

Original Article

# 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

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## Summary

**Background:** Multiple sclerosis (MS) is a neuroinflammatory disease of presumed autoimmune origin. A combination of genetic susceptibility and exposure to certain environmental and lifestyle factors might trigger the onset of MS. The currently known risk factors include a genetic predisposition, infection with the Epstein–Barr virus (EBV), smoking, and an increased body mass index.

**Methods:** In 2021–22, we carried out a case–control study nested within the German National Cohort (NAKO) to investigate associations of potential risk factors with MS.

**Results:** The subjects included 576 persons with MS (cases) and 895 without MS (controls). Beyond the known risk factors, we observed associations between MS and the cumulative number of common childhood infections (odds ratio (OR) 1.14 per additional infection, 95% confidence interval (CI): [1.03; 1.25]), major stressful life events (SLE) (OR 1.25 per additional event, [1.06; 1.48]), being the firstborn child of a mother aged 30 or older (OR 2.11, [1.08; 4.13]); higher amounts of physical activity in the teenage years were associated with a lower risk of MS (OR 0.82 per unit increase

in activity level, [0.71; 0.95]).

**Conclusion:** We confirmed known risk factors for MS and found associations with a number of new ones, e.g., the cumulative number of common childhood infections. These findings may shed light on the etiology of MS and merit further study.

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**M**ultiple sclerosis (MS) is a neuroinflammatory, presumably autoimmune disease causing disability in early adulthood (1), with a considerable impact on the quality of life of those affected (2).

In recent decades, the prevalence and incidence of MS have increased worldwide (1, 3), while its etiology is still only partly known (4–6). Presumably, exposure to environmental and lifestyle factors in genetically susceptible individuals leads to the manifestation of the disease (7, 8).

The odds ratios (OR) of the previously identified MS-associated risk factors body mass index (BMI), vitamin D deficiency (9), and Epstein–Barr virus (EBV) infection range between 1.14 (BMI) (9) and 3.33 (EBV) (10), indicating small to moderate effect sizes. Moderate to vigorous physical activity (PA) during adulthood has been identified as a protective factor (9). Although the majority of observational studies have shown an association between smoking and an elevated risk of MS (11), a causal

relationship has not yet been confirmed (9).

Factors acting prenatally or in (early) childhood/adolescence—i.e., at a time when the immune system is still very susceptible—may be of particular importance in the development of MS (12). These include, for example:

- Number of older siblings
- Maternal age
- Childhood infections
- Passive smoking
- Physical activity (PA)
- Time spent outdoors

A potential association with such factors has already been shown for other autoimmune diseases such as bronchial asthma (13) and type 1 diabetes (T1D) (14). Apart from molecular and environmental factors, psychological factors are increasingly being

Table 1

Characteristics of StERKE participants by sex and case-control status, Germany, 2021–2022

| Variable                                                                                          | Women               |                  | Men                 |                  |
|---------------------------------------------------------------------------------------------------|---------------------|------------------|---------------------|------------------|
|                                                                                                   | Controls<br>n = 638 | Cases<br>n = 396 | Controls<br>n = 257 | Cases<br>n = 180 |
| Age (at time of NAKO baseline survey) (Mean [SD])                                                 | 51 (11.0)           | 49 (10.9)        | 52 (11.3)           | 49 (11.1)        |
| <b>Family history of MS</b>                                                                       |                     |                  |                     |                  |
| No family member with MS                                                                          | 624 (97.8%)         | 358 (90.4%)      | 253 (98.4%)         | 160 (88.9%)      |
| First- or second-degree family member with MS                                                     | 14 (2.2%)           | 38 (9.6%)        | 4 (1.6%)            | 20 (11.1%)       |
| <b>Number of older siblings</b>                                                                   |                     |                  |                     |                  |
| None                                                                                              | 243 (38.1%)         | 166 (41.9%)      | 88 (34.2%)          | 74 (41.1%)       |
| 1 older sibling                                                                                   | 229 (35.9%)         | 143 (36.1%)      | 92 (35.8%)          | 61 (33.9%)       |
| ≥ 2 older siblings                                                                                | 166 (26.0%)         | 87 (22.0%)       | 77 (30.0%)          | 45 (25.0%)       |
| Maternal age at participant's birth (Mean [SD])                                                   | 27 (5.7)            | 28 (5.9)         | 27 (5.6)            | 27 (5.0)         |
| <b>Parents' smoking during pregnancy</b>                                                          |                     |                  |                     |                  |
| Mother smoked during pregnancy <sup>*1</sup>                                                      | 50 (8.8%)           | 22 (6.2%)        | 14 (6.2%)           | 13 (8.2%)        |
| Father smoked during pregnancy <sup>*1</sup>                                                      | 191 (38.7%)         | 129 (41.0%)      | 81 (41.8%)          | 62 (42.2%)       |
| <b>Childhood infections<sup>*1, *2</sup></b>                                                      |                     |                  |                     |                  |
| Chickenpox                                                                                        | 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%)       |
| Rubella                                                                                           | 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%)       |
| Measles                                                                                           | 278 (54.2%)         | 190 (57.4%)      | 113 (59.2%)         | 92 (61.7%)       |
| EBV infection                                                                                     | 22 (4.0%)           | 42 (13.1%)       | 6 (2.8%)            | 8 (5.3%)         |
| Mother and/or father smoked during participant's childhood/adolescence (0–18 years) <sup>*1</sup> | 331 (53.3%)         | 213 (54.8%)      | 137 (55.2%)         | 95 (54.6%)       |
| <b>Time spent outdoors during childhood and adolescence (0–18 years)</b>                          |                     |                  |                     |                  |
| None to a few hours/month                                                                         | 45 (7.1%)           | 19 (4.8%)        | 16 (6.2%)           | 7 (3.9%)         |
| A few hours/week                                                                                  | 112 (17.6%)         | 73 (18.4%)       | 27 (10.5%)          | 30 (16.7%)       |
| A few hours/day                                                                                   | 481 (75.4%)         | 304 (76.8%)      | 214 (83.3%)         | 143 (79.4%)      |
| <b>Physical activity during adolescence (13–19 years)</b>                                         |                     |                  |                     |                  |
| Very low                                                                                          | 19 (3.0%)           | 21 (5.3%)        | 2 (0.8%)            | 4 (2.2%)         |
| Low                                                                                               | 76 (11.9%)          | 60 (15.2%)       | 17 (6.6%)           | 12 (6.7%)        |
| Moderate                                                                                          | 223 (35.0%)         | 134 (33.8%)      | 60 (23.3%)          | 56 (31.1%)       |
| High                                                                                              | 320 (50.2%)         | 181 (45.7%)      | 178 (69.3%)         | 108 (60.0%)      |
| <b>BMI at the age of 18 years (kg/m<sup>2</sup>)<sup>*1, *3</sup></b>                             |                     |                  |                     |                  |
| Underweight (< 18.5)                                                                              | 161 (22.9%)         | 61 (16.6%)       | 24 (6.6%)           | 14 (8.9%)        |
| Normal weight (18.5 to < 25)                                                                      | 485 (68.9%)         | 251 (68.2%)      | 296 (81.1%)         | 113 (71.5%)      |
| Overweight (25 to < 30)                                                                           | 45 (6.4%)           | 44 (12.0%)       | 40 (11.0%)          | 24 (15.2%)       |
| Obesity (≥ 30)                                                                                    | 13 (1.8%)           | 12 (3.3%)        | 5 (1.4%)            | 7 (4.4%)         |
| <b>Stressful life events<sup>*2</sup></b>                                                         |                     |                  |                     |                  |
| Death of partner                                                                                  | 15 (2.3%)           | 13 (3.9%)        | 2 (0.8%)            | 3 (1.7%)         |
| Death of a close person (other than partner)                                                      | 185 (29.0%)         | 144 (36.4%)      | 69 (26.8%)          | 52 (28.9%)       |
| Serious illness of a close person                                                                 | 126 (19.7%)         | 91 (23.0%)       | 32 (12.5%)          | 34 (18.9%)       |
| Own serious illness (other than MS)                                                               | 40 (6.3%)           | 34 (8.6%)        | 13 (5.1%)           | 13 (7.2%)        |
| Ever smoked <sup>*1, *2, *3</sup>                                                                 | 480 (49.2%)         | 252 (51.9%)      | 259 (55.8%)         | 147 (64.2%)      |

<sup>\*1</sup> Percentages refer exclusively to persons for whom information was available. The data for the categories "No" and "Unknown/missing" can be found in *eTable 1*.

