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Gene-environment interactions increase the risk of pediatric-onset multiple sclerosis associated with ozone pollution

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Abstract

Background: We previously reported a relationship between air pollutants and increased risk of pediatric-onset MS (POMS). Ozone is an air pollutant that may play a role in MS pathoetiology. *CD86* is the only non-HLA gene associated with POMS for which expression on antigen-presenting cells (APC) is changed in response to ozone exposure.

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Objective: To examine the association between county-level ozone and POMS, and the interactions between ozone pollution, *CD86* and *HLA-DRB1*15*, the strongest genetic variant associated with POMS.

Methods: Cases and controls were enrolled in the Environmental and Genetic Risk Factors for Pediatric MS study of the US Network of Pediatric MS Centers. County-level modeled ozone data were acquired from the CDC's Environmental Tracking Network. Participants were assigned ozone values based on county of residence. Values were categorized into tertiles based on healthy controls. The association between ozone tertiles and having MS were assessed by logistic regression. Interactions between tertiles of ozone level and the GG genotype of the rs928264 (G/A) single nucleotide polymorphism (SNP) within *CD86*, and the presence of *DRB1*15:01 (DRB1*15)* on odds of POMS was evaluated. Models were adjusted for age, sex, genetic ancestry, and mother's education. Additive interaction was estimated using relative risk due to interaction (RERI) and attributable proportions (AP) of disease were calculated.

Results: 334 POMS cases and 565 controls contributed to the analyses. County-level ozone was associated with increased odds of POMS (odds ratio (OR) 2.47, 95%CI 1.69–3.59 and 1.95, 95%CI 1.32–2.88 for the upper two tertiles, respectively, compared with the lowest tertile). There was a significant additive interaction between high ozone tertiles and presence of *DRB1*15*, with a RERI of 2.21 (95%CI 0.83–3.59) and an AP of 0.56 (95%CI 0.33–0.79). Additive interaction between high ozone tertiles and the *CD86* GG genotype was present, with a RERI of 1.60 (95%CI 0.14–3.06) and an AP of 0.37 (95%CI 0.001–0.75) compared to the lowest ozone tertile. AP results indicated that approximately half of the POMS risk in subjects can be attributed to the possible interaction between higher county-level ozone carrying either *DRB1*15* or the *CD86* GG genotype.

Conclusions: In addition to the association between high county-level ozone and POMS, we report evidence for additive interactions between higher county-level ozone and *DRB1*15* and the *CD86* GG genotype. Identifying gene-environment interactions may provide mechanistic insight of biological processes at play in MS susceptibility. Our work suggests a possible role of APCs for county-level ozone induced POMS risk.

Keywords

Pediatric onset; multiple sclerosis; ozone pollution; gene-environment interaction; *CD86* ; *DRB1*15*

Introduction:

Accumulating evidence points to both genetic and environmental factors contributing to multiple sclerosis (MS) onset (1). The missing heritability reported for MS may partly be explained by gene-environment (GxE) interactions.

The study of GxE interactions in children offers important advantages over adult studies in terms of temporality, i.e. exposure time closer to disease onset, and less irrelevant exposures due to younger age (1,2). We have previously reported a strong association between air pollution and pediatric-onset MS (POMS) (2,3). Ozone which contributes to air pollution is a major global health hazard (4) and may play an important role in MS

pathoetiology through several biological processes (5–8). A single study has reported an association between ozone and adult MS susceptibility (9). Also, In the recent Canadian Census Health and Environment Cohort (CanCHEC) analysis, higher death attributable to ozone exposure is reported in MS compared to other neurological diseases (10). Although individual genetic make-up may increase the biological impact of environmental exposures, the genetic variants increasing the association of air pollutants with MS risk have not been studied. Our Network has previously reported that *HLA-DRB1*15:01 (DRB1*15*) is strongly associated with POMS risk and that absence of *HLA-A*02* has a modest effect. In addition, 28 non-HLA variants reported for adult MS are also associated with POMS risk (11). Among genes with variants associated with POMS, *CD86* is the only one for which expression changes in response to ozone exposure (12).

