

# Pregnancy does not modify the risk of MS in genetically susceptible women

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## Abstract

### Objective

To use the case-only gene-environment ( $G \times E$ ) interaction study design to estimate interaction between pregnancy before onset of MS symptoms and established genetic risk factors for MS among White adult females.

### Methods

We studied 2,497 female MS cases from 4 cohorts in the United States, Sweden, and Norway with clinical, reproductive, and genetic data. Pregnancy exposure was defined in 2 ways: (1)  $\geq 1$  live birth pregnancy before onset of MS symptoms and (2) parity before onset of MS symptoms. We estimated interaction between pregnancy exposure and established genetic risk variants, including a weighted genetic risk score and both HLA and non-HLA variants, using logistic regression and proportional odds regression within each cohort. Within-cohort associations were combined using inverse variance meta-analyses with random effects. The case-only  $G \times E$  independence assumption was tested in 7,067 individuals without MS.

### Results

Evidence for interaction between pregnancy exposure and established genetic risk variants, including the strongly associated *HLA-DRB1\*15:01* allele and a weighted genetic risk score, was not observed. Results from sensitivity analyses were consistent with observed results.

### Conclusion

Our findings indicate that pregnancy before symptom onset does not modify the risk of MS in genetically susceptible White females.

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## Glossary

**EIMS** = Epidemiologic Investigation of Multiple Sclerosis; **G × E** = gene-environment; **GEMS** = Genes and Environment in Multiple Sclerosis; **GERA** = Genetic Epidemiology Research on Adult Health and Aging; **GWAS** = genome-wide association study; **KPNC** = Kaiser Permanente Northern California; **MAF** = minor allele frequency; **NOR** = Norwegian MS Registry and Biobank; **PCA** = principal component analysis; **wGRS** = weighted genetic risk score.

MS is a demyelinating autoimmune disease of the CNS with both environmental and genetic risk factors.<sup>1</sup> This progressive disease results in significant disability and decreased quality of life.<sup>2</sup> MS is more prevalent among females than males, and symptoms typically emerge during child-bearing ages, often soon after pregnancy.<sup>2,3</sup> This has led many to hypothesize that female-specific exposures, such as those related to reproduction, pregnancy, and lactation, have a role in MS. Pregnancy appears to have short-term beneficial effects on existing MS symptoms,<sup>4</sup> but there is no agreement in the scientific literature about the effect of pregnancy on MS risk in general or among women with genetic susceptibility to MS.<sup>1,5</sup>

Gene-environment (G × E) interactions, for which the effect of an environmental exposure is modified by specific genotype(s), are believed to contribute substantially to complex disease risk, and discovery of these interactions can identify subgroups with higher risk of disease.<sup>6</sup> Studies of pregnancy and MS risk have yielded conflicting results; however, no studies, to date, have investigated interaction between pregnancy and genetic susceptibility for risk of MS.<sup>7–16</sup>

We used case-only G × E methods to evaluate interaction between pregnancy before symptom onset and known genetic risk factors for MS, including *HLA-DRB1\*15:01*. Study participants included 2,497 White individuals from 4 established MS cohorts based in California, Sweden, and Norway. Analyses were conducted separately within each cohort and then combined with meta-analytic methods. A separate cohort of females without MS was used to test the case-only G × E independence assumption.

## Methods

### Study participants

Participants were selected from the Kaiser Permanente Northern California (KPNC) MS Research Program, the Kaiser Permanente Southern California MS Sunshine Study, the Norwegian MS Registry and Biobank (NOR), and 2 Swedish MS studies, the Epidemiological Investigation of Multiple Sclerosis (EIMS) and the Genes and Environment in Multiple Sclerosis (GEMS) study.<sup>17–20</sup> MS diagnoses were confirmed by independent neurologists according to the 2005 revised (KPNC, NOR, and EIMS/GEMS) or the 2010 revised McDonald diagnostic criteria (MS Sunshine).<sup>21,22</sup> Additional non-MS female members of KPNC were also studied; these individuals were derived from the Genetic Epidemiology Research on Adult Health and Aging (GERA) study.<sup>23</sup>

KPNC and KPSC are integrated health services systems and are the largest health care providers in California. Their membership is largely representative of their catchment area populations; however, persons of lower socioeconomic status are underrepresented.<sup>24,25</sup>

### Standard protocol approvals, registrations, and patient consents

All study participants provided written informed consent, and the Institutional Review Boards of KPNC and KPSC, regional ethical review boards in Norway and Sweden, and the University of California, Berkeley, approved the study protocols.

