	7	9	6
im Freien verbrachte Zeit in der Kindheit und Jugend (0–18 Jahre)				
keine bis einige Stunden/Monat	45 (7,1 %)	19 (4,8 %)	16 (6,2 %)	7 (3,9 %)
einige Stunden/Woche	112 (17,6 %)	73 (18,4 %)	27 (10,5 %)	30 (16,7 %)
einige Stunden/Tag	481 (75,4 %)	304 (76,8 %)	214 (83,3 %)	143 (79,4 %)
körperliche Aktivität in der Jugend (13–19 Jahre)				
sehr niedrig	19 (3,0 %)	21 (5,3 %)	2 (0,8 %)	4 (2,2 %)
niedrig	76 (11,9 %)	60 (15,2 %)	17 (6,6 %)	12 (6,7 %)
mittel	223 (35,0 %)	134 (33,8 %)	60 (23,3 %)	56 (31,1 %)
hoch	320 (50,2 %)	181 (45,7 %)	178 (69,3 %)	108 (60,0 %)
BMI im Alter von 18 Jahren (kg/m²)*2				
Untergewicht (< 18,5)	161 (22,9 %)	61 (16,6 %)	24 (6,6 %)	14 (8,9 %)
Normalgewicht (18,5 bis < 25)	485 (68,9 %)	251 (68,2 %)	296 (81,1 %)	113 (71,5 %)
Übergewicht (25 bis < 30)	45 (6,4 %)	44 (12,0 %)	40 (11,0 %)	24 (15,2 %)
Adipositas (≥ 30)	13 (1,8 %)	12 (3,3 %)	5 (1,4 %)	7 (4,4 %)
fehlender Wert	310	139	113	81
belastende Lebensereignisse*1				
Tod des Partners	15 (2,3 %)	13 (3,9 %)	2 (0,8 %)	3 (1,7 %)
Tod einer nahestehenden Person (außer Partner)	185 (29,0 %)	144 (36,4 %)	69 (26,8 %)	52 (28,9 %)
schwerwiegende Erkrankung einer nahestehenden Person	126 (19,7 %)	91 (23,0 %)	32 (12,5 %)	34 (18,9 %)
mindestens eines der oben genannten Ereignisse	248 (38,9 %)	183 (46,2 %)	79 (30,7 %)	67 (37,2 %)
eigene schwere Erkrankung (außer MS)*1				
nein	598 (93,7 %)	362 (91,4 %)	244 (94,9 %)	167 (92,8 %)
ja	40 (6,3 %)	34 (8,6 %)	13 (5,1 %)	13 (7,2 %)
jemals geraucht*1,*2				
nein	496 (50,8 %)	234 (48,1 %)	205 (44,2 %)	82 (35,8 %)
ja	480 (49,2 %)	252 (51,9 %)	259 (55,8 %)	147 (64,2 %)
fehlender Wert	38	21	14	10

*1 Fälle: Vor Alter bei Diagnose; Kontrollen: Vor Alter bei Diagnose des gematchten Falls/medianes Alter bei Diagnose der gematchten Fälle, wenn > 1 Fall pro Matchingset
*2 Verteilung aus der Gesamtzahl der in Frage kommenden 746 Fälle und 1 492 Kontrollen
BMI, Body Mass Index; EBV, Epstein-Barr-Virus; MS, multiple Sklerose; NAKO, NAKO Gesundheitsstudie; SD, Standardabweichung

eTabelle 2

Berechnung des Levels körperlicher Aktivität basierend auf der Häufigkeit leichter und intensiver körperlicher Aktivität im Teenageralter (13–19 Jahre)

		intensive körperliche Aktivität			
		nie	< 1-mal/Woche	1- bis 2-mal/Woche	≥ 3-mal/Woche
leichte körperliche Aktivität	nie	sehr niedrig	sehr niedrig	mittel	hoch
	< 1-mal/Woche	sehr niedrig	niedrig	mittel	hoch
	1- bis 2-mal/Woche	niedrig	mittel	mittel	hoch
	≥ 3-mal/Woche	niedrig	mittel	hoch	hoch

eSupplement

Family history, lifestyle and health factors in childhood and adolescence, and multiple sclerosis: Results from a case-control study nested within the German National Cohort

