Smoking and multiple sclerosis: evidence for latitudinal and temporal variation

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Contributory statement

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ABSTRACT

There is growing evidence for the role of smoking in the aetiology of multiple sclerosis. We have expanded existing meta-analyses and further explored the roles of study design, gender, latitude and year of study with regression modelling. We have found a consistent association between smoking and MS with an odds ratio of approximately 1.5, with males at higher risk. This finding is independent of study design. However, latitude and year of study may have unexpected influence. Smoking appeared to confer a greater risk to females living closer to the equator than to females at higher latitudes. The effect of cigarette smoke exposure on MS risk may not be fixed over time, but could be increasing. These results suggest a threshold model of MS risk which includes a fairly constant genetic risk (for Caucasian populations) together with variable environmental risks which are dominated by vitamin D deficiency at higher latitudes and are more significant in women who have an intrinsically lower threshold for development of disease.

MAIN TEXT

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory condition of the central nervous system.[5]

Susceptibility to the disease results from genetic and environmental influences. There is strong evidence for a number of environmental factors including latitude (likely related to exposure to ultraviolet light and vitamin D levels), Epstein-Barr virus and exposure to cigarette smoke.[32] Obesity may also be important.[29]

There has been increasing epidemiological evidence for an association between smoking and MS within the last two decades. Over the same period, there has been an increase in the incidence and prevalence of MS in many countries. This increase is largely attributable to more women developing the disease.[22, 23, 33] Meanwhile the prevalence of smoking has declined in the developed world, whilst the relative number of women who smoke has increased.[2, 11, 12, 37]

A single case-control study that objectively measured tobacco exposure with blood levels of cotinine, a nicotine metabolite, found females had increased risk of MS at levels of exposure equivalent to passive smoking.[43] At these exposure levels men were unaffected. Case-control studies specifically evaluating passive smoke exposure in children[27] and adults[16, 34] found no difference in susceptibility by gender.

There have been two prior meta-analyses evaluating the influence of smoking upon risk of multiple sclerosis, including 14 and 6 studies respectively.[14, 15] We have undertaken a further meta-analysis of case-control and cohort studies, including more recently published material, to

establish cigarette smoking as a risk factor for MS. We have gone on to specifically examine gender-specific risks, and correlation with latitude and year of study to investigate whether smoking habit has contributed to a latitude gradient or the increase in incidence of MS in females.

METHODS

Articles were extracted by a search in all languages in Pubmed (1964-present), Google Scholar, CINAHL and the Cochrane library. Search terms were: “smoking”, “cigarettes”, “multiple sclerosis”, “demyelinating disease”. Bibliographic references from identified studies and references from review articles were checked to identify other eligible studies. The following exclusion criteria for studies were used: a) study did not include a suitable control group (for case-control studies); b) presented data were inadequate to permit reanalysis to obtain relative risk (RR) or odds ratio (OR) with confidence intervals; c) where the population studied was a repeat sampling (only the first published set of results for the population was included in the meta-analysis); d) subjects were not adults; e) diagnosis was not confined to probable or definite MS diagnosed by recognised international diagnostic criteria of the time; and f) study only published as abstract, poster or thesis. Where studies did not quote an OR for ever smoker versus never smoker, this was calculated from available data. The two cohorts of the Nurses’ Health Study (NHS I&II) could not be combined and were treated as two independent studies in the meta-analysis.[18]

Studies for analysis included case-control and cohort studies which varied in measure of effect (OR and RR). By the ‘rare disease assumption’ OR and RR can be considered equivalent.[4] RR and OR were analysed on the log scale in a restricted maximum likelihood estimation random effects model. The primary analysis was of two parts: where smoking status prior to

diagnosis of MS was ascertained (conservative analysis) and where smoking habits were established at time of study (non-conservative analysis).