### KPNC participants

Study recruitment is described in detail elsewhere.<sup>17</sup> Briefly, study participants were recruited from KPNC membership between 2006 and 2014. Prevalent MS cases were the focus of recruitment. Participants were aged between 18 and 69 years and were KPNC members at initial contact.

### MS Sunshine participants

MS Sunshine is a multiethnic case-control study of incident MS and first demyelinating event.<sup>18</sup> Participants in this study were recruited from KPSC membership between 2011 and 2014. At the time of initial contact, participants were KPSC members, aged 18 years or older, and diagnosed with MS within 1.5 years or had MS symptom onset within the 3 years before recruitment.

### NOR participants

NOR participants were recruited from the Oslo MS Registry and DNA biobank in 2011–2012.<sup>20</sup> The Oslo MS Registry and biobank was established in 1990 and includes clinical data and DNA samples from a population-based MS cohort.

### EIMS/GEMS participants

EIMS and GEMS are Swedish population-based case-control studies. At enrollment, EIMS participants were aged 18–70 years and had recently (within 2 years) confirmed MS. GEMS participants were identified from the Swedish National MS registry and recruited between 2009 and 2011. All EIMS participants were distinct from the GEMS study. Details have been described elsewhere.<sup>19</sup>

### GERA participants (noncases)

GERA participants are a subsample of the Research Program on Genes, Environment, and Health longitudinal cohort.<sup>23</sup> Participants were respondents to a mailed health survey that was sent to all adult members of KPNC in 2007 who had been

**Table 1** Characteristics of multiple sclerosis cases (N = 2,497)

	KPNC	MS Sunshine	NOR	EIMS/GEMS
<b>n</b>	814	151	119	1,413
<b>Age at onset, mean (SD)</b>	33.1 (9.1)	39.9 (11.5)	33.4 (9.4)	34.4 (10.1)
<b>Year of onset, median (IQR)</b>	1991 (1983, 1997)	2011 (2009, 2013)	1992 (1986, 1997)	2005 (2001, 2008)
<b>Age first pregnant,<sup>a</sup> mean (SD)</b>	24.6 (5.3)	25.9 (5.8)	27.1 (6.0)	27.2 (5.1)
<b>≥1 pregnancy<sup>a</sup> before symptom onset, n (%)</b>	428 (51.7)	95 (60.1)	69 (58.0)	668 (47.3)
<b>No. pregnancies<sup>a</sup> before symptom onset, n (%)</b>				
<b>0</b>	400 (48.3)	63 (39.9)	50 (42.0)	745 (52.7)
<b>1</b>	146 (17.6)	26 (16.5)	25 (21.0)	180 (12.7)
<b>2</b>	198 (23.9)	41 (25.9)	30 (25.2)	350 (24.8)
<b>3</b>	67 (8.1)	21 (13.3)	14 (11.8)	106 (7.5)
<b>≥4</b>	17 (2.1)	7 (4.4)	0 (0.0)	32 (2.3)
<b>HLA-DRB1*15:01 carrier, n (%)</b>	446 (53.9)	74 (46.8)	71 (59.7)	789 (55.8)
<b>wGRS<sup>b</sup> median [IQR]</b>	23.3 (22.7, 23.9)	23.2 (22.7, 23.8)	18.0 (17.5, 18.6)	22.6 (22.0, 23.3)

Abbreviations: EIMS = Environment in Multiple Sclerosis; GEMS = Genes Environment in Multiple Sclerosis; HLA = human leukocyte antigen; IQR = interquartile range; KPNC = Kaiser Permanente Northern California; NOR = Norway; wGRS = weighted genetic risk score.

<sup>a</sup> Pregnancy defined as pregnancy resulting in live birth.

<sup>b</sup> wGRS calculated from 182 MS risk loci for KPNC, MS Sunshine, EIMS/GEMS, and 144 MS risk loci for NOR cases.

members of KPNC for at least 2 years and were aged 18 years or older. After providing informed consent, participants were asked to submit a saliva sample for DNA genotyping. Additional phenotypic and health condition data were obtained from *International Classification of Diseases, Ninth Revision* codes from KPNC electronic health record data. Participants included in this study were confirmed to not have MS or other autoimmune disease.

