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## Cigarette Smoking and Incidence of Multiple Sclerosis

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Experimental data suggest that cigarette smoking may play a role in the development of multiple sclerosis (MS), but epidemiologic studies have been small and inconclusive. The authors assessed the association between MS incidence and smoking in two cohort studies of US women, the Nurses' Health Study (121,700 women aged 30–55 years at baseline in 1976) and the Nurses' Health Study II (116,671 women aged 25–42 years at baseline in 1989). Smoking history was assessed at baseline and updated on biennial questionnaires. A total of 315 definite or probable cases of MS were documented. Compared with that for women who never smoked, the relative incidence rate was 1.6 (95% confidence interval: 1.2, 2.1) among current smokers and 1.2 (95% confidence interval: 0.9, 1.6) among past smokers after adjustment for age, latitude, and ancestry. The relative rate increased significantly with cumulative exposure to smoking ( $p$  for trend < 0.05), from 1.1 (95% confidence interval: 0.8, 1.6) for 1–9 pack-years to 1.5 (95% confidence interval: 1.2, 2.1) for 10–24 pack-years and 1.7 (95% confidence interval: 1.2, 2.4) for 25 or more pack-years. Similar results were obtained after adjustment for other potential confounding factors. Although these prospective results do not prove a cause-and-effect relation, they suggest that smoking is associated with an increased risk of MS. *Am J Epidemiol* 2001;154:69–74.

cohort studies; multiple sclerosis; smoking

Environmental factors are likely to play a prominent role in the development of multiple sclerosis (MS), but few such factors have been identified in epidemiologic studies (1). In experimental studies with animals and humans, some components of cigarette smoke have neurotoxic (2) or immunomodulatory (3, 4) effects. Moreover, in epidemiologic investigations, cigarette smoking has been associated with optic neuropathy (5) and some autoimmune diseases, including systemic lupus erythematosus (6, 7) and rheumatoid arthritis (8–12). Finally, cigarette smoking increases the frequency and duration of several respiratory infections (13), and infections are likely to be important in the etiology of MS, although specific agents remain unidentified (1).

Early reports described an aggravation of MS symptoms after smoking (14–17). A few case-control studies were also conducted (18–21). The results of these studies were inconsistent, but overall, they supported the existence of a mod-

estly increased risk of MS among smokers. An increased incidence of MS has been found among smokers in the only two prospective cohort studies that examined this association of which we are aware (22, 23), but these results were based on a relatively small number of cases and did not attain traditional statistical significance. Furthermore, they did not assess cumulative exposure to smoking.

We present below the association between smoking and MS in two ongoing cohort studies of US women: the Nurses' Health Study (NHS) and the Nurses' Health Study II (NHS II).

### MATERIALS AND METHODS

#### Population

The NHS was established in 1976, when 121,700 female registered nurses from 11 states who were aged 30–55 years responded to a mailed questionnaire about disease history and lifestyle items. The NHS II was established in 1989, when 116,671 female registered nurses from 14 states aged 25–42 years responded to a similar questionnaire. Follow-up questionnaires are mailed to the participants of both studies every 2 years to update information on potential risk factors for chronic diseases and to ascertain whether major medical events have occurred. A specific question on the lifetime occurrence of MS was first included in the 1992 (NHS) and 1991 (NHS II) questionnaires. A question on MS diagnosis within the last 2 years was included in subsequent questionnaires. Most MS cases diagnosed before 1992 in the NHS had already been reported through an open-ended

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Abbreviations: CI, confidence interval; MS, multiple sclerosis; NHS, Nurses' Health Study; NHS II, Nurses' Health Study II; RR, relative rate.

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question on "Other major illness." For this analysis, we excluded women who had received a diagnosis of MS before they answered the baseline questionnaire.

### Case ascertainment

We requested permission to obtain relevant medical records from all participants who reported a new diagnosis of MS. After obtaining permission, we sent to the treating neurologists a questionnaire on the certainty of the diagnosis (definite, probable, possible, not MS), clinical history, and laboratory tests. If a neurologist was not involved or did not respond, we mailed the questionnaire to the patient's internist.

