Title

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Journal

Journal of neurology, neurosurgery, and psychiatry, 94(7)

ISSN

0022-3050

Authors

Nasr, Zahra
Schoeps, Vinicius Andreoli
Ziaei, Amin
et al.

Publication Date

2023-07-01

DOI

10.1136/jnnp-2022-330713

Peer reviewed

Published in final edited form as:

J Neurol Neurosurg Psychiatry. 2023 July ; 94(7): 518–525. doi:10.1136/jnnp-2022-330713.

Gene-environment interactions increase the risk of pediatric-onset multiple sclerosis associated with household chemical exposures

Zahra Nasr[1], **Vinicius A Schoeps**[1], **Amin Ziaei**[1], **Akash Virupakshaiah**[1], **Cameron Adams**[2], **T Charles Casper**[3], **Michael Waltz**[3], **John Rose**[3], **Moses Rodriguez**[4], **Jan M Tillema**[4], **Tanuja Chitnis**[5], **Jennifer S Graves**[6], **Leslie A Benson**[7], **Mary Rensel**[8], **Lauren Krupp**[9], **Amy T Waldman**[10], **Bianca Weinstock-Guttman**[11], **Tim Lotze**[12], **Benjamin Greenberg**[13], **Gregory Aaen**[14], **Soe Mar**[15], **Teri Schreiner**[16], **Janace Hart**[1], **Steve Simpson-Yap**[17,18,19], **Clementina Mesaros**[20], **Lisa F Barcellos**[21], **Emmanuelle Waubant**[1]

[1]UCSF Weill Institute for Neurosciences, University of California San Francisco, San Francisco, California

[2]School of Public Health, University of California, Berkeley 94720, USA

[3]University of Utah, Salt Lake City, Utah

[4]Mayo Clinic, Rochester, Minnesota

[5]Brigham and Women's Hospital, Harvard Medical school, Boston, Massachusetts

[6]University of California San Diego, San Diego, California

[7]Boston Children's Hospital, Boston, Massachusetts

[8]Cleveland Clinic, Cleveland, Ohio

[9]New York University Medical Center, New York, New York

[10]Division of Child Neurology, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

[11]Jacobs School of Medicine and Biomedical Sciences University of Buffalo, Buffalo, NY

[12]Texas Children's Hospital, Houston, Texas

[13]University of Texas Southwestern Medical Center, Dallas, Texas

[14]Loma Linda University Children's Hospital, Loma Linda, California

[15]Washington University in St. Louis, St. Louis, Missouri

Correspondence to: Dr. Emmanuelle Waubant, UCSF Will Institute for Neurosciences, University of California San Francisco, 675 Nelson Rising Lane, San Francisco, CA 94158, Emmanuelle.Waubant@ucsf.edu, Telephone Number: (415) 514-2468, Fax Number: (415) 514-2468.

Author Contributions

Z.N, A.Z, C.A, C.M, L.F.B, and E.W. contributed to the conception and design of the study; Z.N, V.A.S, A.Z, C.A, T.C.C, M.W, S.S.Y, L.F.B, and E.W. contributed to the acquisition and analysis of data; Z.N, V.A.S, A.Z, A.V, C.A, T.C.C, M.W, J.R, M.R, J.M.T, T.C, J.S.G, L.A.B, M.R, L.K, A.T.W, B.W.G, T.L, B.G, G.A, S.M, T.S, J.H., S.S.Y, C.M, L.F.B, and E.W. contributed to draft the text and preparing the figures.

Potential Conflicts of Interest

E.W has current support from the NIH, NMSS, PCORI, CMSC, and Race to Erase MS.

[16]Denver Children's Hospital, Denver, Colorado

[17]Neuroepidemiology Unit, Melbourne School of Population & Global Health, The University of Melbourne, Carlton, Australia

[18]Clinical Outcomes Research Unit (CORe), Royal Melbourne Hospital, The University of Melbourne, Parkville, Australia

[19]Multiple Sclerosis Flagship, Menzies Institute for Medical Research, University of Tasmania, Hobart, Australia

[20]Department of Systems Pharmacology and Translational Therapeutics (SPATT), University of Pennsylvania, Philadelphia, PA

[21]Department of Integrative Biology, University of California, Berkeley, USA

Abstract

Background: We previously reported an association between household chemical exposures and an increased risk of pediatric-onset multiple sclerosis.

Methods: Using a case-control pediatric multiple sclerosis study, gene-environment interaction between exposure to household chemicals and genotypes for risk of pediatric-onset multiple sclerosis was estimated.

