Published in final edited form as:

Arch Environ Occup Health. 2016 January 2; 71(1): 26–34. doi:10.1080/19338244.2014.937381.

Heavy metals, organic solvents and multiple sclerosis: an exploratory look at gene-environment interactions

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Abstract

Exposure to heavy metals and organic solvents are potential etiologic factors for multiple sclerosis (MS), but their interaction with MS-associated genes is under-studied. We explored the relationship between environmental exposure to lead, mercury, and solvents and 58 single nucleotide polymorphisms (SNPs) in MS-associated genes. Data from a population-based case-control study of 217 prevalent MS cases and 496 age-, race-, gender-, and geographically-matched controls were used to fit conditional logistic regression models of the association between the chemical, gene, and MS, adjusting for education and ancestry. MS cases were more likely than controls to report lead (odds ratio (OR)=2.03; 95% confidence interval (CI): 1.07, 3.86) and mercury exposure (OR=2.06; 95% CI: 1.08, 3.91). Findings of potential gene-environment

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Conflict of Interest Declaration: Dr. Marrie receives research funding from the Canadian Institutes of Health Research, Public Health Agency of Canada, Manitoba Health Research Council, Health Sciences Centre Foundation, Multiple Sclerosis Society of Canada, Multiple Sclerosis Scientific Foundation, Rx & D Health Research Foundation, and has conducted clinical trials funded by Sanofi-Aventis. I and Drs. Poole, Satten, Ashley-Koch, and Williamson have no conflicts of interest regarding the submitted manuscript.

Disclaimer: The results and conclusions in this article are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

interactions between SNPs in *TNF-α, TNF-β, TCA-β, VDR, MBP, and APOE*, and lead, mercury, or solvents should be considered cautiously due to limited sample size.

Keywords

autoimmune diseases; heavy metal exposure; epidemiology; case-control; gene-environment interaction; organic solvents multiple sclerosis

Introduction

Multiple sclerosis (MS) is a complex disease with an etiology that is hypothesized to involve both environmental and genetic factors.[1] Environmental agents are believed to trigger a T-cell-mediated chronic inflammatory response to myelin proteins in individuals with a genetic predisposition, creating the characteristic lesions that cause disease.[2] Therefore, examining environmental stressors and genes that modulate the immune system concurrently represent the best starting point for exploring the etiology of MS. Numerous environmental exposures such as heavy metals, organic solvents, ultraviolet radiation, infection, and diet have been investigated as possible etiologic factors for MS with inconsistent findings.[1,3–10]

The cellular accumulation of lead and mercury has been associated with the development of autoantibodies against neuronal cytoskeletal proteins, neurofilaments, and myelin basic protein in humans and animals.[11–13] Overexposure to lead and mercury ions is known to be neurotoxic, particularly to motor neurons.[14] Low-to-moderate levels of lead exposure can cause functional alterations in T-lymphocytes and macrophages that lead to increased hypersensitivity and can alter cytokine production, which increases risk of inflammation-associated tissue damage.[15] In non-predisposed animals, pretreatment with inorganic mercury can exacerbate autoimmune disease even prior to inducing disease.[16] In genetically susceptible animals, mercury exposure even at low doses accelerates autoimmune disease and leads to disruption in cytokine production.[13] These studies support an etiologic role of metals in autoimmune disease and suggest the importance of interactions between gene and environment, or between environmental factors in understanding metal toxicity.

Likewise, exposure to organic solvents, such as toluene or xylene, has been postulated to affect MS risk[1,8] through altered immune function or neurotoxicity.[8,17,18] Solvents have been linked to neurobehavioral changes, short-term memory impairment and loss of psychomotor functions.[18] There is limited research on the effects of organic solvents on the immune system however a recent meta-analysis concluded that such exposure is significantly associated with an increased risk of autoimmune diseases, including MS, primary systemic vasculitis, and systemic sclerosis.[17] Therefore, organic solvents likely exert their toxic effects through molecular mechanisms involving the immune system.

Studies of multiplex families, half-siblings, and adoptees have provided evidence for a genetic contribution to MS susceptibility.[19] The most convincing evidence for a genetic component comes from twin studies which have shown heritability estimates ranging between 25% and 76%.[20] Collectively, those studies suggest that multiple genes act either

independently or interactively with environmental factors to contribute to overall risk for MS. Several genetic loci for MS have been identified, including human leukocyte antigen (*HLA*) DRB1*1501 allele, interleukin 2 receptor alpha chain (*IL2RA*), and interleukin-7 receptor alpha chain (*IL7RA*).[21] Other immunomodulatory genes, such as the vitamin D receptor (*VDR*)[22] myelin basic protein (*MBP*),[23] tumor necrosis factor alpha and beta (*TNF-α/β*),[24] the T-cell antigen receptor beta (*TCA-β*),[25] and apolipoprotein E (*APOE*)[19] have plausible contributions to MS risk, but findings conflict.