When standardized research criteria (24) for the diagnosis of MS were applied to the clinical and laboratory data provided by the treating physicians in the questionnaire, 93 percent of all definite and probable diagnoses were confirmed. Therefore, for the purpose of the investigation, we confirmed as cases women who had had a diagnosis of definite or probable MS according to their physicians. We considered the categories clinically definite or laboratory-supported definite as definite and the categories clinically probable or laboratory-supported probable as probable. No upper age limit was set for the diagnosis of MS.

### Assessment of exposures

A detailed smoking history was obtained at the beginning of the study and updated every 2 years. Smoking status was defined as never, past, or current (if any amount of current smoking was reported). The cumulative exposure to cigarette smoking was summarized by multiplying the number of packs smoked per day (one pack contains 20 cigarettes) by the number of years over which that amount was smoked (pack-years). We excluded from the analysis the 2-year periods for which a participant did not report her smoking status (<1 percent of total person-time). Analyses restricted to participants who always reported their smoking status yielded virtually identical results. An indirect validity assessment of the information on smoking is provided by the strong dose-response associations found between smoking and several smoking-related diseases in the NHS and the NHS II (25, 26).

Other covariates that were considered in the analyses included latitude and ancestry. Details on the association of MS with latitude and ancestry in these cohorts have been reported previously (27). We divided the continental United States into north (states generally north of 41–42° north latitude), south (states lying south of 37° south latitude), and middle tiers, and into eastern, central, mountain, and Pacific zones. Information on the state of residence at age 15 years was available from 73 percent of the women in the NHS and from 80 percent of those in the NHS II.

The participants were asked whether they had the following ancestries: African, Asian, Hispanic, Scandinavian, southern European/Mediterranean, other Caucasian, or other ancestry. We categorized participants as Southern European/Mediterranean or as Scandinavian when that was the only ancestry reported, as Other White when a mixture

of only Caucasian ancestries was reported, and as non-White when African, Asian, or Hispanic ancestry was reported. Ancestry data were available for 83 percent of women in the NHS and 99 percent in the NHS II.

### Statistical analyses

Each participant contributed person-time of follow-up from the date of return of the baseline questionnaire to the date of MS diagnosis, death from any cause, or end of follow-up, whichever came first. For the rates presented here, end of follow-up was June 1994 for the NHS and June 1995 for the NHS II. Age-specific incidence rates were calculated as the number of MS cases (definite and probable only) divided by person-time of follow-up in each age group. To account for possible changes in smoking habits after the occurrence of neurologic symptoms, we classified women according to smoking status and pack-years of smoking 4 years before the date of diagnosis, when this was used in the analysis. For example, a woman diagnosed between the 1986 and 1988 questionnaires was classified according to her smoking status in 1982. We also performed separate analyses according to the date of first symptoms, defined as the earliest date at which neurologic symptoms attributable to MS were reported by the participant or her physician. In those analyses, women who reported that their symptoms started before the baseline questionnaire were excluded.

We used time-dependent Cox proportional hazards regression to estimate incidence rates and 95 percent confidence intervals for smoking status and pack-years of smoking, adjusted for age, ancestry, and latitude. The proportional hazards assumption was approximately met in all models. A Wald test, in which the squared difference between the log relative rates in the NHS and the NHS II was divided by the sum of the variances of each of the log relative rates, was used to test for heterogeneity of relative rates between the two studies. Log relative rates from the two studies were weighted by the inverse of their variances to obtain a pooled estimate.

## RESULTS

We documented 181 new physician-confirmed diagnoses of MS (127 definite and 54 probable) during the 18 years of follow-up in the NHS and 134 (103 definite and 31 probable) during the 6 years of follow-up in the NHS II. There were 108 MS cases (79 definite and 29 probable) in the NHS and 88 (67 definite, 21 probable) in the NHS II, with first symptoms during the follow-up period.

The age-adjusted distribution of latitude of residence at age 15 years and ancestry by smoking status at baseline is displayed in table 1. Compared with never smokers, there was a higher proportion of smokers that resided in the north tier at age 15 years. No substantial differences regarding ancestry were found.

In both cohorts, smoking 4 years before the diagnosis was associated with an increased incidence of MS (table 2). The pooled relative rate (RR) for current smokers compared with never smokers was 1.6 (95 percent confidence interval (CI): 1.2, 2.1) after adjustment for age, latitude, and ancestry.