Genetic risk factors of interest included the two major HLA multiple sclerosis risk factors, the presence of *DRB1*15* and the absence of *A*02*, and multiple sclerosis risk variants within the metabolic pathways of common household toxic chemicals, including *IL-6* (rs2069852), *BCL-2* (rs2187163) and *NFKB1* (rs7665090).

Results: 490 pediatric-onset multiple sclerosis cases and 716 controls were included in the analyses. Exposures to insect repellent for ticks or mosquitos (OR: 1.47, 95% CI: 1.06–2.04, P = 0.019), weed control products (OR: 2.15, 95% CI: 1.51–3.07, P < 0.001), and plant/tree insect or disease control products (OR: 3.25, 95% CI: 1.92–5.49, P < 0.001) were associated with increased odds of pediatric-onset multiple sclerosis. There was significant additive interaction between exposure to weed control products and *NFKB1* SNP GG (attributable proportions (AP) 0.48, 95% CI: 0.10–0.87), and exposure to plant or disease control products and absence of *HLA-A*02* (AP 0.56; 95% CI: 0.03– 1.08). There was a multiplicative interaction between exposure to weed control products and *NFKB1* SNP GG genotype (OR: 2.30, CI: 1.00–5.30) but not for other exposures and risk variants. No interactions were found with *IL-6* and *BCL-2* SNP GG genotypes.

Conclusions: The presence of gene-environment interactions with household toxins supports their possible causal role in pediatric-onset multiple sclerosis

Introduction

While significant progress has been made toward elucidating distinct genetic and environmental risk factors for multiple sclerosis (MS), there is still a large knowledge gap regarding the contributions of environmental exposures in genetically susceptible individuals[1].

Our preliminary work reported that exposure to insect repellent for tick or mosquitos, weed control products, and plant/tree insect or disease control products during the perinatal period and childhood was strongly associated with MS risk[2]. A growing body of literature suggests that exposure to toxic chemicals may increase the risk of other neurologic diseases, including Parkinson's disease[3], Alzheimer's disease[4], autism spectrum disorder[5], and attention deficit hyperactivity disorder[6]. The molecular basis for the contribution of these chemicals to disease susceptibility remains unclear[7]. Although exposure to a chemical toxin alone may be insufficient to cause neurological diseases such as pediatric-onset multiple sclerosis (POMS), it may be more likely to affect genetically susceptible individuals[1].

We sought to examine a role in POMS risk for the potential interactions between chemical exposures and HLA-*DRB1*15*, *HLA-A*02, and MS* risk variants within genes in metabolic pathways of common household toxic chemicals, including *IL-6* (rs2069852), *BCL-2* (rs2187163), and *NFKB1* (rs7665090).

Methods

Study population

We used data on POMS cases and healthy controls from the US Network of Pediatric Multiple Sclerosis (R01NS071463, PI Waubant). Participants were recruited at 17 participating pediatric MS clinics in the USA between November 1, 2011, and July 1, 2017. POMS cases with symptom onset at less than 18 years of age were enrolled in this study, as previously described[8]. Case ascertainment was established based on the diagnosis of at least two pediatric MS specialists from the US pediatric MS network, using the 2010 McDonald Criteria[9]. The network collects retrospective demographic characteristics and medical history in a centralized database[10]. Healthy controls were recruited from primary care and non-MS pediatric clinics at the participating institutions during case recruitment and were frequency-matched to cases using age and sex. All controls had no history of neurological or autoimmune disease (apart from headache/migraine), no first-degree biological relatives with MS, and were under 22 years of age.

The institutional review board of each participating institution approved the study protocols. Informed consent was obtained from all participants/legal guardians before enrollment. Blood samples were collected upon enrollment.

Environmental data

Information regarding household chemical exposures was collected using a comprehensive environmental questionnaire (http://www.usnpmsc.org/Documents/EnvironmentalAssessment.pdf) completed by parents/legal guardians at the time of enrollment, including prior exposure and timing of exposure to different household chemicals. Subjects were considered exposed to the toxic chemicals if the exposure was before disease onset. Household chemicals previously found to be associated with the risk of POMS were included in gene-environment interaction (GxE) models: 1) insect repellent for ticks or mosquitos, 2) weed control products, and 3) plant/tree insect or disease control products. Commonly used chemicals previously reported to cause neurodevelopmental

and immunological interference were also selected for further investigation, including glyphosate, linuron, chlorpyrifos, diazinon, acephate, malathion, permethrin, bifenthrin, methyl bromide, imidacloprid, and avermectin (5).

Genotyping and definition of genetic risk factors

Study participants were genotyped using the Infinium 660K BeadChip or HumanOmniExpress BeadChip. Stringent quality control measures and comparison of sample genotypes across two Illumina platforms were performed using PLINK version 1.9. The alignment, phasing, imputation, and variant filtering were done as described previously[11].

