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Maternal genetic markers for risk of celiac disease and their potential association with neural tube defects in offspring

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Abstract

Background: We examined the association between the maternal genotype for celiac disease-associated variants and risk of neural tube defects (NTDs).

Methods: We conducted a case–control study, using data from the National Birth Defects Prevention Study. We evaluated 667 cases (women with an offspring with NTD) and 743 controls (women with an offspring without a birth defect). We classified women as having low, intermediate, or high risk of celiac disease based on human leukocyte antigen (HLA) variants. We used logistic regression to assess the relationship between HLA celiac risk group (low, intermediate, high) and risk of NTDs. Fifteen non-HLA variants (identified from genome-wide association studies of celiac disease) were individually evaluated and modeled additively.

Results: There was no association between HLA celiac risk group and NTDs (intermediate vs. low risk: aOR, 1.0; 95% CI, 0.8–1.3; high vs. low risk: aOR, 0.8; 95% CI, 0.5–1.3). Of the fifteen non-HLA variants, we observed five significant associations after accounting for multiple comparisons. Three negative associations were observed with rs10903122, rs13314993, rs13151961 (aOR range: 0.69–0.81), and two

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positive associations were observed with rs13003464 and rs11221332 (aOR range: 1.27–1.73).

Conclusion: If confirmed, our results suggest that the maternal variants related to celiac disease may be involved in the risk of NTDs.

KEYWORDS

candidate genes, celiac disease, human leukocyte antigen, neural tube defects, pregnancy

1 | INTRODUCTION

Celiac disease is a chronic autoimmune condition triggered by the consumption of gluten and affects about 1% of the Caucasians and 0.2% of Mexican-Americans in the United States (Mardini, Westgate, & Grigorian, 2015). Among individuals with celiac disease, consumption of gluten can lead to inflammation in the small intestine and result in nutrient deficiencies because of nutrient malabsorption (Rostami, Steegers, Wong, Braat, & Steegers-Theunissen, 2001). Nutrient deficiencies among pregnant women can lead to adverse fetal outcomes. For example, use of folic acid supplementation periconceptionally among reproductive women has reduced the birth prevalence of neural tube defects (NTDs) from 7–11 per 10,000 to 5–7 per 10,000 (Williams et al., 2015). The treatment for celiac disease is adherence to a gluten-free diet, which also happens to include grain products that are fortified with folic acid in the U.S. (FDA, 1996). Thus, it is possible that women with celiac disease might have a higher risk of NTDs because of low folic acid absorption or intake. In addition, there is some evidence that suggests that immunological factors might be involved in the development of the neural tube (Denny et al., 2013). Therefore, an immune response to celiac disease may also disrupt the immunological factors that regulate neural tube development.

Only a few epidemiological cohort studies have investigated the relationship between maternal celiac disease and risk of NTDs and these individual studies are inconclusive (Ban et al., 2015; Tata et al., 2005; Zugna et al., 2014). All of these studies used medical records to obtain celiac disease status. However, in the United States, most individuals with celiac disease are undiagnosed (Choung et al., 2016); thus, there is a potential for misclassifying undiagnosed women with celiac disease as not having celiac disease.

Over 90% of individuals with celiac disease carry the human leukocyte antigen (HLA) type DQ2 or DQ8 haplotypes (OMIM #212,750) (Sollid et al., 1989). These haplotypes confer risk of developing celiac disease (especially in Caucasians) and can be identified using single nucleotide polymorphisms (SNPs) from HLA genes (Monsuur et al., 2008). In fact, the HLA-DQ haplotypes have an area under the receiver-operating curve of 82% for detecting celiac disease (Romanos et al., 2014). In addition, non-HLA SNPs associated with celiac disease have been identified from genome-wide association studies of celiac disease and were shown to improve the prediction of risk of celiac disease (Dubois et al., 2010; Romanos et al., 2014). Because self-reported celiac disease status is unreliable, using genetic variants may more accurately determine celiac disease status and may allow us to identify both diagnosed and undiagnosed women (i.e., those with subclinical inflammation, which may lead to undetected damage in the small intestine). In this study, we examined the association between maternal genetic risk of celiac disease and NTDs, using HLA and non-HLA genetic variants.

2 | MATERIALS AND METHODS

2.1 | Ethical compliance

The Institutional Review Boards at each of the ten centers that participated in the National Birth Defects Prevention Study (NBDPS) and at the Centers for Disease Control and Prevention approved the study.

