

# Genome-Wide Genetic and Transcriptomic Investigation of Variation in Antibody Response to Dietary Antigens

Rohina Rubicz,<sup>1,2\*</sup> Robert Yolken,<sup>3</sup> Armin Alaedini,<sup>4</sup> Eugene Drigalenko,<sup>1</sup> Jac C. Charlesworth,<sup>5</sup> Melanie A. Carless,<sup>1</sup> Emily G. Severance,<sup>3</sup> Bogdana Krivogorsky,<sup>3</sup> Thomas D. Dyer,<sup>1</sup> Jack W. Kent Jr,<sup>1</sup> Joanne E. Curran,<sup>1</sup> Matthew P. Johnson,<sup>1</sup> Shelley A. Cole,<sup>1</sup> Laura Almasy,<sup>1</sup> Eric K. Moses,<sup>6</sup> John Blangero,<sup>1</sup> and Harald H. H. Göring<sup>1</sup>

<sup>1</sup>Department of Genetics, Texas Biomedical Research Institute, San Antonio, Texas, United States of America; <sup>2</sup>Division of Public Health Sciences, Fred Hutchinson Cancer Research Institute, Seattle, Washington, United States of America; <sup>3</sup>Department of Pediatrics, Johns Hopkins School of Medicine, Baltimore, Maryland, United States of America; <sup>4</sup>Department of Medicine, Columbia University Medical Center, New York, New York, United States of America; <sup>5</sup>Menzies Research Institute, University of Tasmania, Hobart, Tasmania, Australia; <sup>6</sup>Centre for Genetic Epidemiology and Biostatistics, The University of Western Australia, Perth, Australia

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**ABSTRACT:** Increased immunoglobulin G (IgG) response to dietary antigens can be associated with gastrointestinal dysfunction and autoimmunity. The underlying processes contributing to these adverse reactions remain largely unknown, and it is likely that genetic factors play a role. Here, we estimate heritability and attempt to localize genetic factors influencing IgG antibody levels against food-derived antigens using an integrative genomics approach. IgG antibody levels were determined by ELISA in >1,300 Mexican Americans for the following food antigens: wheat gliadin; bovine casein; and two forms of bovine serum albumin (BSA-a and BSA-b). Pedigree-based variance components methods were used to estimate additive genetic heritability ( $h^2$ ), perform genome-wide association analyses, and identify transcriptional signatures (based on 19,858 transcripts from peripheral blood lymphocytes). Heritability estimates were significant for all traits (0.15–0.53), and shared environment (based on shared residency among study participants) was significant for casein (0.09) and BSA-a (0.33). Genome-wide significant evidence of association was obtained only for antibody to gliadin ( $P = 8.57 \times 10^{-8}$ ), mapping to the human leukocyte antigen II region, with *HLA-DRA* and *BTNL2* as the best candidate genes. Lack of association of known celiac disease risk alleles HLA-DQ2.5 and -DQ8 with antigliadin antibodies in the studied population suggests a separate genetic etiology. Significant transcriptional signatures were found for all IgG levels except BSA-b. These results demonstrate that individual genetic differences contribute to food antigen antibody measures in this population. Further investigations may elucidate the underlying immunological processes involved.

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**KEY WORDS:** IgG antibody; gliadin; integrative genomics; association; transcriptional profiling; pedigree study

## Introduction

Adverse reactions to dietary substances are common in the U.S. general population, with most of the affected individuals experiencing gastrointestinal, skin, and/or respiratory symptoms. Reactions include IgE-mediated allergies that affect an estimated 4–6% of children and 1–2% of adults [Patel et al., 2011] and, more commonly, food intolerances, which can be either immune or nonimmune mediated. Food intolerances are often dose-dependent and generally take longer to become symptomatic than allergic reactions [Skypala, 2011]. Food allergies and intolerances have been associated with atopic diseases (e.g., asthma, rhinitis, and eczema) and autoimmune disorders (such as celiac disease, CD) [Briani et al., 2008; Tan

and Corren, 2011]. CD is an autoimmune enteropathy triggered by gluten proteins of wheat and related cereal grains in genetically susceptible individuals that affects an estimated 2–3 million people in the United States [Fasano et al., 2003].