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eSupplement Table 1 Overview of analysis variables by category and their respective data basis

	Variables and covariates	Data basis
Sociodemographic factors	Sex	NAKO
	Birth year	NAKO
	Education	NAKO
Exposures	Family member with an MS diagnosis	StERKE
	Number of older siblings	StERKE
	Maternal age at participant's birth	StERKE
	Maternal/paternal smoking during pregnancy with the participant	StERKE
	Common childhood infections (including: Chickenpox, mumps, pertussis, rubella, measles, EBV infection)	StERKE
	Cumulative number of common childhood infections (including: Chickenpox, mumps, pertussis, rubella, measles)	StERKE
	Parental smoking during participant's childhood/adolescence (0-18 years)	StERKE
	Time spent outdoors during childhood/adolescence (0-18 years)	StERKE
	Physical activity during teenage years (13-19 years)	StERKE
	BMI at the age of 18 years (kg/m²)	NAKO
	Major stressful life events (including: Death of the partner, death of a close person (other than partner), serious illness of a close person, own serious illness (other than MS))	StERKE
	Cumulative number of major stressful life events (including: Death of the partner, death of a close person (other than partner), serious illness of a close person)	StERKE
	Smoking	NAKO

BMI = Body Mass Index; EBV = Epstein-Barr-Virus; MS = Multiple Sclerosis; NAKO = German National Cohort; StERKE = Study on risk factors for the occurrence and progression of Multiple Sclerosis

eSupplement Table 2 Methods for handling of missing values

Method for handling of missing values	Variables
Exposure set to "No"	Family history
	Major stressful life events (including: Death of the partner, death of a close person (other than partner), serious illness of a close person, own serious illness (other than MS))
Additional missing category	Common childhood infections (including: Chickenpox, mumps, rubella, pertussis, measles, EBV infection)
	Smoking
	Passive smoking (maternal and/or paternal smoking during pregnancy with the participant as well as during the participant's childhood/adolescence (0-18 years))
	Time spent outdoors during childhood/adolescence (0-18 years)
	BMI at the age of 18 years (kg/m^2)
Imputation of the median	Number of older siblings
	Maternal age at participant's birth
	Cumulative number of common childhood infections (including: Chickenpox, mumps, pertussis, rubella, measles)
	Cumulative number of major stressful life events (including: Death of the partner, death of a close person (other than partner), serious illness of a close person)

BMI = Body Mass Index; EBV = Epstein-Barr-Virus infection; MS = Multiple Sclerosis

eSupplement Table 3 Conditional logistic regression on the association between family history, prenatal, childhood, adolescence and adulthood factors and multiple sclerosis – total sample and subsets by time of multiple sclerosis diagnosis in relation to the NAKO baseline examination

	Conditional logistic regression – sample sizes											
	Incident cases		***Incident cases + diagnosis ≤ 2 years from BL***		***Incident cases + diagnosis ≤ 5 years from BL***		***Incident cases + diagnosis ≤ 10 years from BL***		***Incident cases + diagnosis ≤ 20 years from BL***		***Total sample***	
Source of variable	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
StERKE	88	206	140	314	194	405	312	609	469	790	576	895
NAKO	118	236	180	360	252	504	399	798	609	1218	746	1492