2.2 | Study population

A population-based, case–control study was conducted, using data from the NBDPS. The study population has been previously described in detail (Cogswell et al., 2009; Reefhuis et al., 2015; Yoon et al., 2001). Briefly, the NBDPS is a large multi-center study of major structural birth defects in the United States. The NBDPS includes four statewide surveillance systems (Arkansas, Iowa, New Jersey, and Utah) and selected surveillance counties from six states (California, Georgia, North Carolina, Massachusetts, New York, and Texas). Clinical geneticists reviewed all clinical information from medical records to identify livebirths or stillbirths with a major structural birth defect. Potential cases identified with a genetic syndrome or chromosome abnormality were excluded. Controls were live births without a major birth defect. Mothers of cases and controls who verbally consented to the study during the interview were mailed collection kits for sampling cheek cells (Rasmussen et al., 2002; Reefhuis et al., 2015; Yoon et al., 2001). DNA was extracted from those who provided written consent and quality

control procedures were conducted, following the NBDPS protocol (Rasmussen et al., 2002).

This study was restricted to a subset of case mothers (hereafter referred as "cases") and control mothers (hereafter referred as "controls") who had an estimated date of delivery from 1 October 1997 to 31 December 2009 and had an available DNA sample. Cases were women who had offspring with NTDs (i.e., anencephaly, spina bifida, encephalocele). Women with self-reported pregestational or gestational diabetes were excluded.

2.3 | Data collection

Women completed a one-hour computer-assisted telephone interview (CATI) within six weeks to two years after the estimated date of delivery. The CATI could be completed in English or Spanish and included questions on maternal race/ethnicity, prepregnancy weight and height, education, age at delivery, and use of folic acid supplementation three months before pregnancy through the first month of pregnancy. Women were also asked to complete a 58-item food frequency questionnaire (FFQ) modified from the FFQ used in The Nurse's Health Study (Willett et al., 1985). The CATI did not include questions specific to celiac disease. Information on the sex of the offspring and the birth year was obtained from vital records.

2.4 | SNP selection

We included the six HLA SNPs in *HLA-DQA1* (OMIM: 146880; NM_002122.3) and *HLA-DQB1* (OMIM: 604305; NM_001243961.1) that define the HLA-DQ2.2 (HLA-DQA1*0201 and HLA-DQB1*0202), HLA-DQ2.5 (HLA-DQA1*0501 and HLA-DQB1*0201), HLA-DQ8 (HLA-DQA1*0301 and HLA-DQB1*0302), and HLA-DQ7 (HLA-DQA1*0505 and HLA-DQB1*0301 haplotypes (Figure 1; Monsuur et al., 2008). In addition, we included 17 of the most significant non-HLA SNPs identified from genome-wide association studies of celiac disease (Supplemental Table S1; Dubois et al., 2010; van Heel et al., 2007).

2.5 | Genotyping and quality control

PCR was conducted using the Qiagen multiplex PCR kit (Qiagen, Hilden, Germany). Two microliters of ExoSAP-IT™ PCR Product Cleanup Reagent (ThermoFisher Scientific, Waltham, MA) was added directly into the PCR product. The resulting mixture was incubated at 37°C for 45 min and 85°C for 15 min to cleanup leftover primers and dNTPs. A mini-sequencing reaction was performed using SNaPshot Multiplex Kit (ThermoFisher Scientific, Waltham, MA), following the SNaPshot Multiplex protocol. Genotypes from the SNaPshot assay were called using GeneMapper 4.0 (ThermoFisher Scientific, Waltham, MA). Because we were unable to genotype rs4713586 using SNaPshot, rs4713586 was genotyped by a custom TaqMan SNP assay (Cat# 4331349, Applied Biosystem), following manufacture's protocol. Briefly, 2.5 μl TaqMan Genotyping Master Mix, 0.125 μl 40X TaqMan probe, 1 μl DNA, and 1.5 μl nuclease free water were mixed for the PCR reaction. PCR was performed using a standard protocol (10 min at 95°C, 40 cycles for denaturation at 92° for 15 s and annealing/extension at 60°C for 90 s). Allelic discrimination was performed on a 7900HT machine, using the Sequence Detection System software.

Quality control procedures were applied to the genotyped data. We excluded SNPs with a minor allele frequency <5%