We searched PubMed for papers published before March 31, 2022, with the following terms: “name of each chemical”, “gene”, “expression”, “alteration”, and “regulation”. Seventeen genes were reported with altered expression by exposure to one of the above chemicals (Table 1). Looking at the largest genome-wide association study (GWAS) of MS conducted by the International Multiple Sclerosis Genetics Consortium (IMSGC) three of the seventeen candidate genes, specifically *IL-6* (rs2069852, G/A), *BCL2* (rs2187163, G/A), and *NFKB1* (rs7665090, G/A), were reported to increase MS risk[12]. The expression of *IL-6, BCL2,* and *NFKB1* were shown to be altered by exposure to glyphosate, which is widely used in weed control products[13–15]. We utilized these three SNPs to evaluate potential interactions with exposure to weed control products for increased risk of POMS. We further investigated the potential interaction between the presence of *HLA-DRB1*15* or absence of *HLA-A*02* (as the strongest genetic variants associated with POMS) and exposure to insect repellent for tick or mosquitos, weed control products, or plant/tree insect or disease control products, separately and in different models.

DNA samples of all subjects have been genotyped for *DRB1* status, as previously published[8]. *DRB1* status was dichotomized according to the presence of one or more *HLA-DRB1*15* alleles versus no carriage. The *HLA-A*02* (rs2975033) tagging SNP was imputed. The allele “A” of rs2975033 has been reported to be in strong linkage disequilibrium ($r^2 = 0.97$) with the *HLA-A*02* allele[16]. Participants were categorized according to no carriage of any *HLA-A*02* allele (absence of *HLA-A*02*) versus any carriage. In addition, the subjects were dichotomized according to GG genotype of *IL-6* SNP (rs2069852 G/A)[17], GG genotype of *BCL2* SNP (rs2187163 G/A)[12], and GG genotype of *NFKB1* SNP (rs7665090 G/A)[18] as risk alleles, versus other (GA and AA genotypes).

We used the SNP weighting method to estimate the percentage of genetic ancestry related to four major populations (European, East Asian, West African, and Native American)[19].

Statistical analysis

The odds of having POMS associated with each environmental and genetic exposure was assessed separately using logistic regression models adjusted for age, sex, genetic ancestry, mother’s education as a proxy of socioeconomic status (SES), and recruiting site. Environmental exposures included three categories of household chemicals, namely (1) insect repellent for ticks or mosquitos, (2) weed control products, and (3) plant/tree insect or disease control products). The presence of *HLA-DRB1*15*, absence of *HLA-A* 02*, and GG

genotype of *IL-6* SNP, GG genotype of *BCL-2* SNP and GG genotype of *NFKB1* SNP were also assessed. We tested for additive interaction between each household chemical exposure and genotype status with logistic regression models calculating the relative excess risk due to interaction (RERI) and the attributable proportion (AP) of disease due to interaction[28]. An interaction term was also added to logistic regression models to assess the presence of multiplicative interaction. In the primary analysis, patients with missing environmental exposures were excluded. Aiming to minimize a possible differential missingness pattern of the environmental exposure, a sensitivity analysis using inverse probability weighting (IPW) was performed, as previously described[29]. All statistical analyses were done in Stata 17.0 (Stata Corp, College Station, TX). We calculated RERI and AP, their CIs, and p-values according to the method described in VanderWeele and Knol et al[30].

Results

Participants characteristics

490 POMS cases and 716 healthy controls were enrolled in this study. Baseline characteristics are shown in (Table 2). Hispanic ethnicity was more frequent in cases than in controls (32.1% vs. 23%, P < 0.001). Mother's education was higher in controls compared to cases (university degree 46.7% vs. 31.9%, P < 0.001). The mean time interval between clinical onset and MS diagnosis was four months (median months [IQR: 0 – 5.13]).

Toxic chemical exposures are associated with higher odds of POMS

As shown in (Table 3), logistic regression analysis adjusting for covariates (age, sex, genetic ancestry, mother's education, and recruiting site), POMS cases had higher odds of exposure to insect repellent for tick or mosquitos (OR 1.47, 95% CI 1.06–2.04, P = 0.019), weed control products (OR 2.15, 95% CI 1.51–3.07, P < 0.001) and plant/tree insect or disease control products (OR 3.25, 95% CI 1.92–5.49, P < 0.001) compared to controls. Sensitivity analyses using IPW resulted in similar results (Tables 4)

Genetic characteristics

POMS cases were more likely to have at least one *HLA-DRB1*15* allele (41% vs. 23.6%, P<0.001). Available imputed genotype data for *HLA-A*02* demonstrated a higher proportion of POMS cases than healthy controls without the *HLA-A*02* allele (61.5% vs. 56.3%, P= 0.29). In addition, a higher proportion of POMS cases had the GG genotype of *NFKB1* risk SNP (rs7665090) (37% vs. 31.5%, P= 0.44).