	Conditional Logistic Regression[a]											
	Incident cases		***Incident cases + diagnosis ≤ 2 years from BL***		***Incident cases + diagnosis ≤ 5 years from BL***		***Incident cases + diagnosis ≤ 10 years from BL***		***Incident cases + diagnosis ≤ 20 years from BL***		***Total sample***	
Variable	***OR***	***95% CI***	***OR***	***95% CI***	***OR***	***95% CI***	***OR***	***95% CI***	***OR***	***95% CI***	***OR***	***95% CI***
Family history[b]												
No family member with MS	Ref.		Ref.		Ref.		Ref.		Ref.		Ref.	
1st and 2nd degree family member with MS	7.49	2.45 – 22.93	5.04	1.93 – 13.15	4.32	1.91 – 9.78	5.28	2.60 – 10.74	6.06	3.24 – 11.34	7.02	3.87 – 12.72
Number of older siblings (per additional sibling)[b] (continuous)	0.98	0.72 – 1.32	0.95	0.76 – 1.19	0.96	0.80 – 1.16	0.87	0.75 – 1.01	0.87	0.77 – 0.97	0.85	0.76 – 0.95
Maternal age (per year) at participant's birth[b] (continuous)	1.03	0.97 – 1.09	1.01	0.97 – 1.06	1.02	0.98 – 1.05	1.03	1.00 – 1.06	1.02	1.00 – 1.05	1.03	1.00 – 1.05
Number of common childhood infections (per additional infection) before the age of 18 years[b,c,d] (continuous)	1.01	0.76 – 1.35	1.22	0.99 – 1.49	1.19	1.01 – 1.41	1.14	1.00 – 1.30	1.14	1.03 – 1.26	1.14	1.04 – 1.25
EBV infection[b,c]												
No	Ref.		Ref.		Ref.		Ref.		Ref.		Ref.	
Yes	6.65	1.63 – 27.22	2.72	1.01 – 7.35	2.70	1.18 – 6.17	3.10	1.61 – 5.96	3.14	1.78 – 5.51	3.04	1.79 – 5.14
Physical activity (per level increase) during teenage years (13-19 years)[b] (continuous)	0.47	0.31 – 0.70	0.67	0.51 – 0.89	0.68	0.54 – 0.86	0.73	0.60 – 0.88	0.79	0.67 – 0.93	0.82	0.71 – 0.95
BMI at the age of 18 years (kg/m²)[e]												
Underweight (<18.5)	0.65	0.25 – 1.71	0.74	0.37 – 1.47	1.05	0.61 – 1.78	0.97	0.64 – 1.47	0.77	0.55 – 1.08	0.86	0.64 – 1.16
Normal weight (18.5 - <25)	Ref.		Ref.		Ref.		Ref.		Ref.		Ref.	
Overweight (25 - <30) or Obesity (≥30)	2.28	1.10 – 4.73	2.21	1.20 – 4.06	1.78	1.07 – 2.98	1.81	1.19 – 2.74	1.89	1.33 – 2.69	1.82	1.32 – 2.50

Number of major stressful life events (per additional event)[b,c,f] (continuous)	1.26	0.85 – 1.87	1.27	0.95 – 1.69	1.21	0.95 – 1.55	1.16	0.95 – 1.43	1.21	1.01 – 1.44	1.26	1.06 – 1.49
Ever smoked[c,e]												
No	Ref.		Ref.		Ref.		Ref.		Ref.		Ref.	
Yes	0.99	0.62 – 1.58	1.00	0.68 – 1.46	0.92	0.67 – 1.26	1.07	0.83 – 1.38	1.17	0.96 – 1.44	1.19	0.99 – 1.43
Model fit (Nagelkerke's R^2)	0.270		0.150		0.127		0.124		0.133		0.142	

BL = NAKO baseline examination; BMI = Body Mass Index; CI = Confidence Interval; EBV = Epstein-Barr-Virus; MS = Multiple Sclerosis; NAKO = German National Cohort; OR = Odds Ratio; StERKE = Study on risk factors for the occurrence and progression of Multiple Sclerosis

[a]Final model obtained from backward selection with $p = 0.10$ as selection limit

[b]OR estimate of the respective analysis based on the sample of all participating StERKE participants. Exact numbers can be found above.

[c]Cases: Before age at diagnosis; Controls: Before age at diagnosis of the matched case/median age of diagnosis if >1 case per matching set

[d]Including: Chickenpox, mumps, rubella, pertussis, and measles

[e]OR estimate of the respective analysis based on the sample of all eligible StERKE participants regardless of their participation in the StERKE study, since information on BMI at the age of 18 years and smoking was available from the NAKO baseline examination. Exact numbers can be found above.

