Interaction between adolescent obesity and HLA risk genes in the etiology of multiple sclerosis

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Anna Karin Hedström, MD

Izaura Lima Bomfim, PhD

Lisa Barcellos, PhD

Milena Gianfrancesco, MPH

Catherine Schaefer, PhD

Ingrid Kockum, PhD

Tomas Olsson, PhD*

Lars Alfredsson, PhD*

Correspondence to
Dr. Hedström:
anna.hedstrom@ki.se

ABSTRACT

Objective: We investigated potential interactions between human leukocyte antigen (HLA) genotype and body mass index (BMI) status in relation to the risk of developing multiple sclerosis (MS).

Methods: We used 2 case-control studies, one with incident cases (1,510 cases, 2,017 controls) and one with prevalent cases (937 cases, 609 controls). Subjects with different genotypes and BMI were compared with regard to incidence of MS by calculating odds ratios (ORs) with 95% confidence intervals (CIs) employing logistic regression. Potential interactions between genotypes and BMI were evaluated by calculating the attributable proportion due to interaction.

Results: In both cohorts, a significant interaction was observed between HLA-DRB1*15 and obesity, regardless of HLA-A*02 status. Similarly, there was a significant interaction between absence of A*02 and obesity, regardless of DRB1*15 status. In the incident cohort, obese subjects with the most susceptible genotype (carriage of DRB1*15 and absence of A*02) had an OR of 16.2 (95% CI 7.5–35.2) compared to nonobese subjects without the genetic risk factors. The corresponding OR in the prevalent study was 13.8 (95% CI 4.1–46.8).

Conclusions: We observed striking interactions between BMI status and HLA genotype with regard to MS risk. Hypothetically, a low-grade inflammatory response inherent to obesity synergizes with the adaptive, HLA molecule–restricted arm of the immune system, causing MS. Prevention of adolescent obesity may thus lower the risk of developing MS, predominantly among people with a genetic susceptibility to the disease. *Neurology®* 2014;82:865–872

GLOSSARY

AP = attributable proportion due to interaction; **BMI** = body mass index; **CI** = confidence interval; **EAE** = experimental autoimmune encephalomyelitis; **EIMS** = Epidemiological Investigation of MS; **HLA** = human leukocyte antigen; **KPNC** = Kaiser Permanente Medical Care Plan, Northern California Region; **MS** = multiple sclerosis; **OR** = odds ratio; **SNP** = single nucleotide polymorphism.

Multiple sclerosis (MS) is an inflammatory demyelinating disorder of the CNS, and the most common nontraumatic cause of acquired neurologic disability affecting young adults. Susceptibility to MS is determined by both genetic and environmental factors. A relationship between obesity in early life and increased MS risk has been demonstrated in 3 previous studies.[1–3] A potential mechanism for the association has been suggested in that obese people have lower levels of vitamin D metabolites than do normal-weight people and decreased levels of serum 25-hydroxyvitamin D appear to increase MS risk. Furthermore, fat-related chronic inflammation may be involved. Adipose tissue produces and releases a variety of proinflammatory cytokines, including leptin, which promotes Th1 responses and reduces regulatory T-cell activity.[4]

The hitherto known lifestyle/environmental factors associated with MS have only modest impact on the risk of developing the disease. The recently demonstrated gene–environment

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interactions with regard to MS risk show that the risk conveyed by lifestyle/environmental factors may substantially differ depending on genetic background.[5,6]

Using a Swedish population-based case-control study as well as an American case-control study, we studied the potential interaction between adolescent obesity and the most strongly associated genes in MS: the human leukocyte antigen (HLA)–DRB1*15 allele, which provides an increased risk with odds ratios (ORs) in the order of ~3, and the HLA-A*02 allele (figure), which reproducibly has shown a protective association to MS.[7,8]