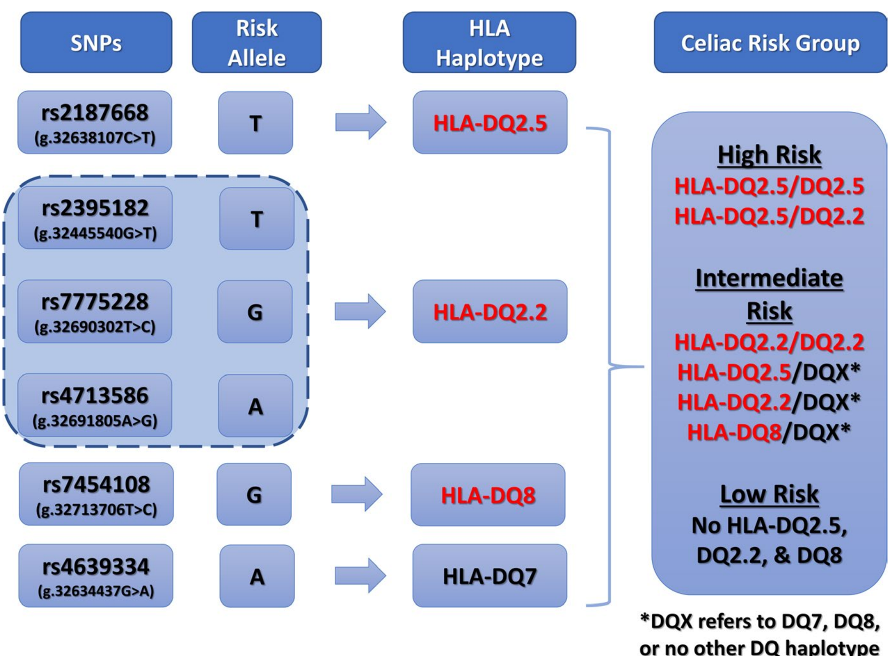

FIGURE 1 Celiac risk group schematic. This figure diagrams how the six single nucleotide polymorphisms were used to determine the human leukocyte antigen haplotype. Then, using the human leukocyte antigen haplotype, the women were classified into celiac risk groups. SNPs, single nucleotide polymorphisms; HLA-DQ, human leukocyte antigen DQ

and a genotyping call rate <90%, as well as samples with a genotyping call rate <80%. We evaluated departure from Hardy–Weinberg equilibrium (HWE) among controls. We considered each SNP to be in HWE if the p-value was >0.05. Because of the potential for population stratification, we evaluated HWE using data from all controls, as well as separately in non-Hispanic white (NHW) and Hispanic controls. SNPs that departed from HWE across all three groups (all, NHW, Hispanic) were excluded, and SNPs that departed in one or two groups were kept for group-specific analyses (discussed below). All quality control procedures were conducted using SNP and Variation Suite version 8.6.0 (Golden Helix Inc., Bozeman, MT).

2.6 | Study variables

Maternal prepregnancy body mass index was calculated in kg/m^2 from self-reported prepregnancy weight and height. Women were categorized into one of the four standard body mass index categories (underweight [<18.5 kg/m^2], normal [18.5 to <25 kg/m^2], overweight [25 to <30 kg/m^2], and obese [≥30 kg/m^2]) (Janssen, Katzmarzyk, & Ross, 2002). We measured diet quality using the Healthy Eating Index 2010 (HEI-2010). Specifically, data from the FFQ were converted to the USDA Food Patterns Equivalents Database 2011–2012 and a score was calculated for each of the twelve dietary components (e.g., refined grain, whole grain) (Guenther et al., 2013). The twelve scores were summed to calculate the HEI-2010 score (ranging from 0 to 100), with higher scores reflecting higher diet quality. Because consumption of gluten triggers the autoimmune response among people with celiac disease, we wanted to measure gluten intake. Because the FFQ did not collect information on intake of gluten-free products, we assumed that the products used to calculate the HEI-2010 components for refined (e.g., bran of all cereals, light rye) and whole grains (e.g., dark rye, oats) contained gluten and used the HEI-2010 estimates for refined and whole grains to calculate a proxy of gluten intake. The total ounces of refined and whole grain were converted to grams based on the Food Patterns Equivalents Database 2011–2012 (i.e., one ounce of refined grain was equivalent to 16 grams and one ounce of whole grain was equivalent to 28.35 grams). Grams of refined and whole grain were summed and adjusted for total energy intake to approximate gluten intake.

2.7 | Statistical analysis

We reported the count and frequency of maternal and infant characteristics for all cases and controls. We also reported the distribution of maternal and infant characteristics separately for NHW and Hispanic women.

2.7.1 | HLA analyses

HLA-DQ haplotypes were used to categorize women into celiac risk groups (low, intermediate, or high) (Figure 1; Monsuur et al., 2008; Romanos et al., 2014). For HLA-DQ2.2, we used SHAPEIT version 2.r644 to phase rs2395182, rs7775228, and rs4713586 (Delaneau, Marchini, & Zagury, 2011) and used the 1,000 Human Genomes Phase I integrated variant set as the reference (Genomes Project et al., 2015). Women missing genotype information for any HLA SNPs were excluded. We reported the median and interquartile range of gluten intake by celiac risk group among all cases and controls. To assess the association between maternal celiac risk group and NTDs, we used logistic regression, controlling for race/ethnicity.

2.7.2 | Non-HLA analyses

For each non-HLA SNP, the allele that was associated with increased risk of celiac disease in Dubois et al.'s study was considered the risk allele (Dubois et al., 2010). Each non-HLA SNP was modeled additively and assessed using logistic regression, adjusting for race/ethnicity. To account for multiple comparisons, we reported the false discovery rate p-value (Anderson, 2008; Benjamini & Hochberg, 1995).