<sup>\*2</sup> Cases: before age at diagnosis; controls: before age at diagnosis of the matched case/median age at diagnosis of the matched cases if > 1 case per matching set

<sup>\*3</sup> Distribution from the totality of eligible persons: 746 cases and 1492 controls

BMI, Body mass index; EBV, Epstein-Barr virus; MS, multiple sclerosis; NAKO, German National Cohort; SD, standard deviation

discussed as potential risk factors for MS. A recently published meta-analysis showed a weak to moderate effect of psychological stressors on the risk of MS (15).

Owing to the limited number of studies on some of these modifiable risk factors for MS, the inconsistent study results, and/or marked methodological heterogeneity, further research on these factors is needed.

To contribute to the understanding of the causes of MS, we conducted a case-control study. Our aim was to identify associations between potential risk factors and the development of MS. The following potential risk factors were investigated:

- Family history of MS
- Infectious diseases in childhood
- Passive smoking
- Number of older siblings
- Maternal age at birth
- Time spent outdoors during childhood and adolescence
- PA during adolescence
- Stressful life events (SLE)
- BMI at the age of 18 years
- Smoking.

## Methods

The analyses are based on data from the baseline survey of the German National Cohort (NAKO) and the StERKE study, a case-control study on the effect of risk factors on the course and onset of MS that is nested within NAKO (16). Cases were defined as persons with a self-reported physician-based MS diagnosis. The controls, randomly selected NAKO participants without MS, were individually matched to the cases (matching ratio 2:1) by birth year, sex, and study center. In the NAKO baseline survey, data was collected by means of an interview, while the StERKE participants completed a questionnaire. In the analyses, participants were considered exposed only if the exposure had occurred before MS diagnosis (for cases) or before the age of the matched case at MS diagnosis (for controls). A conditional logistic regression was performed to assess the association between observed exposure variables and MS onset. Results are displayed as odds ratios (OR) with 95% confidence intervals.

All statistical analyses were performed with R version 4.3.1 (2023-06-16) (17). The methods used are described in detail in the *eMethods* and in *eSupplement Tables 1-5*.

## Results

In total, 576 persons with MS (396 women, 180 men) and 895 controls (638 women, 257 men) participated, corresponding to a response rate of 77.2% for cases and 60.0% for controls.

The study population comprised 70% women and 30% men with a mean age of 50 years. The main characteristics of the StERKE participants and the distribution of exposure variables and covariates by sex and case-control status are shown in *Table 1* and *eTable 1*.

The median age at MS manifestation was 33 and 35 years for women and men, respectively. The median age at diagnosis was 38 years for both sexes. Relapsing-remitting MS (RRMS) was diagnosed in 80% of cases, and 58% had ever received immunotherapy. Among the StERKE participants, 88 (15.3%) were incident cases (*Table 2*).

Table 2

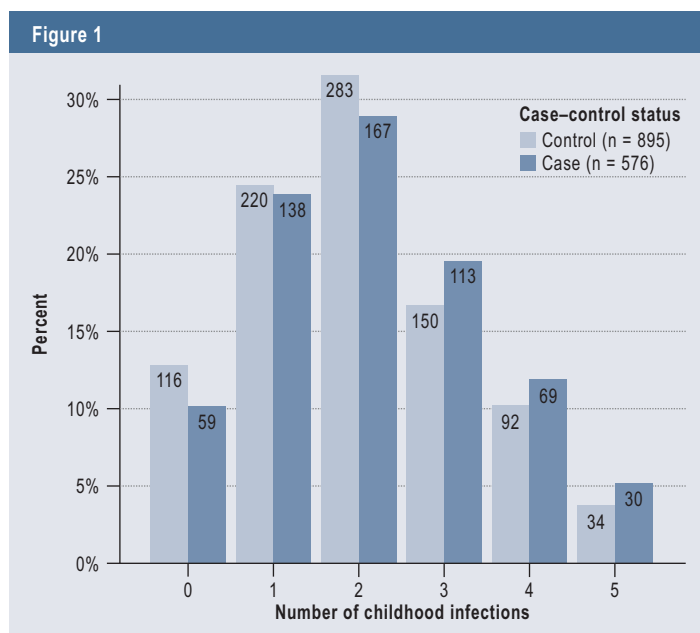
Characteristics of StERKE participants with multiple sclerosis by sex, Germany, 2021-2022

|                                                                              | Women       | Men         |
|------------------------------------------------------------------------------|-------------|-------------|
| Variable                                                                     | n = 396     | n = 180     |
| Age at MS diagnosis (median [IQR])                                           | 38 (29, 47) | 38 (28, 46) |
| Age at MS manifestation (median [IQR])                                       | 33 (26, 42) | 35 (27, 41) |
| Missing                                                                      | 43          | 28          |
| <b>Type of MS</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%)  |
| Unknown                                                                      | 89          | 47          |
| <b>MS-specific medication (up to 7 days before the NAKO baseline survey)</b> |             |             |
| No                                                                           | 219 (65.6%) | 90 (58.4%)  |
| Yes                                                                          | 115 (34.4%) | 64 (41.6%)  |
| No MS at NAKO baseline survey                                                | 62          | 26          |
| <b>Ever received immunotherapy</b>                                           |             |             |
| No                                                                           | 150 (41.7%) | 75 (49.7%)  |
| Yes                                                                          | 210 (58.3%) | 76 (50.3%)  |
| Unknown                                                                      | 36          | 29          |
| <b>Time of MS diagnosis</b>                                                  |             |             |
| Incident case (diagnosis after NAKO baseline survey)                         | 62 (15.7%)  | 26 (14.4%)  |
| Diagnosis ≤ 2 years before NAKO baseline survey                              | 36 (9.1%)   | 16 (8.9%)   |
| 3 to ≤ 5 years before NAKO baseline survey                                   | 35 (8.8%)   | 19 (10.6%)  |
| 6 to ≤ 10 years before NAKO baseline survey                                  | 87 (22.0%)  | 31 (17.2%)  |
| 11 to ≤ 20 years before NAKO baseline survey                                 | 106 (26.8%) | 51 (28.3%)  |
| > 20 years before NAKO baseline survey                                       | 70 (17.7%)  | 37 (20.6%)  |

IQR, Interquartile range; MS, multiple sclerosis; NAKO, German National Cohort

*Figures 1* and *2* show the distribution of the cumulative number of childhood infections and SLE before MS diagnosis, respectively.

