Interaction between passive smoking and two HLA genes with regard to multiple sclerosis risk

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Abstract

Background: The recently described interaction between smoking, human leukocyte antigen (HLA) DRB1*15 and absence of HLA-A*02 with regard to multiple sclerosis (MS) risk shows that the risk conveyed by smoking differs depending on genetic background. We aimed to investigate whether a similar interaction exists between passive smoking and HLA genotype.

Methods: We used one case-control study with incident cases of MS (736 cases, 1195 controls) and one with prevalent cases (575 cases, 373 controls). Never-smokers with different genotypes and passive smoking status were compared with regard to occurrence of MS, by calculating odds ratios (ORs) with 95% confidence intervals (CIs). The potential interaction between different genotypes and passive smoking was evaluated by calculating the attributable proportion (AP) due to interaction.

Results: An interaction was observed between passive smoking and carriage of HLA-DRB1*15 (AP 0.3, 95% CI 0.02–0.5 in the incident study, and AP 0.4, 95% CI 0.1–0.7 in the prevalent study), as well as between passive smoking and absence of HLA-A*02. Compared with non-smokers without any of these two genetic risk factors, non-exposed subjects with the two risk genotypes displayed an OR of 4.5 (95% CI 3.3–6.1) whereas the same genotype for subjects exposed to passive smoking rendered an OR of 7.7 (95% CI 5.5–10.8).

Conclusions: The risk of developing MS associated with different HLA genotypes may be influenced by exposure to passive smoking. The finding supports our hypothesis that priming of the immune response in the lungs may subsequently lead to MS in people with a genetic susceptibility to the disease.

Key words: Multiple sclerosis, smoking, passive smoking, HLA genotype, gene-environment interaction, case-control study, immunology
**Key Messages**

- Exposure to tobacco smoke increases the risk of MS.
- Smoke-induced lung irritation induces inflammation and posttranslational modifications of proteins in the lungs.
- There is an interaction between tobacco smoke exposure and HLA genotypes in the development of MS.
- Tobacco smoke exposure, in the context of HLA MS risk genes, may involve autoimmunity against proteins with post-translational modifications that are cross-reactive with CNS antigens.

**Introduction**

MS is a complex disease where both genetic and environmental factors contribute to disease susceptibility. The strongest genetic associations with MS are presence of the class II allele HLA-DRB1*15, which increases the risk of MS with an odds ratio around 3,\(^2,3\) and the class I allele HLA-A*02, which has a protective effect with an odds ratio of approximately 0.7.\(^2,3\) One of the most established environmental risk factors influencing MS risk is smoking.\(^4\) The recently described interaction between smoking and HLA complex genes with regard to MS risk shows that the risk conveyed by an environmental factor may substantially differ depending on genetic background, and vice versa. A significant interaction was observed between smoking and the genetic risk factors carriage of HLA-DRB1*15 and absence of HLA-A*02. Compared with non-smokers without any of these two genetic risk factors, the odds ratio for MS among non-smokers with both genetic risk factors was 4.9 (3.6–6.6) whereas the OR among smokers with both these genetic risk factors was 13.5 (8.1–22.6).\(^5\) We hypothesize that the interaction between smoking and HLA genotype occurs as a result of smoking-related lung irritation. Using the same Swedish population-based case-control study, and an American prevalent case-control study, we aimed to investigate whether a similar interaction exists between passive smoking and HLA genotype.

**Methods**

**Study design and study subjects**

This report was based on data from two case-control studies on environmental and genetic risk factors for MS. The first study is EIMS (Epidemiological Investigation of Multiple Sclerosis) with a study group comprising large parts of the Swedish population aged 16–70 years. Incident cases of MS were recruited via 40 study centres, including all university hospitals in Sweden. All cases fulfilled the McDonald criteria. For each case, two controls were randomly selected from the national population register, matched by age (5-year age groups), gender and residential area. During the study period April 2005 to March 2012, completed questionnaires were obtained from 1798 cases and 3907 controls, the response proportion being 91% for the cases and 69% for the controls. Ethical approval was obtained from the relevant ethics committee. More details on study design and methods are given elsewhere.\(^5\)

The prevalent case-control study, comprising 1238 cases and 701 controls, used a study population of White non-Hispanic people identified among members of Kaiser Permanente Medical Care Plan, Northern California Region, USA, (KPNC) using electronic medical records. KPNC is an integrated health services delivery system with a membership of 3.2 million that comprises about 25–30% of the population of a 22-county service area in northern California. Cases of MS were required with an MS diagnosis by a neurologist, self-identified White race/ethnicity, age 18 through 69 years and KPNC membership. Controls were randomly selected from current KPNC members who did not have a MS diagnosis or related conditions, and were individually matched to cases on gender, birth date, race/ethnicity and ZIP code of the case residence. The study participation proportion was 79% for cases and 58% for controls. The study protocol was approved by the Institutional Review Boards (IRB) of KP Division of Research and the University of California, Berkeley.