Assessment of G × E interactions

Results from *G* × *E* analyses of environmental toxic chemical exposures and genetic variants of interest are presented in (Table 5). In those without exposure to weed control products, the *NFKB1* SNP GG genotype did not significantly increase the odds of POMS (OR 0.77, 95% CI: 0.48–1.21). When both *NFKB1* SNP GG genotype and exposure to weed control products were present, the odds of exposure in cases compared to controls increased to 3.32 (95% CI: 1.74–6.36), which is higher than the product of the two respective ratios (0.77 and 1.86). The AP indicated that 50% (AP: 0.50, 95% CI: 0.13–0.88) of the POMS risk in individuals with *NFKB1* SNP GG genotype and exposure to weed control products

were attributable to their interaction. In addition, greater than 50% (AP: 0.56, 95% CI: 0.06–1.07) of the POMS risk in individuals without *HLA-A*02* and exposure to plant/tree insect or disease control products were attributable to the interaction between these risk factors. Without exposure to plant/tree insect or disease control products, the absence of *HLA-A**02 did not significantly increase the odds of having POMS. When both absences of *HLA-A**02 and exposure to plant/tree insect or disease control products were present, the OR of POMS increased to 4.93 (95% CI: 2.23–10.88), indicating an additive interaction with an AP of 0.56 (95% CI: 0.06–1.07). Sensitivity analyses using IPW resulted in similar results (Tables 6)

We also detected an increased odds of exposure to insect repellent for ticks or mosquitos and the absence of *HLA-A*02* (OR: 1.85, CI: 1.09–3.12), although the AP and RERI were not significant. Similarly, a significant increase in OR was observed for exposure to weed control products and the absence of *HLA-A*02* (OR: 2.65, CI: 1.60–4.39), although the AP and RERI were not significant. There was no interaction of *DRB1* with environmental exposures.

(Table 7) shows multiplicative interaction analysis results for toxic chemical exposures with genetic risk factors in various logistic regression models adjusted for age, sex, genetic ancestry, and mother's education. There was evidence of a multiplicative interaction between exposure to weed control products and *NFKB1* SNP GG genotype (OR: 2.30, CI: 1.00–5.30) but not for other exposures and risk variants. Sensitivity analyses using IPW resulted in similar results (Table 8).

Discussion

In this study, we expanded on results from a previous study finding evidence of associations between household toxic chemical exposures and risk of POMS using GxE interactions (2). More specifically, we identified evidence of additive and multiplicative GxE interaction between MS risk variant *NF-κB1* SNP GG genotype (rs7665090) and exposure to glyphosate.

The mechanisms through which toxic chemical exposure may contribute to MS onset remains are not completely understood. Exposure to weed control agents can alter immune response, potentially resulting in inflammatory diseases, including rheumatoid arthritis and systematic lupus erythematosus[31,32]. Glyphosate (RoundUp®) can induce the nuclear factor-kappa B (*NF-κB*) signaling pathway in several human cell types[14,15,21] inducing gene expression of proinflammatory cytokines like TNF- α, IL-1, and IL-6. Activation of *NF-κB* in multiple cell types in the CNS and peripheral blood mononuclear cells of MS patients has been reported[33]. Furthermore, genome-wide studies have identified the contribution of *NF-κB* to MS risk[34].

MS risk variant rs7665090^{G}, located near *NF-κB*, has been associated with the upregulation of *NF-κB* in human astrocytes[18]. Here, we report evidence for both additive and multiplicative interactions between the *NF-κB* SNP and exposure to weed control products influencing POMS risk. The presence of additive and specifically multiplicative interaction

suggests the potential role for synergically induced *NF-κB* signaling pathway through environmental exposure in genetically susceptible individuals.

Studies of GxE interactions in MS have been limited by the sample size and have mainly focused on the two strongest MS risk variants, *HLA-DRB1*15* and the absence of *HLA-A02*[1]. While the interaction between *HLA-DRB1*15* and EBV, HSV, smoking, and adolescent obesity has been reported in whites, the interaction of *HLA-DRB1*15* and EBV has also been reported in blacks[1,28,35]. *HLA-DRB1*15* and the absence of HLA-A*02 may also interact with occupational exposure to inhaled organic solvents[36]. We did not find any additive or multiplicative interaction between *HLA-DRB1*15* and the absence of *HLA-A*02* and the studied environmental exposures. The gene-environment interactions in MS may be attributed to the various epigenetic factors that affect the downstream biological effects due to molecular modifications[1]. This could explain why we did not observe any interaction between our candidate environmental risk factors and *HLA-DRB1*15* and the absence of *HLA-A*02*.