In this study, we sought to assess the association between county-level ozone (hereafter referred to as ozone) and POMS risk and determine possible genetic interactions with ozone levels, focusing on the two top HLA risk factors (*DRB1*15* and *A*02*) and *CD86* which expression is modified by ozone exposure. Identifying GxE interactions might provide insight into biological mechanisms that are involved in MS pathoetiology.

Method:

Study Population:

Pediatric MS cases who had disease onset before the age of 18 years were enrolled in this multi-center case-control study of pediatric MS risk factors between 2011 and 2017 (3). All the cases were ascertained by at least two pediatric MS experts from the US pediatric MS network. All samples were tested for MOG-IgG and AQP4 antibodies at Mayo Clinic, and positive cases were excluded. Frequency matched healthy controls younger than 20 years were recruited from the general and subspecialty pediatric clinics at participating institutions during the same period of time as the cases. Inclusion criteria for controls included the absence of autoimmune disease and no first-degree relatives with MS. Participants and their families completed a detailed environmental questionnaire (http://www.usnpmsc.org/Documents/EnvironmentalAssessment.pdf) including places of residence since birth and provided blood samples for genetic analysis (9). The institutional review boards at the participating sites have approved the study. Consent and assent (when appropriate) were obtained for each subject and their parent.

Environmental Data:

The CDC's Environmental Health Tracking Network provides modelled US county-level values of ozone (13). Each measure contains a population-weighted mean value for the county. In this study, we used the population-weighted mean values for each county in 2014, the mid-point for our study enrollment. SAS statistical software (version: 9.3) was used to assign ozone level values to each participant based on their county of residence. Population-weighted indexes for air pollutants provide a refined representation of the exposure of the study population since they give proportionately greater weight to the air pollution experienced where most people live (14,15). Ozone levels were categorized into tertiles based on healthy control values with the lowest tertile serving as the reference category.

Genetic data:

Each study participant has been genotyped by Infinium 660K BeadChip or HumanOmniExpress BeadChip. Stringent quality control (QC) measures and comparison of sample genotypes across two Illumina platforms were performed using PLINK v.1.9. The alignment, phasing, imputation, and variant filtering were done as described previously (11). The *HLA-A*02* (rs2975033) tagging SNP was imputed. The allele 'A' of rs2975033 has been reported to be in perfect linkage disequilibrium (r^2=0.97) with the *HLA-A*02* allele (14). Therefore, the GG genotype of rs2975033 has been considered as absence of *HLA-A*02* in subjects. The participants were categorized based on no carriage of any *HLA-A*02* allele vs any carriage. In addition, the subjects were classified according to presence of two risk alleles 'G' of *CD86* SNP (rs928264 G/A) which is associated with POMS risk (11). Furthermore, carrying the GG genotype of rs928264 has been reported to increase the expression of *CD86* (15). Therefore, *CD86* SNP genotyping was dichotomized according to the presence of GG genotype vs. other (GA and AA genotypes).

In addition, DNA samples of all subjects have also been genotyped for *DRB1* status, as previously published (16). *DRB1* status was dichotomized according to the presence of one or more *HLA-DRB1*15* alleles vs no carriage.

SNP weighting method (17) has been used to estimate the percentage of genetic ancestry related to four major populations (European, East Asian, West African, and Native American).