**TABLE 1. Distribution of residence and ancestry among women in the Nurses' Health Study (1976–1994) and the Nurses' Health Study II (1989–1995), by smoking status 4 years before baseline\***

	Smoking status		
	Never smoker	Past smoker	Current smoker
<b>NHS†</b>			
No. of women	52,889	21,918	46,342
Age at baseline (mean)	42.9	43.6	42.8
Residence in north tier at age 15 years (%)	37.6	45.0	44.9
Scandinavian ancestry (%)	4.4	4.4	3.9
Southern European-Mediterranean ancestry (%)	15.2	15.1	14.7
<b>NHS II‡</b>			
No. of women	75,749	16,909	23,457
Age at baseline (mean)	34.0	36.6	34.0
Residence in north tier at age 15 years (%)	31.7	39.5	38.7
Scandinavian ancestry (%)	4.6	4.1	4.3
Southern European-Mediterranean ancestry (%)	13.5	14.9	14.4

\* Each category is directly standardized to the baseline age distribution of the corresponding cohort.

† NHS, Nurses' Health Study; NHS II, Nurses' Health Study II.

The pooled RRs were 1.7 (95 percent CI: 1.1, 2.4) for smokers of 1–14 cigarettes per day, 1.2 (95 percent CI: 0.8, 1.8) for smokers of 15–24 cigarettes per day, and 2.2 (95 percent CI: 1.5, 3.2) for smokers of 25 or more cigarettes per day

compared with never smokers. The pooled RR for past smokers was 1.2 (95 percent CI: 0.9, 1.6), and there were no differences by time since quitting smoking. Similar relative rates were obtained for first symptoms of MS: 1.5 (95 percent CI: 1.0, 2.1) for current smokers and 1.0 (95 percent CI: 0.7, 1.5) for past smokers versus never smokers.

The incidence of MS increased with the number of pack-years smoked up to 4 years before the diagnosis ( $p$  for trend < 0.05, table 3). Smokers of 25 or more pack-years had the highest increase in the incidence of MS compared with never smokers (pooled RR = 1.7, 95 percent CI: 1.2, 2.4) after adjustment for age, latitude, and ancestry. The comparable RR for first symptoms of MS was 1.5 (95 percent CI: 0.9, 2.3).

When the analyses were restricted to women under age 50 years in the NHS (all NHS II participants were under age 50), the association between MS and smoking was somewhat strengthened. The pooled RR for smokers of 25 pack-years or more was 2.0 (95 percent CI: 1.3, 3.0). Neither duration of smoking nor age of starting smoking was significantly associated with the risk of MS in pooled analyses.

We repeated all analyses with a more restrictive case definition (i.e., only definite cases of MS) and found associations similar to those reported above. Compared with never smokers, the pooled RRs were 1.6 (95 percent CI: 1.1, 2.2) for current smokers, 1.4 (95 percent CI: 1.0, 2.0) for past smokers, 1.2 (95 percent CI: 0.8, 1.8) for smokers of 1–9 pack-years, 1.6 (95 percent CI: 1.2, 2.3) for smokers of 10–24 pack-years, and 1.9 (95 percent CI: 1.3, 2.8) for smokers of 25 or more pack-years (data not shown).

Further adjustment for longitude zone, alcohol and caffeine intakes, and body mass index exerted little change on the results. The association between smoking status and diagno-

**TABLE 2. Relative rates and 95% confidence intervals for diagnosis of multiple sclerosis by smoking status 4 years before the diagnosis, Nurses' Health Study (1976–1994) and Nurses' Health Study II (1989–1995)**

Smoking status	No. of cases	Person-years	Age-adjusted RR*	Multivariate analysis†	
				RR	95% CI*
<b>NHS*,‡</b>					
Never smoker	62	916,954	1.0	1.0	
Past smoker	57	559,769	1.5	1.5	1.0, 2.1
Current smoker	61	638,197	1.4	1.4	1.0, 2.1
<b>NHS II*,§</b>					
Never smoker	77	422,734	1.0	1.0	
Past smoker	22	114,527	0.9	0.9	0.6, 1.5
Current smoker	35	111,429	1.7	1.8	1.2, 2.7
<b>Pooled¶</b>					
Never smoker			1.0	1.0	
Past smoker			1.3	1.2	0.9, 1.6
Current smoker			1.5	1.6	1.2, 2.1

\* RR, relative rate; CI, confidence interval; NHS, Nurses' Health Study; NHS II, Nurses' Health Study II.