**Data collection**

In EIMS, information regarding lifestyle factors and different exposures was collected using a standardized questionnaire. Information on smoking was obtained by asking about current and previous smoking habits, and information on exposure to passive smoking (i.e. exposure to environmental tobacco smoke) was obtained by asking if the subject had been daily exposed to passive smoking at home or at work (Supplementary Table 1, available as Supplementary data at IJE online). For each case, the time of the initial appearance of MS symptoms was used as an
estimate of the disease onset, and the year in which this occurred was defined as the index year. The corresponding controls were given the same index year.

Smoking and exposure to passive smoking were considered prior to and during the index year in the cases and during the same period of time in the corresponding controls. Subjects who had smoked before or during index were defined as ever-smokers, whereas those who had never smoked were defined as never-smokers. All subjects who had smoked prior to or during the index year were excluded (965 cases and 1766 controls). Never-smokers who had been exposed to passive smoking before or during index were defined as exposed to passive smoking, whereas never-smokers who had never been exposed to passive smoking were defined as not exposed.

In the KPNC study, participants completed a computer-assisted telephone interview regarding lifestyle factors and various exposures. Information on smoking was obtained by asking about current and previous smoking habits, and information on passive smoking was obtained by asking detailed questions regarding exposure to passive smoking at home. Smoking and exposure to passive smoking were considered prior to disease onset in the cases, and before the age of 32 (median age at disease onset among cases) in the controls. All cases who had smoked prior to disease onset, and all controls who had smoked before the age of 32, were excluded (559 cases and 274 controls).

Never-smokers who had been exposed to passive smoking were defined as exposed to passive smoking, whereas never-smokers who had never been exposed to passive smoking were defined as not exposed.

### Genotyping and definition of genetic risk factors

All EIMS participants who filled out the questionnaire were asked to provide a blood sample. Among never-smokers, blood samples were available for 736 cases (89%) and 1195 controls (56%). Allelic dosages of HLA-DRB1*15 and HLA-A*02 were obtained by one of three methods: (i) polymerase chain reaction (PCR) amplification with sequence-specific primers (Olerup SSP low resolution typing kits, Olerup SSP AB, Stockholm, Sweden); (ii) imputation using HLA*IMP:02 based on single nucleotide polymorphisms (SNPs) genotyped on the Immunochip custom array in an overlapping Swedish cohort; or (iii) TaqMan® allelic discrimination of the SNPs rs9271366 (assay ID: C__33416976_20), which tags HLA-DRB1*15, or rs2975033 (assay ID: C__15962692_20) which tags for HLA-A*02 allele has reproducibly shown a protective association to MS. Absence of HLA-A*02 is thus a risk factor of developing the disease, and the participants were classified according to no carriage of any HLA-A*02 allele vs any HLA-A*02 allele.

### Statistical analysis

Never smoking subjects with different genotypes and exposures to passive smoking were compared with regard to occurrence of MS, by calculating odds ratios (ORs) with 95% confidence intervals (CIs) employing logistic regression.

The potential interaction between passive smoking and HLA-DRB1*15 and HLA-A*02, respectively, was examined by calculation of departure from additivity of effects and evaluated by calculating attributable proportion (AP) due to interaction, as suggested by Rothman, together with a 95% CI. In addition, an alternative version of AP recently suggested by Vanderweele, that uses a different denominator, was calculated. The data from the two studies were analysed separately and in combination.

In EIMS, we performed matched analyses based on all available case-control duplets/triplets, as well as...
unmatched analyses of the data based on all available cases and controls. Only the results from the unmatched analyses are presented in this report since these were in close agreement with those from the matched analyses but in general had a higher degree of precision (due to a higher number of controls).