[f]Including: Death of the partner, death of a close person (other than partner), serious illness of a close person

eSupplement Table 4 Conditional logistic regression on the association between family history, prenatal, childhood, adolescence, and adulthood factors and multiple sclerosis in cases with relapsing-remitting MS

Variable	*Conditional Logistic Regression*[a] *Cases with RRMS (248 cases and 502 controls)*	
	OR	***95% CI***
Family history		
No family member with MS	Ref.	
1st and 2nd degree family member with MS	5.01	2.09 – 11.99
Number of older siblings (per additional sibling) (continuous)	0.69	0.57 – 0.84
Maternal age (per year) at participant's birth (continuous)	1.03	0.99 – 1.07
Number of common childhood infections (per additional infection) before the age of 18 years[b,c] (continuous)	1.10	0.96 – 1.27
EBV infection[b]		
No	Ref.	
Yes	2.97	1.52 – 5.82
Physical activity (per level increase) during teenage years (13-19 years) (continuous)	0.89	0.71 – 1.12
BMI at the age of 18 years (kg/m²)		
Underweight (<18.5)	0.67	0.38 – 1.19
Normal weight (18.5 - <25)	Ref.	
Overweight (25 - <30)	2.06	1.07 – 3.95
Obesity (≥30)	2.12	0.54 – 8.24
Number of major stressful life events (per additional event)[b,d] (continuous)	1.27	0.98 – 1.64
Ever smoked[b]		
No	Ref.	
Yes	0.82	0.58 – 1.16

BMI = Body Mass Index; CI = Confidence Interval; EBV = Epstein-Barr-Virus; MS = Multiple Sclerosis; OR = Odds Ratio; RRMS = Relapsing-remitting MS

[a]Final model obtained from backward selection with $p = 0.10$ as selection limit; Nagelkerke's $R^2 = 0.155$

[b]Cases: Before age at diagnosis; Controls: Before age at diagnosis of the matched case/median age of diagnosis if >1 case per matching set

[c]Including: Chickenpox, mumps, rubella, pertussis, and measles

[d]Including: Death of the partner, death of a close person (other than partner), serious illness of a close person

eSupplement Table 5 Conditional logistic regression on the association between family history, prenatal, childhood, adolescence, and adulthood factors and multiple sclerosis – stratified by sex

Variable	*Conditional Logistic Regression*[a]			
	Women (396 cases and 638 controls)		*Men (180 cases and 257 controls)*	
	OR	*95% CI*	*OR*	*95% CI*
Family history				
No family member with MS	Ref.		Ref.	
1st and 2nd degree family member with MS	6.18	3.13 – 12.22	10.16	2.80 – 36.89
Number of older siblings (per additional sibling) (continuous)	0.85	0.74 – 0.96	0.87	0.71 – 1.06
Maternal age (per year) at participant's birth (continuous)	1.04	1.01 – 1.07	1.00	0.96 – 1.05
Number of common childhood infections (per additional infection) before the age of 18 years[b,c] (continuous)	1.16	1.03 – 1.30	1.10	0.93 – 1.31
EBV infection[b]				
No	Ref.		Ref.	
Yes	3.40	1.88 – 6.15	2.16	0.60 – 7.79
Physical activity (per level increase) during teenage years (13-19 years) (continuous)	0.81	0.68 – 0.96	0.84	0.62 – 1.14
BMI at the age of 18 years (kg/m²)[d]				
Underweight (<18.5)	0.72	0.52 – 1.01	1.58	0.78 – 3.18
Normal weight (18.5 - <25)	Ref.		Ref.	
Overweight (25 - <30)	1.86	1.20 – 2.87	1.62	0.90 – 2.91
Obesity (≥30)	1.86	0.84 – 4.13	3.78	1.06 – 13.38
Number of major stressful life events (per additional event)[b,e] (continuous)	1.25	1.02 – 1.52	1.29	0.93 – 1.79
Ever smoked[b,d]				
No	Ref.		Ref.	
Yes	1.09	0.88 – 1.37	1.38	0.98 – 1.94
Model fit (Nagelkerke's R²)	0.163		0.148	

BMI = Body Mass Index; CI = Confidence Interval; EBV = Epstein-Barr-Virus; MS = Multiple Sclerosis; NAKO = German National Cohort; OR = Odds Ratio; StERKE = Study on risk factors for the occurrence and progression of Multiple Sclerosis