*Table 3* summarizes the results of the main analysis. Having a first- or second-degree relative with MS showed an OR of 7.08 [3.90; 12.86] compared with no family member with MS. Maternal age at birth of the participant was associated with MS (OR 1.03 per year [1.00; 1.05]), whereas an inverse association with MS was observed for the number of older siblings (OR 0.85 [0.77; 0.95]). The combination of both factors, having no older siblings and being born to a mother ≥ 30 years of age at delivery, was associated with a higher likelihood of MS (OR 2.11 [1.08; 4.13]). We observed a direct association between the cumulative number of childhood infections (OR 1.14 per additional infection [1.03; 1.25]) and having MS. Persons with the maximum of five such infections had an estimated OR of 1.93 (= 1.14<sup>5</sup>) [1.18; 3.06]) compared with those without any reported infection. Persons who had contracted an EBV infection had a 3.05-fold [1.80; 5.16] likelihood of having MS compared with persons who did not. Further, the higher the PA level during teenage years, the lower the likelihood of having MS (OR 0.82 [0.71; 0.95]). Compared with normal weight, overweight and obesity at the



**Distribution of the cumulative number of childhood infections** (including: chickenpox, mumps, rubella, pertussis, measles) among StERKE participants before multiple sclerosis diagnosis by case-control status, Germany, 2021–2022

age of 18 years were associated with having MS (OR 1.73 [1.22; 2.44] and OR 2.29 [1.18; 4.46], respectively). Moreover, the cumulative number of SLE (OR 1.25 per additional event [1.06; 1.48]) was associated with MS. Finally, we observed a weak association between smoking prior to the diagnosis of MS and MS (OR 1.19 [0.99; 1.43]).

The remaining factors—own serious illness (other than MS) before diagnosis; passive smoking, i.e., parental smoking during pregnancy and in the participant's childhood/adolescence; and time spent outdoors—were not associated with MS. The estimates of the subgroup analysis and the sex-stratified analysis differed only marginally from the results of the final model (*eSupplement Tables 3, 4 and 5*).

## Discussion

This analysis was based on the StERKE study, a case-control study nested within the population-based cohort study NAKO. Our study yielded several novel findings. We found that the cumulative number of childhood infections and SLE are both associated with MS risk. Higher maternal age at first childbirth is known to be associated with various negative consequences for the child (18), and in this study we found an association with MS risk. Recent studies have shown that PA in adulthood is a protective factor against MS (9). The results of our analyses extend this finding to PA during adolescence. Moreover, we confirmed already known risk factors for MS, including a family history of MS (7), an EBV infection (10, 19), and an elevated BMI in childhood/adolescence (9).

Regarding higher maternal age at first childbirth, a smaller study did not observe an association with having MS (20). However, our finding is in line with studies investigating other autoimmune diseases, e.g., T1D (21), although the underlying pathomechanisms need to be further elucidated.

Previous studies yielded conflicting results regarding the cumulative number of childhood infections and the age at which the infectious diseases occurred (22, 23). We observed an association between the cumulative number of childhood infections and the risk of MS, but

this was not the case for the individual diseases. The majority of StERKE participants were born and raised before the introduction of standard vaccinations against childhood infections (24) and were accordingly exposed to the risks associated with contracting an infection. Given the increasing prevalence of MS (1) despite vaccination efforts, future studies including younger, predominantly vaccinated persons or even birth cohorts might help to clarify the relevance of this finding. Our results regarding childhood infections contradict the hygiene hypothesis (25). However, the effect of childhood infections may depend on the age at infection or the type of pathogen. With regard to MS, conclusive evidence for the hygiene hypothesis is lacking to date (26).

We observed an association between the cumulative number of SLE and an increased likelihood of MS. Stress is a common risk factor for chronic, non-communicable diseases (NCD) and has been linked with the development of both mental and somatic illnesses (27). SLE have been shown to have a direct effect on the immune system (28). The specific immune-modulating mechanisms underlying this association remain to be explored.

Our results on PA during adolescence corroborate the results of a large multiregional study reporting an inverse association between PA in adolescence and MS risk (29). PA contributes to the regulation of body weight and has an anti-inflammatory effect, which may be relevant in the development of chronic inflammatory diseases such as MS (30). This is also reflected in our results on the association between overweight/obesity at the age of 18 years and MS, confirming elevated BMI in youth as a risk factor for MS (31).

Exposure to tobacco smoke leads to irritation of the lungs with increased pro-inflammatory cell activation and alteration of proteins up to the point of increased auto-antigenic activation, which in turn may be associated with MS risk (11, 32). In our study we found a weak association between smoking and MS. While the majority of observational studies suggest an effect of smoking on MS risk (11), a recently published systematic review summarizing mendelian randomization studies found no causal effect (9).

Our study has several strengths. We were able to investigate a large study sample and use high-quality data acquired in the course of extensive data collection in the NAKO baseline survey. Moreover, the stringent recruitment management in the StERKE study, with up to three reminders per participant, led to a high proportion of responses (77% for cases, 60% for controls).

Nevertheless, our study also has some limitations. The majority of cases were prevalent cases, which is commonly regarded as a potential source of survival bias (33). To account for this, we investigated the estimates of our final model by first including only incident cases and their controls in our analyses, and then adding successive subgroups of sets with increasing intervals between diagnosis and recruitment. We generally found no trend in the estimates (*eSupplement Table 3*). Although this is not a formal proof, we nevertheless believe our results are sufficiently valid. In addition, the survival of persons with MS after diagnosis is high, in contrast to, for example, some kinds of cancer.

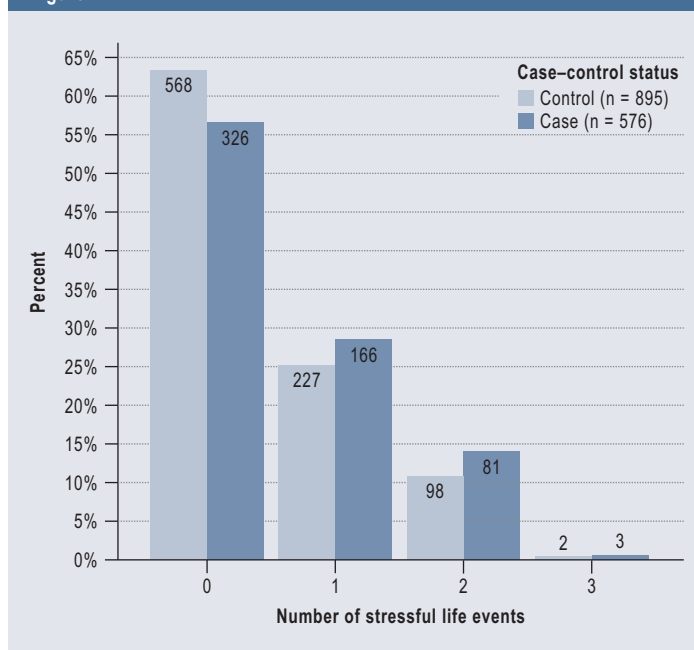
Our analyses are based on self-reported physician-based MS diagnoses, which may have led to possible misclassification. However, we assume that a self-report is reliable in view of the psychological impact of such a diagnosis. For a plausibility check, we conducted a subgroup analysis restricted to the participants with RRMS who reported treatment with MS-specific medication or immunotherapy. The results were very similar to the findings in the complete set of cases (*eSupplement Table 4*). While we acknowledge the limitation of the self-reported diagnosis of MS, we consider additional statements on MS type or specific medication as a clear indication of a valid diagnosis. Furthermore, persons with chronic diseases might have a greater interest in participating in a study that aims to investigate potential causes of diseases. Considering the frequent multimorbidity of persons with MS, there may have been overrepresentation of persons with MS in the NAKO.