### Ancestry determination

KPNC, MS Sunshine, and GERA participants with average European ancestry proportions greater than 80% estimated from ancestry informative markers who did not self-report Hispanic ethnicity were included.<sup>26</sup> ALL NOR and EIMS/GEMS participants had self-reported White ancestry. Population outliers were identified with principal components analysis (PCA) and excluded.<sup>27</sup>

### Genotype and exposure assessment

All participants in this study completed an interview or self-reported questionnaires related to MS disease events, reproductive history, and environmental exposures.<sup>17–20</sup> Participants provided blood or saliva samples for genotyping. Genome-wide single nucleotide polymorphism (SNP) genotyping was performed using the Illumina Infinium 660K BeadChip Array and Human Omni Express Array (KPNC, MS Sunshine), Illumina ImmunoArray BeadChip Array (NOR, EIMS/GEMS), and Affymetrix Axiom Array (GERA). Genome-wide SNP imputation was performed using SHAPEIT2 and IMPUTE2.<sup>28</sup> HLA alleles were imputed using

SNP2HLA.<sup>29</sup> Participants with missing genotypes that met QC thresholds (info score >0.8, missingness per SNP <0.05, missingness per cohort <0.05, and minor allele frequency [MAF] >0.05) were imputed using the average MAF within each cohort.

Pregnancy exposure was evaluated as dichotomous variable, having at least 1 live birth pregnancy before reported age at first MS symptom onset, and as an ordinal variable, the number of live birth pregnancies before reported age at first MS symptom onset (table 1). Age of pregnancy was defined as mother's age at birth, and age at symptom onset for MS cases was determined through review of medical records and/or comprehensive clinical histories for each participant collected through interview or questionnaire.

### Candidate genes and weighted genetic risk score

To maximize power and to identify variants with functional relevance to MS, we conducted a 2-tiered analysis. In the primary analysis, we assessed G × E interaction between pregnancy exposure and (1) a weighted genetic risk score (wGRS) comprised of recently established non-HLA MS genome-wide association study (GWAS) risk variants, (2) *HLA-DRB1\*15:01* alleles, and (3) *HLA-A\*02:01* alleles.<sup>30</sup> We also evaluated evidence for effect modification of the interaction between *HLA-A\*02:01* and *HLA-DRB1\*15:01* for MS susceptibility by pregnancy exposure.<sup>31</sup> In the secondary discovery analysis, we individually tested established MS-associated variants (HLA: 2 variants) and (non-HLA: 144 variants) that passed QC criteria in all 4 cohorts. Individual

genotypes were modeled assuming a linear effect of each additional risk allele (0, 1, or 2 risk alleles).

The wGRS was derived by multiplying the log OR for each risk allele from recent GWAS by the number of risk alleles carried by each participant and summing across GWAS variants (table e-1, [links.lww.com/NXI/A320](https://links.lww.com/NXI/A320)).<sup>30</sup> Scores for each individual were calculated using non-HLA risk variants that passed genotype QC thresholds within each cohort (KPNC, MS Sunshine, and EIMS/GEIMS: 182 non-HLA risk variants, NOR: 144 non-HLA risk variants, and GERA: 161 non-HLA risk variants). Within each cohort, the wGRS was modeled as a continuous variable and with categorical quartiles (reference: 1st quartile).

## Statistical analysis

Genome-wide SNP genotyping data were available for 990 KPNC, 409 MS Sunshine, 153 NOR, and 1,462 EIMS/GEMS female MS cases. Following exclusion of MS cases with European ancestry proportions <80% (KPNC and MS Sunshine) and population outliers identified from PCA analysis (NOR and EIMS/GEMS), 814 KPNC, 151 MS Sunshine, 119 NOR, and 1,413 EIMS/GEMS cases had age at onset  $\geq 18$  years, complete pregnancy history data, and genome-wide genetic profiles. The final data set for G  $\times$  E analysis included 2,497 MS cases (table 1, figure e-1, [links.lww.com/NXI/A319](https://links.lww.com/NXI/A319)).

Case-only G  $\times$  E models rely on the assumption that the genotype and environmental exposure are uncorrelated in the source population. If that assumption is valid, the association between genotype and exposure in a case-only model estimates the departure of the joint of effects on the OR scale. If the disease is rare and the above assumption is valid, the case-only model estimates the departure of joint effects on the risk ratio scale.<sup>32</sup> We used logistic regression to model having at least 1 live birth pregnancy before symptom onset as a function of genotypes or wGRS:

$$\text{logit}[P(E = 1|G)] = \beta_0 + \beta G$$

$E$  is an indicator for having at least 1 live birth pregnancy before symptom onset or not,  $G$  is 0, 1, or 2 alleles for a risk variant or continuous wGRS, and  $\beta$  is the estimate of the interaction parameter measuring the departure of the joint effects of  $E$  and  $G$  on the multiplicative scale.<sup>32</sup>

Proportional odds regression was used to model parity before symptom onset,<sup>32</sup> where the probability of an equal or less than  $k$  number of pregnancies before symptom onset,  $E \leq k$ , to the probability of more than  $k$  number of pregnancies,  $E > k$ , as a function of genotypes or wGRS:

$$\log \frac{P(E \leq k|G)}{P(E > k|G)} = \gamma_k - \gamma G, \text{ for } k \in 0, 1, 2, 3, 4$$