[a]Final model obtained from backward selection with $p = 0.10$ as selection limit

[b]Cases: Before age at diagnosis; Controls: Before age at diagnosis of the matched case/median age of diagnosis if >1 case per matching set

[c]Including: Chickenpox, mumps, rubella, pertussis, and measles

[d]The OR estimate for BMI at the age of 18 years and smoking were calculated based on the original matching with all cases ($n = 746$, female = 507, male = 239) and controls ($n = 1492$, female =1014, male = 478) regardless of their participation in the StERKE study, since information on BMI and smoking was available from the NAKO baseline examination.

[e]Including: Death of the partner, death of a close person (other than partner), serious illness of a close person

9. SUMMARY in German and English

Im Rahmen dieser Dissertation wurde der Einfluss potenzieller Risikofaktoren auf die Entstehung der Multiplen Sklerose (MS) untersucht. Von besonderem Interesse waren hierbei vor allem Faktoren, die in der (frühen) Kindheit und Jugend auftreten. Grundlage dieser Arbeit bildeten sowohl ein systematisches Review mit Metaanalyse als auch zwei Beobachtungsstudien, die „NAKO Gesundheitsstudie (NAKO)“ und die „Studie zum Einfluss von Risikofaktoren auf den Krankheitsverlauf und die Entstehung der Multiplen Sklerose (StERKE)“.

Die Ergebnisse dieser Arbeit zeigen deutlich die multifaktorielle Ätiologie der MS, die einerseits eine genetische Komponente aufzuweisen, aber auch maßgeblich durch Umwelt- und Lebensstilfaktoren beeinflusst zu sein scheint sowie das sich daraus ableitende Präventionspotenzial. So war es uns möglich, die in der Literatur berichteten Ergebnisse zu bereits etablierten Risikofaktoren wie z.B. einer EBV-Infektion, Übergewicht in der Kindheit und Jugend und einem Vitamin D-Mangel zu reproduzieren. Darüber hinaus konnten wir zahlreiche neue Erkenntnisse in Hinblick auf die MS-Ätiologie generieren. Wir beobachteten Assoziationen zwischen der kumulativen Anzahl häufiger Infektionserkrankungen (Windpocken, Mumps, Röteln, Pertussis und Masern) im Kindesalter, der kumulativen Anzahl belastender Lebensereignisse (Tod des/r Partners/Partnerin, Tod einer nahestehenden Person, schwere Erkrankung einer nahestehenden Person), sowie dem Umstand das Kind einer Erstgebärenden zu sein, die bei der Geburt ≥ 30 Jahre alt war, und einem erhöhten MS-Risiko. Für körperliche Aktivität in der Jugend sowie im Säuglingsalter gestillt worden zu sein beobachteten wir einen protektiven Effekt auf das Risiko, im weiteren Leben an MS zu erkranken.

Diese Arbeit ergänzt die bestehende Literatur dahingeheng, dass i) die MS-Prävention bereits im frühen Kindes- und Jugendalter beginnen sollte, ii) sich diese bedingt durch die Überlappung mit anderen chronischen Erkrankungen in Hinblick auf potenzielle Risikofaktoren, an Präventionsprogrammen, wie z.B. Rauchentwöhnungprogrammen oder aber der Förderung körperlicher Aktivität, für diese Erkrankungen orientieren sollte und iii) es weiterhin einen hohen Bedarf an Studien gibt, die vor allem MS-spezifische Faktoren wie z.B. die Erkrankungsschwere, aber auch die Geschlechtsverteilung in der Prävalenz der MS zwischen Männern und Frauen berücksichtigen.

This dissertation investigated the influence of potential risk factors on the development of multiple sclerosis (MS). Factors that occur in (early) childhood and adolescence are of particular interest. This work was based on a SR-MA and two observational studies, the "German National Cohort (NAKO Gesundheitsstudie, NAKO)" and the "Study on the Influence of Risk Factors on Disease Progression and the Development of Multiple Sclerosis (StERKE)".