In addition to evaluating each non-HLA SNP individually, we used the non-HLA SNPs to create an unweighted and weighted genetic risk score. The genetic risk score only included SNPs that were in HWE among all women. The unweighted genetic risk score was created by counting the number of risk alleles. For the weighted genetic risk score, we first calculated the beta coefficients by taking the natural logarithm of the odds ratio reported in Dubois et al. (2010). (For a protective association, the inverse of the odds ratio was determined before taking the natural logarithm.) For each study subject, a genetic risk score was calculated by summing the product of the beta coefficients and the number of risk alleles for each corresponding SNP. Therefore, SNPs with stronger magnitudes of association with celiac disease increased the score more than those with weaker magnitudes of association.

We repeated all HLA and non-HLA analyses separately for NHW and Hispanic women. For the non-HLA analyses, the SNP and genetic risk score analyses only included the non-HLA SNPs that were in HWE within each race/ethnicity. We repeated the genetic risk score analyses, adjusting for celiac risk group in the subset of women who were categorized into a celiac risk group. In addition, all HLA and non-HLA analyses were repeated for spina bifida and anencephaly.

Among all women, we assessed the potential for interaction between each exposure (celiac risk group or each non-HLA SNP) and gluten intake, as well as between each exposure and

use of folic acid supplementation. Each model included terms for the exposure, the third variable of interest (gluten intake, HEI-2010 score, use of folic acid supplementation), race/ethnicity, and an interaction term between the exposure and the third variable of interest. We compared a model with the interaction term to a model without the interaction term and evaluated the significance of the interaction using a likelihood ratio test. The interaction term was considered significant if the likelihood ratio test p-value was <0.05 when the exposure was celiac risk group. When the exposure was the non-HLA SNPs, significance of the interaction was assessed using a Bonferroni corrected p-value. All analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC).

3 | RESULTS

We genotyped 766 controls and 695 cases. We excluded 38 women with an overall call rate <80% (21 controls, 17 cases) and 13 women with self-reported pregestational diabetes (two controls, 11 cases) (Figure 2). The maternal and infant characteristics of all controls and cases and by race/ethnicity are reported in Table 1. Compared to all controls, all cases had a lower frequency of use of folic acid supplementation (28% vs. 32%), similar median HEI-2010 score (73 vs. 75), and similar gluten intake (26 vs. 25 g/1,000 kcal).

3.1 | HLA SNPs

Of the HLA variants, rs4639334 (g.32634437G > A) had a minor allele frequency <5% and rs7454108 (g.32713706T > C) appeared to depart from HWE in the full group and in the Hispanic subset (Supplemental Table S1). Because these SNPs tag the HLA-DQ7 and HLA-DQ8 haplotypes respectively, they were included in order to categorize women into HLA celiac risk groups. Among all women, gluten intake was similar across celiac risk group in cases and controls (Table 2).

After adjusting for maternal race/ethnicity, cases had lower odds of being in the HLA high celiac risk group versus the HLA low celiac risk group, but this difference was not significant (aOR, 0.78; 95% CI, 0.46, 1.32) (Table 3). The odds of being in the intermediate versus low celiac risk group was not statistically different between cases and controls (aOR, 1.03; 95% CI, 0.82, 1.29). Because rs7454108 (marker for HLA-DQ8) was out of HWE, we removed rs7454108 and recategorized women into celiac risk groups in a sensitivity analysis. The associations with NTDs were similar with and without rs7454108. We considered conducting a similar analysis for rs4639334 (HLA-DQ7 with minor allele frequency <5%), but excluding this SNP did not change any woman's celiac risk group. Similar results were observed in the analyses of the NHW and Hispanic subgroups and in the analyses of NTD subtypes (spina bifida and anencephaly). Among all women, no significant interaction was observed between celiac risk group and gluten intake, HEI-2010 score, or use of folic acid supplementation (data not shown).

3.2 | Non-HLA SNPs

Two of the non-HLA SNPs (rs2816316, rs10806425) were excluded because they departed from HWE in the full group, as well as in the NHW and Hispanic subgroups (Supplemental Table S1). The genotypic distribution of the 15 eligible non-HLA SNPs by NTD subtype is available in Supplemental Table S2.

After accounting for multiple comparisons and adjusting for maternal race/ethnicity, five non-HLA SNPs were associated with NTDs among all women. Three SNPs (rs10903122, rs13314993, rs13151961) were negatively associated with NTDs (range of aORs, 0.69–0.81), and two SNPs (rs13003464, rs11221332) were positively associated with NTDs (range of aORs, 1.27–1.73) (Table 4). When restricted to NHW women, only rs11221332 remained significantly associated with NTDs (aOR, 1.76). Among Hispanic women, the negative association with rs13151961 remained significant and rs13010713 became significant.