Although it is believed that MS is multifactorial in etiology, little research has examined the joint role of environmental exposure to heavy metals or organic solvents and susceptibility to genes associated with an immune response and the subsequent development of MS.[26] The objectives of this study were: 1) to estimate the independent associations between MS and heavy metals (lead, mercury)/organic solvents; and 2) to explore potential gene-environment interactions between each heavy metal/solvent and nine previously identified MS susceptibility genes.

Methods

Study design and population

The study population consisted of 866 individuals (276 cases and 590 controls) who participated in a multisite population-based case-control study led by the Agency for Toxic Substances and Disease Registry (ATSDR) in 2005-2009. The details of the study population and design have been previously described.[27] Briefly, the study was conducted in four geographic areas of the US: the 28 counties comprising metropolitan Atlanta, Georgia; Lorain County, Ohio; the cities of Independence and Sugar Creek, Missouri; and the 19-county area surrounding Lubbock, Texas. Cases were those with a diagnosis of definite MS under both the Poser[28] and McDonald[29] criteria. Controls were individuals without MS selected by random-digit-dialing methods from the same geographic areas as the cases and matched by gender, race, age, and year of birth. Response rates ranged from 36% to 43%; further details can be found in our previous paper[27]. For this analysis, 153 participants were excluded for the following reasons: Forty-five non-white and 12 Hispanic participants were excluded to reduce heterogeneity in the genetic results that would result due to differing allele frequencies and disease risk among racial/ethnic groups. An additional three (3) participants were excluded because they reported having an ancestor from Africa, Asia or the Middle East (1 case; 2 controls). Eighty-four (84) participants that did not provide a blood sample for genotyping (due to refusals, scheduling conflicts, and unsuccessful blood draws) were also excluded. Finally, nine (9) participants were excluded because of missing exposure data. Therefore, the final study population for the present analysis included 713 participants (217 cases, 496 controls). Due to challenges in recruiting controls, the case: control ratio varied in the number of cases (1 to 4) and controls (1 to 12) per stratum.

Human subjects

Written informed consent was obtained from all participants. The study was approved by the Institutional Review Boards of the Centers for Disease Control and Prevention (CDC), Duke

University Medical Center, Michigan Public Health Institute, the Texas Department of State Health Services, and the Cleveland Clinic.

Data collection and exposure assessment

Interview and data collection has also been described previously.[27] Briefly, trained interviewers used computer-assisted telephone interviewing to collect information through questionnaires regarding demographics; lead, mercury, and solvent exposure history; established and hypothesized MS risk factors; family and medical histories; immunizations; residential and occupational histories; smoking; hobbies; and recreational activities. Questions from previous studies conducted by ATSDR, CDC, and our state health department collaborators were used to build the questionnaire. The questionnaire and study materials were refined and pilot-tested through focus groups of individuals with MS ineligible for participation or identified in a previous study of a metals smelter in EL Paso, Texas.[27] For each chemical exposure, participants reported their exposure to it in any form (e.g. inhalation, skin contact, ingestion, other) on a regular basis at any time (e.g. "Were you exposed to lead in any form (fumes, dust, particles)?), specified whether exposure occurred at work or outside of work (e.g. recreation), and reported the year(s) of exposure. Exposure was dichotomized as present or absent for lead, mercury and solvents because exposure was not quantified, nor was intensity or duration captured with self-report responses. Cases were considered exposed if exposure occurred at least one year before symptom onset and unexposed if they had no history of exposure at the time of symptom onset. Controls were considered exposed if exposure occurred at least one year before the date of interview and unexposed if they had no history of exposure before date of interview. Cases (n=2) who reported exposure after MS symptom onset and controls (n=0) who reported exposure after interview date were reclassified as unexposed.

Specimen collection and genotyping

Participants provided a blood sample (3 EDTA Starstedt tubes of whole blood) after completing the questionnaire. Samples were frozen at -80°C before shipment on dry ice to the Center for Human Genetics at Duke University Medical Center for DNA extraction and genotyping. DNA was extracted from lymphocytes using the PUREGENE system (QIAGEN, Germantown, MD). Genotyping was performed using the TaqMan single nucleotide polymorphisms (SNP) genotyping assay (Applied Biosystems, Foster City, California, USA).