Statistical Analysis:

The odds of having POMS associated with ozone level tertiles, presence of *HLA-DRB1*15*, absence of *HLA-A*02* and GG genotype of *CD86* SNP were assessed separately using logistic regression models adjusted for age, sex, genetic ancestry, and mother's education as a proxy of socioeconomic status (SES). We tested for additive interaction between high ozone levels (tertiles 2 and 3) and genotype status in several logistic regression models calculating the relative excess risk due to interaction (RERI) and the attributable proportion (AP) of disease due to interaction. Confidence intervals for all estimates were calculated using published methods (18,19). A multiplicative interaction term was also created and added to logistic regression models to assess for the presence of multiplicative interaction. All statistical analyses were performed using Stata 15.0 (Stata Corp, College Station, TX). To calculate RERI and AP and their confidence intervals, we used adjusted odds ratio from our logistic regression models. We first ran our adjusted logistic regression models in Stata and then used the "nlcom" command according to the formula of RERI and AP as described in (19). The output included the adjusted estimates of RERI and AP, their 95% confidence interval, and P-value.

Results:

Participant's characteristics

A total of 334 POMS cases and 565 healthy controls with valid US zip codes contributed to this analysis. Table 1 depicts the characteristics of the study participants. Healthy controls

were overall frequency matched to POMS cases for age, sex, and race but more were Hispanics and more had mother's education less than high school in the POMS group (Table 1). POMS cases were significantly more likely to live in a county with assigned "tertiles 2 and 3" ozone level (82% of POMS cases vs. 66% in healthy controls). There were 16 POMS cases and ten controls drop-out in our logistic regression model because of missing genetic ancestry data estimations.

Genetic characteristics

Compared to healthy children, children with MS were more likely to have at least one *HLA-DRB1*15* allele (Table 2). In addition, higher proportion of POMS participants had the GG genotype of *CD86* POMS-risk SNP (rs9282641) compare to healthy controls (Table 2). Based on the available imputed genotype for *HLA-A*02*, the proportion of participants without any *HLA-A*02* alleles was higher in POMS (61%) than healthy subjects (56%).

High ozone levels associated with higher odds of POMS

Figure 1 displays odds ratios (ORs) based on logistic regression models adjusted for age, sex, genetic ancestry and mother's education. High ozone levels (tertiles 2 and 3) more than doubled the odds of having MS (adjusted OR 2.47, 95%CI: 1.69–3.59 and 1.95, 95%CI: 1.32–2.88, respectively). In addition, the presence of *HLA-DRB1*15* and the *CD86* SNP GG genotype were also associated with POMS risk (adjusted OR of 2.28, 95%CI: 1.75–2.96 and 1.96, 95%CI: 1.32–2.93, respectively). The absence of *HLA-A*02* alleles tended to increase odds of POMS (adjusted OR of 1.22, 95%CI: 0.93–1.59).

Furthermore, using a continuous ozone variable in our adjusted logistic regression model, each unit increase in population-weighted ozone levels increase POMS risk by 2%.

Supplementary Figure 1 illustrates predicted probabilities in an adjusted logistic regression model based on the marginal standardization method implemented by the 'margins' command in Stata. Standard errors have been calculated by delta methods (20). The risk of having POMS increased in two upper tertiles (high ozone exposure) in comparison to low ozone exposure (tertile 1). Supplementary Figure 2 indicates comparable odds ratios of developing POMS with different models adjusted for genetic background.

Assessment of gene-environment interactions

In Table 3, we stratified analyses of high ozone levels (tertiles 2 and 3) in comparison to the other genetic risk factors (presence of *HLA-DRB1*15*, the GG genotype of *CD86* SNP, and absence of *HLA-A*02*) in POMS cases compared to healthy controls. Without high ozone exposure, the presence of *HLA-DRB1*15* alleles did not significantly increase the odds of POMS. When both *HLA-DRB1*15* alleles and high ozone exposure were present, the odds of having POMS increased to 3.89 (95% CI: 2.47–6.12). The RERI for high ozone category and presence of *HLA-DRB1* alleles (2.21, 95% CI: 0.83–3.59) suggested an additive interaction and indicates that the risk fraction in presence of both ozone and *HLA-DRB1*15* exceeds the addition of risks. The attributable proportion (AP) indicated that approximately 50% (AP 0.56, 95% CI: 0.33–0.79) of the POMS risk in those with *DRB1* haplotype and high ozone levels was attributable to the interaction between these risk