A significant strength of our study is the large POMS cohort, with cases and controls enrolled nationally at the same sites. We adjusted the analyses for multiple potential confounders, including genetic ancestry estimations, mother's education as a proxy of SES, age, sex, and recruiting site. There were several limitations to our study. The questionnaire did not address the specific time, dose, and brand name of chemical exposures. We considered that all the participants had been exposed to the commonly used chemicals as we did not have the specific type of chemicals to which each subject was exposed. Furthermore, this is a retrospective study of exposures; however, focusing on pediatric rather than adult onset MS improves recall quality due to the much shorter time between exposure and outcome. Our results will need to be replicated in a larger study. We considered repeating similar analyses in the Ausimmune and the Swedish cohorts, but the age range, type of exposure, and questionnaires were too different.

Taken together, exposure to herbicides may be a risk factor for POMS, especially among individuals with genetic susceptibility to the disease. G×E interactions between weed control products and *NF-κB* SNP (rs7665090, GG genotype) suggest a possible role of *NF-κB* signaling pathway in susceptibility to POMS. To fill the gap in the underlying biological pathways, international collaborative studies with diverse genetic and environmental backgrounds can be helpful.

Disclosures/Acknowledgements:

Funding for this work includes R01NS071463 (PI Waubant), NMSS HC-1509-06233 (PI Casper), and MSIF (PI Nasr). None of the authors have any conflict of interest with this study. We thank Dr. Robyn Lucas for her assistance in considering duplication of the results in Aussies cohort. We thank the patients and families who participated in the study.

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Key messages:

- Identifying gene-environment interactions contributing to the risk of MS is critical in advancing the understanding of molecular processes at play.
- We report a novel gene-environment interaction association with household toxic chemical exposures increasing the risk of pediatric-onset MS.
- We identified both additive and multiplicative interactions with MS risk variant *NF-κB1* SNP GG genotype (rs7665090) and likely exposure to glyphosate as one of the commonly used herbicides.
- We take one step forward toward unanswered research questions for the concurrent role of genetic and environmental factors in susceptibility to MS

Table 1.

Gene expression alterations by exposure to toxic chemicals.

Author (Year of publish)	Toxic chemical	Altered gene
Gui[20] (2012)	Glyphosate	*BECN1*
Martinez[15] (2020)		*BAX, BCL2, CASP3, CASP9, TP53, NFKB, SNCA, IL-6, TNFα*
Tang[14] (2020)		*IL-1β, IL-6, TNF-α, NFKB, CASP3*
Wozniak[13] (2020)		*TP53, BCL2*
Zheng[21] (2021)		*NRF2, NFKB2, IL-1β*
Gargouri[22] (2018)	Bifenthrin (pyrethroid)	*NFKBp65, TNF-α, NRF2*
Ibrahim[23] (2021)	Avermectin	*BAX, CASP3, CASP9*
Mense[24] (2006)	Chlorpyrifos	*IL6R*
Zhao[25] (2019)		*NRF2, IL-1β, IL-18*
Chorfa[26] (2013)	Rotenone, Paraquat, Maneb	*SNCA*
Wheeler[27] (2019)	Linuron	*SIGMAR, XBP1*

Table 2.

Characteristics of study participants.

Characteristics	POMS (n=490)	Healthy controls (n=716)	P-value
Age, median years (IQR)	16 (3)	15 (5)	0.034[a]
Female, n (%)	316 (64.3)	420 (59.1)	0.07[b]
Ratio, F:M	1.8:1	1.44:1	
Self-reported race, n (%)			
White	312 (68.9)	439 (66.4)	0.398[b]
Self-reported ethnicity, n (%)			
Hispanic	150 (32.1)	156 (23)	<0.001[b]
Genetic ancestry estimation, mean (SD)			
European ancestry percentage	65.6 (0.3)	66.7 (0.3)	0.57[c]
East Asian ancestry percentage	3.6 (0.2)	8 (0.2)	0.007[c]
Native American ancestry percentage	10.2 (0.2)	7.6 (0.2)	0.006[c]
West African ancestry percentage	19.5 (0.3)	17.6 (0.3)	0.23[c]
Mother's education, n (%)			
Less than high school	52 (12.1)	40 (6.4)	<0.001[d]
High school and college	240 (56)	295 (46.9)	
University degree	137 (31.9)	293 (46.7)	

POMS: Pediatric-onset multiple sclerosis; IQR: interquartile range; SD: standard deviation.