† Adjusted for age (5-year categories), latitude tier (north, middle, south), and ancestry (Scandinavian, southern European, other White, and non-White).

‡ Smoking status was missing for 5,455 person-years, including one case.

§ Smoking status was missing for 940 person-years.

¶  $p$  values from Wald tests for heterogeneity of multivariate RRs were 0.13 and 0.44 for past and current smokers, respectively.

**TABLE 3. Relative rates and 95% confidence intervals for diagnosis of multiple sclerosis by pack-years of smoking up to 4 years before the diagnosis for current and past smokers combined, Nurses' Health Study (1976–1994) and Nurses' Health Study II (1989–1995)**

Pack-years	No. of cases	Person-years	Age-adjusted RR*	Multivariate analysis†		<i>p</i> for trend‡	<i>p</i> for trend§
				RR	95% CI*		
<b>NHS*,¶</b>							
Never smoker	62	916,954	1.0	1.0			
1–9	26	270,505	1.3	1.2	0.8, 1.9		
10–24	50	378,951	1.8	1.8	1.2, 2.6		
≥25	42	533,737	1.4	1.4	0.9, 2.0	0.07	0.61
<b>NHS II*,¶</b>							
Never smoker	77	422,734	1.0	1.0			
1–9	17	97,235	1.0	1.0	0.6, 1.6		
10–24	25	102,067	1.3	1.3	0.8, 2.0		
≥25	15	25,029	2.7	2.7	1.5, 4.8	<0.01	<0.01
<b>Pooled#</b>							
Never smoker			1.0	1.0			
1–9			1.1	1.1	0.8, 1.6		
10–24			1.6	1.5	1.2, 2.1		
≥25			1.7	1.7	1.2, 2.4	<0.01	0.04

\* RR, relative rate; CI, confidence interval; NHS, Nurses' Health Study; NHS II, Nurses' Health Study II.

† Adjusted for age (5-year categories), latitude tier (north, middle, south), and ancestry (Scandinavian, southern European, other White, and non-White).

‡ Test for trend determined by modeling pack-years as a continuous variable with never smokers as zero.

§ Test for trend determined by modeling pack-years as a continuous variable after excluding never smokers.

¶ NHS: 20,588 person-years, including one case with missing pack-years information. NHS II: 2,565 person-years with missing pack-years information.

# *p* values from Wald tests for heterogeneity of multivariate RRs were 0.51, 0.29, and 0.06 for 1–9, 10–24 and ≥25 pack-years, respectively.

sion of MS was slightly strengthened when information on smoking at baseline or 8 years before the diagnosis was used.

## DISCUSSION

In these two large, prospective studies of US women, we found a 60 percent greater incidence rate of MS among current smokers compared with never smokers. The incidence of MS increased with the cumulative exposure to smoking.

The prospective design ensures that smoking history was assessed before the diagnosis of MS was made and thus prevents a differential reporting of exposure to smoking between cases and noncases. There remains the concern, though, that undiagnosed cases who experienced their first symptoms of MS might have increased their smoking as a consequence of the symptoms. In fact, the opposite was true. The age-adjusted rate of quitting smoking between first symptoms and diagnosis among women with MS was higher than that among women without MS symptoms. This is consistent with the attenuation of the risk we found when the most recent information on smoking prior to diagnosis was used as the exposure. Further, when the analyses were restricted to cases with first symptoms after the baseline, we found similar relative rates.

The majority of the diagnosing physicians were certified neurologists, which makes misclassification of the diagnosis unlikely. A predisposition among neurologists toward diag-

nosing MS more frequently in former and current smokers than in never smokers is also unlikely, given that smoking is not generally considered a risk factor for the disease. We controlled for known and proposed MS risk factors (especially latitude of residence) to minimize the possibility that the above association could be spuriously induced by a common cause of both smoking and MS.