A further weakness of our study is the investigation of the association between an exposure to EBV and MS by asking the participants whether they had ever had infectious mononucleosis (34). EBV infection in childhood is most often asymptomatic, so there may have been underreporting of the frequency of EBV infection. This might explain the relatively low OR of 3.05 compared with other studies (10, 19).

Since most factors investigated in this study concern events that occurred on average 40–50 years ago, recall bias may have differed between cases and controls, in that persons with MS explain their disease by reference to potential risk factors and thus remember past events more clearly. This may apply to smoking, the amount of time spent outdoors, and particularly infectious diseases in childhood. However, we assume, given the clear nature of the factors assessed, e.g., SLE, that participants answered most questions adequately (35). An objective measure of childhood infections would be an antibody test to confirm a past infection. However, even with antibody tests it is not possible to determine the precise time of infection, which could be important for assessing the effect on the maturing immune system.

In the course of our analyses we investigated a variety of potential risk factors for MS that have received little attention to date. We found associations between MS and the following factors: cumulative number of childhood infections, major SLE, higher maternal age at first pregnancy, and low levels of PA during adolescence. Furthermore, we confirmed known risk factors for MS, including a family history of MS, EBV infection, overweight and obesity in childhood/adolescence, and smoking. These results contribute to the evidence base for existing preventive measures for other NCD, such as cardiovascular diseases childhood infections, and suggest that such measures may also be useful in the context of MS. Vaccination programs, smoking cessation programs, and initiatives to encourage PA and healthy eating habits to prevent overweight and obesity might be promising strategies with regard to MS prevention. Furthermore, our results may inform further research, e.g., the investigation of on one hand dose–response relationships between the newly suggested risk factors and the severity of MS sever-

Figure 2



Distribution of the cumulative number of stressful life events (including: death of the partner, death of a close person [other than partner], serious illness of a close person) among StERKE participants before multiple sclerosis diagnosis by case-control status, Germany, 2021–2022

ity and on the other hand the MS prodrome, assuming a lag time. The NAKO offers many possibilities to:

- Investigate exclusively incident MS cases
- Conduct sex-specific analyses
- Use secondary data to confirm self-reported MS diagnoses
- Use biosamples, for example to demonstrate the presence of antibodies against infectious diseases.

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Table 3

Conditional logistic regression on the association of family history and factors occurring in the prenatal period, childhood, adolescence, and adulthood with multiple sclerosis, Germany, 2021–2022

|                                                                                                                                  | Conditional logistic regression* <sup>1</sup><br>(576 cases and 895 controls) |               |
|----------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|---------------|
|                                                                                                                                  | OR                                                                            | [95% CI]      |
| <b>Family history and prenatal factors</b>                                                                                       |                                                                               |               |
| <b>Family history</b>                                                                                                            |                                                                               |               |
| No family member with MS                                                                                                         | Ref.                                                                          |               |
| First- or second-degree family member with MS                                                                                    | 7.08                                                                          | [3.90; 12.86] |
| Number of older siblings (per additional sibling) (continuous)                                                                   | 0.85                                                                          | [0.77; 0.95]  |
| Maternal age (per year) at participant's birth (continuous)                                                                      | 1.03                                                                          | [1.00; 1.05]  |
| <b>Childhood diseases</b>                                                                                                        |                                                                               |               |
| Number of childhood infections (per additional infection) before the age of 18 years* <sup>2</sup> , * <sup>3</sup> (continuous) | 1.14                                                                          | [1.03; 1.25]  |
| <b>EBV infection*<sup>2</sup></b>                                                                                                |                                                                               |               |
| No                                                                                                                               | Ref.                                                                          |               |
| Yes                                                                                                                              | 3.05                                                                          | [1.80; 5.16]  |
| <b>Lifestyle factors in adolescence</b>                                                                                          |                                                                               |               |
| Physical activity (per level increase) during adolescence (13–19 years) (continuous)                                             | 0.82                                                                          | [0.71; 0.95]  |
| <b>BMI at the age of 18 years (kg/m<sup>2</sup>)*<sup>4</sup></b>                                                                |                                                                               |               |
| Underweight (< 18.5)                                                                                                             | 0.86                                                                          | [0.64; 1.16]  |
| Normal weight (18.5 to < 25)                                                                                                     | Ref.                                                                          |               |
| Overweight (25 to < 30)                                                                                                          | 1.73                                                                          | [1.22; 2.44]  |
| Obesity (≥ 30)                                                                                                                   | 2.29                                                                          | [1.18; 4.46]  |
| <b>Other factors</b>                                                                                                             |                                                                               |               |
| Number of stressful life events (per additional event)* <sup>2</sup> , * <sup>5</sup> (continuous)                               | 1.25                                                                          | [1.06; 1.48]  |
| <b>Ever smoked*<sup>1</sup>, *<sup>2</sup>, *<sup>4</sup></b>                                                                    |                                                                               |               |
| No                                                                                                                               | Ref.                                                                          |               |
| Yes                                                                                                                              | 1.19                                                                          | [0.99; 1.43]  |

\*<sup>1</sup> Final model as obtained from backward selection with  $p = 0.10$  as selection limit; Nagelkerke's  $R^2 = 0.142$

\*<sup>2</sup> Cases: before age at diagnosis; controls: before age at diagnosis of the matched case/median age at diagnosis if  $> 1$  case per matching set

\*<sup>3</sup> Including: Chickenpox, mumps, rubella, pertussis, and measles

\*<sup>4</sup> The OR for BMI at the age of 18 years and smoking were calculated based on the original matching with all cases ( $n = 746$ ) and controls ( $n = 1492$ ) regardless of their participation in the StERKE study, since information on BMI and smoking was available from the NAKO baseline survey.

\*<sup>5</sup> Including: Death of partner, death of a close person (other than partner), serious illness of a close person

BMI, Body mass index; CI, confidence interval; EBV, Epstein–Barr virus; MS, multiple sclerosis; OR, odds ratio

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#### Ethics

The Study on Risk Factors for the Occurrence and Progression of Multiple Sclerosis (StERKE) was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the ethics committee of the Hamburg Medical Association (PV7292).

Written informed consent was obtained from all participants in the German National Cohort (NAKO) and the Study on Risk Factors for the Occurrence and Progression of Multiple Sclerosis (StERKE).

#### Data sharing

The data underlying this article were provided by NAKO e.V. by permission. Data will be shared on request to the corresponding author with permission of NAKO e.V.

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#### Conflict of interest statement

CH has received research funding and speaker honoraria from Novartis, Merck, and Roche.

KB is a member of the editorial board of Deutsches Ärzteblatt.

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AK is spokesperson of NAKO's Infectious Diseases Expert Group and acting spokesperson of the NAKO Use & Access Committee.

TP is a member of the executive committee of NAKO e.V., which coordinates the NAKO study.

The remaining authors declare that no conflict of interest exists.