Gene and SNP selection

Based on evidence at the time the study was designed, a total of 58 SNPs in nine candidate genes were selected for analysis because of their potential role in MS development and association with an immune response. Seven genes were associated with MS in a previous genome-wide association study (GWAS) or association study (*HLA* DRB1*1501,[21,30] *IL2RA*,[21] *IL7RA*,[21] *TCA-β*,[25] *TNF-α*,[30] *TNF-β*,[30] and *APOE*.[19] Two additional genes — *VDR* and *MBP* — were selected because there was biological plausibility of an association between each one and MS in the literature.[23,30,31] For all nine genes, we selected SNPs associated with MS from the literature or used a tag SNP approach to select SNPs.[32]

Haplotype tagging SNPs were identified using LDSelect v1.0[33] based on data from the CEU population (Utah residents with ancestry from northern and western Europe) in the HapMap project (www.hapmap.org). To minimize redundancy among SNPs in high linkage disequilibrium, a single SNP was selected to represent each haplotype block, as defined by $r^2 > 0.64$. SNPs were prioritized based on the potential for biological effect (coding SNPs, 5′/3′ untranslated and regulatory regions), physical position, and allele frequency.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was tested in controls for all SNPs using PLINK 1.07[34] with an alpha level of 0.005 to determine statistical significance. Conditional logistic regression models were fit to account for the matched design.[34] We calculated the odds ratio (OR) with 95% confidence intervals (CI) for the association between lead and MS, mercury and MS, and organic solvents and MS.

Gene-environment interaction was examined multiplicatively and additively using conditional logistic regression. To assess interaction on a multiplicative scale, we coded genotypes to count the number of minor alleles at each locus. We fit a model containing SNP and toxin main effects as well as a SNP-by-toxin interaction term. We calculated the 95% CI for the interaction term, and performed a Wald chi-square test using an alpha level of 0.05 to determine its statistical significance. To assess interaction on an additive scale, we constructed binary genotype variables using a dominant model. We estimated stratum-specific ORs and the relative excess risk due to interaction (RERI) and a 95% CI for each combination of joint effects according to Hosmer and Lemeshow.[35] If the combined effect of risk factors was larger (or smaller) than the sum of the stratum-specific effects, there was interaction on an additive scale. RERI estimates the strength of the interactive effect compared to the effect without exposure.[35] RERI values $\neq 0$ indicate additive interaction (alpha=0.05).

All analyses were adjusted for educational level (≤ high school graduate vs. post-high school education) and ancestry using questionnaire data regarding paternal and maternal ancestry. Participants were asked to list up to three countries where ancestors came from and responses were grouped into geographic areas (Western Europe, Eastern Europe, Mediterranean, Scandinavia, US/Canada, Don't know). The proportion of ancestry from each geographic region was entered as a covariate in the analysis. Statistical analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, North Carolina).

Results

The characteristics of the 713 participants in the present study are provided in Table 1. Four times as many participants were female as male, two-thirds of participants came from Georgia and Ohio, and 78% of participants had some college education. Respondents most commonly reported exposure to lead (13%), followed by mercury (9%), and organic solvents (6%). Approximately 62% of all participants reported no exposure to heavy metals or organic solvents while 12% reported exposure to one chemical and 5% reported exposure to more than one chemical. No SNPs were excluded from further analyses because of deviation from HWE.

Environmental exposures and MS

After adjustment for education and ancestry, MS cases were more likely to report exposure to lead (adjusted OR (AOR)=2.03; 95% CI: 1.07,3.86) or mercury (AOR=2.06; 95% CI: 1.08, 3.91) than controls. MS cases were less likely to report organic solvent exposure than controls (AOR=0.65; 95% CI: 0.28, 1.51) but this association was not statistically significant. Lead and mercury odds ratio estimates were more precise (i.e., had narrower confidence intervals) than solvent estimates.

Gene-environment interactions

Statistically significant ($p<0.05$) environmental interactions were identified on the multiplicative scale with SNPs in five of the genes examined (*TNF-α, TNF-β, VDR, MBP*, and *APOE*) (Table 2). For lead, there were six statistically significant environmental interactions identified: rs1799964/C (*TNF-α*), rs769178/T (*TNF-β*), rs2189480/T (*VDR*), rs3782905/C (*VDR*), rs4890785/T (*MBP*), and rs7412/T (*APOE*). For mercury, there were four statistically significant environmental interactions: rs1540339/T (*VDR*), rs2189480/T (*VDR*), rs2239186/G (*VDR*), and rs8096433/A (*MBP*). Three statistically significant environmental interactions were also identified with reported exposure to solvents: rs2238136/T (*VDR*), rs8096433/A (*MBP*), and rs17660901/G (*MBP*). One SNP modified the association of more than one exposure: rs8096433/A (*MBP*, with mercury and solvents); the estimates were on the same side of the null. The effect estimates for lead, mercury, and solvents were generally imprecise.