$E$  is the number of live birth pregnancies before MS symptom onset,  $G$  is 0, 1, or 2 alleles for a risk variant or continuous

wGRS,  $k$  is the threshold for live birth pregnancies before symptom onset for each ordinal comparison, and  $\gamma$  is the estimate of the interaction parameter measuring the departure of the joint effects of  $E$  and  $G$  on the multiplicative scale.<sup>32</sup>

G  $\times$  E interaction was estimated within each study, and combined estimates of G  $\times$  E interaction were obtained with random-effects meta-analysis using restricted maximum likelihood estimation with weights proportional to the inverse of the variance for each cohort-specific association.  $I^2\%$  was used to study assess heterogeneity between within-study association. All models were adjusted for age at MS onset and population stratification using components from PCA. wGRS quartiles were modeled using dummy variables with the first quartile as the reference category. Secondary discovery analysis  $p$  values were adjusted for the false discovery rate using the Benjamini-Hochberg method, and adjusted  $p$  values <0.05 were considered significant.<sup>33</sup>

Effect modification of the interaction between *HLA-A\*02:01* and *HLA-DRB1\*15:01* by pregnancy exposure was evaluated separately by stratifying case-only regression models for pregnancy exposure and *HLA-DRB1\*15:01* by the absence and presence of *HLA-A\*02:01* alleles adjusting for age at MS onset and population stratification.

We tested the assumption of G  $\times$  E independence between genotype and pregnancy using healthy female GERA participants ( $N = 7,067$ ).<sup>6</sup> Logistic and proportional odds regression models described above were used to test for associations between MS genetic risk factors, including wGRS and *HLA-DRB1\*15:01*, and having  $\geq 1$  pregnancy or not and parity. All statistical analyses were conducted using Plink 1.9 and R 3.5.1.

## Sensitivity analyses

We estimated G  $\times$  E association between live or non-live birth pregnancy before symptom onset (binary and ordinal models) and genetic variants among KPNC and MS Sunshine cases (data not available for NOR and EIMS/GEMS) to identify bias from exclusion of non-live birth pregnancies in primary analyses. To determine whether there was a short-term effect of pregnancy on MS risk, we altered the definition of pregnancy exposure to only consider first pregnancies that occurred within 5 years before age at onset as exposed. Next, to rule out reverse causality from MS disease latency or recall bias, we considered age at symptom onset at 5 years before reported age at onset. To check for bias from differing numbers of variants used in wGRSs between cohorts, G  $\times$  E interactions for wGRSs derived from risk variants only found in NOR data (144 variants, table e-1, [links.lww.com/NXI/A320](https://links.lww.com/NXI/A320)) were estimated for each cohort and combined with meta-analysis.

## Data availability

Anonymized data used in this study from KPNC, MS Sunshine, NOR, and EIMS/GEMS will be shared upon request by

any qualified investigator pending Institutional Review Board approval at each site. GERA data are publicly available on dbGaP (phs000674.v2.p2).

## Results

Characteristics of MS cases are summarized in table 1. Age at MS onset among the cohorts that recruited predominantly prevalent cases (KPNC, NOR, and EIMS/GEMS) was similar (mean = 33.1, 33.4, and 34.4 years, respectively), but occurred later among the cohort that recruited incident cases

only (MS Sunshine, mean = 39.9 years). Year of symptom onset occurred earlier among KPNC and NOR (median = 1992) than EIMS/GEMS (median = 2005) and MS Sunshine (median = 2011). The average age at first pregnancy (live birth) ranged between 24.6 and 27.2 years, with KPNC and MS Sunshine participant pregnancies occurring earlier than NOR and EIMS/GEMS. More than half of cases were *HLA-DRB1\*15:01* carriers; NOR and EIMS/GEMS had a higher proportion of carriers (60% and 56%, respectively) than KPNC and MS Sunshine (54% and 47%, respectively). The median wGRS was similar across KPNC, MS Sunshine, and EIMS/GEMS. NOR cases had lower scores than the others, which is likely because fewer SNPs were available to calculate the NOR wGRS. Approximately half (49.8%) of the cases had a live birth pregnancy before onset of MS symptoms. Parous cases were most likely to have 2 live births before symptom onset.