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#### Supplementary material

Complete list of full references, eReferences, eMethods, eTables, eSupplement:  
[www.aerzteblatt-international.de/m2025.0069](http://www.aerzteblatt-international.de/m2025.0069)

Supplementary material to accompany the article

# 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

by Anja Holz, Nadia Obi, [...]\*, Christoph Heesen\*, and Heiko Becher\*

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## eMETHODS

## Study design and participants

These analyses are based on data from the StERKE study, a case-control study on the effect of risk factors on the course and onset of MS, and the German National Cohort (NAKO).

The NAKO is a large population-based cohort study covering 18 regions of Germany. The baseline investigation, conducted between 2014 and 2019, comprised a total of 205 053 participants (103 471 women and 101 582 men) aged 19–74 years: The proportion of 40- to 74-year-olds was higher than that of 19- to 39-year-olds. A more detailed description of the design of the NAKO can be found elsewhere (e1). The StERKE study is a case-control study nested within the NAKO that took place between November 2021 and March 2022 at 16 of the 18 NAKO study centers. Persons who had an incident MS diagnosis at the questionnaire-based interim survey—on average two years after the baseline survey—or at the first follow-up examination up to recruitment of StERKE, or who reported a prevalent physician-based MS diagnosis at the NAKO baseline interview, were defined as cases. The controls were randomly selected NAKO participants without MS, matched individually to each MS case on the basis of birth year, sex, and study center (matching ratio 2:1).

During the recruitment process, the selected and eligible persons (746 cases, 1492 controls) were contacted either by mail or by e-mail, depending on whether they had provided an e-mail address at the NAKO baseline survey. Written informed consent for the StERKE study was obtained from all participants at study onset. The ethics committee of the Hamburg Chamber of Physicians granted approval for the conduct of the study (PV7292). Participants who initially failed to respond received up to three reminders during the course of the study. The StERKE study questionnaire included questions on the following:

- The participant's medical history and family history
- Stressful life events (SLE)
- Passive smoking during childhood and adolescence
- Time spent outdoors and physical activity during teenage years

Persons in the group “Cases” were additionally asked questions about their disease, impairments associated with the disease, and any immunotherapy received.

## Outcomes

*eSupplement Table 1* provides an overview of the exposure variables and covariates included in the analyses. Sociodemographic variables, smoking status, and body mass index (BMI) at the age of 18 years were recorded during the NAKO baseline face-to-face interview conducted by trained study staff or by completion of a self-administered touch-screen questionnaire. The remaining variables were ascertained with the aid of the StERKE questionnaire.

On the basis of the International Standard Classification of Education (ISCED 97), education was categorized as low (ISCED level 0–2), medium (ISCED level 3/4), or high (ISCED level 5/6) (e1). The level of physical activity during teenage years was derived from two questions on light and vigorous physical activity, based on the Godin Leisure-Time Exercise Questionnaire (e2) and categorized as: very low, low, moderate, and high physical activity (*eTable 2*). BMI at the age of 18 years was calculated from self-reported weight at that age and measured height and was divided into the following categories: underweight (< 18.5 kg/m<sup>2</sup>), normal weight (18.5 to < 25 kg/m<sup>2</sup>), overweight (25 to < 30 kg/m<sup>2</sup>), and obesity (≥ 30 kg/m<sup>2</sup>). The questions on major SLEs were based on questions from the Robert Koch Institute's German-language “Health Questionnaire 18 to 64 Years” (German Health Interview and Examination Survey for Adults – DEGS) (e3). The data on childhood infections came from self-reports. Participants were asked to state the year in which each infection occurred and whether they were under or over 18 years of age at the time. The cumulative number of childhood infections was calculated from the individual infections and included as sum score in the analyses. In the analyses, participants were considered as exposed to one of the potential risk factors only if exposure occurred before MS diagnosis (for cases) or before the age of the matched case at MS diagnosis (for controls).

## Statistical analyses

Sociodemographic factors and exposure variables were portrayed descriptively with respect to case-control status. MS-specific information such as age at MS diagnosis, age at MS manifestation—defined as first occurrence of symptoms—MS disease type, and MS medication were described stratified by sex.

The percentage of missing values across predictor variables ranged from 0% to 28.0% in the group with MS and from 0% to 27.0% in the group without MS (*eTable 1*). For information on the handling of missing values in subsequent analyses see *eSupplement Table 2*.

We performed a conditional logistic regression to assess the association between the exposure variables described above and the occurrence of MS. The results are displayed as odds ratios (OR) with corresponding 95% confidence intervals. The outcome was defined as the self-report of a physician-based MS diagnosis. Participants from the group “Cases” and participants from the group “Controls” with identical values of the matching variables were combined into a joint matching set according to the method described by Neuhäuser and Becher (e4). If no control of the same sex, study center, and year of birth was available for a case, the age condition was relaxed to  $\pm 2$  years, which resulted in at least one control being assigned to each case. The mean absolute difference in years of birth in the matching sets was 0.2 years. In 75% of matching sets, cases and controls had the same birth year. For variables that were available from the NAKO baseline investigation, i.e., BMI at the age of 18 years and smoking status, the OR was calculated on the basis of a separate model with all StERKE-eligible 746 cases and 1492 controls to increase the precision of the estimates. The full model included all described exposure variables and covariates. The final model was obtained by means of backward selection with a selection threshold of  $p = 0.10$ .

In order to address a possible survival bias caused by the inclusion of prevalent cases, we conducted five subgroup analyses. These included initially

- Only incident cases, and then
- Incident cases in combination with various subsamples of prevalent cases according to disease duration (time between MS diagnosis and NAKO baseline examination of 2, 5, 10, or 20 years)

In additional analyses, we investigated:

- Only cases with relapsing–remitting MS who reported the use of MS-specific medication or who had received immunotherapy in the past.

Furthermore, an analysis stratified by sex was conducted. For all subgroup analyses, the final model was used.

All statistical analyses were performed with R version 4.3.1 (2023–06–16) (17) using the packages sjPlot (e5), survival (e6), and tidyverse (e7).

eTable 1

Characteristics of StERKE participants by sex and case-control status, Germany, 2021–2022