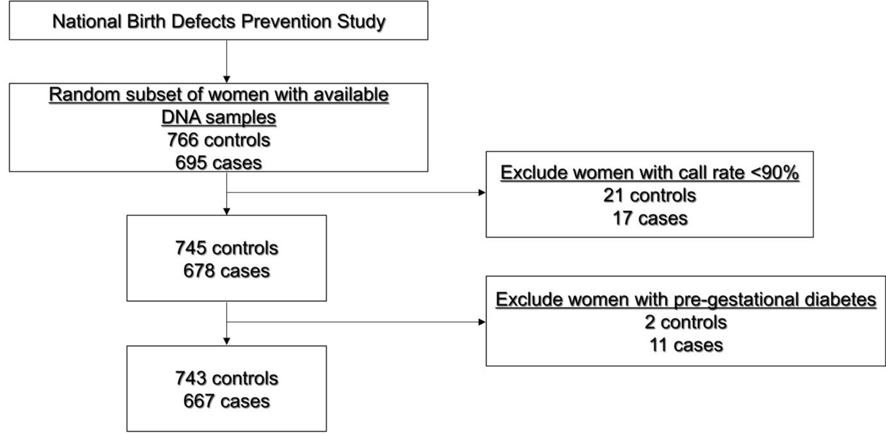

FIGURE 2 Flowchart of study population

TABLE 1 Maternal and infant characteristics of cases and controls, National Birth Defects Prevention Study, 1997–2009

	All		Non-Hispanic white		Hispanic	
	Case N = 667	Control N = 743	Case N = 381	Control N = 497	Case N = 211	Control N = 172
Maternal characteristics	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Maternal race/ethnicity						
Non-Hispanic white	381 (57.1)	497 (66.9)	—[a]	—[a]	—[a]	—[a]
Non-Hispanic black	40 (6.0)	42 (5.7)	—[a]	—[a]	—[a]	—[a]
Hispanic	211 (31.6)	172 (23.2)	—[a]	—[a]	—[a]	—[a]
Other	35 (5.3)	32 (4.3)	—[a]	—[a]	—[a]	—[a]
Body mass index (kg/m^2)						
Underweight	24 (3.8)	47 (6.6)	9 (2.4)	30 (6.1)	9 (5.0)	10 (6.9)
Normal	299 (47.2)	363 (51.1)	189 (49.9)	267 (54.1)	83 (46.4)	67 (46.5)
Overweight	167 (26.4)	162 (22.8)	99 (26.1)	102 (20.7)	49 (27.4)	39 (27.1)
Obese	143 (22.6)	138 (19.4)	82 (21.6)	95 (19.2)	38 (21.2)	28 (19.4)
Missing	34	33	2	3	32	28
Education (years)						
<12	124 (18.6)	110 (14.8)	22 (5.8)	25 (5.0)	85 (40.5)	75 (43.6)
12	171 (25.7)	157 (21.1)	84 (22.1)	86 (17.3)	69 (32.9)	48 (27.9)
13–15	192 (28.8)	225 (30.3)	118 (31.0)	166 (33.4)	48 (22.9)	34 (19.8)
>15	179 (26.9)	251 (33.8)	157 (41.2)	220 (44.3)	8 (3.8)	15 (8.7)
Missing	1	0	0	0	1	0
Age (years)						
<20	97 (14.5)	80 (10.8)	34 (8.9)	34 (6.8)	42 (19.9)	32 (18.6)
20–34	501 (75.1)	587 (79.0)	296 (77.7)	409 (82.3)	154 (73.0)	130 (75.6)
>=35	69 (10.3)	76 (10.2)	51 (13.4)	54 (10.9)	15 (7.1)	10 (5.8)
Folic supplementation						
Yes	185 (27.7)	234 (31.5)	160 (42.0)	202 (40.6)	16 (7.6)	23 (13.4)
No	482 (72.3)	509 (68.5)	221 (58.0)	295 (59.4)	195 (92.4)	149 (86.6)
HEI-2010 score[b,c]	73.4 (67.7–77.9)	74.9 (68.9–79.5)	73.6 (68.4–78.7)	75.1 (68.8–79.7)	73.4 (68.8–77.2)	75.7 (70.4–79.3)
Gluten (g/1,000 kcal)[b]	25.9 (19.4–36.7)	25.4 (19.0–33.7)	24.8 (18.7–33.9)	24.5 (18.5–32.7)	31.3 (22.4–43.1)	29.8 (22.1–37.9)
Infant characteristics						
Type of NTD						
Anencephaly	233 (34.9)	—[a]	122 (32.0)	—[a]	85 (40.3)	—[a]
Spina bifida	354 (53.1)	—[a]	218 (57.2)	—[a]	104 (49.3)	—[a]
Encephalocele	80 (12.0)	—[a]	41 (10.8)	—[a]	22 (10.4)	—[a]
Sex						
Male	317 (49.8)	371 (49.9)	184 (50.8)	240 (48.3)	104 (50.5)	92 (53.5)
Female	320 (51.2)	372 (50.1)	178 (49.2)	257 (51.7)	102 (49.5)	80 (46.5)
Missing	30	0	0	0	5	0
Year of birth						
1997–1998[d]	18 (2.7)	10 (1.3)	16 (4.2)	7 (1.4)	1 (0.5)	3 (1.7)
1999–2004[e]	301 (45.1)	320 (43.1)	187 (49.1)	210 (42.3)	70 (33.2)	90 (52.3)
2005–2009[e]	348 (52.2)	413 (55.6)	178 (46.7)	280 (56.3)	121 (57.3)	79 (45.9)