Individuals are thought to be universally exposed to a wide variety of food proteins (although exposure to some food items varies by culture). Differences in the level of antibodies produced in response to food antigens are therefore likely due in part to genetic differences. Previous research indicates that a hyperactive immune response to certain food proteins tends to run in families, and genetic factors have been implicated in some instances [Hong et al., 2009; Liu et al., 2009; Tsai et al., 2009]. For example, certain human leukocyte antigen (HLA) alleles are present in higher frequencies in allergic individuals than in controls (e.g., peanut allergy (*HLA-DRB1*, *HLA-DQB1*, and *HLA-DPB1* gene polymorphisms) and apple allergy (*HLA-DRB1\*07* allele) [Howell et al., 1998; Senechal et al., 1999]) and *HLA-DQ* variants

Supporting Information is available in the online issue at wileyonlinelibrary.com.

\*Correspondence to: Dr. Rohina Rubicz, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Mailstop: M4-A402, 1100 Fairview Avenue North, P.O. Box 19024, Seattle, WA 98109-1024. E-mail: rrubicz@fhcrc.org

have long been known to predispose to CD [Louka and Solid, 2003]. There is also evidence suggesting that *CD14*, *IL10*, *IL13*, and *SPINK5* gene polymorphisms may predispose to food allergy and/or sensitization in general [Campos Alberto et al., 2008; Kusunoki et al., 2005; Woo et al., 2003]. However, the underlying disease processes contributing to adverse reactions to many food proteins, especially those of nonallergic etiology, remain largely unknown.

## Materials and Methods

### Participants

Individuals participating in this study consisted of 1,367 members of randomly ascertained, extended Mexican-American families from San Antonio, TX, who were recruited for participation in the San Antonio Family Heart Study, which seeks to identify cardiovascular disease risk factors [Mitchell et al., 1996]. Up to six generations and 63 families are represented in the sample, as previously described [Rubicz et al., 2013]. Initial recruitment took place during the years 1991–1995. Participants range in age from 16 to 94 years (with a mean of 39 years) and they consist of 816 women and 551 men. The Institutional Review Board of the University of Texas Health Science Center at San Antonio approved the study protocols, and all participants signed statements of informed consent.

### Serology

Following an overnight fast, blood samples were collected from participants using EDTA vacutainers at the time of recruitment (1991–1995). Frozen plasma aliquots were obtained as previously described [Cheng et al., 1986] and carefully stored at  $-80^{\circ}\text{C}$ , until their recent use for antibody determinations. ELISA kits were used to determine immunoglobulin G (IgG) antibody titers to: gliadin [Samaroo et al., 2010]; bovine casein [Niebuhr et al., 2011; Severance et al., 2011]; and two forms of bovine serum albumin consisting of complete BSA (BSA-a) and a more-purified form derived from Cohn Fraction V (BSA-b) [Sheridan and Simmons, 1983]. Antigens for these assays were obtained from the Sigma-Aldrich Chemical Company, St. Louis, MO (catalog numbers are: casein C7078; gliadin G3375; BSA-a A9647; and BSA-b 85040C).

### SNP Genotyping

DNA samples extracted from lymphocytes were typed for SNPs using several versions of Illumina's SNP genotyping BeadChip microarrays (HumanHap550v3, HumanExon510Sv1, Human1Mv1, Human1M-Duov3) according to the Illumina Infinium Protocol (Illumina, San Diego, CA) and underwent stringent quality control measures prior to analysis, as previously described [Rubicz et al., 2013]. SNPs were excluded if they had a low call rate, were monomorphic, had a minor allele in  $<10$  individuals, and if Hardy-Weinberg

test statistics (calculated in SOLAR [Almasy and Blangero, 1998]) were  $P \leq 10^{-4}$ . SNPs overlapping between the different microarray versions were kept for further processing. SNP genotypes were checked for Mendelian consistency with SimWalk2 [Sobel et al., 2002], and the most likely incorrect genotype was blanked. Subsequently, the blanked genotypes were re-imputed using MERLIN [Abecasis et al., 2002] (based on local relatives' genotypes, or if uncertain then using a weighted average of possible genotypes). Allele frequencies were calculated for the resulting 944,565 SNPs using maximum likelihood estimates in SOLAR [Almasy and Blangero, 1998].

### Statistical Methodology

Variance components (VCs) methods were used to estimate heritability ( $h^2$ ), defined as the aggregate additive autosomal genetic effects, on the antibody levels to each food antigen using SOLAR [Almasy and Blangero, 1998]. Prior to analysis, the quantitative antibody level measurements were transformed using a rank-based inverse normalization, as VC analysis can be sensitive to non-normality, particularly high kurtosis. The covariates sex, age, and their interactions were included in all statistical analyses. In addition, a household VC (based on cohabitation of family members at the time of the study, including both genetically related and unrelated individuals) was used to model the influence of shared environmental factors [Spence et al., 1977].