Additive interaction was identified between lead exposure and the rs17243/G allele in the *TCA-β* gene (See Supplemental Material, Table S1. The combined effect of having the rs1243/G allele and lead exposure was larger than the sum of the stratum-specific effects ($OR_{observed}$=2.05; 95% CI: 0.90, 4.67 vs. $OR_{expected}$=0.44), however the RERI was not significantly different from 0 (RERI=1.61; 95% CI: -0.02, 3.24). No evidence of additive interaction was observed between SNPs and mercury or SNPs and solvents (See Supplemental Material, Table S2 and S3). Similar to multiplicative interaction effect estimates, RERI values were generally imprecise.

Comment

Although MS is the most common non-traumatic neurologic disease disabling young adults in the United States,[36] its cause remains unknown. Evidence indicates that it is a complex autoimmune disease caused by both environmental factors and genetic susceptibility.[1] However, limited research has focused on potential gene-environment interactions especially with regard to exposure to heavy metals and organic solvents. In this study, we examined exposure to lead, mercury, and solvents, and 58 SNPs in genes associated with MS, and identified statistically significant multiplicative interactions with SNPs in five of the genes (*TNF-α, TNF-β, VDR, MBP*, and *APOE*) and additive interaction with a SNP in *TCA-β*. Interestingly, although *HLA* DRB1*1501 (rs3135388) is the strongest genetic susceptibility allele for MS and SNPs for *IL2RA* and *IL7RA* (rs2104286 and rs6897932, respectively) exert modest effects on risk (ORs=1.1-1.3),[21] none of those SNPs appeared to modify the relationship between any of the exposures and MS. Because of their role in

immune regulation and demonstrated association with MS risk, we had considered them the most plausible candidates for an interaction effect.

Existing epidemiologic evidence for the association between metals/solvents and MS is inconsistent partly due to small sample sizes, varying definitions of MS, exposure measurement, and whether they account for effect measure modification.[7,17] We found that participants with MS were more likely to report exposure to lead and mercury than controls, which is consistent with some prior studies,[5,37] but contrary to others.[38] For the organic solvent-MS association, we found no association between organic solvents and MS in contrast to two meta-analyses suggesting an increase in risk with exposure (relative risk=1.7; 95% CI: 1.1, 2.4 and OR=1.53; 95%CI: 1.02, 2.29).[8,17] Our study may have lacked the power to detect an association because the prevalence of self-reported exposure to organic solvents in the study population was 6%.

Our results indicating possible multiplicative interaction between metals/solvents and SNPs in the *MBP, VDR, APOE, TNF-α*, and *TNF-β* genes are novel. Interestingly, joint effects were identified between SNPs tagging *VDR* and *MBP* and lead, mercury, and solvents, although the individual SNPs varied. If confirmed, these results may suggest that *MBP* and *VDR* are important modifiers of the association between lead, mercury, solvents, and MS. The protein encoded by the *MBP* gene is a major constituent of the myelin sheath of oligodendrocytes and Schwann cells in the nervous system and an obvious candidate for exploration of MS etiology. In animal models, inorganic mercury exposure and other heavy metals has been shown to produce antibodies against *MBP*.[11–13] Vitamin D deficiency has been consistently associated with increased MS risk[3] and functional variations in genes in the vitamin D pathway, including *VDR*, are expected to also influence MS risk, but evidence so far is mixed.[39] A recent review suggested that the inconsistent associations between *VDR* polymorphisms and MS might reflect differences in environmental exposures that may modify a *VDR*-MS association.[3] Genetic variability among *VDR* polymorphisms may also influence the way that vitamin D produces an effect on MS.[39] Our results suggest that exposure to metals/solvents may help to explain these findings.

We identified joint effects between SNPs tagging *APOE, TNF-α*, and *TNF-β*, and lead exposure. As a major lipid carrier protein in the central nervous system, *APOE* has a putative role in immunomodulation and myelin repair. *APOE* has been identified as a genetic risk factor for increased disease severity, but inconsistently associated with disease susceptibility. Recent GWAS and large meta-analytic studies concluded that there is no association between *APOE* SNPs rs429358 (ε4) and rs7412 (ε2) and MS susceptibility.[40,41] Pro-inflammatory cytokines *TNF-α* and *TNF-β* located within the MHC III region are implicated in MS susceptibility and disease progression.[42,43] Both have been detected in MS lesions and are associated with the deterioration of myelin, apoptosis of oligodendrocytes, activation of astrocytes, lymphocyte infiltration, and triggering of T-cell responses in MS.[42–44]

We also identified interactions between a SNP tagging *TCA-β* (rs17243/G) and solvents on the additive scale. On the additive scale, the presence of both lead exposure and the rs17243/G allele resulted in an increase in odds ($OR_{observed}$=2.05 (95% CI: 0.90, 4.67))

rather than the decrease expected under the additive model ($OR_{expected}$=0.62+0.82-1=0.44). Had the RERI estimate been significantly different from zero, we would have been more confident in further quantifying the magnitude of the combined effect of the rs17243/G allele and lead exposure as 161% greater than the odds among those without the rs17243/G allele or lead exposure. T-cells are believed to be the main driver of autoimmune responses and inflammation in MS, but the effect of T-cell receptor polymorphisms, such as *TCA-β*, on MS pathogenesis is unclear.[25] Our results suggest further exploration is warranted.