HEI, Healthy Eating Index; NTD, neural tube defect.

[a]Not applicable. [b]Median (IQR). [c]Score can range from 0 to 100. [d]Pre-folic acid fortification. [e]Post-folic acid fortification.

TABLE 2 Gluten intake by celiac risk group (defined in Figure 1) among all cases and controls, National Birth Defects Prevention Study, 1997–2009

Celiac risk group	Cases		Controls	
	N	Median (IQR)	*N*	Median (IQR)
All	587	25.9 (19.5–36.7)	695	25.3 (18.9–33.8)
Low	253	25.0 (19.8–36.5)	304	25.0 (19.2–33.3)
Intermediate	308	26.1 (19.3–37.2)	351	25.5 (18.8–33.9)
High	26	26.9 (19.7–36.0)	40	26.8 (20.0–34.3)

IQR, interquartile range.

For spina bifida, seven significant associations were observed among all women (the same five non-HLA SNPs identified from the NTD analyses and two additional SNPs) (Supplemental Table S3). When the spina bifida analyses were restricted by race/ethnicity, only the positive association with rs11221332 among NHWs and the negative association with rs13151961 among Hispanics were significant. For anencephaly, the only significant association was observed with rs11221332 among all women (Supplemental Table S3). There were no significant associations between the unweighted or weighted genetic risk score and risk of NTDs among all women or by race/ethnicity (Table 4). Results were similar after adjusting for celiac risk group (data not shown).

Of the fifteen non-HLA SNPs, two SNPs (rs13010713 and rs1893217) deviated from HWE among all women and were not included in the interaction analyses. After Bonferroni correction (based on $p < 0.0038$, considering 0.05/13 SNP comparisons), no significant interactions with gluten intake, HEI-2010 score, or use of folic acid supplementation were observed (Supplemental Tables S4–S6).

4 | DISCUSSION

Our findings provided little evidence that maternal celiac risk related to HLA SNPs is associated with NTDs in offspring in most women. There was some evidence that certain non-HLA SNPs related to celiac disease may be positively or negatively associated with NTDs. Additionally, we did not find any significant interactions between maternal celiac risk group and gluten intake, HEI-2010 score, or use of folic acid supplementation, as well as between maternal non-HLA SNPs and gluten intake or use of folic acid supplementation.

The few epidemiological cohort studies that have investigated the association between maternal celiac disease and NTDs reported null findings, partially because most of the studies are underpowered due to the rarity of both celiac disease and NTDs (Ban et al., 2015; Tata et al., 2005; Zugna et al., 2014). For example, in two of the studies that have assessed this relationship, the number of women with celiac

TABLE 3 Association between celiac risk group (defined in Figure 1) and neural tube defects by race/ethnicity, National Birth Defects Prevention Study, 1997–2009

	Celiac Risk Group	All				Non-Hispanic White				Hispanic			
		N cases	*N* controls	OR[a]	95% CI	*N* cases	*N* controls	OR	95% CI	*N* cases	*N* controls	OR	95% CI
NTD	Low	253	304	1.00	Ref.	156	214	1.00	Ref.	70	53	1.00	Ref.
	Intermediate	308	352	1.03	0.82, 1.29	178	22	1.10	0.83, 1.46	94	99	0.72	0.46, 1.13
	High	26	40	0.78	0.46, 1.32	15	29	0.71	0.37, 1.37	6	7	0.65	0.21, 2.04
Spina Bifida	Low	130	304	1.00	Ref.	88	214	1.00	Ref.	31	53	1.00	Ref.
	Intermediate	171	352	1.11	0.84, 1.47	106	222	1.16	0.83, 1.63	51	99	0.88	0.50, 1.54
	High	15	40	0.88	0.47, 1.64	8	29	0.67	0.30, 1.53	3	7	—[b]	—[b]
Anencephaly	Low	91	304	1.00	Ref.	50	214	1.00	Ref.	30	53	1.00	Ref.
	Intermediate	102	352	0.92	0.66, 1.27	56	22	1.08	0.71, 1.65	33	99	0.59	0.32, 1.07
	High	9	40	0.74	0.35, 1.60	6	29	0.89	0.35, 2.25	3	7	—[b]	—[b]

CI, confidence interval; NTD, neural tube defect; OR, odds ratio.
[a]Adjusted for maternal race/ethnicity. [b]Not calculated due to < 5 observed exposed cases.