To determine if there is overlap in the additive genetic factors influencing the food antigen IgG measurements, bivariate heritability analyses were conducted in SOLAR for each pair of antibody traits [Almasy and Blangero, 1998; Boehnke et al., 1986; Lange and Boehnke, 1983]. Genome-wide association analyses were run in SOLAR [Almasy and Blangero, 1998] using 944,565 SNPs. Association analyses were conducted using an additive measured genotype model, allowing for nonindependence of family members due to shared genes and shared environment. We empirically estimated the genome-wide significance threshold (at  $\alpha = 0.05$ ) for our sample to be  $P \leq 1.3 \times 10^{-7}$  using a large number of genome-wide analyses on simulated traits unlinked to the genotyped SNPs, using the true pedigree structures and genome-wide SNP genotypes for analysis of each replicate. Principal components analysis (PCA) was conducted on SNP genotypes to identify principal components (PCs) that can be used to correct for differences in ancestral ethnic contributions to study participants, as population substructure may produce spurious results in association analyses [Price et al., 2006]. PCA (using R princomp) [R Development Core Team, 2011] was run on all individuals contributing independent haplotypes to the sample, including all founders; offspring were assigned the average of both their parents' values for each PC. This was done so that the PCA would not unknowingly capture differences between pedigrees per se, as real differences between pedigrees (in particular among rare variation) may exist and aid in the localization of genetic effects. We included the first five PCs as additional covariates in all statistical analyses.

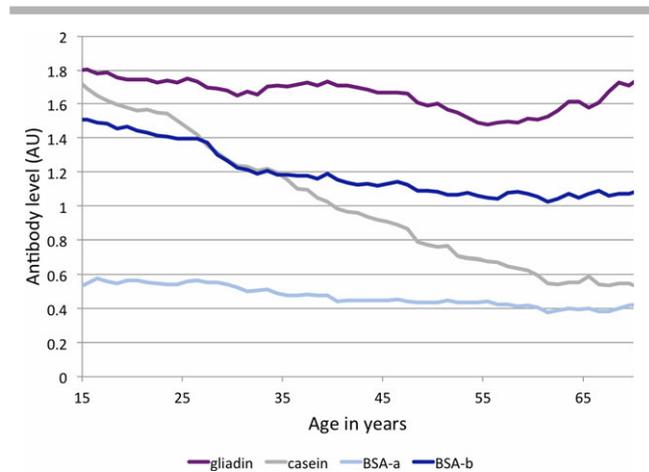
## Transcriptional Profiles

Gene expression data that were generated from peripheral blood lymphocyte samples collected from participants at the same time as the plasma samples used for antibody determinations (as previously described [Goring et al., 2007]) were also analyzed. Microarray-based expression data were available for 1,243 study participants, and the raw and normalized expression values are available under accession number E-TABM-305 at <http://www.ebi.ac.uk/arrayexpress>. Sample quality was assessed by comparing the number of expressed probes (at  $P \leq 0.05$ ), the mean expression across expressed probes, and the mean correlation across expressed probes with other samples. 20,643 significantly expressed probes were identified at a false discovery rate (FDR) of  $\leq 0.05$ , using a one-sided binomial test based on counts of successful compared to unsuccessful detection at  $P \leq 0.05$ . Significantly expressed transcripts next underwent background noise correction, log2 transformation, and quantile normalization. Of these, 19,858 transcripts mapped to known genes and were included in the analyses here. We examined whether any SNPs that were significantly associated with the food antigen antibody traits were also associated with expression levels of neighboring transcripts, which would suggest these SNPs may be *cis*-regulatory variants. In addition, we tested more generally whether the expression levels of any transcripts were significantly correlated with food antigen antibody levels. A regression model was implemented in SOLAR [Almasy and Blangero, 1998] with transcript level as the focal covariate for IgG antibody level, while simultaneously taking into account the nonindependence of related individuals. Significance was tested using a likelihood ratio statistic. Prior to correlating expression levels with the food antigen antibody level traits, the relationship between each of the top 50 expression PCs and the antibody level traits were examined (by regression analysis) and were regressed out except for those that were significantly correlated, so that any true connections between transcripts and food antigen antibody traits were not removed. Transcripts significantly correlated with the antibody traits (using an FDR of 0.05 to account for multiple testing) were analyzed using Ingenuity Pathway Analysis (IPA) version 6.3 [Ingenuity Systems] to identify possible connections (pathways) between identified transcripts.