METHODS Design and study population. This report is based on data from 2 case-control studies on environmental and genetic risk factors for MS. The first study is the Epidemiological Investigation of MS (EIMS), with a study base comprising the Swedish population aged 16 to 70 years. Incident cases were recruited via 40 clinics, including all university hospitals in Sweden. A case was defined as a person in the study base who had received a diagnosis of MS according to the McDonald criteria.[9] For each case, 2 controls

were randomly selected from the national population register, frequency matched by age (5-year age groups), sex, and residential area. Information regarding exposures and other circumstances was collected using a standardized questionnaire that was filled in at home. The study period for this report was April 2005 to March 2012. Completed questionnaires were obtained from 1,798 cases and 3,907 controls, which is equivalent to 91% for the case group and 69% for the controls. Since the impact of adolescent obesity was investigated in this study, cases with disease onset before age 20, and their corresponding controls, were excluded (110 cases, 218 controls).

The American case-control study used a study population of white non-Hispanic people identified among members of Kaiser Permanente Medical Care Plan, Northern California Region (KPNC), using electronic medical records. KPNC is an integrated health services delivery system with a membership of 3.2 million that comprises about 25% to 30% of the population of a 22-county service area in northern California. Cases of MS were required to have an MS diagnosis by a neurologist, self-identified white race/ethnicity, age 18 through 69 years, and KPNC membership at initial contact. 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 sex, birth date, race/ethnicity, and zip code of the case residence. In total, 1,087 cases and 687 controls were included in this report; 128 cases with disease onset before age 20 were excluded, and 23 cases and 14 controls were excluded due to missing data on body mass index (BMI). The response rate was 79% for cases

Figure   Interaction among adolescent obesity, carriage of HLA-DRB1*15, and absence of HLA-A*02
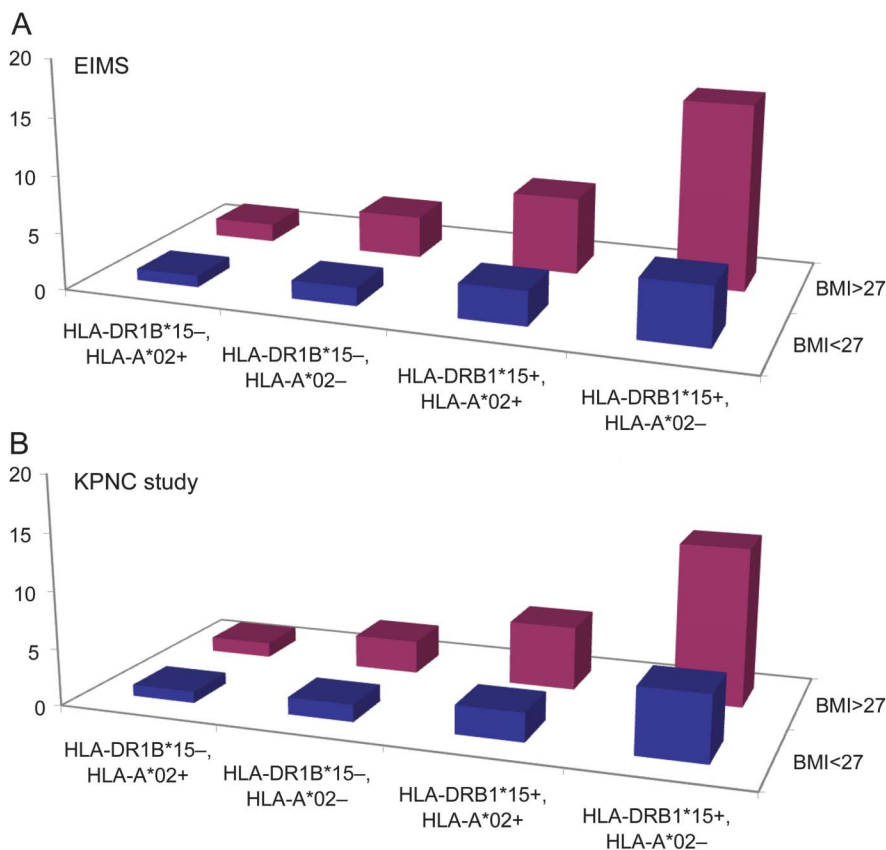
(A) Epidemiological Investigation of MS (EIMS) study. (B) Kaiser Permanente Medical Care Plan, Northern California Region (KPNC) study. Odds ratios for different combinations of body mass index (BMI), human leukocyte antigen (HLA)–DRB1*15, and HLA-A*02 status, with regard to the risk of developing multiple sclerosis. Statistics are shown in table 4.

and 58% for controls. All participants completed a computer-assisted telephone interview regarding lifestyle factors and exposures.