| Variable                                                 | Women               |                  | Men                 |                  |
|----------------------------------------------------------|---------------------|------------------|---------------------|------------------|
|                                                          | Controls<br>n = 638 | Cases<br>n = 396 | Controls<br>n = 257 | Cases<br>n = 180 |
| <b>Birth year</b>                                        |                     |                  |                     |                  |
| < 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%)        |
| <b>Age (at time of NAKO baseline survey) (mean [SD])</b> | 51 (11.0)           | 49 (10.9)        | 52 (11.3)           | 49 (11.1)        |
| <b>Education</b>                                         |                     |                  |                     |                  |
| Low                                                      | 9 (1.5%)            | 2 (0.6%)         | 2 (0.8%)            | 1 (0.6%)         |
| Medium                                                   | 457 (76.9%)         | 286 (79.2%)      | 205 (85.4%)         | 131 (81.9%)      |
| High                                                     | 128 (21.5%)         | 73 (20.2%)       | 33 (13.8%)          | 28 (17.5%)       |
| Unknown/missing                                          | 44                  | 35               | 17                  | 20               |
| <b>Family history of MS</b>                              |                     |                  |                     |                  |
| No family member with MS                                 | 624 (97.8%)         | 358 (90.4%)      | 253 (98.4%)         | 160 (88.9%)      |
| First- or second-degree family member with MS            | 14 (2.2%)           | 38 (9.6%)        | 4 (1.6%)            | 20 (11.1%)       |
| <b>Number of older siblings</b>                          |                     |                  |                     |                  |
| None                                                     | 243 (38.1%)         | 166 (41.9%)      | 88 (34.2%)          | 74 (41.1%)       |
| 1 older sibling                                          | 229 (35.9%)         | 143 (36.1%)      | 92 (35.8%)          | 61 (33.9%)       |
| ≥ 2 older siblings                                       | 166 (26.0%)         | 87 (22.0%)       | 77 (30.0%)          | 45 (25.0%)       |
| <b>Maternal age at participant's birth (mean [SD])</b>   | 27 (5.7)            | 28 (5.9)         | 27 (5.6)            | 27 (5.0)         |
| <b>Maternal smoking during pregnancy</b>                 |                     |                  |                     |                  |
| No                                                       | 515 (91.2%)         | 331 (93.8%)      | 212 (93.8%)         | 146 (91.8%)      |
| Yes                                                      | 50 (8.8%)           | 22 (6.2%)        | 14 (6.2%)           | 13 (8.2%)        |
| Unknown/missing                                          | 73                  | 43               | 31                  | 21               |
| <b>Paternal smoking during pregnancy</b>                 |                     |                  |                     |                  |
| No                                                       | 302 (61.3%)         | 186 (59.0%)      | 113 (58.2%)         | 85 (57.8%)       |
| Yes                                                      | 191 (38.7%)         | 129 (41.0%)      | 81 (41.8%)          | 62 (42.2%)       |
| Unknown/missing                                          | 145                 | 81               | 63                  | 33               |
| <b>Chickenpox infection*1</b>                            |                     |                  |                     |                  |
| No                                                       | 93 (16.6%)          | 59 (16.9%)       | 54 (26.6%)          | 36 (23.7%)       |
| Yes                                                      | 466 (83.4%)         | 291 (83.1%)      | 149 (73.4%)         | 116 (76.3%)      |
| Unknown/missing                                          | 79                  | 46               | 54                  | 28               |
| <b>Mumps infection*1</b>                                 |                     |                  |                     |                  |
| No                                                       | 277 (53.5%)         | 162 (49.5%)      | 87 (46.0%)          | 65 (44.5%)       |
| Yes                                                      | 241 (46.5%)         | 165 (50.5%)      | 102 (54.0%)         | 81 (55.5%)       |
| Unknown/missing                                          | 120                 | 69               | 68                  | 34               |
| <b>Rubella infection*1</b>                               |                     |                  |                     |                  |
| No                                                       | 298 (61.3%)         | 172 (56.0%)      | 111 (63.4%)         | 74 (58.3%)       |
| Yes                                                      | 188 (38.7%)         | 135 (44.0%)      | 64 (36.6%)          | 53 (41.7%)       |
| Unknown/missing                                          | 152                 | 89               | 82                  | 53               |

|                                                                                                 |             |             |             |             |
|-------------------------------------------------------------------------------------------------|-------------|-------------|-------------|-------------|
| <b>Pertussis infection*<sup>1</sup></b>                                                         |             |             |             |             |
| No                                                                                              | 396 (79.0%) | 242 (76.3%) | 147 (75.8%) | 103 (76.9%) |
| Yes                                                                                             | 105 (21.0%) | 75 (23.7%)  | 47 (24.2%)  | 31 (23.1%)  |
| Unknown/missing                                                                                 | 137         | 79          | 63          | 46          |
| <b>Measles infection*<sup>1</sup></b>                                                           |             |             |             |             |
| No                                                                                              | 235 (45.8%) | 141 (42.6%) | 78 (40.8%)  | 57 (38.3%)  |
| Yes                                                                                             | 278 (54.2%) | 190 (57.4%) | 113 (59.2%) | 92 (61.7%)  |
| Unknown/missing                                                                                 | 125         | 65          | 66          | 31          |
| <b>EBV infection*<sup>1</sup></b>                                                               |             |             |             |             |
| No                                                                                              | 530 (96.0%) | 278 (86.9%) | 206 (97.2%) | 142 (94.7%) |
| Yes                                                                                             | 22 (4.0%)   | 42 (13.1%)  | 6 (2.8%)    | 8 (5.3%)    |
| Unknown/missing                                                                                 | 86          | 76          | 45          | 30          |
| <b>Maternal and/or paternal smoking during participant's childhood/adolescence (0–18 years)</b> |             |             |             |             |
| No                                                                                              | 290 (46.7%) | 176 (45.2%) | 111 (44.8%) | 79 (45.4%)  |
| Yes                                                                                             | 331 (53.3%) | 213 (54.8%) | 137 (55.2%) | 95 (54.6%)  |
| Unknown/missing                                                                                 | 17          | 7           | 9           | 6           |
| <b>Time spent outdoors during childhood and adolescence (0–18 years)</b>                        |             |             |             |             |
| None to a few hours/month                                                                       | 45 (7.1%)   | 19 (4.8%)   | 16 (6.2%)   | 7 (3.9%)    |
| A few hours/week                                                                                | 112 (17.6%) | 73 (18.4%)  | 27 (10.5%)  | 30 (16.7%)  |
| A few hours/day                                                                                 | 481 (75.4%) | 304 (76.8%) | 214 (83.3%) | 143 (79.4%) |
| <b>Physical activity during adolescence (13–19 years)</b>                                       |             |             |             |             |
| Very low                                                                                        | 19 (3.0%)   | 21 (5.3%)   | 2 (0.8%)    | 4 (2.2%)    |
| Low                                                                                             | 76 (11.9%)  | 60 (15.2%)  | 17 (6.6%)   | 12 (6.7%)   |
| Moderate                                                                                        | 223 (35.0%) | 134 (33.8%) | 60 (23.3%)  | 56 (31.1%)  |
| High                                                                                            | 320 (50.2%) | 181 (45.7%) | 178 (69.3%) | 108 (60.0%) |
| <b>BMI at the age of 18 years (kg/m<sup>2</sup>)*<sup>2</sup></b>                               |             |             |             |             |
| Underweight (< 18.5)                                                                            | 161 (22.9%) | 61 (16.6%)  | 24 (6.6%)   | 14 (8.9%)   |
| Normal weight (18.5 to < 25)                                                                    | 485 (68.9%) | 251 (68.2%) | 296 (81.1%) | 113 (71.5%) |
| Overweight (25 to < 30)                                                                         | 45 (6.4%)   | 44 (12.0%)  | 40 (11.0%)  | 24 (15.2%)  |
| Obesity (≥ 30)                                                                                  | 13 (1.8%)   | 12 (3.3%)   | 5 (1.4%)    | 7 (4.4%)    |
| Missing                                                                                         | 310         | 139         | 113         | 81          |
| <b>Stressful life events*<sup>1</sup></b>                                                       |             |             |             |             |
| Death of partner                                                                                | 15 (2.3%)   | 13 (3.9%)   | 2 (0.8%)    | 3 (1.7%)    |
| Death of a close person (other than partner)                                                    | 185 (29.0%) | 144 (36.4%) | 69 (26.8%)  | 52 (28.9%)  |
| Serious illness of a close person                                                               | 126 (19.7%) | 91 (23.0%)  | 32 (12.5%)  | 34 (18.9%)  |
| At least one of the above events                                                                | 248 (38.9%) | 183 (46.2%) | 79 (30.7%)  | 67 (37.2%)  |
| <b>Own serious illness (other than MS)*<sup>1</sup></b>                                         |             |             |             |             |
| No                                                                                              | 598 (93.7%) | 362 (91.4%) | 244 (94.9%) | 167 (92.8%) |
| Yes                                                                                             | 40 (6.3%)   | 34 (8.6%)   | 13 (5.1%)   | 13 (7.2%)   |
| <b>Ever smoked*<sup>1, *2</sup></b>                                                             |             |             |             |             |
| No                                                                                              | 496 (50.8%) | 234 (48.1%) | 205 (44.2%) | 82 (35.8%)  |
| Yes                                                                                             | 480 (49.2%) | 252 (51.9%) | 259 (55.8%) | 147 (64.2%) |
| Missing                                                                                         | 38          | 21          | 14          | 10          |