factors. Without high ozone exposure, the presence of the GG genotype of *CD86* SNP did not significantly increase the odds of having POMS. Conversely, without the GG genotype of *CD86* SNP, high ozone exposure was not associated with increased odds of POMS. When both GG genotype of *CD86* SNP and high ozone exposure were present, the odds ratio of POMS increased to 4.06 (95% CI: 1.36–12.14), indicating an additive interaction with a RERI of 1.60 (95%CI: 0.14–3.06). We also detected a statistically significant interaction between high ozone levels and absence of *HLA-A*02* with regard to POMS risk (RERI 1.55, 95% CI: 0.78–2.32, AP 0.52, 95% CI: 0.15–0.89).

Table 4 shows multiplicative interactions for high ozone levels with genetic risk factors in various logistic regression models adjusted for age, sex, genetic ancestry, and mother's education. Although there was evidence for multiplicative interactions between high county-ozone values and presence of *HLA-DRB1* and also absence of *HLA-A*02* (p=0.03 and 0.02, respectively), the interaction term in the logistic regression model adjusted for the presence of the *CD86* SNP GG genotype was not statistically significant suggesting that the interaction present between high ozone levels and this genetic risk factor (CD86 SNP GG genotype) is not multiplicative. According to Log-likelihood and Pseudo R2 values, the best logistic regression model to predict POMS risk is model 2 (with the interaction term, Ozone X DRB1*15 +). In addition, higher Log-likelihood, and Pseudo R2 values indicate that model 3 (with the interaction term, Ozone X CD86 GG +) is a better model than model 1 (without any interaction terms).

Discussion:

In this study, we demonstrated that children who live in areas with high ozone pollution may be at higher risk of having POMS. Furthermore, we identified the potential for gene-environment interactions with high ozone levels in POMS for three genetic risk factors (presence of *HLA-DRB15*, absence of *HLA-A*02 and* the GG genotype of *CD86* SNP).

In a large population-based cohort, the ozone level was the only air pollutant associated with increased adult MS incidence (9). In another multi-pollutant study, ozone levels was associated with increased risk of relapse in adult MS patients (21). In the present study as the first study in children, we showed that high ozone levels increased the risk of POMS (OR of ~2), expanding our previous work that air pollution is strongly associated with POMS susceptibility (2). Ozone pollution might play an important role in MS pathoetiology. Not only can ozone induce inflammation and oxidative insult in the central nervous system (CNS) and modulate the immune response (5), it can also impact myelin ultrastructure in *in vitro studies* (6). Furthermore, we demonstrate evidence for additive and multiplicative interactions between *HLA-DRB1*15,* the strongest genetic risk factor for POMS(11), and high ozone levels. POMS risk associated with high ozone level in those carrying *HLA-DRB1*15* exceeds the addition of the separate risks (RERI~2 and AP~0.6). Multiplicative interactions were also detected for these risk factors that highlight strong biological association. Long-term ozone exposure may lead to inflammation and oxidative stress which in turn prime microglia and damage the blood brain barrier (BBB) (8). This lung-brain axis eventually may contribute to CNS autoimmunity (7,8). This is intriguing as the *HLA-DRB15:01*-binding pocket has a high affinity for CNS autoantigens (22) and

could conceptually explain the interaction of high ozone levels with the *HLA-DRB1*15:01* haplotype. Several interactions between environmental risk factors and *HLA-DRB1* have been reported in MS (1). The most relevant to our study are those related to various sources of lung irritation that may make individuals susceptible to MS such as cigarette smoking, secondhand smoking (23) and exposure to organic solvents (24).