Standard protocol approvals, registrations, and patient consents. EIMS was approved by the Regional Ethical Review Board at Karolinska Institutet. The KPNC study protocol was approved by the institutional review boards of KP Division of Research and the University of California, Berkeley.

Genotyping. In EIMS, blood samples were available from 1,510 cases (89%) and 2,017 controls (55%). Allelic dosage of HLA-DRB1*15 and HLA-A*02 was obtained by 1 of 3 methods: (1) PCR amplification with sequence-specific primers (Olerup SSP low resolution typing kits, Olerup SSP AB, Stockholm, Sweden); (2) imputation using HLA*IMP:02[10] based on single nucleotide polymorphisms (SNPs) genotyped on the Immunochip custom array[11] in an overlapping Swedish cohort; or (3) TaqMan allelic discrimination of the SNPs rs9271366 (assay ID: C__33416976_20), which tags HLA-DRB1*15 or rs2975033 (assay ID: C__15962692_20) and rs12206499 (assay ID: C__31338281_10), which combined tags for HLA-A*02. The distribution of method for classification of HLA-DRB1*15 status was as follows: 67% of cases/64% of controls were genotyped with the Olerups SSP kit, 4% cases/1% controls had imputed genotypes, and 29% cases/35% controls were genotyped with TaqMan genotyping assays. The figures for distribution of method for classification of HLA-A*02 status were the same with the exception of percentage of cases that were genotyped with Olerups kit, which was 66% for HLA-A*02 status. In the KPNC study, information on HLA genotype was available for 937 (86%) cases and 609 (87%) controls. HLA-DRB1 genotypes were determined by use of a sequence-specific PCR method, as previously described.[12] Allelic dosage of HLA-A*02 was obtained by TaqMan allelic discrimination of the SNPs rs2975033 (assay ID: C__15962692_20), which tags for HLA-A*02. The validity of imputing HLA types from SNPs has been studied against classical methods of HLA typing and congruence between methods is very good for most alleles, including HLA-DRB1*15 and HLA-A*02, studied herein.[10,13] Participants in both studies were classified according to the carriage of any HLA-DRB1*15 allele vs no carriage, and according to carriage of any HLA-A*02 allele vs no carriage (where absence of HLA-A*02 is the risk factor).

Body mass index. The questionnaire in EIMS contained questions regarding demographic factors, lifestyle factors, and socio-economic circumstances. Information was obtained regarding current body height and body weight at age 20. Using current height, we calculated BMI at age 20 by dividing weight in kilograms by height in meters squared.

In the KPNC study, participants reported their current height at the time of the interview, as well as their highest and lowest (nonpregnancy) weight during their 20s. Using current height, BMI during their 20s was calculated by dividing mean weight in kilograms by height in meters squared.

In order to analyze the influence of BMI on the risk of developing MS, we categorized the subjects into the following groups based on BMI: <18.5, 18.5–<21, 21–<23, 23–<25, 25–<27, and ≥27 kg/m². The typical standard for obesity is BMI >30 kg/m². However, in EIMS, fewer than 2% had an adolescent BMI exceeding 30 kg/m². The OR among subjects with BMI 27–30 and >30 was fairly similar (BMI 27–30: OR 2.1, 95% confidence interval [CI] 1.4–3.0; and BMI >30: OR 2.5, 95% CI 1.7–3.7). Therefore, we chose to combine these BMI groups into one group defined as obese.