TABLE 4 Association between maternal non-HLA single nucleotide polymorphisms and neural tube defects by race/ethnicity, National Birth Defects Prevention Study, 1997–2009

			All					Non-Hispanic White				
Chr	SNP	Risk Allele	*N* ca.	*N* co.	OR[a]	95% CI	*p-Value*[b]	*N* ca.	*N* co.	OR	95% CI	*p-Value*[b]
1	rs3748816	A	665	738	0.85	0.71, 1.01	0.10	381	493	0.76	0.61, 0.95	0.06
1	rs10903122	G	663	733	0.81	0.69, 0.95	0.02	380	490	0.82	0.67, 0.99	0.08
2	rs13003464	G	666	739	1.27	1.07, 1.52	0.02	381	493	1.33	1.07, 1.66	0.05
2	rs13010713	G	666	739	—[c]	—[c]	—[c]	380	494	—[c]	—[c]	—[c]
3	rs13314993	G	666	736	0.80	0.69, 0.94	0.02	380	490	0.89	0.73, 1.08	0.29
3	rs13098911	T	667	739	0.79	0.58, 1.08	0.22	381	494	0.74	0.50, 1.09	0.18
3	rs11712165	G	666	741	0.97	0.82, 1.15	0.90	381	495	0.85	0.69, 1.05	0.18
3	rs17810546	G	665	741	1.05	0.82, 1.34	0.90	379	496	1.11	0.82, 1.52	0.50
4	rs13151961	A	664	739	0.69	0.55, 0.85	0.004	381	493	0.76	0.59, 0.98	0.07
6	rs802734	G	664	734	1.01	0.85, 1.20	0.94	381	492	1.13	0.91, 1.40	0.29
6	rs1738074	T	667	743	0.98	0.84, 1.41	0.91	381	497	—[c]	—[c]	—[c]
7	rs9792269	A	667	743	0.83	0.69, 0.99	0.08	381	497	0.78	0.62, 0.97	0.07
11	rs11221332	T	663	733	1.73	1.43, 2.09	<0.002[d]	380	491	1.76	1.39, 2.24	<0.002[e]
12	rs653178	C	664	740	1.21	1.01, 1.44	0.08	379	494	—[c]	—[c]	—[c]
18	rs1893217	G	665	740	—[c]	—[c]	—[c]	379	494	—[c]	—[c]	—[c]
Risk Score[e]												
Unweighted[g]			650	699	0.97	0.92, 1.03		374	463	0.96	0.89, 1.03	
Weighted[h]			650	699	0.86	0.64, 1.18		374	463	0.80	0.53, 1.23	

Chr, chromosome; CI, confidence interval; HLA, human leukocyte antigen; NTD, neural tube defect; OR, odds ratio.
[a]Adjusted for maternal race/ethnicity. [b]FDR adjusted *p*-value. [c]Not calculated because SNP deviated from HWE ($p < 0.05$). [d]SAS provided an unadjusted *p*-value of <0.0001, so we were unable to provide an exact adjusted *p*-value. [e]Calculated using SNPs that were in HWE ($p \geq 0.05$). [f]accession number for the genes are as following: MMEL1:NM_033467.4; PUS10:NM_144709.4; LINC01934:NR_130784.1; ARHGAP31:NM_020754.3; KIAA1109:NM_015312.3; NRF1:NM_001293164.1; ETS1:NM_001143820.2; PTPN2:NM_002828.4. [g]Based on risk allele count. [h]Weighted by beta coefficients for each risk alleles from Dubois et al. (2010).

disease and offspring with NTDs was either zero or one (Ban et al., 2015; Tata et al., 2005). Additionally, because celiac disease status was determined from medical records in all three studies, it is possible that some women with undiagnosed celiac disease were misclassified as not having celiac disease.

In our study, we attempted to reduce the potential for such misclassification by using genetic variants associated with celiac disease to determine risk of celiac disease. We did not find evidence to support that celiac risk based on the HLA-DQ haplotypes was associated with NTDs, which may be because the HLA-DQ haplotypes are better suited for screening risk of celiac disease than for diagnosing celiac disease (Rubio-Tapia, Hill, Kelly, Calderwood, & Murray, 2013). For example, only 0.2%–13% of people with HLA-DQ2 or HLA-DQ8 haplotypes (i.e., the two most predictive haplotypes) will develop celiac disease (Romanos & Wijmenga, 2010). Additionally, because the HLA-DQ2 and HLA-DQ8 haplotypes are common in the general population and celiac disease is rare, the women categorized to the intermediate and high risk groups were probably heterogenous with respect to celiac disease (e.g., most women probably did not have celiac disease) and several comparisons in our study were also probably underpowered (e.g., analysis of anencephaly among Hispanics). Because the genetic variants do not clinically determine celiac disease, it may be one of the reasons that we did not find any significant interactions with gluten intake.