Two other prospective cohort studies have assessed the association between MS and smoking. In the Oxford Family Planning Association Study (28), 17,032 White, married British women who attended family planning clinics between 1968 and 1974 were followed through 1991. Although there were only 63 new MS cases during the follow-up, a borderline significant linear trend between the number of cigarettes smoked at baseline and the risk of MS was detected ( $p = 0.05$ ) (22). The estimated risk for developing MS for women who smoked more than 15 cigarettes per day at baseline was 1.8 (95 percent CI: 0.8, 3.6) times greater than that for never smokers. The Royal College of General Practitioners' Oral Contraception Study was a cohort study conducted between 1968 and 1996 that identified 114 new MS cases among the 46,000 married British women initially recruited (23). The estimated risk for developing MS among women who smoked more than 15 cigarettes per day at baseline was 1.4 (95 percent CI: 0.9, 2.2) times greater than that among never smokers. These numbers are similar to that found in the NHS/NHS II.

On the other hand, the results of case-control studies have been conflicting. An Israeli study (18) that included 241 prevalent MS cases (92 percent of all the cases identified in the country in 1960) and randomly selected population controls found a significantly higher proportion of smokers before age at onset among cases than among controls (44 vs. 36 percent,  $p = 0.02$ ). Prevalence of current smoking was similar in MS patients and controls in a British study (19), but this result is minimally informative since many patients were likely to quit smoking because of the disease. No significant differences regarding smoking within the year before onset of the disease were found between 100 MS patients and 100 controls in Alberta, Canada (20). The control group, however, partly comprised patients with arthritis, a condition associated with smoking (8–12). A case-control study from Ferrara, Italy, did not find statistically significant differences regarding smoking in adolescence between 104 prevalent MS cases and 150 controls (21).

An accentuation of MS symptoms after smoking has been reported by several authors (14–17, 29–32) and was described in the first edition of the classical textbook, *Multiple Sclerosis* (33), but the etiologic relevance of these observations is unknown.

How smoking may be related to the increased incidence of MS is unclear. The link might depend on the immunomodulatory effects of smoking, although the evidence is sketchy. Different components of cigarette smoke may cause either immunosuppression (e.g., nicotine) or immunostimulation (e.g., tobacco glycoprotein) (3, 4). A predisposition to autoimmune responses in smokers has been suggested (34), and indeed, smoking has been found to be associated with an elevated risk of developing some autoimmune diseases, such as rheumatoid arthritis (8–12), systemic lupus erythematosus (6, 7), Graves' disease (35, 36), and Crohn's disease (37), but not others, such as Hashimoto's thyroiditis (36) and ulcerative colitis (37).

An alternative mechanism could involve a direct effect of cigarette smoke components on the blood-brain barrier. Nicotine has been shown to increase microvascular blood flow on the brain (38, 39) and to raise the influx of permeable solutes across the blood-brain barrier in rats (40). Leakage of the blood-brain barrier has been suggested as an initiating event in the development of MS (41).

Another possibility is that some components of cigarette smoke may have direct toxic effects on the central nervous system. Cyanide, a component of cigarette smoke (42), for which levels in the blood—and levels of its main metabolite, thiocyanate—are strongly correlated with amount of smoking (43, 44), has long been known to cause demyelination in the central nervous system of animals administered comparatively large (45–55), and possibly also lower (2, 56), doses. Demyelination is produced more successfully with repeated doses of cyanide than with one single, massive dose. This compound has also been implicated in epidemics of tropical spastic paraparesis (57, 58) and optic neuropathy (5, 59), diseases that share some clinical features with MS but that are characterized by symmetric bilateral symptoms and no history of relapses (5, 60).

Smoking might also increase the risk of MS by increasing the frequency and persistence of respiratory infections (13). For example, some reports (61) describe an association between *Chlamydia pneumoniae* infection and MS, and it has been shown that *C. pneumoniae*-specific antibodies are higher in smokers than in nonsmokers (62, 63). However, this association has not been confirmed (64), and the relevance of any of these or other mechanisms to the association between smoking and MS remains to be established.

In summary, in two prospective studies of US women, we have found that the incidence of MS was higher for cigarette smokers compared with never smokers and increased with cumulative exposure to smoking. Although the biologic basis for the link between smoking and MS remains to be elucidated, these results suggest that smoking may increase the risk of developing MS.

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