Statistical analysis. By means of unconditional logistic regression, the incidence of MS in subjects belonging to different BMI groups was compared with that of normal-weight subjects with BMI between 18.5 and 21 kg/m,² by calculating ORs with 95% CIs. Trend test for a dose-response relationship regarding adolescent BMI and risk of MS was performed by using a continuous variable for BMI (kg/m²) in a logistic regression model. Analyses of interaction between BMI and HLA-DRB1-15 as well as between BMI and HLA-A*02 were performed, and the possible gene–environment interaction was evaluated by estimating departure from additivity of effects using attributable proportion due to interaction (AP) as described.[14,15]

In EIMS, we also performed conditional logistic regression. However, 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 had a higher degree of precision (due to a higher number of controls). In the KPNC study, there were far more cases than controls, and in order to use information from the whole group we conducted unconditional logistic regression with adjustment for the matching factors.

In EIMS, all analyses were adjusted for age, sex, and residential area as well as for ancestry and smoking. Age was categorized into the following 8 strata: 16–19, 20–24, 25–29, 30–34, 35–39, 40–45, 45–49, and 50–70 years of age. Assessment of ancestry was based on whether the subject was born in Sweden or not, and whether either of the subject's parents had immigrated to Sweden. A subject who was born in Sweden, whose parents had not immigrated, was classified as Swedish. Smoking was dichotomized as ever or never-smokers. Smoking was considered prior to the index year in the cases (i.e., the year of disease onset) and during the same period of time in the corresponding controls. Adjustments were also made for heredity (having a first- or second-degree relative with MS or not), educational level (university degree or not), socioeconomic status (according to an established socioeconomic classification),[16] physical activity during ages 18 to 22 (yes/no), oral contraceptive use (ever/never), parity (yes/no), EBNA1–immunoglobulin G (high/low), vitamin D status (more or less than 50 ng/mL), and ultraviolet radiation exposure habits (high/low), but these factors had minor influence on the results of the study and were not retained in the final analyses. All analyses were conducted using SAS version 9.2.

In the KPNC study, adjustments were made for age, sex, ancestry, and smoking. Age was categorized in the same manner as in EIMS. Ancestry was categorized into the following stratas: Northern Europe, Western Europe, Southern Europe, Eastern Europe, other, and unknown. Smoking was dichotomized as smokers or nonsmokers at age 20.

RESULTS Selected characteristics of subjects by study and BMI at age 20 are presented in table e-1 on the *Neurology®* Web site at Neurology.org.

In both studies, subjects with adolescent BMI ≥27 kg/m² had an increased risk of developing MS compared to normal-weight subjects with BMI between 18.5 and 21 kg/m² (OR 2.2 [95% CI 1.7–3.0] in EIMS and 1.8 [95% CI 1.2–2.6] in the KPNC study) (table 1). MS risk in subjects with BMI between 25 and 27 kg/m² was modestly increased (OR 1.4 [95% CI 1.05–1.9] in EIMS and 1.3 [95% CI 0.9–2.0] in the KPNC study). The observed trend of a higher BMI resulting in a higher risk of developing MS was statistically significant in both studies. The ORs remained unchanged when the analyses were restricted to those who had been genotyped.

Table 1 Odds ratios with 95% confidence intervals of developing multiple sclerosis for subjects in different categories according to body mass index at age 20 compared with normal-weight subjects

BMI, kg/m²	EIMS			KPNC study		
	Ca/co[a]	OR (95% CI)[b]	p	Ca/co[a]	OR (95% CI)[c]	p
<18.5	126/197	1.0 (0.8-1.3)	0.9	86/65	1.0 (0.7-1.4)	0.8
18.5-21	462/711	1.0 (reference)		278/208	1.0 (reference)	
21-23	381/550	1.1 (0.9-1.3)	0.5	214/147	1.0 (0.8-1.4)	0.9
23-25	283/337	1.3 (1.05-1.6)	0.01	155/96	1.1 (0.8-1.5)	0.6
25-27	110/121	1.4 (1.05-1.9)	0.02	84/44	1.3 (0.9-2.0)	0.2
≥27	148/101	2.2 (1.7-3.0)	<0.0001	120/49	1.8 (1.2-2.6)	0.006
p for trend			<0.0001			0.005