[a] Mann Whitney test

[b] Fisher's exact test

[c] Unpaired t-test

[d] Chi-squared test

Table 3.

Toxic chemical exposure analysis.

	POMS (N = 490)	Healthy controls (N = 716)	Odds ratio (95% CI)	P-Value
Insect repellent for ticks or mosquitos, x/n (%)	179/293 (61)	262/479 (55)	1.47 (1.06 – 2.04)	0.019
Weed control products, x/n (%)	114/309 (37)	130/498 (26)	2.15 (1.51 – 3.07)	<0.001
Plant/tree insect or disease control products, x/n (%)	46/307 (15)	35/510 (7)	3.25 (1.92 – 5.49)	<0.001

Adjusted for age, sex, genetic ancestry, mother's highest level of education, recruiting site

Table 4.

Toxic chemical exposure analysis (sensitivity analysis: using inverse probability weighting).

	POMS (N = 490)	Healthy controls (N = 716)	Odds ratio (95% CI)	P-Value
Insect repellent for ticks or mosquitos, x/n (%)	179/293 (61)	262/479 (55)	1.55 (1.11 – 2.16)	0.009
Weed control products, x/n (%)	114/309 (37)	130/498 (26)	2.20 (1.54 – 3.15)	<0.001
Plant/tree insect or disease control products, x/n (%)	46/307 (15)	35/510 (7)	3.35 (1.99 – 5.65)	<0.001

Adjusted for age, sex, genetic ancestry, mother's highest level of education, recruiting site

Table 5.

Additive interaction between household chemical exposure and genotype. Stratified analysis assessing the interactions between household chemical exposure and presence of *HLA-DRB1*15* alleles, absence of *HLA-A*02, IL-6* SNP GG genotype, *BCL2* SNP GG genotype and *NFKB1* SNP GG genotype

Household chemical exposure	Genetic exposure	N Case/ N Control	OR (95% CI)	RERI (95% CI)	AP (95% CI)
Exposure to Insect repellent	**Presence of *HLA-DRB1*15***				
–	–	67/172	reference		
–	+	44/45	2.92 (1.69 – 5.03) ***		
+	–	102/187	1.69 (1.12 – 2.53) *		
+	+	74/74	2.91 (1.82 – 4.66) ***	-0.69 (−2.47 – 1.09)	-0.23 (−0.88 – 0.40)
Exposure to Insect repellent	**Absence of *HLA-A*02***				
–	–	35/73	reference		
–	+	64/99	1.31 (0.76 – 2.24)		
+	–	64/92	1.50 (0.86 – 2.61)		
+	+	91/112	1.85 (1.09 – 3.12) *	0.03 (−0.91 – 0.98)	0.01 (−0.49 – 0.53)
Exposure to weed control products	**Presence of *HLA-DRB1*15***				
–	–	109/280	reference		
–	+	77/87	2.62 (1.74 – 3.93) ***		
+	–	61/94	2.42 (1.56 – 3.76) ***		
+	+	47/33	4.79 (2.72 – 8.43) ***	0.74 (−1.89 – 3.38)	0.15 (−0.32 – 0.64)
Exposure to weed control products	**Absence of *HLA-A*02***				
–	–	74/130	reference		
–	+	93/164	0.99 (0.65 – 1.49)		
+	–	32/37	1.80 (0.98 – 3.30)		
+	+	64/54	2.65 (1.60 – 4.39) ***	0.86 (−0.54 – 2.26)	0.32 (−0.13 – 0.78)
Exposure to weed control products	**Presence of *IL-6* SNP,GG**				
–	–	32/47	reference		
–	+	129/229	0.77 (0.42 – 1.42)		
+	–	11/9	2.16 (0.69 – 6.74)		
+	+	84/81	1.84 (0.92 – 3.66)	-0.09 (−2.46 – 2.27)	-0.05 (−1.34 – 1.23)
Exposure to weed control products	**Presence of *BCL2* SNP, GG**				
–	–	63/104	reference		
+	–	35/29	3.46 (1.78 – 6.71)		
+	+	57/60	2.36 (1.34 – 4.17) **	-1.38 (−3.64 – 0.87)	-0.58 (−1.59 – 0.42)