Heterogeneity between trials was assessed with calculation of I^2 and τ^2 as well as by production of forest and funnel plots.[7] I^2 is a percentage estimate of how much of the observed variability between study estimates can be attributed to heterogeneity. τ^2 is an estimate of between-study variance in random-effects model meta-analysis, and τ ($\sqrt{\tau^2}$) is an estimate of standard deviation of underlying effect across studies. In meta-regression, inclusion of moderators that reduce between study heterogeneity will reduce τ^2 . [7]

Funnel plots are scatter plots of exposure effects against study size. With increasing study size there is increasing precision; smaller studies scatter at the bottom of the graph, with larger studies converging near the top.[10] Asymmetry results from bias, true heterogeneity, data irregularities, artefact and chance. True heterogeneity may reflect difference in size of effect according to study size (e.g. in an observational study - ascertainment bias) or differences in underlying risk.[8] For observational studies, a skew in a funnel plot is likely due to bias or confounding, rather than publication bias.[9] For these analyses, funnel plots were constructed for OR or RR against standard error, generating plots most useful to assess possible bias in studies of similar size.[41]

The pre-specified variables for further analysis were gender, latitude and year of study, with the null hypotheses of no effect of these variables on magnitude of risk with smoking. Subgroup analysis was based upon the categories used in prior meta-analysis. Latitude was determined by either the site of study centre or geographical midpoint of the region or country surveyed as appropriate. Latitude and year were included as continuous variables in a mixed-effects analysis (meta-regression) and predictive models from these analyses were developed. The effects of

each moderator was plotted against risk estimates, whilst the other was held constant.

Subgroup analyses were performed by the same methods. Categorical variables were included in meta-regression using dummy variables to reduce the total number of analyses. Examination of externally standardised residuals and case deletion diagnostics was performed to identify those studies which were influential but statistical outliers within the analysis.[45] Analyses were repeated with outliers removed. All analyses were conducted in R[44] with the metafor package.[45]

RESULTS

There were 26 included studies (summarised in Table e1 and listed in Appendix 1). In total there were 8,615 cases of MS and 392,352 controls. The cohort studies contributed an additional 792 cases of MS from a total cohort population of 601,492 individuals, representing more than 9 million person-years. The nineteen excluded studies are detailed in Appendix 2.

Table e1 – Included studies in meta-analysis

There were 17 studies suitable for inclusion in the conservative analysis. The summary OR was 1.54, 95% CI 1.41-1.67 ($I^2=16\%$, $p<0.0001$). The non-conservative analysis included 9 studies and gave a summary OR of 1.49, 95% CI 1.24-1.80 ($I^2=77\%$, $p<0.0001$). There was no significant difference between these results ($p=0.6309$). Combining these analyses gave a summary OR of 1.51, 95% CI 1.38-1.65 ($I^2=56\%$, $p<0.0001$) (figure 1). The estimates for I^2 suggest low to moderate levels of between-study heterogeneity and was higher in the non-conservative analysis.

Inspection of the funnel plot (figure e1) shows four studies lying outside the 95% credibility intervals [6, 24, 40] and one in contact.[34] When segregated for gender, the summary OR for males was 1.79, 95% CI 1.57-2.03 ($I^2=34\%$, $p<0.0001$) from ten studies. Twelve studies, including both cohorts from the Nurses' Health Study[18] gave a summary OR for females of 1.36, 95% CI 1.18-1.56 ($I^2=56\%$, $p<0.0001$). In each case, the estimates for I^2 suggested moderate between-study heterogeneity.

Subgroup analyses based upon study design were performed (table 1) and showed no significant departures from the reported overall result. There were no significant differences between subgroups.

Table 1: Subgroup Analyses

Subgroup	OR	(95% CI)	Number of studies	p-value
Case-control	1.50	(1.36 - 1.66)	20	0.7641
Cohort	1.56	(1.27 - 1.90)	6	
Interview / medical records smoking data	1.45	(1.26 - 1.66)	13	0.2055
Questionnaire smoking data	1.56	(1.35 - 1.80)	12	
Diagnostic criteria	1.49	(1.35 - 1.65)	21	0.9266
Self / Physician reported	1.65	(1.27 - 2.13)	4	
Current vs ever/never comparison	1.29	(1.03 - 1.61)	4	0.1292
Ever vs never comparison	1.55	(1.42 - 1.69)	22	