The results of this study clearly show the multifactorial aetiology of MS, which on the one hand has a genetic component and on the other hand also appears to be influenced by environmental and lifestyle factors, as well as the resulting potential for prevention. We were able to reproduce the results reported in the literature on already established risk factors such as an EBV infection, obesity in childhood and adolescence, and vitamin D deficiency. In addition, we were able to generate numerous new findings concerning MS aetiology. We observed associations between the cumulative number of common childhood infections (chickenpox, mumps, rubella, pertussis and measles), the cumulative number of stressful life events (death of a partner, death of a close person (other than partner), serious illness of a close person), and being the first-born child of a mother who was ≥ 30 years old at birth and an increased risk of MS. We observed a protective effect of PA during teenage years and having been breastfed on the risk of developing MS later in life.

This work complements the existing literature in that i) MS prevention should begin in early childhood and adolescence, ii) due to the overlap with other chronic diseases in terms of potential risk factors, it should be based on prevention programs, such as smoking cessation programs or the promotion of PA, for these diseases, and iii) there is still a great need for studies that focus on MS-specific factors such as disease severity, but also the sex distribution in the prevalence of MS between men and women.

10. STATEMENT OF OWN CONTRIBUTIONS TO PUBLICATIONS

Holz, A., Riefflin, M., Heesen, C., Riemann-Lorenz, K., Obi, N., & Becher, H. (2022). **Breastfeeding and Risk of Multiple Sclerosis: A Systematic Review and Meta-Analysis of Observational Studies.** *Neuroepidemiology*, 56(6), 391–401. doi: 10.1159/000526895.

- Conceptualization of research idea
- Development and implementation of methodology
- Data collection and analysis
- Visualization and interpretation of results
- Preparation of original manuscript draft

Holz, A., Obi, N., Ahrens, W., Berger, K., Bohn, B., Brenner, H., Fischer, B., Fricke, J., Führer, A., Gastell, S., Greiser, K.H., Harth, V., Heise, J-K., Holleczek, B., Keil, T., Klett-Tammen, C.J., Leitzmann, M., Lieb, W., Meinke-Franze, C., Michels, K.B., Mikolajczyk, R., Nimptsch, K., Peters, A., Pischon, T., Riedel, O., Schikowski, T., Schipf, S., Schmidt, B., Schulze, M.B., Stang, A., Hellwig, K., Riemann-Lorenz, K., Heesen, C., Becher, H. (2024). **Childhood and adolescence factors and multiple sclerosis: results from the German National Cohort (NAKO).** BMC Neurol, 24(1), 123. https://doi.org/10.1186/s12883-024-03620-4.

- Conceptualization of research idea
- Development and implementation of methodology
- Data analysis, visualisation, and interpretation of results
- Preparation of original manuscript draft

Holz, A., Obi, N., Pischon, T., Schulze, M.B., Ahrens, W., Berger, K., Bohn, B., Brenner, H., Emmel, C., Fischer, B., Greiser, K.H., Harth, V., Holleczek, B., Kaaks, R., Karch, A., Katzke, V., Keil, T., Krist, L., Leitzmann, M., Meinke-Franze, C., Michels, K.B.; Nimptsch, K., Peters, A., Riedel, O., Schikowski, T., Schipf, S., Schmidt, B., Thierry, S., Hellwig, K., Riemann-Lorenz, K., Heesen, C., Becher, H. (2025). **The Relation of Multiple Sclerosis to Family History, Lifestyle, and Health Factors in Childhood and Adolescence: Findings of a Case-Control Study Nested Within the German National Cohort (NAKO) study.** Dtsch Arztebl Int. 122: online first. doi: 10.3238/arztebl.m2025.0069.

- Conceptualization, planning, and conduct of the nested case-control study StERKE
- Conceptualization of research idea
- Development and implementation of methodology
- Data analysis, visualisation, and interpretation of results
- Preparation of original manuscript draft

11. ACKNOWLEDGEMENTS

Acknowledgements not shown due to data protection.

12. CURRICULUM VITAE

Curriculum vitae not shown due to data protection.