All analyses were adjusted for age, gender, residential area and ancestry (Northern European ancestry or not). In EIMS, additional adjustments were further made for heredity (yes/no), educational level (university degree or not), socioeconomic status (eight categories according to an established socioeconomic classification13), ultraviolet radiation exposure habits (high/low) and body mass index at age 20 (more or less than 27 kg/m2). However, these factors had minor influence on the results and were not retained in the final analyses. All analyses were conducted using Statistical Analysis System (SAS) version 9.2.

Results

Our analyses of passive smoking and HLA genotype among never-smokers with regard to MS risk included 736 cases and 1195 controls from EIMS, and 575 cases and 373 controls from the KPNC study. In EIMS, almost all cases were recruited within 1 year after the diagnosis and the mean duration between onset of the disease and inclusion in the study was 4.6 years. The median of the disease duration in the KPNC study was 10.0 years. Characteristics of cases and controls by study and smoking status are presented in Table 1.

Among never-smokers, subjects who had been exposed to passive smoking had an increased risk of MS compared with those who had never been exposed (OR 1.3, 95% CI 1.1–1.6 in EIMS, and OR 1.7, 95% CI 1.3–2.3 in the KPNC study). There was a dose-response correlation between duration of exposure (years) and MS risk (P value for trend 0.006 in EIMS and <0.0001 in the KPNC study). There were no significant gender or age related differences (data not shown).

We observed a statistically significant interaction between smoking and carriage of HLA-DRB1*15 with regard to MS risk (AP 0.3, 95% CI 0.02–0.5 in EIMS, and AP 0.4, 95% CI 0.1–0.7 in the KPNC study). The result remained similar whether or not the analysis of interaction was adjusted for HLA-A*02 status (Table 2). Similarly, an interaction was observed between smoking and absence of HLA-A*02 with regard to MS risk (AP 0.3, 95% CI 0.03–0.5 in EIMS, and AP 0.3, 95% CI –0.02–0.5 in the KPNC study). Adjustment for HLA-DRB1*15 status did not affect the result (Table 3). In a combined analysis considering all three factors, we calculated the ORs associated with the most susceptible genotype (carriage of DRB1*15 but not HLA-A*02) among those who had and had not been exposed to passive smoking, compared with non-exposed subjects without these genetic risk factors (Table 4). Non-exposed subjects with the two risk genotypes displayed an OR of 4.5 (95% CI 3.3–6.1) whereas the same genotype for subjects exposed to passive smoking rendered an OR of 7.7 (95% CI 5.5–10.8). The interaction between carriage of the risk factor HLA-DRB1*15, absence of HLA-A*02 and passive smoking is illustrated in Figure 1.

Discussion

We herein describe an interaction between presence of HLA-DRB1*15 and passive smoking, and between absence of HLA-A*02 and passive smoking, with regard to MS risk. Subjects exposed to passive smoking carrying HLA-DRB1*15 and lacking HLA-A*02 had an 8-fold increased risk compared with non-exposed subjects without the genetic risk factors (OR 7.7, 95% CI 5.5–10.8). The impact of exposure to passive smoking on MS risk is weaker than the influence of active smoking. Consequently, the interaction that takes place between passive smoking and HLA risk genes is less pronounced than the recently described interaction between smoking and the same risk genes.5 However, our study suggests that also lower degrees of lung irritation may contribute to the triggering of the disease, in particular among subjects with a genetic susceptibility to MS.

Both studies retrospectively collected information regarding lifestyle factors and other exposures. Theoretically, this

| Characteristics | Cases | Controls |
|-----------------|-------|----------|
| EIMS            |       |          |
| Women (%)       | 539 (73) | 862 (72) |
| Men (%)         | 197 (27) | 333 (28) |
| Scandinavian origin (%) | 640 (87) | 997 (83) |
| Passive smoking (%) | 283 (38) | 399 (33) |
| <10 years       | 77 (10)  | 106 (9)  |
| 10–20 years     | 131 (18) | 195 (16) |
| ≥20 years       | 75 (10)  | 98 (8)   |
| Total           | 736 (100) | 1195 (100) |
| KPNC study      |       |          |
| Women (%)       | 470 (82) | 320 (86) |
| Men (%)         | 105 (18) | 54 (14)  |
| Scandinavian origin (%) | 359 (62) | 242 (65) |
| Passive smoking (%) | 387 (67) | 206 (55) |
| Total           | 575 (100) | 373 (100) |
could lead to biased estimates of the ORs associated with passive smoking (recall bias). However, this potential bias would not be differential with regard to genotype and thus would not seriously affect the estimated interaction between passive smoking and the two HLA alleles studied. This EIMS used incident cases of MS in order to minimize recall bias. Furthermore, the questionnaire contained a wide range of questions regarding many potential environmental risk factors and no section in the questionnaire was given prime focus. The KPNC study was based on prevalent cases of MS. Thus, recall bias may be more substantial in this study than in EIMS.