## Results

### Antibody Levels

IgG antibody levels to four food antigens (gliadin, casein, BSA-a, BSA-b) were measured in a sample of 1,367 Mexican Americans participating in the San Antonio Family Heart Study. Figure 1 shows antibody levels across individuals of different ages in this cross-sectional study, with most antibody levels decreasing over the examined age range of 16 to 94 years, and a slight increase for some antibody levels in the older age categories. IgG levels to casein demonstrated the largest difference between young and old age categories. All four antibodies were detected in a substantial number



**Figure 1.** The antibody level (AU) for food antigen IgG antibodies averaged across individuals for each age bracket in this cross-sectional study. AU = arbitrary unit (representing the optical density ratio: raw optical density measurement divided by negative control). Sliding 15-year-age windows were used to smooth the curves, and the age shown is the midpoint of each age interval.

**Table 1. Heritability estimates of food antigen IgG measurements with household effects**

Trait	$h^2$ (heritability)	$P$ -value ( $h^2$ )	$c^2$ (household effects)	$P$ -value ( $c^2$ )
Gliadin	<b>0.53</b>	$2.3 \times 10^{-25}$	0.06	0.04
Casein	<b>0.44</b>	$2.4 \times 10^{-13}$	<b>0.09</b>	<b>0.01</b>
BSA-a	<b>0.15</b>	$1.5 \times 10^{-3}$	<b>0.33</b>	$1.0 \times 10^{-7}$
BSA-b	<b>0.21</b>	$1.1 \times 10^{-4}$	0.09	0.02

Bold = significant after correction for multiple testing ( $P = 0.05/4$  traits = 0.0125).

of study participants, suggesting that food intolerances to these four food antigens are not rare in the studied Mexican-American population.

### Heritability Estimates

We investigated whether additive genetic factors in the aggregate influenced the levels of antibodies. Heritability estimates were significant for antibodies against all of the food antigens (Table 1). Estimates ranged from  $h^2 = 0.15$  for BSA-a to  $h^2 = 0.53$  for gliadin. Shared household, a proxy for shared environmental exposures based on cohabitation at the same residence at the time of sample collection, was significant for only casein (0.09) and BSA-a (0.33). With the exception of BSA-a, the shared household effects were smaller in magnitude than the estimated heritabilities. We performed bivariate heritability analysis to determine whether and to what extent genetic factors influencing the different IgG antibody levels might overlap. Genetic factors are significantly positively correlated for most pairs of food antigen antibody measures (supplementary Table S1), ranging from 0.22 to 0.52, indicating that a substantial proportion of the underlying genetic variation is shared among these four food IgG antibodies.