13. LIST OF PUBLICATIONS

Publications Related to Thesis

Holz, A., Riefflin, M., Heesen, C., Riemann-Lorenz, K., Obi, N. & Becher, H. (2022) **Breastfeeding and Risk of Multiple Sclerosis: A Systematic Review and Meta-Analysis of Observational Studies.** *Neuroepidemiology.* 56(6): 391-401. dOI:10.1159/000526895.

Holz, A., Obi, N., Ahrens, W., Berger, K., Bohn, B., Brenner, H., Fischer, B., Fricke, J., Führer, A., Gastell, S., Greiser, K.H., Harth, V., Heise, J-K., Holleczek, B., Keil, T., Klett-Tammen, C.J., Leitzmann, M., Lieb, W., Meinke-Franze, C., Michels, K.B., Mikolajczyk, R., Nimptsch, K., Peters, A., Pischon, T., Riedel, O., Schikowski, T., Schipf, S., Schmidt, B., Schulze, M.B., Stang, A., Hellwig, K., Riemann-Lorenz, K., Heesen, C., Becher, H. (2024). **Childhood and adolescence factors and multiple sclerosis: results from the German National Cohort (NAKO).** *BMC Neurol.* 24(1), 123. doi: 10.1186/s12883-024-03620-4.

Holz, A., Obi, N., Pischon, T., Schulze, M.B., Ahrens, W., Berger, K., Bohn, B., Brenner, H., Emmel, C., Fischer, B., Greiser, K.H., Harth, V., Holleczek, B., Kaaks, R., Karch, A., Katzke, V., Keil, T., Krist, L., Leitzmann, M., Meinke-Franze, C., Michels, K.B.; Nimptsch, K., Peters, A., Riedel, O., Schikowski, T., Schipf, S., Schmidt, B., Thierry, S., Hellwig, K., Riemann-Lorenz, K., Heesen, C., Becher, H. (2025). **The Relation of Multiple Sclerosis to Family History, Lifestyle, and Health Factors in Childhood and Adolescence: Findings of a Case-Control Study Nested Within the German National Cohort (NAKO) study.** Dtsch Arztebl Int. 122: online first. doi: 10.3238/arztebl.m2025.0069.

Other Publications

Holz, A., Herold, R., Friemert, D., Hartmann, U., Harth, V. & Terschüren, C. (2021). **Datenbrillen am Arbeitsplatz. Informationsdichte am Auge.** *Zentralblatt für Arbeitsmedizin, Arbeitsschutz und Ergonomie.* 71(6): 24-28. doi: 10.1007/s40664-020-00394-7.

Lange-Drenth, L., Willemer, H., Banse, M., Ernst, A., Daubmann, A., Holz, A., Bleich, C., Weg-Remers, S. and Schulz, H. (2025) **Development and effectiveness evaluation of an interactive e-learning environment to enhance digital health literacy in cancer patients: study protocol for a randomized controlled trial.** *Front. Digit. Health* 7:1455143. doi: 10.3389/fdgth.2025.1455143.

Vanella, P., Wiessner, C., Holz, A., Krause, G., Möhl, A., Wiegel, S., Lange, B., Becher, H. (2022) **Pitfalls and solutions in case fatality risk estimation – A multi-country analysis on the effects of demographics, surveillance, time lags between case reports and deaths and healthcare system capacity on COVID-19 CFR estimates.** *Vienna Yearbook of Population Research.* doi:10.1553/populationyearbook2022.res1.4.

14. DECLARATION ON OATH

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe, insbesondere ohne entgeltliche Hilfe von Vermittlungs- und Beratungsdiensten, verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe. Das gilt insbesondere auch für alle Informationen aus Internetquellen.
Soweit beim Verfassen der Dissertation KI-basierte Tools („Chatbots“) verwendet wurden, versichere ich ausdrücklich, den daraus generierten Anteil deutlich kenntlich gemacht zu haben. Die „Stellungnahme des Präsidiums der Deutschen Forschungsgemeinschaft (DFG) zum Einfluss generativer Modelle für die Text- und Bilderstellung auf die Wissenschaften und das Förderhandeln der DFG“ aus September 2023 wurde dabei beachtet.
Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe.
Ich erkläre mich damit einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

Datum:…… Unterschrift: ……