Abbreviations: BMI = body mass index; CI = confidence interval; EIMS = Epidemiological Investigation of MS; KPNC = Kaiser Permanente Medical Care Plan, Northern California Region; OR = odds ratio.
[a] Exposed cases and controls.
[b] Adjusted for age, sex, residential area (according to study design), ancestry, and smoking.
[c] Adjusted for age, sex, ancestry, and smoking.

A significant interaction was observed between the HLA-DRB1*15 allele and obesity with regard to risk for MS (table 2). The interaction was restricted to those with BMI ≥27 kg/m² (AP 0.6, 95% CI 0.3–0.8 in EIMS and AP 0.5, 95% CI 0.2–0.9 in the KPNC study). When the analysis of interaction between HLA-DRB1*15 and obesity (in categories: DRB1*15−/BMI <27, DRB1*15+/BMI <27, DRB1*15−/BMI ≥27, and DRB1*15+/BMI ≥27) was performed by HLA-A*02 status in both materials combined, the AP was statistically significant in each strata (p value 0.0003 among HLA-A*02-positive subjects and <0.0001 among HLA-A*02-negative subjects).

Table 2 Odds ratios with 95% confidence intervals of developing multiple sclerosis for subjects with different combinations of HLA-DRB1*15 status and BMI status

DR15 status	BMI status	EIMS			KPNC study		
		Ca/co[a]	OR (95% CI)[b]	p	Ca/co[a]	OR (95% CI)[c]	p
−	<27	608/1,345	1.0 (reference)		370/399	1.0 (reference)	
+	<27	754/571	3.0 (2.6-3.5)	<0.0001	447/161	3.2 (2.5-4.0)	<0.0001
−	≥27	68/77	1.9 (1.3-2.7)	0.0003	60/40	1.6 (1.0-2.6)	0.04
+	≥27	80/24	7.9 (4.9-12.7)	<0.0001	60/9	7.0 (3.4-14.7)	<0.0001
AP			0.5 (0.3-0.7)	<0.0001		0.5 (0.1-0.9)	0.03
−	18.5-21	192/473	1.0 (reference)		127/140	1.0 (reference)	
+	18.5-21	270/238	2.9 (2.3-3.7)	<0.0001	151/68	2.7 (1.8-4.0)	<0.0001
−	25-<27	51/87	1.5 (1.0-2.2)	0.05	38/32	1.1 (0.6-2.0)	0.7
+	25-<27	59/33	4.5 (2.8-7.3)	<0.0001	46/12	3.9 (1.9-8.1)	0.0002
AP			0.3 (−0.1-0.6)	0.2		0.3 (−0.3-0.8)	0.3
−	18.5-21	192/473	1.0 (reference)		127/140	1.0 (reference)	
+	18.5-21	270/238	2.9 (2.3-3.8)	<0.0001	151/68	2.6 (1.8-4.0)	<0.0001
−	≥27	68/77	2.1 (1.4-3.1)	0.0001	60/40	1.5 (0.9-2.6)	0.1
+	≥27	80/24	9.1 (5.5-15.1)	<0.0001	60/9	6.9 (3.2-15.0)	<0.0001
AP			0.6 (0.3-0.8)	<0.0001		0.5 (0.2-0.9)	0.004

Abbreviations: AP = attributable proportion due to interaction between HLA-DRB1*15 and obesity; BMI = body mass index; CI = confidence interval; EIMS = Epidemiological Investigation of MS; HLA = human leukocyte antigen; KPNC = Kaiser Permanente Medical Care Plan, Northern California Region; OR = odds ratio.
[a] Number of cases and controls.
[b] Adjusted for age, sex, residential area (according to study design), ancestry, smoking, and HLA-A*02 status.
[c] Adjusted for age, sex, ancestry, smoking, and HLA-A*02 status.