Among non-HLA genes with variants associated with POMS risk (11), *CD86* is the only one for which expression is reportedly influenced by ozone exposure. Ozone exposure increases *CD86* expression on antigen-presenting cells (APCs) (12). Carrying the GG genotype of *CD86* related POMS-risk SNP (rs928264) is associated with increased expression as well (15). *CD86* is a co-stimulatory ligand expressed on APCs contributing to T cells proliferation, cytokine secretion (25) and severity of experimental autoimmune encephalitis (EAE) (26). Furthermore, in MS patients higher expression of *CD86* has been reported in B cells (27,28), macrophages (29) and astrocytes (30) that can all serve as APCs. Taken together, high ozone exposure increases the expression of *CD86* and could increase susceptibility to POMS, especially in those carrying the GG genotype (RERI~1.6 and AP~0.4). The GxE interaction we reported, highlights the possible critical role of APCs in high ozone exposure-induced POMS risk.

Although *HLA- A*02* is the second major allele associated with adult MS (31), its absence has only modest effect on susceptibility to POMS (11). Individuals without *HLA-A*02* tended to have a higher risk of POMS (adjusted OR 1.22, 95%CI: 0.93–1.59). *HLA-A*02* may modulate the immune response contributing the MS onset (32). In addition, the absence of *HLA-A*02* may increase the negative selection of CNS autoreactive T cells (33). This could explain why we observed a slight additive interaction between high ozone levels and absence of *HLA-A*02* (RERI: 1.55 and AP: 0.52). Furthermore, the absence of *HLA-A*02* may interact with other environmental MS risk factors such as Epstein-Barr virus (EBV) infection, cigarette smoking, passive smoking and obesity (1).

The risk of developing POMS among subjects with genetic susceptibility and exposed to high level of ozone is higher than expected risk based on the sum of the risks for each one individually. These additive interactions suggest a potential role for biological synergism of these risk factors (34). From a public health point of view, our results suggest that decreasing ozone exposure in individuals with the genetic risk factors we studied may help decrease MS onset.

The strengths of our study include the large size of our cohort of POMS with frequency-matched controls, the diversity of geographic origin, the careful case ascertainment and the exclusion of confirmed MOG-IgG associated disease (MOGAD) by pediatric MS specialists. The US network of pediatric MS centers are tertiary referral centers; this may result in a selection bias toward more severe or complicated POMS cases. Although those centers serve regional patients from all socioeconomic groups, we adjusted our analyses for mother's education as a proxy of socioeconomic status. In addition, we adjusted our models for genetic ancestry estimations. Other strengths include that we focused the study of GxE interactions on the 2 strongest HLA risk factors for MS and on another genetic variant on the

pathway of ozone exposure. As the genetic risk factors we studied are unlikely to influence environmental exposure habits, they should not affect gene-environment interaction findings.

One of the limitations of our study is the use of population-weighted ozone value as a proxy of long-term ozone exposure rather than a measurement of direct ozone exposure. Individual level factors such as time spent outdoors and travel between counties are less varied than adults but still might impact actual ozone exposure. However, population-weighted indexes are reported to provide acceptable exposure estimation of air pollutants (35). In this study, we used 2014 ozone levels, mid-year of study enrolment, and assigned them to each individual based on county of residence at the time of study enrolment.

Future study with larger sample size would be required to enable three-way interaction analyses. Finally, we were not able to identify another geographically diverse MS cohort to try to replicate our findings as other deeply phenotyped cohorts did not include information on ozone exposure.