Effect of latitude and year of study

Incorporating latitude and year separately as moderators in a mixed-effects model showed that latitude had a significant effect within the model ($p=0.0054$) and reduced heterogeneity ($I^2=42\%$, $\tau^2=0.0145$). Year also had a significant effect ($p=0.0185$) with reduced heterogeneity ($I^2=43\%$, $\tau^2=0.0161$). A combined model found that both latitude ($p=0.0005$) and year ($p=0.0011$) each had significant effects on the risk estimate. Estimated residual heterogeneity was very low ($I^2=15\%$, $\tau^2=0.0038$), suggesting that these moderators account for >99% of the observed heterogeneity. Accordingly, incorporation of these variables in the model brought all studies within the bounds of 95% credibility intervals (Figure 2).

Predicted OR by latitude, holding median year as a fixed variable (2008), shows an increase in OR from 1.39, 95% CI 1.27-1.52 at 60 degrees to 1.83, 95% CI 1.63-2.06 at 30 degrees. This data is illustrated graphically in figure 3. Similarly, predicted OR by year, holding median latitude as a fixed variable (45 degrees), shows an increase in OR from 1.17, 95%CI 0.99-1.39 in 1960 to 1.61, 95% CI 1.50-1.73 in 2010. This relationship is illustrated in figure 4. To explore these relationships further the analyses were repeated, segregated by gender. For males, there was no significant association with year ($p=0.25$) or latitude ($p=0.45$). For females, latitude ($p=0.0161$) remained significant and year had borderline significance ($p=0.057$). These relationships are illustrated in figures e2 and e3.

Examination of externally standardised residuals and case deletion diagnostics found two studies[24, 40] that were outliers in the meta-analysis. Repeating the analysis without these studies did not change the overall result or the underlying relationship to latitude ($p=0.0054$) but association with year was lost. When segregated by gender, the relationships with latitude ($p=0.0116$) and year ($p=0.0364$) were present in females. Repeating the analyses with the two earliest studies excluded showed preserved association with latitude ($p=0.0027$) but not with

year, in separate and combined models. Gender segregated results were unchanged but association with year was lost in females.[1, 40]

DISCUSSION

This meta-analysis has shown there is an increased risk of developing MS in ex-smokers and current smokers. We have significantly expanded upon previous meta-analyses, with 26 included studies.[14, 15] The results are robust, with conservative and non-conservative analyses returning similar results, despite higher levels of between study heterogeneity in the non-conservative model. Subgroup analysis failed to reveal any significant source of between study heterogeneity in the variables analysed. Risk estimates were higher for males than females, with the bounds of 95% confidence intervals just overlapping.

The underlying mechanism for smoking as a risk factor for MS is unclear. Cigarette smoke exposure induces pro-inflammatory changes,[20] has numerous immunological effects, including increasing susceptibility to respiratory viruses[13] and causes changes to post-translational processing of immunogenic proteins[25] that could plausibly increase MS susceptibility. Several components of cigarette smoke are particularly toxic to oligodendrocytes and neurons.[32] Evidence of interaction between smoking and EBV infection[39] has not been replicated.[36, 42] Interactions between susceptibility alleles and smoking have been found in a population-based case-control study,[17] but there was no interaction in a study of multiplex families.[21] Smokers tend to have lower serum vitamin D levels[28, 38] and it is reasonable to assume that choice to smoke is unrelated to underlying MS susceptibility (genetic risk). Smokers may partake in other risk taking behaviours, and patients with MS may have a higher intake of alcohol than the general population.[26] However, there are no other data to support underlying 'genetic' risk taking in MS susceptibility alleles.