Table 3 Odds ratios with 95% confidence intervals of developing multiple sclerosis for subjects with different combinations of HLA-A*02 status and BMI status

A2 status	BMI status	EIMS Ca/co[a]	OR (95% CI)[b]	p	KPNC study Ca/co[a]	OR (95% CI)[c]	p
+	<27	582/1,050	1.0 (reference)		306/282	1.0 (reference)	
−	<27	780/866	1.7 (1.5-2.1)	<0.0001	511/278	1.7 (1.4-2.2)	<0.0001
+	≥27	60/57	1.8 (1.2-2.7)	0.002	44/25	1.6 (0.9-2.9)	0.1
−	≥27	88/44	4.1 (2.8-3.6)	<0.0001	76/24	3.4 (2.0-5.9)	<0.0001
AP			0.4 (0.09-0.7)	0.009		0.3 (−0.1-0.8)	0.2
+	18.5-21	200/382	1.0 (reference)		106/99	1.0 (reference)	
−	18.5-21	262/329	1.6 (1.3-2.1)	<0.0001	172/109	1.4 (0.9-2.1)	0.1
+	25-<27	46/61	1.6 (1.0-2.5)	0.04	31/21	1.0 (0.5-2.1)	0.9
−	25-<27	64/59	2.4 (1.6-3.6)	<0.0001	53/23	2.0 (1.1-3.8)	0.03
AP			0.05 (−0.4-0.5)	0.8		0.3 (−0.2-0.8)	0.3
+	18.5-21	200/382	1.0 (reference)		106/99	1.0 (reference)	
−	18.5-21	262/329	1.6 (1.3-2.2)	<0.0001	172/109	1.4 (0.9-2.0)	0.1
+	≥27	60/57	2.1 (1.4-3.2)	0.0007	44/25	1.4 (0.7-2.7)	0.3
−	≥27	88/44	4.8 (3.1-7.3)	<0.0001	76/24	3.1 (1.7-5.6)	0.0002
AP			0.4 (0.1-0.7)	0.004		0.4 (0.01-0.8)	0.04

Abbreviations: AP = attributable proportion due to interaction between HLA-A*02 and obesity; BMI = body mass index; CI = confidence interval; EIMS = Epidemiological Investigation of MS; HLA = human leukocyte antigen; KPNC = Kaiser Permanente Medical Care Plan, Northern California Region; OR = odds ratio.
[a] Number of cases and controls.
[b] Adjusted for age, sex, residential area (according to study design), ancestry, smoking, and HLA-DRB1*15 status.
[c] Adjusted for age, sex, ancestry, smoking, and HLA-DRB1*15 status.

Similarly, a significant interaction was observed between absence of HLA-A*02 and obesity (AP 0.4, 95% CI 0.1–0.7 in EIMS and AP 0.4, 95% CI 0.01–0.8 in the KPNC study) (table 3). When the analysis of interaction between absence of HLA-A*02 and obesity (in categories: A*02+/BMI <27, A*02−/BMI <27, A*02+/BMI ≥27, and A*02−/BMI ≥27) was performed by HLA-DRB1*15 status in both materials combined, the AP was statistically significant in each strata (p value 0.02 among HLA-DRB1*15-negative subjects and 0.01 among HLA-DRB1*15-positive subjects).

The magnitude of interaction between HLA-DRB1*15 and adolescent obesity remained the same regardless of which reference group was used (table e-2). Finally, we calculated the ORs associated with the most susceptible genotype (carriage of DRB1*15 but not HLA-A*02) in subjects with BMI more or less than 27 kg/m², respectively, compared with nonobese subjects without these genetic risk factors. Subjects with BMI less than 27 kg/m² with the 2 risk genotypes displayed an OR of 5.1 (95% CI 4.1–6.3), whereas the same genotype for subjects with BMI ≥27 kg/m rendered an OR of 16.2 (95% CI 7.5–35.2). The corresponding ORs in the KPNC study were 5.7 (4.0–8.0) and 13.8 (4.1–46.8) (table 4).