**Table 2. Significant and suggestive results of genome-wide association analysis for food antigen IgG measurements**

Trait	SNP	SNP location: chromosome	SNP location: base pair	Nearest gene	Minor allele	Minor allele frequency*	P-value ( $\beta_{SNP}$ )**	
Gliadin	rs4848780	2	122940870	<i>TSN</i>	T	0.335	$8.25 \times 10^{-7}$ (-0.21)	
	rs3130320	6	32331236	<i>NOTCH4</i>	T	0.197	$4.48 \times 10^{-7}$ (0.27)	
	rs3117116	6	32474995	<i>BTNL2</i>	C	0.069	$1.00 \times 10^{-6}$ (0.42)	
	<b>rs3135352</b>	<b>6</b>	<b>32500884</b>	<b><i>HLA-DRA/BTNL2</i></b>	<b>G</b>	<b>0.049</b>	<b><math>1.12 \times 10^{-7}</math> (0.50)</b>	
	<b>rs3135350</b>	<b>6</b>	<b>32500959</b>	<b><i>HLA-DRA/BTNL2</i></b>	<b>G</b>	<b>0.046</b>	<b><math>8.57 \times 10^{-8}</math> (0.51)</b>	
	rs3129971	6	32501213	<i>HLA-DRA/BTNL2</i>	G	0.049	$3.40 \times 10^{-7}$ (0.48)	
	<b>rs3129860</b>	<b>6</b>	<b>32509057</b>	<b><i>HLA-DRA</i></b>	<b>A</b>	<b>0.060</b>	<b><math>8.76 \times 10^{-8}</math> (0.47)</b>	
	rs3135391	6	32518965	<i>HLA-DRA</i>	T	0.051	$1.39 \times 10^{-7}$ (0.49)	
	rs8084	6	32519013	<i>HLA-DRA</i>	A	0.403	$6.34 \times 10^{-7}$ (0.21)	
	rs7192	6	32519624	<i>HLA-DRA</i>	T	0.389	$6.50 \times 10^{-7}$ (0.21)	
	rs7194	6	32520458	<i>HLA-DRA</i>	G	0.391	$4.31 \times 10^{-7}$ (0.21)	
	rs7195	6	32520517	<i>HLA-DRA</i>	A	0.391	$4.31 \times 10^{-7}$ (0.21)	
	<b>rs3135388</b>	<b>6</b>	<b>32521029</b>	<b><i>HLA-DRA</i></b>	<b>T</b>	<b>0.049</b>	<b><math>1.12 \times 10^{-7}</math> (0.50)</b>	
	rs2213586	6	32521072	<i>HLA-DRA</i>	T	0.391	$4.31 \times 10^{-7}$ (0.21)	
	rs2213585	6	32521128	<i>HLA-DRA</i>	C	0.391	$4.31 \times 10^{-7}$ (0.21)	
	rs2227139	6	32521437	<i>HLA-DRA</i>	C	0.391	$4.31 \times 10^{-7}$ (0.21)	
	rs3129889	6	32521523	<i>HLA-DRA</i>	G	0.049	$4.31 \times 10^{-7}$ (0.50)	
	Casein	rs11635085	15	90847640	<i>C15orf32</i>	G	0.107	$6.47 \times 10^{-7}$ (-0.30)
	BSA-a	rs7562116	2	231816490	<i>ARMC9</i>	G	0.223	$1.30 \times 10^{-6}$ (0.22)
		rs9677476	2	231834534	<i>ARMC9</i>	G	0.274	$1.11 \times 10^{-6}$ (0.21)
rs13415839		2	231834778	<i>ARMC9</i>	T	0.270	$1.29 \times 10^{-6}$ (0.21)	
BSA-b	rs2360969	2	208081241	<i>CREB1</i>	T	0.413	$1.24 \times 10^{-6}$ (-0.21)	
	rs1372356	14	87585702	<i>GPR65</i>	C	0.489	$5.81 \times 10^{-7}$ (-0.20)	
	rs2236267	14	87702074	<i>KCNK10</i>	C	0.462	$4.13 \times 10^{-7}$ (0.20)	

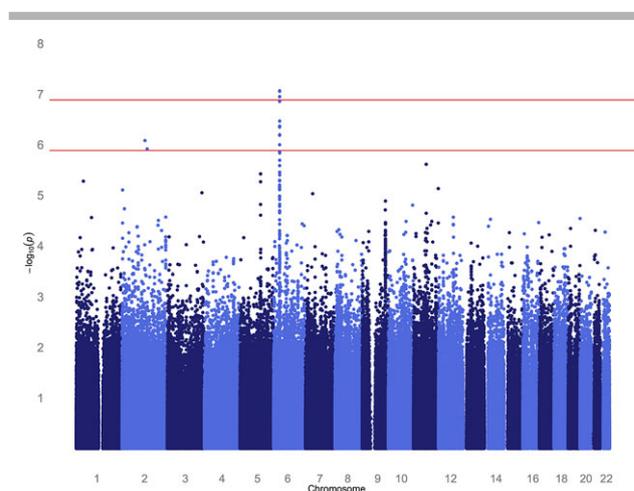
Bold = genome-wide significant at  $P \leq 1.3 \times 10^{-7}$ ; significance threshold was estimated based on our sample of Mexican-American participants. The threshold for suggestive evidence for association used here is  $P \leq 1.3 \times 10^{-6}$ .

\*Minor allele frequency was estimated for our study sample using maximum likelihood methods and taking family relationships into account.

\*\*The direction of effect of the regression coefficient is relative to the minor SNP allele (e.g., for SNP rs3135350, the minor allele is associated with an increase in IgG anti-gliadin antibody level).