The associations suggested by this study may also have relevance for other autoimmune diseases because some autoimmune diseases may share a common origin. It has been demonstrated that genetic susceptibilities, pathologic mechanisms, clinical manifestations, and environmental risk factors may be common to multiple autoimmune diseases, an idea referred to as the autoimmune tautology.[45] For example, smoking is of interest as an environmental risk factor for MS,[46] irritable bowel syndrome,[47] and rheumatoid arthritis.[48] In another example, *PTPN2* and *CTLA4*, genes highly expressed in T lymphocytes, have been associated with type 1 diabetes and celiac disease.[49] Thus, heavy metal and solvent exposure, and the genetic polymorphisms identified in this study, may similarly influence other autoimmune diseases and improve our understanding of mechanisms common to them. For autoimmune disease phenotypes in which a particular environmental factor plays a causative role, it would be helpful to distinguish these phenotypes as environmentally-associated rather than idiopathic. However, there is lack of consensus on how to define these two types of autoimmune disease, and on the amount of evidence necessary and sufficient to classify an environmental exposure as a risk factor for disease.[50] A strong exposure history that captures lifetime history of exposure across a variety of industries would be an integral part of such criteria.

Among the limitations of the study are a potential for recall error and exposure misclassification because exposure to heavy metals and solvents were assessed using self-report. MS cases may be more likely to remember past exposure to chemicals than controls as they search their past for potential causes for their disease. In an effort to reduce potential recall error, interviewers provided participants with a worksheet of questions to answer before the phone interview and read off a list of chemicals by name during the interview, rather than asking open-ended questions. Both strategies are believed to improve subjects' reporting accuracy.[51] Interestingly, there is evidence of little-to-no difference in the validity or reliability of self-reported occupational exposure assessments between cases and controls, so recall error may not be a significant source of bias if the exposure information is high quality.[51] Environmental and occupational chemical exposures are often successfully retrospectively ascertained using questionnaires and interviews, despite recognized concerns with misclassification and recall bias.[52–54] However, self-report is a broad assessment method that requires the participant to be aware of the specific chemicals to which they have been exposed occupationally and recreationally. Thus, self-reported exposure is likely an underestimation of the true exposure experience of our study population. While it was not possible to compare self-reported exposure with another metric in this study, self-reported exposure may correspond better to long-term exposure than other metrics (e.g. biomarkers) because it captures exposure from past residences and recreational activities.

A second limitation of this study is that there may have been other interactions we could not detect due to the small sample size and low prevalence of environmental exposures in our population. Epigenetic mechanisms, which include changes in DNA methylation and histone modification, may independently affect aspects of MS disease such as inflammation and demyelination, but also may mediate the effects of environmental risk factors on MS and its clinical course. Thus, environmental risk factors and epigenetic changes likely interact in complex ways to modulate MS. Despite our best efforts in this present analysis, it is possible that the lack of association we observed between some environmental factors and genetic variants may be because our study focused exclusively on association with disease and was not designed to capture intermediate epigenetic mechanisms at play. As much as 75% of MS heritability is estimated to be unexplained by known genetic variants.[55,56] Gene-environment interactions may well account for some of this missing heritability. These interactions may be mediated by epigenetic mechanisms; if this is the case, studying epigenetic changes directly may result in higher power to detect gene × environment interactions.

A third limitation of this study is that incident MS cases were unavailable. Although we used prevalent cases, we restricted analysis to participants who reported exposure before symptom onset to maintain exposure-disease temporality. Finally, we note that adjusting for multiple comparisons using the Bonferonni correction (by adjusting the p value cutoff to correspond to a size of 0.05/45) would render all our reported interactions non-significant. Although customary in genetic epidemiology, the application of multiple testing methods is still an area of debate with no clear resolution.[57–62] For the above reasons, our results may be considered exploratory until additional studies are conducted.

The main strength of this study is that it examines both the role of environmental exposure to metals/solvents and genetic factors in the development of MS using a population-based sample. Additionally, cases were identified using uniform ascertainment, an important consideration in multi-site studies, and were included in the study only if they were classified as having definite MS under both the McDonald and Poser criteria.[28,29] Finally, because MS has a variable latency period, we only counted cases as exposed if that exposure occurred at least one year before symptom onset, thereby ensuring the temporality is maintained.