\*<sup>1</sup> Cases: before age at diagnosis; controls: before age at diagnosis of the matched case/median age at diagnosis of the matched cases if > 1 case per matching set

\*<sup>2</sup> Distribution from the totality of eligible persons: 746 cases and 1492 controls

BMI, Body mass index; EBV, Epstein–Barr virus; MS, multiple sclerosis; NAKO, German National Cohort; SD, standard deviation

| eTable 2                                                                                                                                 |                |                            |              |                |               |
|------------------------------------------------------------------------------------------------------------------------------------------|----------------|----------------------------|--------------|----------------|---------------|
| Calculation of physical activity level based on the frequency of light and vigorous physical activity during teenage years (13–19 years) |                |                            |              |                |               |
| Light physical activity                                                                                                                  |                | Vigorous physical activity |              |                |               |
|                                                                                                                                          |                | Never                      | <1 time/week | 1–2 times/week | ≥3 times/week |
|                                                                                                                                          | Never          | Very low                   | Very low     | Moderate       | High          |
|                                                                                                                                          | < 1 time/week  | Very low                   | Low          | Moderate       | High          |
|                                                                                                                                          | 1–2 times/week | Low                        | Moderate     | Moderate       | High          |
|                                                                                                                                          | ≥ 3 times/week | Low                        | Moderate     | High           | High          |

## 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 |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| <i>Sociodemographic factors</i>                                                                                                                                                                  | Sex                                                                                                                                                                                 | NAKO       |
|                                                                                                                                                                                                  | Birth year                                                                                                                                                                          | NAKO       |
|                                                                                                                                                                                                  | Education                                                                                                                                                                           | NAKO       |
| <i>Exposures</i>                                                                                                                                                                                 | 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/m <sup>2</sup> )                                                                                                                                     | 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                                                                                                                                                                                                                                                                                                                                                                                                |
|------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Exposure set to "No"</i>                                                        | Family history<br>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))                                                                                                                                                                                                    |
| <i>Additional missing category</i>                                                 | Common childhood infections (including: Chickenpox, mumps, rubella, pertussis, measles, EBV infection)<br>Smoking<br>Passive smoking (maternal and/or paternal smoking during pregnancy with the participant as well as during the participant's childhood/adolescence (0-18 years))<br>Time spent outdoors during childhood/adolescence (0-18 years)<br>BMI at the age of 18 years (kg/m <sup>2</sup> ) |
| <i>Imputation of the median</i>                                                    | Number of older siblings<br>Maternal age at participant's birth<br>Cumulative number of common childhood infections (including: Chickenpox, mumps, pertussis, rubella, measles)<br>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                                                                                                       |                      |                            |  |                                                    |                            |  |                                                    |                            |  |                                                     |                            |  |                                                     |                            |  |                      |                            |
|------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|----------------------------|--|----------------------------------------------------|----------------------------|--|----------------------------------------------------|----------------------------|--|-----------------------------------------------------|----------------------------|--|-----------------------------------------------------|----------------------------|--|----------------------|----------------------------|
| Source of variable                                                                                                                                   | Incident cases       |                            |  | Incident cases +<br>diagnosis ≤ 2 years from<br>BL |                            |  | Incident cases +<br>diagnosis ≤ 5 years from<br>BL |                            |  | Incident cases +<br>diagnosis ≤ 10 years from<br>BL |                            |  | Incident cases +<br>diagnosis ≤ 20 years from<br>BL |                            |  | Total sample         |                            |
|                                                                                                                                                      | Cases                | Controls                   |  | Cases                                              | Controls                   |  | Cases                                              | Controls                   |  | Cases                                               | Controls                   |  | Cases                                               | Controls                   |  | Cases                | Controls                   |
|                                                                                                                                                      | 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 <sup>a</sup>                                                                                                         |                      |                            |  |                                                    |                            |  |                                                    |                            |  |                                                     |                            |  |                                                     |                            |  |                      |                            |
| Variable                                                                                                                                             | Incident cases       |                            |  | Incident cases +<br>diagnosis ≤ 2 years from<br>BL |                            |  | Incident cases +<br>diagnosis ≤ 5 years from<br>BL |                            |  | Incident cases +<br>diagnosis ≤ 10 years from<br>BL |                            |  | Incident cases +<br>diagnosis ≤ 20 years from<br>BL |                            |  | Total sample         |                            |
|                                                                                                                                                      | OR                   | 95% CI                     |  | OR                                                 | 95% CI                     |  | OR                                                 | 95% CI                     |  | OR                                                  | 95% CI                     |  | OR                                                  | 95% CI                     |  | OR                   | 95% CI                     |
|                                                                                                                                                      | Ref.                 |                            |  | Ref.                                               |                            |  | Ref.                                               |                            |  | Ref.                                                |                            |  | Ref.                                                |                            |  | Ref.                 |                            |
| Family history <sup>b</sup><br>No family member with MS<br>1 <sup>st</sup> and 2 <sup>nd</sup> degree family member<br>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<br>additional sibling) <sup>b</sup> (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<br>participant's birth <sup>b</sup> (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<br>infections (per additional<br>infection) before the age of 18<br>years <sup>b,c,d</sup> (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 <sup>b,c</sup><br>No<br>Yes                                                                                                            | Ref.<br>6.65         | 1.63 – 27.22               |  | Ref.<br>2.72                                       | 1.01 – 7.35                |  | Ref.<br>2.70                                       | 1.18 – 6.17                |  | Ref.<br>3.10                                        | 1.61 – 5.96                |  | Ref.<br>3.14                                        | 1.78 – 5.51                |  | Ref.<br>3.04         | 1.79 – 5.14                |
| Physical activity (per level<br>increase) during teenage years<br>(13–19 years) <sup>b</sup> (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<br>(kg/m <sup>2</sup> )<br>Underweight (<18.5)<br>Normal weight (18.5 - <25)<br>Overweight (25 - <30) or<br>Obesity (≥30) | 0.65<br>Ref.<br>2.28 | 0.25 – 1.71<br>1.10 – 4.73 |  | 0.74<br>Ref.<br>2.21                               | 0.37 – 1.47<br>1.20 – 4.06 |  | 1.05<br>Ref.<br>1.78                               | 0.61 – 1.78<br>1.07 – 2.98 |  | 0.97<br>Ref.<br>1.81                                | 0.64 – 1.47<br>1.19 – 2.74 |  | 0.77<br>Ref.<br>1.89                                | 0.55 – 1.08<br>1.33 – 2.69 |  | 0.86<br>Ref.<br>1.82 | 0.64 – 1.16<br>1.32 – 2.50 |