## Genome-Wide Association Analysis

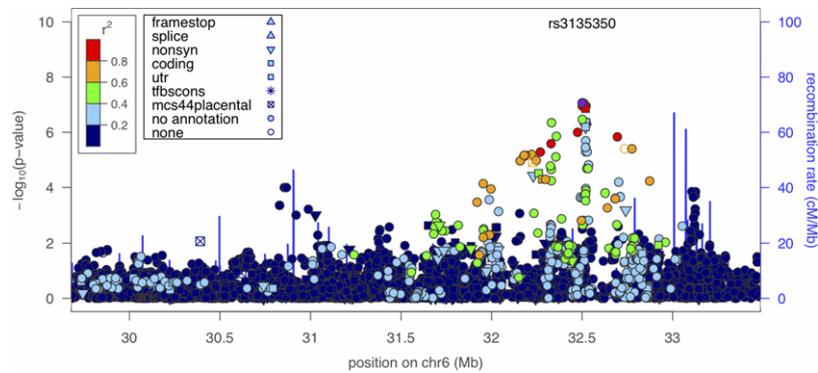
To localize specific genetic factors influencing these traits, we conducted genome-wide association analysis. We estimated empirically that a pointwise  $P \leq 1.3 \times 10^{-7}$  corresponds to a genome-wide  $P$ -value of  $\leq 0.05$  for our Mexican-American cohort, which consists of large multigenerational families. Genome-wide significant results were obtained for antibodies against gliadin in the HLA II region of chromosome 6, with *HLA-DRA* and *BTNL2* being the closest candidate genes (top SNP rs3135350,  $P = 8.6 \times 10^{-8}$ ; Table 2, Fig. 2). As seen in the regional plot of the extended HLA region, several other SNPs are in LD with rs3135350, including rs3129860 ( $r^2 = 0.88$ ,  $P = 8.8 \times 10^{-8}$ , Fig. 3). After conditional association analysis on rs3135350, no other SNPs are significantly associated with antibody to gliadin, suggesting a single locus rather than multiple nearby loci. The quantile-quantile plot of observed  $P$ -values shows that the HLA region accounts for the deviation from the diagonal line of observed vs. expected  $P$ -values under the null hypothesis, as there is no evidence of inflation of significant levels after excluding the HLA region from the plot (supplementary Fig. S1). We examined whether the four HLA SNPs that were significantly associated with anti-gliadin IgG levels were also significantly associated with the other food antigens. Nonsignificant  $P$ -values, after correcting for multiple testing, were obtained for casein and BSA-a for two gliadin SNPs (rs3135350 and rs3129860; Table 3). However, the  $P$ -values for casein and BSA-a were quite small ( $<0.1$ ) and the effect was in the same direction, suggesting that the locus may not be unique to gliadin.



**Figure 2.** Plot of genome-wide association analysis of IgG antibody level against wheat antigen (gliadin).

## Transcript Analysis

To pinpoint genes that may influence the measures of antibody to food antigens, we took an integrative genomics approach in which we analyzed gene expression profiles from peripheral blood lymphocytes collected at the same time as the plasma samples used for antibody determinations. We investigated whether the four significant SNPs associated with the anti-gliadin antibody trait (rs3135352, rs3135350, rs3129860, and rs3135388) were also significantly associated



**Figure 3.** Association analysis results of extended HLA region for antigliadin antibody level. The linkage disequilibrium (LD) pattern was estimated based on SNP genotypes from study participants. SNPs in red are highly correlated with the top SNP associated with the antibody trait (rs3135350).

**Table 3.** Association results for food antigen IgG traits for significant gliadin SNPs

Trait	rs3135352 $P$ -value ( $\beta_{\text{SNP}}$ )*	rs3135350 $P$ -value ( $\beta_{\text{SNP}}$ )*	rs3129860 $P$ -value ( $\beta_{\text{SNP}}$ )*	rs3135388 $P$ -value ( $\beta_{\text{SNP}}$ )*
Gliadin	$1.12 \times 10^{-7}$ (0.50)	$8.57 \times 10^{-8}$ (0.51)	$8.76 \times 10^{-8}$ (0.47)	$1.12 \times 10^{-7}$ (0.50)
Casein	0.07 (0.16)	0.04 (0.19)	0.05 (0.16)	0.07 (0.16)
BSA-a	0.07 (0.16)	0.05 (0.18)	0.05 (0.17)	0.07 (0.16)
BSA-b	0.24 (0.11)	0.30 (0.10)	0.13 (0.14)	0.24 (0.11)

Note that results for casein and BSA-a antigen traits are not significant after applying a stringent Bonferroni correction for multiple testing ( $P = 0.05/4$  food antigen traits = 0.0125). However, the fact that the  $P$ -values are fairly small, with effect sizes in the same direction, suggests that it may be possible that the locus is not unique to gliadin.