Conclusion

The present study addresses the lack of research about (1) the association between lead, mercury, organic solvents and MS, and (2) the joint association that MS susceptibility genes and heavy metals/organic solvents have on MS prevalence. Results from this study are a critical starting point for exploring the validity of the immunological hypothesis of MS pathogenesis for metal/solvent exposure. However, additional studies with a larger sample size and objective exposure measurement are needed to confirm our findings. Assuming our results are replicated in prospective studies that are not subject to the possible biases of retrospective studies such as recall bias, understanding how immunomodulatory genes and heavy metals/organic solvents interact to affect risk of MS may be important for dissecting

disease mechanisms in the future and informing prevention and treatment strategies for this debilitating disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported by the Agency for Toxic Substances and Disease Registry [contract # 200-2003-01396].

The authors thank the individuals who participated in the study as well as Carolyn Harris, Sara Hatch, Janine Trottier, Laurie Wagner, the Michigan Public Health Institute and McKing Consulting.

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Table 1

Demographic characteristics and self-reported exposure information of MS cases and controls (*n*=713)

	Case (n=217) *n* (%)	Control (n=496) *n* (%)
State of Residence [a,b]		
Georgia	68 (31)	149 (30)
Missouri	18 (8)	51 (10)
Ohio	75 (35)	161 (32)
Texas	56 (26)	135 (27)
Sex[b]		
Male	43 (20)	106 (21)
Female	174 (80)	390 (79)
Education Attained[c]		
Less than High School Graduate	8 (4)	19 (4)
High School Graduate	46 (21)	84 (17)
Some Colleg e/Technical School	69 (32)	153 (31)
Graduated College	94 (43)	240 (48)
Lead Exposure [d]		
Yes	36 (17)	54 (11)
No	139 (64)	368 (74)
Don't know	42 (19)	74 (15)
Mercury Exposure [d]		
Yes	25 (12)	36 (7)
No	161 (74)	417 (84)
Don't know	31 (14)	43 (9)
Organic Solvents Exposure[d]		
Yes	10 (5)	30 (6)
No	188 (86)	446 (90)
Don't know	19 (9)	20 (4)
No. of Exposures		
0	119 (55)	323 (65)
1	34 (16)	55 (11)
>1	11 (5)	23 (5)
Don't know	53 (24)	95 (19)

[a] State lived in between Jan 1998 and Dec 2000 based on self-report.

[b] Matching factor. Age at interview and race/ethnicity were also matching factors (not shown).

[c] Highest level of education completed based on self-report.

[d] Self-reported exposure. Among MS cases, exposure was at least 1 year prior to MS symptom onset. Among controls, exposure was at least 1 year prior to interview.

Table 2

Associations between environmental exposures, SNPs, and MS case-control status adjusted for education and ancestry