Household chemical exposure	Genetic exposure	N Case/ N Control	OR (95% CI)	RERI (95% CI)	AP (95% CI)
Exposure to Insect repellent	**Presence of *HLA-DRB1*15***				
Exposure to weed control products	**Presence of *NFKB1* SNP, GG**				
−	−	114/199	reference		
−	+	52/92	0.77 (0.48 – 1.21)		
+	−	62/69	1.86 (1.18 – 2.93) **		
+	+	34/21	3.32 (1.74 – 6.36) ***	1.68 (−0.44 – 3.82)	0.50 (0.13 – 0.88) **
Exposure to plant/tree insect control products	**Presence of *HLA-DRB1*15***				
−	−	147/360	reference		
−	+	110/115	2.33 (1.64 – 3.32) ***		
+	−	25/22	3.93 (2.01 – 7.71) ***		
+	+	19/13	5.08(2.23 – 11.57) ***	-0.19 (−4.95 – 4.57)	-0.03 (−0.99 – 0.92)
Exposure to plant/tree insect control products	**Absence of *HLA-A*02***				
−	−	94/159	reference		
−	+	130/212	1.03 (0.72 – 1.47)		
+	−	12/11	2.10 (0.81 – 5.42)		
+	+	26/13	4.93(2.23 – 10.88) ***	2.79 (−1.34 – 6.94)	0.56 (0.06 – 1.07)

ref: reference group; RERI: Relative Excess Risk due to Interaction; AP: Attributable Proportion of disease; SI: Synergic Index; CI: Confidence Intervals

* : P-value <0.05

** : P-value <0.01

*** : P-value <0.00

Table 6.

Additive interaction between household chemical exposure and genotype sensitivity analysis: using inverse probability weighting). Stratified analysis assessing the interactions between household chemical exposure and presence of *HLA-DRB1*15* alleles, absence of *HLA-A*02, IL-6* SNP GG genotype, *BCL2* SNP GG genotype and *NFKB1* SNP GG genotype

Exposureto Insect repellent	**Presence of *HLA-DRB1*15***	**Case/ Control**	**OR (95% CI)**	**RERI (95% CI)**	**AP (95% CI)**
−	−	67/172	reference		
−	+	44/45	2.94 (1.69 – 5.13) ***		
+	−	102/187	1.75 (1.15 – 2.65) *		
+	+	74/74	3.13 (1.93 – 5.05) ***	-0.56 (−2.40 – 1.27)	-0.18 (−0.79 – 0.43)
Exposure to Insect repellent	**Absence of *HLA-A*02***				
−	−	35/73	reference		
−	+	64/99	1.24 (0.71 – 2.17)		
+	−	64/92	1.48 (0.84 – 2.62)		
+	+	91/112	1.90 (1.10 – 3.27) *	0.17 (−0.76 – 1.10)	0.09 (−0.40 – 0.58)
Exposure to weed control products	**Presence of *HLA-DRB1*15***				
−	−	109/280	reference		
−	+	77/87	2.67 (1.77 – 4.02) ***		
+	−	61/94	2.50 (1.61 – 3.88) ***		
+	+	47/33	4.86 (2.72 – 8.67) ***	0.68 (−2.03 – 3.40)	0.14 (−0.35 – 0.63)
Exposure to weed control products	**Absence of *HLA-A*02***				
−	−	74/130	reference		
−	+	93/164	0.97 (0.63–1.47)		
+	−	32/37	1.76 (0.97–3.21)		
+	+	64/54	2.70 (1.61–4.51) ***	0.96 (−0.43–2.36)	0.35 (−0.07 – 0.79)
Exposure to weed control products	**Presence of *IL-6* SNP,GG**				
−	−	32/47	reference		
−	+	129/229	0.76 (0.41–1.39)		
+	−	11/9	2.47 (0.73–8.30)		
+	+	84/81	1.83 (0.93–3.59)	-0.39 (−3.27 – 2.47)	-0.21 (−1.78 – 1.34)
Exposure to weed control products	**Presence of *BCL2* SNP, GG**				
−	−	63/104	reference		
−	+	101/182	1.24 (0.78–1.97)		
+	−	35/29	3.40 (1.75–6.62) ***		

Exposureto Insect repellent	**Presence of *HLA-DRB1*15***	**Case/ Control**	**OR (95% CI)**	**RERI (95% CI)**	**AP (95% CI)**
+	+	57/60	2.38 (1.34–4.22) **	-1.27 (−3.49 – 0.94)	-0.53 (−1.51 – 0.44)
Exposure to weed control products	**Presence of *NFKB1* SNP, GG**				
–	–	114/199	reference		
–	+	52/92	0.75 (0.46 – 1.20)		
+	–	62/69	1.92 (1.21 – 3.05) **		
+	+	34/21	3.27 (1.74 – 6.13) ***	1.60 (−0.44 – 3.64)	0.48 (0.10 – 0.87) *
Exposure to plant/tree insect control products	**Presence of *HLA-DRB1*15***				
–	–	147/360	reference		
–	+	110/115	2.36 (1.65 – 3.36) ***		
+	–	25/22	4.06 (2.08 – 7.93) **		
+	+	19/13	5.33 (2.36 −12.02) ***	-0.09 (−5.03 – 4.84)	-0.02 (−0.95 – 0.92)
Exposure to plant/tree insect control products	**Absence of *HLA-A*02***				
–	–	94/159	reference		
–	+	130/212	1.02 (0.71 – 1.47)		
+	–	12/11	2.22 (0.83 – 5.98)		
+	+	26/13	5.08(2.36 – 10.94) ***	2.82 (−1.41 – 7.06)	0.56 (0.03 – 1.08)