A potential selection bias may arise when recruiting cases and controls. The proportion of respondents with regard to participation in EIMS was 91% for cases and 69% for controls. Considering the structure of the public Swedish health care system, which provides equal, free-of-charge access to medical services for all Swedish citizens, it is most likely that almost all cases of MS are referred to neurological units. Selection bias among controls is likely to be modest since the prevalence of smoking among controls was in line with that of the general population at equivalent ages. Furthermore, the increased risk for MS associated with passive smoking was similar among those who provided blood and those who did not, indicating that selection did not take place in this step. We consider it unlikely that our main finding of an interaction between HLA genotype and exposure to passive smoking would be affected by bias to a large extent, especially since such a bias would then depend on HLA types.

In the KPNC study, smoking and exposure to passive smoking were considered prior to disease onset in the cases, and before the age of 32 in the controls (i.e. the median age at disease onset among cases). The rationale for this is that controls were not matched to cases on time of diagnosis (or onset). By applying this procedure, all controls could be included in the analysis without creating significant bias in the estimation of the studied associations.

We hypothesize that the mechanism linking smoking/passive smoking to MS susceptibility involves autoimmunity against proteins with post-translational modifications that are cross-reactive with central nervous system (CNS) proteins.

### Table 2. A–C Adjusted ORs with 95% CIs of developing MS for never-smoking subjects exposed to different combinations of passive smoking and HLA-DRB1*15 status compared with subjects exposed to neither of these risk factors. Attributable proportion due to interaction (AP) between HLA-DRB1*15 and passive smoking

| A. Total | Passive smoking | OR (95% CI) | P | AP |
|----------|-----------------|-------------|---|----|
| DR15 status | ca/co | | | |
| – – | 279/660 | 1.0 (–) | | |
| – + | 286/436 | 1.4 (1.1–1.7) | 0.006 | |
| + – | 362/303 | 2.9 (2.3–3.6) | <0.0001 | |
| + + | 384/169 | 4.7 (3.7–6.0) | <0.0001 | |
| B. EIMS | Passive smoking | OR (95% CI) | P | AP |
| DR15 status | ca/co | | | |
| – – | 192/544 | 1.0 (–) | | |
| – + | 123/287 | 1.3 (1.0–1.7) | 0.09 | |
| + – | 261/252 | 2.9 (2.3–3.7) | <0.0001 | |
| + + | 160/112 | 4.3 (3.2–5.8) | <0.0001 | |
| C. KPNC study | Passive smoking | OR (95% CI) | P | AP |
| DR15 status | ca/co | | | |
| – – | 87/116 | 1.0 (–) | | |
| – + | 163/149 | 1.6 (1.1–2.3) | 0.02 | |
| + – | 101/51 | 2.7 (1.7–4.3) | <0.0001 | |
| + + | 224/57 | 5.6 (3.6–8.5) | <0.0001 | |

aNumber of cases and controls.

bAP as defined by Rothman.11 bbas defined by Wanner-Weele.12

cAdjusted for age, gender, ancestry, HLA-A*02 status and study.

dAdjusted for age, gender, residential area, ancestry and HLA-A*02 status.

eAdjusted for age, gender, ancestry and HLA-A*02 status.
The use of oral tobacco in the form of moist snuff is not associated with increased risk for MS, the critical effects of smoking and exposure to passive smoking may be caused by irritation in the lungs. Exposure to tobacco smoke results in increased pro-inflammatory cell activation in the lungs and post-translational modifications of proteins which may render them more autoantigenic. Autoimmune memory cells are present and available for triggering in the lungs. In EAE experimental autoimmune encephalomyelitis studies, these cells