Chr.	Gene	rs number	Minor Allele[a]	MAF	Lead OR (95% CI)[b]	*p*	Mercury OR (95% CI)[b]	*p*	Solvents OR (95% CI)[b]	*p*
5	*IL7RA*	rs6897932	A	0.25	1.06 (0.40,2.80)	0.90	1.34 (0.43,4.17)	0.61	0.51 (0.08,3.23)	0.48
6	*TNF-α*	rs 799964	C	0.21	0.35 (0.12,0.98)*	0.05	0.53 (0.14,1.96)	0.34	0.80 (0.22, 2.82)	0.72
		rs 800629	A	0.15	1.41 (0.36,5.53)	0.62	1.12 (0.33,3.82)	0.86	0.97 (0.17,5.55)	0.97
		rs3093671	A	0.02	-----	-----	-----	-----	-----	-----
	TNF-β	rs769178	T	0.08	4.76 (1.00,22.55)	0.05	1.51 (0.38,5.98)	0.56	3.81 (0.42,34.89)	0.24
		rs769177	T	0.02	_____*	----	----	----	-----	---
		rs909253	A	0.30	1.31 (0.50,3.45)	0.58	2.29 (0.81,6.47)	0.12	1.80 (0.54,6.01)	0.34
	*HLADRB1*1501	rs3135388	T	0.21	0.80 (0.29,2.18)	0.66	0.43 (0.15,1.23)	0.12	1.87 (0.29,11.88)	0.51
7	*TCA receptor β*	rs17133575	G	0.16	0.53 (0.20,1.43)	0.21	1.63 (0.65,4.10)	0.30	0.42 (0.05,3.85)	0.44
		rs17243	G	0.43	1.99 (0.83,4.79)	0.12	2.43 (0.81,7.23)	0.11	2.28 (0.70,7.41)	0.17
10	*IL2RA*	rs2104286	C	0.25	0.80 (0.29,2.21)	0.66	1.22 (0.39,3.83)	0.74	0.73 (0.15,3.65)	0.71
12	*VDR*	rs7975128	A	0.43	1.21 (0.55,2.65)	0.63	0.99 (0.37,2.68)	0.99	0.89 (0.26,3.02)	0.85
		rs2248098	A	0.47	0.78 (0.32,1.91)	0.59	1.02 (0.38,2.77)	0.97	0.57 (0.16,2.00)	0.38
		rs2239182	T	0.49	0.59 (0.25,1.37)	0.22	1.20 (0.48,3.00)	0.70	0.63 (0.16,2.42)	0.50
		rs2107301	A	0.26	0.45 (0.19,1.10)	0.08	1.54 (0.57,4.13)	0.39	0.49 (0.12,1.96)	0.32
		rs1540339	T	0.36	0.56 (0.24,1.30)	0.18	3.00 (1.01,8.91)*	0.05	0.38 (0.09,1.55)	0.18
		rs2239179	C	0.44	1.71 (0.70,4.16)	0.24	0.94 (0.38,2.33)	0.90	2.56 (0.68,9.57)	0.16
		rs2189480	T	0.38	0.39 (0.15,0.98)*	0.04	3.07 (1.13,8.36)*	0.03	1.20 (0.42,3.45)	0.73
		rs3819545	G	0.38	0.53 (0.22,1.24)	0.14	1.28 (0.48,3.44)	0.62	0.66 (0.20,2.19)	0.50
		rs3782905	C	0.34	2.77 (1.08,7.11)*	0.03	0.78 (0.26,2.36)	0.66	1.06 (0.31,3.60)	0.93
		rs2239186	G	0.20	0.88 (0.29,2.69)	0.82	3.96 (1.21, 13.01)*	0.02	2.38 (0.69,8.16)	0.17
		rs2228570	T	0.39	0.84 (0.35,1.98)	0.69	1.52 (0.60,3.86)	0.38	0.71 (0.24, 2.07)	0.53
		rs2254210	A	0.38	1.42 (0.60,3.39)	0.42	1.13 (0.45,2.84)	0.79	0.81 (0.26,2.52)	0.71
		rs2238136	T	0.25	0.69 (0.23,2.05)	0.50	1.02 (0.34,3.09)	0.97	6.76 (1.58,28.92)*	0.01
		rs2853564	G	0.40	1.94 (0.78,4.81)	0.15	1.41 (0.53,3.77)	0.49	0.55 (0.16,1.93)	0.35
		rs4760648	T	0.42	0.90 (0.38,2.13)	0.80	0.88 (0.35,2.23)	0.79	1.30 (0.45,3.71)	0.63