In summary, ozone exposure may be an important risk factor for pediatric MS onset, especially among individuals with genetic susceptibility to the disease. Interactions between high ozone levels and the presence of *HLA-DRB1*15*, *CD86* SNP rs928264 GG genotype, and absence of *HLA-A*02* suggest a possible role of APCs and immune-related mechanisms for ozone induced MS risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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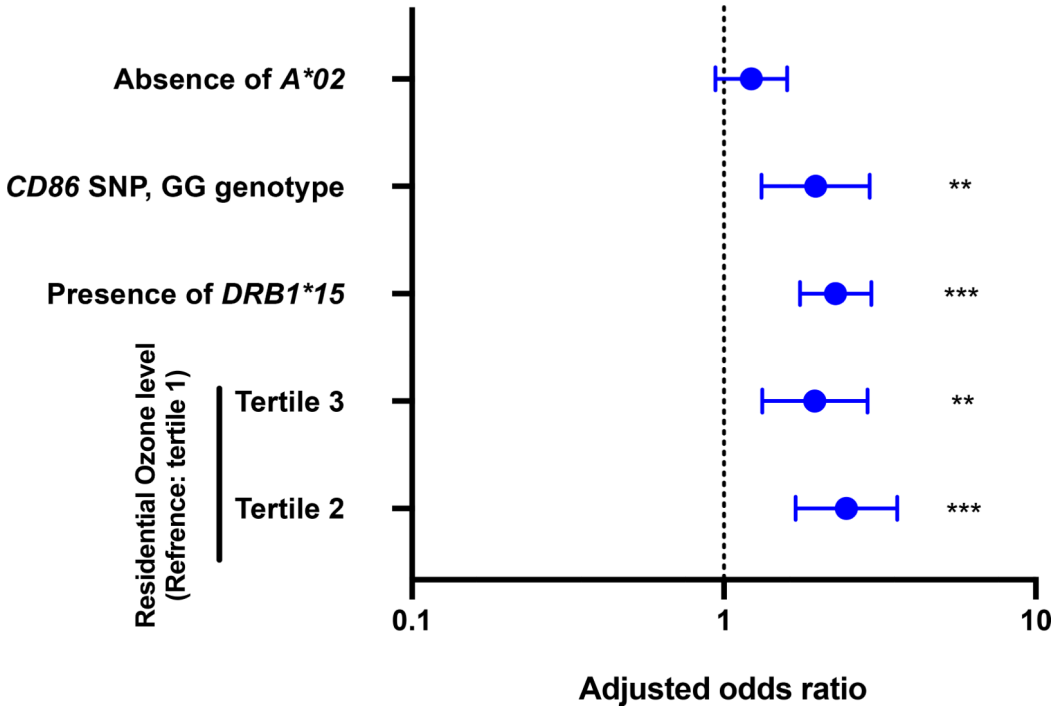

Figure 1: High ozone levels, *DRB1* and *CD86* SNP GG genotype are associated with pediatric onset MS risk.

Odds ratio with 95 % confidence interval of developing MS. Adjusted odds ratios are calculated based on separate logistic regression models adjusted for age, sex, genetic ancestry, and mother's education. **: P-value <0.01, ***: P-value <0.001

Table 1.

Characteristics of study participants.

Characteristics	POMS (n=334)	Healthy controls (n=565)	P-value
Age, median years (IQR)	15 (3)	15 (5)	0.09[a]
Female, n (%)	216 (64.6)	336 (59.5)	0.13[b]
Ratio, F:M	1.8:1	1.46:1	
Self-reported race, n (%)			
White	227 (67.9)	368 (65.2)	0.42[b]
Non-White	107 (32.0)	196 (34.7)	
Self-reported ethnicity, n (%)			
Hispanic	97 (30.0)	115 (20.8)	0.002[b]
Non-Hispanic	226 (69.9)	437 (79.1)	
Genetic ancestry estimation, mean (SD)			
European ancestry percentage	68.4 (0.3)	67.7 (0.3)	0.72[c]
East Asian ancestry percentage	3.5 (0.1)	7.5 (0.2)	0.003[c]
Native American ancestry percentage	10.1 (0.1)	6.6 (0.1)	0.003[c]
West African ancestry percentage	17.8 (0.2)	18.1 (0.2)	0.85[c]
Mother's education, n (%)			
Less than high school	17 (6.1)	5 (1.0)	
High school and college	156 (56.3)	240 (48.0)	<0.001[d]
University degree	104 (37.5)	255 (51.0)	
County-level ozone, n (%)			
Tertile 1	60 (17.9)	190 (33.6)	
Tertile 2	148 (44.3)	187 (33.0)	<0.001[d]
Tertile 3	126 (37.7)	188 (33.2)	

POMS: Pediatric onset multiple sclerosis; IQR: interquartile range.