**Table 2** Results from case-only meta-analyses estimating multiplicative interaction between *HLA-DRB1\*15:01*, *HLA-A\*02:01*, and weighted genetic risk scores with pregnancy before symptom onset

Variant	Crude		Adjusted	
	OR <sup>a</sup>	95% CI	OR <sup>a</sup>	95% CI
<b>≥1 live birth before symptom onset<sup>b</sup></b>				
<i>HLA-DRB1*15:01</i>	0.93	0.82, 1.07	0.98 <sup>e</sup>	0.77, 1.25
<i>HLA-A*02:01</i>	1.06	0.93, 1.21	0.93	0.79, 1.09
wGRS <sup>c</sup>	0.95	0.87, 1.04	1.04 <sup>e</sup>	0.89, 1.21
wGRS Q1 <sup>d</sup>	Ref		Ref	
wGRS Q2	1.07	0.86, 1.34	1.15	0.88, 1.50
wGRS Q3	1.07	0.85, 1.33	1.25	0.95, 1.64
wGRS Q4	0.90	0.72, 1.12	1.11	0.84, 1.45
<b>Parity before symptom onset<sup>b</sup></b>				
<i>HLA-DRB1*15:01</i>	0.96	0.85, 1.07	1.01 <sup>e</sup>	0.84, 1.21
<i>HLA-A*02:01</i>	1.04	0.93, 1.18	0.92	0.81, 1.05
wGRS <sup>c</sup>	0.97	0.89, 1.05	1.05	0.96, 1.15
wGRS Q1 <sup>d</sup>	Ref		Ref	
wGRS Q2	1.09	0.88, 1.34	1.18	0.94, 1.48
wGRS Q3	1.12	0.91, 1.37	1.29	1.00, 1.66
wGRS Q4	0.90	0.73, 1.11	1.06	0.84, 1.33

Abbreviations: HLA = human leukocyte antigen; OR = gene-environment interaction odds ratio; wGRS = weighted genetic risk score.

<sup>a</sup> ORs estimate the departure of the multiplicative joint effects of pregnancy and risk variants on the risk ratio scale for susceptibility to MS. Cohort-specific associations were combined with inverse variance meta-analysis with random effects.

<sup>b</sup> ≥1 pregnancy before symptom onset cohort-specific ORs and 95% CIs estimated with logistic regression models. Parity before symptom onset cohort-specific ORs and 95% CIs estimated with proportional odds regression models. Adjusted models included age at MS onset and principal components for genetic ancestry.

<sup>c</sup> wGRS was modeled as a continuous variable. For KPNC, MS Sunshine, and EIMS/GEMS cases, wGRS calculated from 182 non-HLA MS risk variants and for NOR wGRS calculated from 144 non-HLA risk variants.

<sup>d</sup> wGRS modeled with categorical quartiles with the first quartile used as the reference category.

<sup>e</sup> 25% ≤ *I*<sup>2</sup> < 50%.

In this study, the ORs from case-only regression models estimate the departure of the multiplicative joint effects of E and G on the risk ratio scale for susceptibility to MS. We did not find evidence for G × E interaction between pregnancy exposure and primary genetic risk factors (wGRS, *HLA-DRB1\*15:01*, and *HLA-A\*02:01*) (table 2, figure e-2, links.lww.com/NXI/A319). Point estimates were close to the null or had CIs that contained the null. Estimates were similar for both pregnancy exposures. Significant evidence for effect modification of interaction between *HLA-DRB1\*15:01* and pregnancy exposure by carriage of *HLA-A\*02:01* alleles was not observed, although estimates indicate possible protective effects (presence *HLA\*02:01*: OR: 0.89, 95% CI 0.70, 1.13; absence *HLA\*02:01*: OR: 1.09, 95% CI 0.71, 1.66) (table 3). No variants tested in the secondary discovery analysis had multiple testing adjusted *p* values <0.05 (tables e-2 and e-3, links.lww.com/NXI/A320).

Among GERA controls, the wGRS and *HLA-DRB1\*15:01* were not associated with having at least 1 pregnancy or with parity (table 4), and none of the MS GWAS loci were significantly associated with pregnancy in GERA after correcting for multiple testing (results not shown). Results from sensitivity analyses investigating live or non-birth pregnancy, pregnancy within 5 years of MS onset, and bias from latent disease were consistent with observed results (table 5). Estimates for interaction between pregnancy exposures and the wGRSs derived from variants present in NOR data were similar to observed results (table e-2 and e-3, links.lww.com/NXI/A320).