Chr.	Gene	rs number	Minor Allele[a]	MAF	Lead OR (95% CI)[b]	*p*	Mercury OR (95% CI)[b]	*p*	Solvents OR (95% CI)[b]	*p*
		rs11168287	G	0.49	0.54 (0.24,1.23)	0.14	0.59 (0.24,1.47)	0.26	1.13 (0.38,3.40)	0.83
		rs4328262	G	0.41	0.86 (0.35,2.12)	0.74	0.34 (0.11,1.03)	0.06	1.98 (0.64,6.08)	0.23
		rs4237855	G	0.36	0.92 (0.38,2.22)	0.86	0.85 (0.31,2.31)	0.75	2.39 (0.76,7.48)	0.13
		rs7136534	T	0.25	0.43 (0.16,1.16)	0.10	0.60 (0.22,1.61)	0.31	1.04 (0.25,4.22)	0.96
		rs7299460	A	0.30	0.57 (0.23,1.41)	0.22	0.87 (0.35,2.19)	0.77	0.91 (0.24,3.43)	0.89
		rs4760658	G	0.35	1.85 (0.73,4.72)	0.20	1.67 (0.65,4.31)	0.29	0.37 (0.10,1.46)	0.16
		rs4516035	C	0.44	1.51 (0.65,3.55)	0.34	1.67 (0.69,4.05)	0.26	0.53 (0.15,1.81)	0.31
18	*MBP*	rs 17026	C	0.29	2.27 (0.84,6.16)	0.11	2.16 (0.68,6.87)	0.19	1.83 (0.56,5.95)	0.32
		rs470724	T	0.31	1.66 (0.68,4.05)	0.27	1.82 (0.60,5.57)	0.29	2.48 (0.83,7.47)	0.11
		rs470550	T	0.50	1.04 (0.49,2.22)	0.91	0.43 (0.17,1.11)	0.08	0.68 (0.21,2.21)	0.52
		rs9676113	G	0.26	0.42 (0.16,1.10)	0.08	0.84 (0.31,2.28)	0.74	0.64 (0.18,2.33)	0.50
		rs11661054	A	0.46	1.24 (0.54,2.85)	0.62	0.61 (0.27,1.37)	0.23	1.63 (0.54,4.98)	0.39
		rs4890785	T	0.22	0.29 (0.09,0.88)*	0.03	0.92 (0.29,2.93)	0.88	0.78 (0.19,3.24)	0.74
		rs11661755	A	0.40	0.67 (0.28,1.56)	0.35	0.50 (0.20,1.28)	0.15	1.18 (0.37,3.71)	0.78
		rs9675994	T	0.23	1.11 (0.41,2.99)	0.84	0.50 (0.17,1.45)	0.20	1.62 (0.54,4.90)	0.39
		rs8090438	T	0.43	0.64 (0.27,1.49)	0.30	0.63 (0.25,1.62)	0.34	1.19 (0.42,3.40)	0.74
		rs8094402	G	0.34	0.83 (0.33,2.07)	0.69	0.83 (0.31,2.20)	0.70	0.57 (0.17,1.99)	0.38
		rs12456341	G	0.38	1.21 (0.54,2.68)	0.64	0.98 (0.40,2.38)	0.96	2.15 (0.80,5.81)	0.13
		rs17576751	T	0.39	0.76 (0.31,1.86)	0.54	1.32 (0.54,3.21)	0.54	0.69 (0.20, 2.46)	0.57
		rs3794848	A	0.30	1.25 (0.52,3.00)	0.61	0.94 (0.34,2.55)	0.90	1.21 (0.35,4.14)	0.76
		rs4890875	G	0.33	1.39 (0.55,3.47)	0.49	0.50 (0.16,1.58)	0.24	1.42 (0.36,5.61)	0.62
		rs595997	G	0.22	0.59 (0.23,1.52)	0.27	1.58 (0.54,4.66)	0.41	0.48 (0.11,2.13)	0.33
		rs2974260	T	0.43	1.19 (0.55,2.61)	0.66	0.73 (0.29,1.83)	0.50	0.62 (0.19,2.01)	0.43
		rs2051344	A	0.31	1.63 (0.67,3.98)	0.29	1.67 (0.58,4.77)	0.34	1.88 (0.53,6.64)	0.33
		rs470681	C	0.26	0.47 (0.18,1.19)	0.11	1.23 (0.49,3.08)	0.65	1.14 (0.35,3.68)	0.83
		rs12967023	A	0.30	1.41 (0.58,3.43)	0.45	0.86 (0.31,2.38)	0.76	0.41 (0.10,1.65)	0.21
		rs4890788	T	0.36	1.04 (0.44,2.47)	0.93	1.47 (0.53,4.06)	0.46	1.71 (0.55,5.28)	0.35
		rs7232502	A	0.39	1.07 (0.43,2.68)	0.88	1.45 (0.61,3.47)	0.40	0.40 (0.12,1.32)	0.13
		rs8096433	A	0.44	1.20 (0.46,3.13)	0.71	0.38 (0.14,0.99)*	0.05	0.23 (0.07, 0.72)*	0.01
		rs17660901	G	0.34	0.96 (0.38,2.43)	0.93	1.62 (0.63,4.16)	0.32	3.36 (1.18,9.56)*	0.02

Chr.	Gene	rs number	Minor Allele[a]	MAF	Lead OR (95% CI)[b]	*p*	Mercury OR (95% CI)[b]	*p*	Solvents OR (95% CI)[b]	*p*
19	*APOE*	rs429358	C	0.14	1.30 (0.38,4.42)	0.68	3.04 (0.72, 12.87)	0.13	2.27 (0.33,15.74)	0.41
		rs7412	T	0.08	5.45 (1.06,28.10)*	0.04	1.70 (0.35,8.27)	0.51	2.59 (0.33,20.14)	0.36

Abbreviations: *APOE*, Apolipoprotein E; CI=confidence interval; *HLA*, Human leukocyte antigen; *IL2RA*, Interleukin-2 receptor alpha; *IL7RA*, Interleukin-7 receptor alpha; MAF, minor allele frequency; *MBP*, Myelin basic protein; SNP, single nucleotide polymorphism; *TCA-β*, T cell antigen receptor beta; *TNFα*, Tumor necrosis factor alpha; *TNFβ*, Tumor necrosis factor beta; *VDR*, Vitamin D receptor.

[a] The major allele in controls was used as the reference category in all models.

[b] OR = Ratio of odds ratios

* p<0.05. After adjusting for multiple comparisons none of these results remained significant.