| Number of major stressful life events (per additional event) <sup>b,c,f</sup><br>(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 <sup>e,e</sup>                                                                                                                                                                                                                                                                                         |      |             |             |       |             |       |             |       |             |       |             |       |             |
| No                                                                                                                                                                                                                                                                                                                 | Ref. |             |             | Ref.  |             | Ref.  |             | Ref.  |             | Ref.  |             | Ref.  |             |
| Yes                                                                                                                                                                                                                                                                                                                | 0.99 | 0.62 – 1.58 | 1.00        | 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 <sup>2</sup> )                                                                                                                                                                                                                                                                           |      | 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                                        |      |             |             |       |             |       |             |       |             |       |             |       |             |
| <sup>a</sup> Final model obtained from backward selection with $p = 0.10$ as selection limit                                                                                                                                                                                                                       |      |             |             |       |             |       |             |       |             |       |             |       |             |
| <sup>b</sup> OR estimate of the respective analysis based on the sample of all participating StERKE participants. Exact numbers can be found above.                                                                                                                                                                |      |             |             |       |             |       |             |       |             |       |             |       |             |
| <sup>c</sup> Cases: Before age at diagnosis; Controls: Before age at diagnosis of the matched case/median age of diagnosis if >1 case per matching set                                                                                                                                                             |      |             |             |       |             |       |             |       |             |       |             |       |             |
| <sup>d</sup> Including: Chickenpox, mumps, rubella, pertussis, and measles                                                                                                                                                                                                                                         |      |             |             |       |             |       |             |       |             |       |             |       |             |
| <sup>e</sup> 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. |      |             |             |       |             |       |             |       |             |       |             |       |             |
| <sup>f</sup> 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

| <i>Variable</i>                                                                                                                                        | <i>Conditional Logistic Regression<sup>a</sup></i> |               |
|--------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------|---------------|
|                                                                                                                                                        | <i>Cases with RRMS</i>                             |               |
|                                                                                                                                                        | <i>(248 cases and 502 controls)</i>                |               |
|                                                                                                                                                        | <i>OR</i>                                          | <i>95% CI</i> |
| <b>Family history</b>                                                                                                                                  |                                                    |               |
| <i>No family member with MS</i>                                                                                                                        | Ref.                                               |               |
| <i>1<sup>st</sup> and 2<sup>nd</sup> degree family member with MS</i>                                                                                  | 5.01                                               | 2.09 – 11.99  |
| <b>Number of older siblings (per additional sibling) (continuous)</b>                                                                                  | 0.69                                               | 0.57 – 0.84   |
| <b>Maternal age (per year) at participant's birth (continuous)</b>                                                                                     | 1.03                                               | 0.99 – 1.07   |
| <b>Number of common childhood infections (per additional infection) before the age of 18 years<sup>b,c</sup> (continuous)</b>                          | 1.10                                               | 0.96 – 1.27   |
| <b>EBV infection<sup>b</sup></b>                                                                                                                       |                                                    |               |
| <i>No</i>                                                                                                                                              | Ref.                                               |               |
| <i>Yes</i>                                                                                                                                             | 2.97                                               | 1.52 – 5.82   |
| <b>Physical activity (per level increase) during teenage years (13-19 years) (continuous)</b>                                                          | 0.89                                               | 0.71 – 1.12   |
| <b>BMI at the age of 18 years (kg/m<sup>2</sup>)</b>                                                                                                   |                                                    |               |
| <i>Underweight (&lt;18.5)</i>                                                                                                                          | 0.67                                               | 0.38 – 1.19   |
| <i>Normal weight (18.5 - &lt;25)</i>                                                                                                                   | Ref.                                               |               |
| <i>Overweight (25 - &lt;30)</i>                                                                                                                        | 2.06                                               | 1.07 – 3.95   |
| <i>Obesity (≥30)</i>                                                                                                                                   | 2.12                                               | 0.54 – 8.24   |
| <b>Number of major stressful life events (per additional event)<sup>b,d</sup> (continuous)</b>                                                         | 1.27                                               | 0.98 – 1.64   |
| <b>Ever smoked<sup>b</sup></b>                                                                                                                         |                                                    |               |
| <i>No</i>                                                                                                                                              | Ref.                                               |               |
| <i>Yes</i>                                                                                                                                             | 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     |                                                    |               |
| <sup>a</sup> Final model obtained from backward selection with $p = 0.10$ as selection limit; Nagelkerke's $R^2 = 0.155$                               |                                                    |               |
| <sup>b</sup> Cases: Before age at diagnosis; Controls: Before age at diagnosis of the matched case/median age of diagnosis if >1 case per matching set |                                                    |               |
| <sup>c</sup> Including: Chickenpox, mumps, rubella, pertussis, and measles                                                                             |                                                    |               |
| <sup>d</sup> 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 <sup>a</sup> |              |                                     |              |
|----------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------|--------------|-------------------------------------|--------------|
|                                                                                                                                  | Women<br>(396 cases and 638 controls)        |              | Men<br>(180 cases and 257 controls) |              |
|                                                                                                                                  | OR                                           | 95% CI       | OR                                  | 95% CI       |
| <b>Family history</b>                                                                                                            |                                              |              |                                     |              |
| No family member with MS                                                                                                         | Ref.                                         |              | Ref.                                |              |
| 1 <sup>st</sup> and 2 <sup>nd</sup> degree family member with MS                                                                 | 6.18                                         | 3.13 – 12.22 | 10.16                               | 2.80 – 36.89 |
| <b>Number of older siblings (per additional sibling)</b><br>(continuous)                                                         | 0.85                                         | 0.74 – 0.96  | 0.87                                | 0.71 – 1.06  |
| <b>Maternal age (per year) at participant's birth</b><br>(continuous)                                                            | 1.04                                         | 1.01 – 1.07  | 1.00                                | 0.96 – 1.05  |
| <b>Number of common childhood infections (per additional infection) before the age of 18 years<sup>b,c</sup></b><br>(continuous) | 1.16                                         | 1.03 – 1.30  | 1.10                                | 0.93 – 1.31  |
| <b>EBV infection<sup>b</sup></b>                                                                                                 |                                              |              |                                     |              |
| No                                                                                                                               | Ref.                                         |              | Ref.                                |              |
| Yes                                                                                                                              | 3.40                                         | 1.88 – 6.15  | 2.16                                | 0.60 – 7.79  |
| <b>Physical activity (per level increase) during teenage years (13-19 years)</b><br>(continuous)                                 | 0.81                                         | 0.68 – 0.96  | 0.84                                | 0.62 – 1.14  |
| <b>BMI at the age of 18 years (kg/m<sup>2</sup>)<sup>d</sup></b>                                                                 |                                              |              |                                     |              |
| 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 |
| <b>Number of major stressful life events (per additional event)<sup>b,e</sup></b> (continuous)                                   | 1.25                                         | 1.02 – 1.52  | 1.29                                | 0.93 – 1.79  |
| <b>Ever smoked<sup>b,d</sup></b>                                                                                                 |                                              |              |                                     |              |
| No                                                                                                                               | Ref.                                         |              | Ref.                                |              |
| Yes                                                                                                                              | 1.09                                         | 0.88 – 1.37  | 1.38                                | 0.98 – 1.94  |
| <b>Model fit (Nagelkerke's R<sup>2</sup>)</b>                                                                                    | 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

<sup>a</sup>Final model obtained from backward selection with  $p = 0.10$  as selection limit

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

<sup>c</sup>Including: Chickenpox, mumps, rubella, pertussis, and measles

<sup>d</sup>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.

<sup>e</sup>Including: Death of the partner, death of a close person (other than partner), serious illness of a close person