## Discussion

We hypothesized that pregnancy before MS symptom onset modifies the risk of MS in genetically susceptible females. Using data from 4 study populations, we did not find evidence to support G × E interaction between established genetic risk factors for MS and exposure to pregnancy before symptom

**Table 3** Estimates of multiplicative interaction between *HLA-DRB1\*15:01* and pregnancy exposure stratified by the presence and absence of *HLA-A\*02:01* alleles

	<i>HLA-A*02:01</i> alleles	Crude		Adjusted	
		OR <sup>a</sup>	95% CI	OR <sup>a</sup>	95% CI
<b>≥1 live birth before symptom onset<sup>b</sup></b>					
<i>HLA-DRB1*15:01</i>	+	0.85	0.70, 1.03	0.89	0.70, 1.13
	-	1.08 <sup>d</sup>	0.78, 1.48	1.09 <sup>d</sup>	0.71, 1.66
<b>Parity before symptom onset<sup>b</sup></b>					
<i>HLA-DRB1*15:01</i>	+	0.91	0.76, 1.09	0.97	0.80, 1.18
	-	1.01 <sup>c</sup>	0.83, 1.23	1.04 <sup>d</sup>	0.77, 1.41

Abbreviations: OR = multiplicative interaction odds ratio; HLA = human leukocyte antigen.

<sup>a</sup> ORs estimate the departure of the multiplicative joint effects of pregnancy exposure and *HLA-DRB1\*15:01* on the risk ratio scale for susceptibility to MS stratified by the absence and presence of *HLA-A\*02:01* alleles. Cohort-specific associations were combined with inverse variance meta-analysis with random effects.

<sup>b</sup> ≥1 pregnancy before symptom onset cohort-specific ORs and 95% CIs estimated with logistic regression models. Parity before symptom onset cohort-specific ORs and 95% CIs estimated with proportional odds regression models. Adjusted models included age at MS onset and principal components for genetic ancestry.

<sup>c</sup> 25% ≤ *I*<sup>2</sup> < 50%.

<sup>d</sup> 50% ≤ *I*<sup>2</sup> < 75%

onset. The *HLA-A\*02:01* ± stratified point estimates indicate a protective effect of *HLA-A\*02:01* alleles in the relationship between *HLA-DRB1\*15:01* and pregnancy exposure, but our study was not sufficiently powered to detect these associations. Evidence for interaction between pregnancy and non-HLA or HLA variants considered individually was also not observed.

Although we considered pregnancy as a single environmental exposure, pregnancy is a complex and heterogeneous combination of physiologic changes that result in weight gain, increases in lipid levels, and changes in basal metabolic rate, among others.<sup>5</sup> These physiologic changes are the product

of pregnancy-induced modifications in hormones, such as estradiol, progesterone, prolactin, early pregnancy growth factor, alpha-fetoprotein, and leptin as well as elevated levels of other growth factors. There are increases in circulating regulatory T cells and B cells, increased Th2 responses, and decreased Th-1 and Th-17 immune responses.<sup>5</sup> These immune changes are important for fetal tolerance, as the maternal immune system and endocrine pathways respond to fetal antigens that circulate in the mother.<sup>34</sup> Following pregnancy, hormone levels and immune adaptations quickly return to prepregnancy states.<sup>35</sup> The reduction of MS relapse rate during pregnancy is

**Table 4** Evidence for G × E independence among n = 7,067 healthy genetic epidemiology research on aging participants

	Crude			Adjusted		
	OR <sup>a</sup>	95% CI	<i>p</i> Value	OR <sup>a</sup>	95% CI	<i>p</i> Value
<b>≥1 live birth pregnancy or not<sup>b</sup></b>						
<i>HLA-DRB1*15:01</i>	0.93	0.81, 1.11	0.37	0.89	0.77, 1.04	0.15
wGRS <sup>c</sup>	1.02	0.93, 1.11	0.71	1.04	0.95, 1.13	0.41
<b>No. of live birth pregnancies<sup>b</sup></b>						
<i>HLA-DRB1*15:01</i>	0.95	0.86, 1.04	0.23	0.92	0.84, 1.01	0.08
wGRS <sup>c</sup>	0.95	0.90, 1.00	0.04	0.96	0.91, 1.01	0.14

Abbreviations: G × E = gene-environment; HLA = human leukocyte antigen; OR = gene-environment interaction odds ratio; wGRS = weighted genetic risk score. Results from crude and adjusted regression analyses for association between weighted genetic risk score and *HLA-DRB1\*15:01* and having at least 1 live birth pregnancy or not and number of live birth pregnancies.

Results from crude and adjusted regression analyses for association between weighted genetic risk score and *HLA-DRB1\*15:01* and having at least 1 live birth pregnancy or not and number of live birth pregnancies.

<sup>a</sup> ORs estimate the departure of the multiplicative joint effects of pregnancy exposure and wGRS/*HLA-DRB1\*15:01* on the risk ratio scale for susceptibility to MS among healthy female controls from Genetic Epidemiology Research on Aging participants.