In the non-HLA analyses, we hypothesized that increased risk of celiac disease would be positively associated with NTDs. We observed five significant associations, but only two associations were in the positive direction. These two SNPs provide some evidence that maternal variants related celiac disease may be associated with NTDs in offspring. However, we also observed three negative associations, which suggests that there may be other pathways independent of celiac disease that might be associated with NTDs (i.e., pathways related to the immune system [Supplemental Table S7]). There is some evidence that certain immunological factors (i.e., proinflammatory cytokines) may be regulated by levels of folate (Denny et al., 2013). Because we did not find any significant interactions with use of folic acid

Hispanic							
N ca.	*N* co.	OR	95% CI	*p*-Value[b]	Gene name[f]	HGVS nomenclature	Function
209	172	0.81	0.58, 1.13	0.32	*MMEL1*	g.2595307A > G	missense
208	170	0.75	0.55, 1.03	0.23	n/a	g.24977085A > G	intergenic
210	172	1.30	0.92, 1.94	0.27	*PUS10*	g.60959694A > G	intron
211	172	0.60	0.43, 0.84	0.02	*LINC01934*	g.181131318A > G	intron
211	172	0.75	0.55, 1.02	0.23	n/a	g.32973977G > T	intergenic
211	171	1.26	0.67, 2.37	0.59	n/a	g.46193709C > T	regulatory
210	172	1.25	0.91, 1.72	0.29	*ARHGAP31*	g.119399949T > G	intron
211	171	0.97	0.63, 1.48	0.89	n/a	g.159947262A > G	regulatory
208	172	0.41	0.24, 0.70	0.01	*KIAA1109*	g.122194347A > G	intron
208	169	0.87	0.61, 1.23	0.59	n/a	g.127957653A > G	intergenic
211	172	0.80	0.60, 1.08	0.28	n/a	g.159044945T > C	regulatory
211	172	0.98	0.70, 1.36	0.89	*NRF1*	g.128252343A > G	intron
209	169	1.49	1.04, 2.13	0.14	*ETS1*	g.128511079C > T	intron
210	172	1.30	0.92, 1.84	0.28	n/a	g.111569952C > T	regulatory
211	172	0.85	0.53, 1.38	0.59	*PTPN2*	g.12809341A > G	intron
201	166	0.93	0.85, 1.02		n/a		n/a
201	166	0.67	0.38, 1.19		n/a		n/a

supplementation, if the immune system is involved in the development of the neural tube, our results suggest that the variants in our study are involved in pathways independent of the folate metabolism. Given that previous studies have not evaluated the association between genetic variants related to celiac disease and NTDs, our findings need to be replicated in an independent sample and perhaps studied in animal or in vitro studies.

This study had some limitations. Two of the genetic variants used to determine HLA celiac risk group did not meet our quality control procedures, which may have nondifferentially misclassified women's HLA celiac risk group by case–control status. Thus, it is possible that our results for the HLA celiac risk group might be biased towards the null. Our results may not be generalizable to all reproductive women. The mean HEI-2010 score among women ≥20 years old who participated in NHANES from 2003 to 2004 was 58 (Guenther et al., 2014), whereas the median HEI-2010 score in this study was 75. Additionally, women who were not categorized into a celiac risk group were slightly different from women who were categorized into a celiac risk group. We did not have additional genetic data to control for potential residual population stratification. Although we stratified our analyses by self-reported race/ethnicity, some residual population stratification is possible. Stratifying by race/ethnicity revealed that NHWs had more non-HLA SNPs out of HWE in than Hispanics. The associations for the non-HLA SNPs that were in HWE among all women but were out of HWE in NHWs should be interpreted with caution. We did not collect information on whether the products women consumed were gluten-free, which should be considered in future studies.

This study was the first to use genetic variants to assess the association between genetic risk of celiac disease and NTDs. By using genetic variants to assess risk of celiac disease, we attempted to identify women with diagnosed and undiagnosed celiac disease to limit the potential for exposure misclassification of celiac disease status. Our study provides some evidence that certain non-HLA genetic variants related to celiac disease may be associated with NTDs in offspring. In addition, the negative associations observed with the non-HLA genetic variants provide some

evidence for potential candidate genes and other pathways that are independent of celiac disease for consideration in future studies. If our results are confirmed, it will allow us to identify women for counseling and targeted prevention strategies to reduce their risk of NTDs, as well as improve our understanding of NTDs.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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