\*The direction of effect of the regression coefficient is relative to the minor SNP allele (e.g., for SNP rs3135352, the minor allele is associated with an increase in IgG antigliadin antibody level).

with the expression levels of any neighboring transcripts, which would indicate that these SNPs are potential *cis*-acting regulatory variants. There were 150 expressed transcripts in the HLA region, yielding 600 SNP-transcript pairs. After correcting for multiple testing ( $P = 0.05/600 = 8.33 \times 10^{-5}$ ) there were no significantly associated SNP-transcript pairs. The smallest  $P$ -value was  $P = 2.20 \times 10^{-3}$  for rs3129860 and the expression of *HLA-DQB1*.

We also used the transcriptional profiles as a separate way to identify genes related (though not necessarily causally) to the measured antibodies, by investigating whether transcript levels were correlated with IgG level, while simultaneously modeling shared genetic and environmental factors among relatives and household members. We identified significant transcriptional correlates for three antibody traits (supplementary Table S2). After applying Bonferroni correction for multiple testing ( $P = 0.05/19858$  transcripts =  $2.52 \times 10^{-6}$ ), 21 transcripts were significantly associated with antigliadin antibody levels, two with anticasein antibodies, 175 with anti-BSA-a antibody level, and none for BSA-b. There was little overlap of significant transcripts among the different IgG antibody measures. At a less stringent 0.05 FDR, there were 387 significantly correlated transcripts for gliadin, 413 for casein, 10,402 for BSA-a, and 0 for BSA-b. The discrepancy between the two different measures of IgG antibodies against BSA may be due to BSA-a containing more impurities.

The FDR 0.05 significant transcripts were then subjected to network analysis using IPA. An FDR 0.01 level was used

for BSA-a to deal with a more manageable number of transcripts (1,231) for pathway analysis. Supplementary Table S3 gives highly significant functional categories of transcripts and numbers of genes involved. Highly significant functional categories across the food antigen traits were inflammatory response, cell death, cellular proliferation and differentiation, and autoimmunity (including rheumatoid arthritis for BSA-a). Results of IPA canonical pathways assignments (according to generalized pathways from the IPA library) for the transcript analyses are provided in supplementary Table S4, along with the number of genes in each pathway. Several canonical pathway categories are involved in inflammatory response, including IL-10 and CD40 signaling.

### Relationship of Gliadin Trait to CD

Given that gliadin is the prime environmental trigger for CD and the condition is closely associated with antibody response to gliadin, we investigated whether published genome-wide significant susceptibility loci for CD show evidence for association with antigliadin antibodies in this study (supplementary Table S5). After applying a Bonferroni correction for examining 55 CD-related SNPs, a single SNP (rs10188217,  $P = 3.5 \times 10^{-4}$ ) was significantly associated with the gliadin trait. This SNP, rs10188217, is located on chromosome 2, in the *PUS10* (pseudouridylylase 10) gene. A nearby SNP, rs13003464, also showed a fairly small  $P$ -value ( $P = 5.2 \times 10^{-3}$ ), and is in LD with rs10188217 ( $r^2 = 0.87$ ).

## Discussion

This study demonstrates that individual genetic differences contribute to antifood antigen IgG levels in this sample of Mexican Americans. Shared household is also a significant contributing factor to some antibody measures, probably due to similar exposure to dietary antigens given that individuals residing in the same household are likely to share meals and dietary habits. Our results are in agreement with other studies that demonstrate adverse reaction to food antigens is due to both genetic and environmental factors [Nistico et al., 2006]. However, to our knowledge, heritability estimates have not been published for these IgG measures. IgG antibody response to dietary antigens has been suggested as a measure of food sensitivity in some individuals. However, it contrasts greatly with IgE antibody response, which is involved in classic allergic reaction to food antigens (i.e., immediate immune reaction that occurs within minutes to hours of exposure) [Vojdani, 2009]. Previously published significant heritability estimates for IgE antibodies against milk ( $h^2 = 0.15$ ) and wheat ( $h^2 = 0.35$ ) antigens [Tsai et al., 2009] are somewhat lower than our estimates for IgG antibodies to casein ( $h^2 = 0.44$ ) and gliadin ( $h^2 = 0.53$ ). Our bivariate analysis indicates that some of the underlying genetic factors appear to be shared between these IgG antibody traits.

Individuals with food sensitivities are reported to be at greater risk for atopic diseases, including asthma, allergic rhinitis and eczema, which tend to cluster in families [Kiyohara et al., 2008; Kusunoki et al., 2005; Liu et al., 2009; Vojdani, 2009]. In addition, there is overlap between genetic risk factors for adverse food reactions and autoimmune diseases such as inflammatory bowel disease [Parmar et al., 2012]. The inflammatory response and symptoms associated with CD, another autoimmune condition, are triggered by the ingestion of gluten proteins, and it is estimated that 15–20% of individuals with CD will develop other autoimmune diseases [Cosnes et al., 2008]. Our analysis of gene expression data in this study provides further support for common mechanisms of these conditions, as we identified significant functional pathways related to autoimmune disease (rheumatoid arthritis) for the BSA-a IgG antibody trait, and dermatological conditions for antigliadin IgGs.