<sup>b</sup> ≥1 pregnancy before symptom onset ORs and 95% CIs estimated with logistic regression models. Parity before symptom onset ORs and 95% CIs estimated with proportional odds regression models. Adjusted models included principal components for genetic ancestry.

<sup>c</sup> wGRS calculated 161 non-HLA risk variants in Genetic Epidemiology Research on Adult Health and Aging data after QC and modeled as a continuous variable.

**Table 5** Sensitivity analysis results for pregnancy defined as live or non-live birth pregnancy, first pregnancy within 5 years before symptom onset, and latent MS onset models

Variant/risk score	Model	≥1 live birth before symptom onset		Parity before symptom onset	
		OR <sup>a</sup>	95% CI	OR <sup>a</sup>	95% CI
<b>HLA-A*02:01</b>	Observed <sup>b</sup>	0.93	0.79, 1.09	0.92	0.81, 1.05
	Live or nonlive <sup>c</sup>	0.84 <sup>g</sup>	0.53, 1.34	0.93	0.76, 1.13
	Short term <sup>d</sup>	0.96	0.76, 1.21	0.84	0.69, 1.03
	Latent <sup>e</sup>	0.96	0.81, 1.14	0.98	0.85, 1.13
<b>HLA-DRB1*15:01</b>	Observed <sup>b</sup>	0.98 <sup>g</sup>	0.77, 1.25	1.01 <sup>g</sup>	0.84, 1.21
	Live or nonlive <sup>c</sup>	0.79	0.62, 1.00	0.85	0.71, 1.03
	Short term <sup>d</sup>	1.03 <sup>g</sup>	0.73, 1.45	0.97	0.78, 1.21
	Latent <sup>e</sup>	0.97	0.82, 1.14	1.00	0.87, 1.15
<b>wGRS<sup>f</sup></b>	Observed <sup>b</sup>	1.04 <sup>g</sup>	0.89, 1.21	1.05	0.96, 1.15
	Live or nonlive <sup>c</sup>	0.94	0.79, 1.11	1.06 <sup>g</sup>	0.84, 1.33
	Short term <sup>d</sup>	1.02	0.87, 1.19	1.07	0.94, 1.21
	Latent <sup>e</sup>	1.08 <sup>h</sup>	0.83, 1.41	1.05	0.96, 1.16

Abbreviations: OR = gene-environment interaction odds ratio; HLA = human leukocyte antigen; wGRS = weighted genetic risk score.  
<sup>a</sup> ORs estimate the departure of the multiplicative joint effects of pregnancy and risk variants on the risk ratio scale for susceptibility to MS. Cohort-specific associations were combined with inverse variance meta-analysis with random effects. ≥1 pregnancy before symptom onset cohort-specific ORs and 95% CIs estimated with logistic regression models. Parity before symptom onset cohort-specific ORs and 95% CI estimated with proportional odds regression models. Adjusted models included age at MS onset and principal components for genetic ancestry.  
<sup>b</sup> Estimates from observed data as presented in table 2.  
<sup>c</sup> Estimate of multiplicative interaction between pregnancy exposure defined as have at least 1 live or non-live birth before MS symptom onset. Data on non-live births only available for Kaiser Permanente and MS Sunshine cases, n = 968.  
<sup>d</sup> Estimate of multiplicative interaction between pregnancy exposure defined as have at least 1 live pregnancy and parity within 5 years before MS symptom onset.  
<sup>e</sup> Estimate of multiplicative interaction between pregnancy exposure with MS age at onset adjusted by -5 years.  
<sup>f</sup> wGRS modeled as a continuous variable.  
<sup>g</sup> 25% ≤  $I^2$  < 50%.  
<sup>h</sup> 50% ≤  $I^2$  < 75%.

attributed to the dynamic immune and endocrine alterations that result from maternal-fetal crosstalk during pregnancy.<sup>36</sup> Little is known about how physiologic changes during pregnancy affect the risk of developing MS, but it is hypothesized that the pregnancy-induced changes in endocrine pathways and immune system have protective effects.<sup>5</sup>

Epidemiologic studies investigating the effect of pregnancy on MS risk have reported conflicting results.<sup>1</sup> Five studies reported a protective association between parity and risk of MS; however, 2 of these studies attributed their protective associations to reverse causality from reduced fertility and increased likelihood of miscarriage among women with latent MS.<sup>7,8,10,11,15</sup> Five additional studies reported no association between pregnancy and risk of MS.<sup>9,12-14,16</sup> A recent study investigating breastfeeding, ovulatory years, and risk of MS found evidence that cumulative duration of breastfeeding is associated with a decreased risk of MS.<sup>37</sup> The authors suggest that breastfeeding duration confounds the association between parity/pregnancy and risk of MS and may explain previously reported conflicting findings.