Table 3. A–C Adjusted ORs with 95% CIs of developing MS for never-smoking subjects exposed to different combinations of passive smoking and HLA-A*02 status compared with subjects exposed to neither of these risk factors. Attributable proportion due to interaction (AP) between HLA-A*02 and passive smoking

| A. Total | Passive smoking | OR (95% CI) | P | AP |
|----------|-----------------|-------------|---|----|
| +        | –               | 264/510     | 1.0 (–) | 0.03 | 0.3 (0.1–0.5) |
| –        | –               | 244/328     | 1.3 (1.0–1.6) | 0.03 |
| +        | +               | 377/453     | 1.6 (1.3–2.0) | <0.0001 |
| –        | +               | 426/277     | 2.6 (2.0–3.2) | <0.0001 |

B. EIMS

| Passive smoking | OR (95% CI) | P | AP |
|-----------------|-------------|---|----|
| -               | 1.0 (–)     | 0.3 (0.07–0.8) |
| 194/423         | 1.3 (1.0–1.6) | 0.03 |
| 116/226         | 1.2 (0.9–1.6) | 0.3 |
| 259/373         | 1.5 (1.2–2.0) | 0.0005 |
| 167/173         | 2.4 (1.8–3.1) | <0.0001 |

C. KPNC study

| Passive smoking | OR (95% CI) | P | AP |
|-----------------|-------------|---|----|
| -               | 1.0 (–)     | 0.3 (0.02–0.5) |
| 70/87           | 1.2 (0.9–1.6) | 0.03 |
| 128/102         | 1.6 (1.1–2.5) | 0.02 |
| 1.7 (1.2–2.7)   | 1.7 (1.2–2.7) | 0.008 |
| 3.3 (2.2–4.9)   | 3.3 (2.2–4.9) | <0.0001 |

Table 4. Adjusted ORs with 95% CIs of developing MS for never-smoking subjects exposed to different combinations of passive smoking and the genetic risk factors carriage of the HLA-DRB1*15 allele and absence of the HLA-A*02 allele, compared with subjects exposed to neither of these risk factors. Both studies combined

| HLA-DRB1*15 | HLA-A*02 | Passive smoking | OR (95% CI) | P |
|-------------|----------|-----------------|-------------|---|
| -           | +        | –               | 118/343     | 1.0 (–) | 0.02 |
| -           | –        | –               | 161/317     | 1.4 (1.1–1.9) | 0.02 |
| +           | +        | –               | 146/167     | 2.5 (1.9–3.5) | <0.0001 |
| +           | –        | –               | 216/136     | 4.5 (3.3–6.1) | <0.0001 |
| -           | +        | +               | 101/233     | 1.1 (0.8–1.5) | 0.5 |
| -           | –        | +               | 185/203     | 2.3 (1.7–3.0) | <0.0001 |
| +           | +        | +               | 143/95      | 3.9 (2.8–5.5) | <0.0001 |
| +           | –        | +               | 241/74      | 7.7 (5.5–10.8) | <0.0001 |

Numbers of cases and controls. AP as defined by Rothman, as defined by WanderWeele. Adjusted for age, gender, ancestry and HLA-DRB1*15 status and study. Adjusted for age, gender, ancestry and HLA-DRB1*15 status. Adjusted for age, gender, ancestry and HLA-DRB1*15 status. The analysis was adjusted for age, gender, residential area (EIMS), ancestry and study.
strongly proliferate after local stimulation of the lungs and, after assuming migratory properties, reach the CNS with inflammation as a consequence. Preferences in peptide binding by allelic variants of class II molecules are likely to be critical for the HLA class II influences on autoimmune diseases. With regard to risk of MS and of rheumatoid arthritis, interactions may exist between smoking, HLA alleles and autoimmunity to post-translationally modified proteins. We thus hypothesize that smoke-induced lung-irritation in the context of HLA-MS risk genes may post-translationally modify peptides cross-reactive with CNS antigens, promoting a CNS-directed autoaggressive immunity that results in MS.

In conclusion, the observed interaction between exposure to passive smoking, presence of HLA-DRB1*15 and absence of HLA-A*02 with regard to MS risk may be considered as a replication of our recently observed interaction between smoking and the same genes. This gives support to the hypothesis of the pivotal role of the lungs in smoking-associated MS susceptibility. Further studies would be valuable in order to clarify whether other forms of lung irritation contribute to the triggering of MS.

Supplementary Data
Supplementary data are available at IJE online.

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Figure 1. ORs with 95% CIs for never-smokers with different combinations of HLA-DRB1*15 and HLA-A*02 status and exposure to passive smoking. Based on EIMS and the KPNC study. Statistics are shown in Table 4.
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