Our genome-wide investigation for genetic factors influencing food antigen IgG antibody levels identified a significant association of HLA class II genes *HLA-DR* and *BTNL2* at 6p21.3 with antigliadin antibodies. Although gliadin is the main environmental trigger for CD and elevated levels of antibodies against gluten are associated with the disease, the genes that we identify here are not known CD risk factors: *HLA-DRA* is involved in binding peptides derived from antigens for recognition by CD4 T-cells; and *BTNL2* has been identified as a risk factor for sarcoidosis and ulcerative colitis (both are diseases with an autoimmune background) that likely functions to costimulate T-cells [Rybicki et al., 2005]. CD requires priming of CD4 T-cells by gliadin peptides, which then accumulate in the lamina propria where they produce the cytokine IFN- $\gamma$  that contributes to intestinal inflammation, and it is possible that the genes identified

here are involved in this process. On the other hand, these genetic factors may be associated with the IgG antibody response to gluten in nonceliac gluten sensitivity [Lundin and Alaedini, 2012]. We did not successfully map major loci using our modest sample size (by GWAS standards for commonly measured traits and diseases) for the remaining food antigen IgG measures, which suggests a complex genetic architecture underlying these traits.

Among individuals with CD, an estimated 95% are known to carry at least one copy of the HLA-DQ2.5 and -DQ8 risk haplotypes, coded for by alleles DQA1\*05/DQB1\*02 and DQA1\*0505/DQB1\*0301, respectively [Monsuur et al., 2008; Sollid et al., 1989; Sollid et al., 2012]. In our study, we did not find a significant association of level of antibodies against gliadin antigen with these CD risk haplotypes using HLA tagging SNPs rs2187668 (for DQ2.5) and rs7454108 (for DQ8) as described by Monsuur et al., 2008. As most individuals with these risk alleles will not develop CD upon exposure to gluten, other factors must also be involved, and additional loci, particularly in the HLA region, are implicated in CD development and severity [Cosnes et al., 2008; Henderson et al., 2007; Kim et al., 2004]. Meta-analyses and dense genotyping using the Immunochip have increased the number of CD risk loci to >40 [Dubois et al., 2010; Trynka et al., 2011]. We found evidence for association of the antigliadin antibody trait with CD susceptibility locus rs10188217 on chromosome 2 in *PUS10* (also a risk locus for ulcerative colitis and Crohn's disease [Festen et al., 2011; McGovern et al., 2010]) and our analysis of the gene expression data identified an overlap with CD susceptibility gene *BACH2* [McGovern et al., 2010]. These results point to some genetic overlap between antigliadin antibody levels and CD. However, the nonsignificant findings for the major CD risk haplotypes indicates that although there may be similar underlying mechanisms leading to these disease states, they in fact likely have largely separate etiologies. Shared factors such as increased intestinal permeability could contribute to both gluten sensitivity and CD, but the immune response in these two disease processes might be directed at different antigenic determinants (i.e., different gluten proteins and/or epitopes) [Samaroo et al., 2010].

To summarize, here we demonstrated that genetic and environmental (e.g., shared residence) factors significantly contributed to levels of IgG antibodies against several food antigens, and many pairs of antibody traits appear to be influenced by shared genetic factors. We identified a significant association of antigliadin IgG with the HLA II region, which is involved in immune function, and we report some genetic overlap between antigliadin antibodies and CD risk alleles. Our analysis of gene expression data (from peripheral blood lymphocytes) identified significant correlations between transcripts with several IgG traits, as well as a number of functional categories that appeared to be related to levels of these antibodies, though the directionality of effect is uncertain at the moment. Our study has a number of limitations. It would be useful to have dietary information to examine the relationship between antibody presence and level and exposure to the studied food antigens, but

detailed exposure information was not available. Also, the size of our study is moderate, in particular when compared to current meta-analytic GWAS on common diseases and commonly measured quantitative traits. Hopefully this investigation will be a stepping-stone toward future investigations and combining different data sources to increase the sample size, permitting identification of the specific causal variants and the mechanisms by which they contribute to the food antigen sensitivity and possibly atopic and/or autoimmune diseases.

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