Genes within the HLA complex contribute substantially to MS, with the *HLA-DRB1\*15:01* allele conferring the largest known genetic risk for disease.<sup>30</sup> Interactions between this allele and established environmental exposures such as tobacco smoking, Epstein-Barr Virus infection, and adolescent obesity have previously demonstrated large effect sizes.<sup>1</sup> A recent GWAS identified approximately 200 non-HLA genetic variants associated with MS risk.<sup>30</sup>

Previous research reported that protective associations between pregnancy and MS onset were attributable to reverse causality and that pregnancy was more likely among women with less severe disease or without latent disease.<sup>7,8</sup> However, a registry-based study in Norway found that pregnancies among women with MS before symptom onset have birthweights and outcomes similar to those in women without MS.<sup>38</sup> Furthermore, changes in counseling and availability of safer disease-modifying therapies and new diagnostic criteria that allow for earlier MS diagnosis have likely contributed to a recent reported increase in the rate of pregnancy among women with MS.<sup>39,40</sup> Results from our sensitivity analyses did not indicate evidence for bias from latent disease. Furthermore, restricting

analyses to cases reporting their first pregnancy within 5 years before symptom onset did not change our findings. Including data on non-live births in the pregnancy exposure did appear to move point estimates further from the null; however, data on non-live births were only available for KPNC and MS Sunshine cases and the CIs still contained the null.

Case-only  $G \times E$  methodologies were developed to address one of the largest challenges with studying interactions, statistical power.<sup>6</sup> Since they were first introduced, advances in case-only  $G \times E$  methodology have focused on combining the increased power from case-only methods with evidence for the all-important  $G \times E$  independence assumption from case-control data.<sup>6</sup> Healthy controls with reproductive history matched on case symptom onset and genetic data were not available for our large combined data set of MS cases; however, we used female participants without MS from GERA to formally test for evidence of independence between MS genetic risk factors and pregnancy. Results from this analysis support the validity of our findings. Our models were adjusted for age at MS onset and population stratification. Although there may be additional variables that confound the relationship between both pregnancy and MS and genetic risk factors and MS, case-only  $G \times E$  analyses only need to adjust for confounders of the  $G \times E$  relationship. We cannot rule out that our findings may be due to confounding from unknown factors.

The objective of this study was to investigate  $G \times E$  interaction between pregnancy and established MS genetic risk variants in MS. A primary strength of the current study was the large sample size with complete genetic and reproductive history data. In addition, cases used in this study were largely representative of their respective surrounding populations, providing support for external validity, and identification of cases from integrated health services delivery systems and national registries reduced the probability of selection bias. Furthermore, we found evidence to support the independence of  $G$  and  $E$  factors among the KPNC source population. Results from sensitivity analyses suggest that our findings are not attributable to reverse causality; however, given the uncertainty regarding disease latency and first MS symptoms, we cannot conclusively rule out reverse causality. Our study was subject to some limitations. Because of the absence of suitable controls, we were unable to evaluate the association between pregnancy and MS. Our  $G \times E$  associations were consistent between studies; however, differences between the study populations may have biased our findings. With the exception of MS Sunshine, the other studies relied on older MS diagnostic criteria, which may have excluded milder MS cases. Because of the case-only study design, we were not able to assess interaction on the additive scale and our null findings on the multiplicative scale cannot rule out the presence of interaction on the additive scale. In addition, we were not able to assess  $G$  and  $E$  independence in non-KPNC source populations. Future studies should investigate the effects of pregnancy on MS risk among non-European populations. The current study

was focused on White individuals to achieve the statistical power required for analyses. It is possible that interaction between MS genetic risk factors and pregnancy may differ by ethnic/ancestral group. If controls with reproductive data matched to case age at symptom onset are available,  $G \times E$  interaction should be assessed among cases and controls with methods used to combine case-only and case-control interactions.

Our findings suggest that genetic susceptibility to MS does not modify the association between pregnancy and MS. This information may be useful for counseling women who have genetic susceptibility to MS about decisions to pursue pregnancy, although further research is needed to determine the effect of pregnancy on MS susceptibility.

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### Disclosure

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<b>Sean Wu, MPH</b>	University of California, Berkeley, Berkeley, CA	Data analysis



## Appendix (continued)

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<b>Catherine Schaefer, PhD</b>	Kaiser Permanente Northern California, Oakland, CA	Data collection
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