The results of the mixed-effects model regression were interesting. Incorporating year and latitude into the analysis reduced the residual heterogeneity to <1% and moved all studies within the 95% credibility intervals on the funnel plot, suggesting that these variables may explain much of the observed between study heterogeneity. We found increasing risk of MS for smokers with decreasing latitude, an effect that seems to be specific to females. This runs contrary to the usual pattern of increased MS susceptibility with latitude. Meta-analysis of observational studies has limitations and interpretation of these results should be cautious. Combining the data from studies assumes similarity of study populations, ethnicity, tobacco composition, and unmeasured variables that may be confounded with smoking habit. However, if these findings are valid, there may be a confounder specific to females in operation with differential effect by latitude. MS susceptibility can be seen as a threshold that needs to be passed to develop disease, with any combination of risk factors being sufficient to meet or exceed that threshold. We have previously shown that genetic risk in multiplex families seems constant at different latitudes, with the differences in prevalence being attributable to environmental factors, but that the relative contributions of hereditary and environmental factors does vary with latitude.[31] The strongest genetic susceptibility allele, HLA-DRB1*1501, is up-regulated by vitamin D.[35] This effect may only contribute to susceptibility in conditions of vitamin D deficiency.

At any given latitude, females will have a higher absolute risk to develop MS than males.[31] Prevalence studies have shown that the female:male sex ratio in MS decreases with increasing latitude, suggesting that latitudinally dependent environmental factors may have differential effects on males and females (e.g. increasing female susceptibility at lower latitudes). In a threshold model, individuals at higher latitudes may reach MS susceptibility through vitamin D deficiency or lack of ultraviolet (UV) radiation exposure alone. If smoking has an independent

effect on increasing susceptibility it may be less relevant in those conditions. Whereas, at lower latitudes where relative vitamin D deficient may be uncommon, smoking may have a greater contribution to MS susceptibility. This would create an inverse association between smoking and MS risk and latitude. This effect might be more clearly seen in those whose baseline susceptibility is higher (i.e. females).

Other possibilities include that smoking habits are confounded by other factors that differ with latitude such as lifestyle, occupation, socioeconomic status or other environmental exposures. It has been shown that change in smoking habits in females accompanied population movement from rural to urban areas, and this could be expected to change other environmental exposures including vitamin D and UV radiation exposure.[23] Smokers tend to have lower levels of serum vitamin D than non-smokers.[28, 38] The magnitude of risk in Tasmania, Australia (where the majority of the population have functional vitamin D deficiency for much of the year) (OR 1.5, 95% CI 1.0 – 2.4)[39] is comparable to the risk estimate for the Queensland population at lower latitude (OR 1.9, 95% CI 1.5-2.5)[30]. Smoking associated vitamin D deficiency might be relevant in regions where vitamin D deficiency is not the norm.

The mixed-effects model regression also found a positive association between risk and year of study. This association was more tenuous being lost with exclusion of the outliers from meta-analysis or with exclusion of the two earliest studies. If real, this might result from a change in relative importance of risk factors over time (assuming a threshold model), a change in the composition and design of cigarettes [19] or confounding by other variables measured by smoking habit. For example, the socioeconomic status of smokers has changed over time.[3, 46]

The present study adds to existing data confirming a very clear association between smoking and risk of developing MS. Whilst association does not prove causation, there are clear pathophysiological mechanisms by which smoking can affect both the central nervous system and immune function, and further research on these potential mechanisms is warranted. We have shown that the effects of smoking on MS susceptibility have increased over the period of time studied. The effects of smoking seem to be increased at lower latitudes in females. This is consistent with a threshold model for MS susceptibility. Changes in lifestyle, occupation and socioeconomic status may play a role in the increase in MS prevalence in women over the last 40 years.

COMPETING INTERESTS

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ETHICAL STANDARDS

The manuscript does not contain any patient data.

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APPENDICES

Appendix 1 – Included studies

Appendix 2 – Excluded studies

FIGURE LEGENDS

Figure 1 – Forest plot of smoking and MS risk

Figure 2 – Funnel plot of mixed-effect model

Figure 3 – Latitude against Odds Ratio*

Figure 4 – Year against Odds Ratio*

*: odds ratios plotted proportional to inverse of standard error

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Figure e1 – Funnel plot Log OR or RR against standard error

Figure e2 – Latitude against Odds Ratio (Females)*

Figure e3 – Year against Odds Ratio (Females)*

*: odds ratios plotted proportional to inverse of standard error