DISCUSSION We demonstrate striking interactions between adolescent obesity and 2 potent MS risk genes, properly replicated in an independent material, similar to the interaction between carriage of HLA-DRB1*15, absence of HLA-A*02, and smoking in the development of MS.[5] The biological explanations for these interactions are far from clear, but the data open up for mechanistically oriented studies. It should be emphasized that interaction measured this way does not necessarily imply evidence for direct protein–protein interactions such as in a ligand–receptor pair, but rather indicates that the factors act synergistically in the same pathogenic pathway. In case of the BMI–HLA MS risk gene interaction, we primarily consider a low-grade chronic inflammation with activation of the innate immune system, promoted by the fat tissue,[4] which may increase the options for HLA-restricted activation of autoreactive T cells attacking the CNS in MS.

It is important to consider potential drawbacks, confounders, or biases in our study. Both studies retrospectively gathered information regarding lifestyle factors and personal information such as weight and height. EIMS primarily included cases who had received the diagnosis within the past year in order to minimize recall bias. Moreover, the questionnaire

Table 4 Odds ratios with 95% confidence intervals of developing multiple sclerosis for subjects with different combinations of BMI and the genetic risk factors carriage of the HLA-DRB1*15 allele and absence of the HLA-A*02 allele, compared with nonobese subjects carrying none of the genetic risk factors

DR15	A2	BMI ≥27	EIMS			KPNC study		
			Ca/co[a]	OR (95% CI)[b]	p	OR (95% CI)[c]	Ca/co[a]	OR (95% CI)[d]	p
−	+	−	249/723	1.0 (reference)		1.0 (reference)	136/192	1.0 (reference)	
−	−	−	359/622	1.6 (1.4–2.0)	<0.0001	1.7 (1.4–2.1)	234/207	1.5 (1.1–2.0)	0.009
+	+	−	333/327	3.0 (2.4–3.7)	<0.0001	3.1 (2.5–3.9)	170/90	2.5 (1.8–3.6)	<0.0001
+	−	−	421/244	5.1 (4.1–6.3)	<0.0001	5.3 (4.3–6.7)	277/71	5.7 (4.0–8.0)	<0.0001
−	+	+	23/41	1.6 (0.9–2.7)	0.1	1.4 (0.8–2.4)	18/19	1.3 (0.6–2.7)	0.5
−	−	+	45/36	3.7 (2.3–5.8)	<0.0001	3.5 (2.1–5.6)	42/21	2.9 (1.6–5.3)	0.0007
+	+	+	37/16	6.8 (3.7–12.5)	<0.0001	6.5 (2.1–12.0)	26/6	5.6 (2.2–14.4)	0.0003
+	−	+	43/8	16.2 (7.5–35.2)	<0.0001	15.9 (7.3–34.7)	34/3	13.8 (4.1–46.8)	<0.001

Abbreviations: BMI = body mass index; CI = confidence interval; EIMS = Epidemiological Investigation of MS; HLA = human leukocyte antigen; KPNC = Kaiser Permanente Medical Care Plan, Northern California Region; OR = odds ratio.
[a] Number of cases and controls.
[b] Adjusted for age, sex, residential area (according to study design), ancestry, and smoking.
[c] Adjusted for age, sex, residential area (according to study design), ancestry, smoking, heredity, educational level, socioeconomic status, physical activity during ages 18 to 22, oral contraceptive use, parity, EBNA1–immunoglobulin G, vitamin D status, and ultraviolet radiation habits.
[d] Adjusted for age, sex, ancestry, and smoking.