[a]Mann Whitney test

[b]Fisher's exact test

[c]Unpaired t test, test

[d]Chi-squared test

Table 2.

Distribution of genetic covariates between POMS and healthy control participants.

Genetic covariates	POMS	Healthy controls
*HLA-DRB1*15*	n=321	n=556
Presence of one or two alleles, n (%)	127 (39.6)	139 (25.0)
CD86 SNP (rs9282641, G/A)	n=318	n=555
Presence of GG genotype, n (%)	290 (91.1)	465 (83.7)
*HLA-A*02*	n=285	n=442
Absence of one or two alleles, n (%)	175 (61.4)	248 (56.1)

POMS: Pediatric-Onset Multiple Sclerosis; SNP: Single Nucleotide Polymorphism

Table 3.

Stratified analyses assessing additive interactions between high ozone levels and presence of *HLA-DRB1*15* allele, *CD86* SNP GG genotype, and absence of *HLA-A*02*.

High ozone levels (Tertiles 2 and 3)	Presence of *HLA-DRB1*15*	Ca/Co	OR (95% CI)	RERI (95% CI)	AP (95% CI)
−	−	44/140	1 (reference)		
−	+	15/48	0.99 (0.50–1.95)		
+	−	150/277	1.68 (1.12–2.53) *		
+	+	112/91	3.89 (2.47–6.12) ***	2.21 (0.83–3.59) **	0.56 (0.33–0.79) ***
High ozone levels (Tertiles 2 and 3)	Presence of *CD86* SNP, GG.				
−	−	4/20	1 (reference)		
−	+	55/168	1.72 (0.56–5.34)		
+	−	24/70	1.79 (0.55–5.81)		
+	+	235/297	4.06 (1.36–12.14) *	1.60 (0.14–3.06) *	0.37 (0.001–0.75) *
High ozone levels (Tertiles 2 and 3)	Absence of *HLA-A*02*				
−	−	22/49	1 (reference)		
−	+	32/95	0.67 (0.35–1.30)		
+	−	88/145	1.22 (0.68–2.19)		
+	+	143/153	1.90 (1.08–3.35) *	1.55 (0.78–2.32) ***	0.52 (0.15–0.89) **

Ca/Co: Number of POMS cases and healthy controls; RERI: Relative Excess Risk due to Interaction; AP: Attributable Proportion of disease; CI: Confidence Intervals.

* P-value <0.05

** P-value <0.01

*** P-value <0.001

Logistic regression models were adjusted for age, sex, genetic ancestry, and mother's education.

Table 4.

Models assessing multiplicative interactions for odds of POMS.

	Model 1	**Model 2**	**Model 3**	**Model 4**
	No interaction term	**Interaction term, Ozone X *DRB1*15* +**	**Interaction term, Ozone X *CD86* GG +**	**Interaction term, Ozone X *A*02* −**
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
High ozone exposure (Tertiles 2&3)	2.22 (1.57– 3.13) ***	1.68 (1.12–2.53) *	1.79 (0.55–5.81)	1.22 (0.68–2.19)
Secondary risk factors (*DRB1*15* +, *CD86* GG+, *A*02* −)	-	0.99 (0.50–1.95)	1.72 (0.56–5.34)	0.67 (0.35–1.30)
High ozone X *HLA-DRB1*15 (+)*	-	2.32 (1.08–4.99) *	-	-
High ozone X *CD86* SNP, GG (+)	-	-	1.31 (0.38–4.48)	-
High ozone X *HLA-A*02 (−)*	-	-	-	2.29 (1.08–4.84) *
Pseudo R^2	0.044	0.063	0.054	0.043
Log likelihood	−546.74	−535.13	−540.25	−464.95

*: P-value <0.05

**: P-value <0.01

***: P-value <0.001

The logistic regression models were adjusted for age, sex, genetic ancestry, and mother's education.