ref: reference group; RERI: Relative Excess Risk due to Interaction; AP: Attributable Proportion of disease; SI: Synergic Index; CI: Confidence Intervals

* : P-value <0.05

** : P-value <0.01

*** : P-value <0.0

Table 7.

Models assessing multiplicative interactions for odds of POMS

Model	Interaction term	Exposure to toxic chemical, OR (95% CI)	Secondary risk factors (*DRB1*15* +, A*2 -, *IL-6* GG+, *BCL2* GG+, *NFKB1* GG+), OR (95% CI)	Interaction, OR (95% CI)
1	Exposure to Insect repellent for ticks or mosquitos X *DRB1*15*	1.69 (1.122.53)*	2.92 (1.695.03)***	0.59(0.20 – 1.18)
2	Exposure to Insect repellent for ticks or mosquitos X *A*02*	1.50 (0.86–2.61)	1.31 (0.76–2.24)	0.93(0.46–1.88)
3	Exposure to weed control products X *DRB1*15*	2.42(1.56–3.76)	2.62(1.74–3.93)	0.75(0.36–1.54)
4	Exposure to weed control products X *A*02*	1.80 (0.98–3.30)	0.85 (0.65–1.49)	1.48(0.69–3.17)
5	Exposure to weed control products X *IL-6* GG +	2.16 (0.60–6.74)	0.77 (0.42–1.42)	1.09(0.33–3.65)
6	Exposure to weed control products X *BCL2* GG +	3.46(1.78–6.71)	1.29 (0.82–2.02)	0.52(0.23–1.16)
7	Exposure to weed control products X *NFKB1* GG +	1.86(1.18–2.93)**	0.77 (0.48–1.21)	2.30(1.00–5.30)*
8	Exposure to plant/tree insect or disease control products X *DRB1*15*	3.93(2.01–7.71)	2.33 (1.63–3.32)	0.55(0.18–1.60)
9	Exposure to plant/tree insect or disease control products X *A*02*	1.03 (0.72–1.47)	2.10 (0.81–5.42)	2.27(0.67–7.67)

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

* p-Value

Table 8.

Models assessing multiplicative interactions for odds of POMS (sensitivity analysis: using inverse probability weighting)

Model	Interaction term	Exposure to toxic chemical, OR (95% CI)	Secondary risk factors (*DRB1*15* +, A*2 -, *IL-6* GG+, *BCL2* GG+, *NFKB1* GG+), OR (95% CI)	Interaction, OR (95% CI)
1	Exposure to Insect repellent for ticks or mosquitos X *DRB1*15*	1.75 (1.15–2.65) *	2.94 (1.69–5.11)***	0.60 (0.30–1.22)
2	Exposure to Insect repellent for ticks or mosquitos X *A*02*	1.48 (0.84–2.62)	1.24 (0.71–2.17)	1.02 (0.50–2.09)
3	Exposure to weed control products X *DRB1*15*	2.50 (1.61–3.88)***	2.67 (1.77–4.02)***	0.72 (0.35–1.49)
4	Exposure to weed control products X *A*02*	1.76 (0.97–3.21)	0.96 (0.63–1.47)	1.58 (0.74–3.36)
5	Exposure to weed control products X *IL-6* GG +	2.47 (0.73–8.30)	0.76 (0.41–1.39)	0.97 (0.27–3.46)
6	Exposure to weed control products X *BCL2* GG +	3.40 (1.75–6.62)***	1.24 (0.78–1.97)	0.56 (0.25–1.24)
7	Exposure to weed control products X *NFKB1* GG +	1.92 (1.21–3.05)	0.75 (0.46–1.20)	2.26 (0.99–5.17)
8	Exposure to plant/tree insect or disease control products X *DRB1*15*	4.06 (2.08–7.93)***	2.36 (1.65–3.36)***	0.55 (0.19–1.60)
9	Exposure to plant/tree insect or disease control products X *A*02*	1.02 (0.71–1.47)	2.22 (0.83–5.98)	2.21 (0.64–7.65)

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

* p-Value