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 have distorted the results. However, a validation study among women in the Nurses' Health Study found high correlations between recalled and measured past weight, and between reported current height and measured past height.[17] Participants slightly underreported weight at 18 years of age with a mean difference of 1.4 kg. The underreporting of weight probably does not differ between cases and controls. There is no reason to believe that the results would be different in other populations. Furthermore, body weight at age 30 was available in the American study, and no association was observed between BMI at age 30 and MS risk, which makes recall differences between cases and controls unlikely. Any recall bias regarding weight would tend to bias toward the null, thus underestimating the OR.

The proportion of respondents with regard to participation in EIMS was 91% for cases and 69% for controls. A potential selection bias may result from the relatively high proportion of nonresponders among the controls. However, this bias is most likely to be modest because the prevalence of smoking among the controls, seen as an indicator of lifestyle, was in line with that of the general population at equivalent ages.[18] Furthermore, the increased risk for MS associated with overweight and obesity was similar among those who provided blood and those who did not.

The molecular pathways responsible for the observed association between adolescent obesity and MS are still unknown, but there are different hypotheses attempting to explain the association. High levels of circulating 25-hydroxyvitamin D have been associated with reduced risk of MS in early adulthood.[19] Total body fat is inversely related to the levels of circulating 25-hydroxyvitamin D and consequently, obese people have lower levels of this metabolite than normal-weight people.[20] The higher risk of MS among subjects who were obese during adolescence is consistent with a protective effect of vitamin D. However, our results based on EIMS remained unchanged when the analyses were adjusted for sun exposure habits and vitamin D status. Furthermore, both sun exposure habits and vitamin D status seem to affect MS risk in adults independently of HLA risk genes.[21]

We hypothesize that obesity-related inflammatory/immunologic mechanisms contribute to explain the association between adolescent obesity and increased MS risk. Obesity induces a state of chronic, low-grade inflammation[22] that arises from the production and secretion of inflammatory mediators driven by adipose tissue macrophages. The number of macrophages present in adipose tissue is directly correlated with adiposity.[23] Upon high fat feeding, adipose tissue macrophages undergo a phenotypic switch from an anti-inflammatory M2 polarization state to a proinflammatory M1 polarization.[24] A recently published study demonstrated that the M1/M2 equilibrium in blood and CNS favors mild experimental autoimmune encephalomyelitis (EAE), whereas imbalance toward M1 promotes relapsing EAE.[25]

Leptin is produced mainly by adipose tissue in proportion to body fat mass[26] and has been considered a

link among obesity, metabolic state, and autoimmunity. Leptin promotes proliferation of effector T cells and constrains expansion of regulatory T cells, generally switching the phenotype toward a Th1 response,[4] promoting the onset and progression of autoimmune responses. In the early stages of MS, an inverse correlation has been demonstrated between serum leptin concentration and the number of regulatory T-cells, which are known to dampen autoreactive responses mediated by CD4+ cells.[27] Leptin-deficient mice have been shown to be resistant to EAE. This protection is reversed by leptin administration and is associated with a switch from Th2- to Th1-type responses.[28]

Obesity has been associated with increased susceptibility to inflammatory and autoimmune diseases such as psoriasis, autoimmune thyroiditis, diabetes, and MS. The increased incidence of both diabetes[29] and MS[30] has been parallel with the rise in childhood obesity.[31,32] The finding of an interaction between obesity and HLA genotype with regard to MS supports the hypothesis that the Th1-promoting effects of obesity increase the risk of developing MS, in particular among subjects with a genetic susceptibility to the disease. An increased expression of HLA class II genes has been observed in adipose tissue macrophages specifically recruited to adipose tissue.[33] The prime function of the molecules encoded by these genes is antigen presentation to T cells, and strong experimental data suggest that the association between HLA genotype and MS risk depends on preferences in peptides allowing CNS-directed autoimmunity.[34]

We thus hypothesize that by increasing the release of proinflammatory cytokines and promoting Th1 responses, and decreasing the number of regulatory T cells, obesity may increase the risk of recruitment of autoaggressive CD4+ cells that target CNS autoantigens. Obesity in the context of HLA risk genes may then further increase the risk of autoaggressive immunity that results in MS.

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