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GLUTEN INTAKE AND RISK OF THYROID PEROXIDASE AUTOANTIBODIES IN THE DIABETES AUTOIMMUNITY STUDY IN THE YOUNG (DAISY)

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Abstract

Purpose—Autoimmune diseases co-occur, perhaps due to common risk factors. The age at gluten introduction and gluten intake in early childhood have been associated with the autoimmunity preceding celiac disease (CD) and type 1 diabetes (T1D). We explored their associations with the development of thyroid autoimmunity.

Methods—DAISY has prospectively followed children at increased risk for T1D and CD since 1993. During follow-up, 107 children developed thyroid autoimmunity, defined as positivity for autoantibodies against thyroid peroxidase on at least two study visits. Age at gluten introduction

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Authors' contributions

JAG and RKJ contributed equally to this work as first authors; they designed the study, performed statistical analyses, interpreted results, drafted and approved the final manuscript. FD performed statistical analyses and critically reviewed and approved the final manuscript. MH, AKS, and BIF acquired study data, and critically reviewed and approved the final manuscript. MR and JMN designed the DAISY study, interpreted results, and critically reviewed and approved the final manuscript.

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Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest.

Ethics approval

The Colorado Multiple Institutional Review Board approved all DAISY study protocols (COMIRB 92-080). Informed consent was obtained from the parents/legal guardians of all children. Assent was obtained from children age 7 years and older. All research was performed in accordance with relevant guidelines/regulations.

Consent to participate

Not Applicable

Consent for publication

Not Applicable

Availability of data and/or Code

Study data and code can be made available upon reasonable request to the corresponding author.

was ascertained from food history interviews every 3 months until 15 months of age. Gluten intake (grams/day) at age 1-2 years was estimated using a food frequency questionnaire.

Results—From multivariable Cox regression, there was no association between the age of gluten introduction nor the amount of gluten intake and development of thyroid autoimmunity. However, females (Hazard Ratio=2.19, 95%CI: 1.46, 3.27) and cases of islet autoimmunity (HR=2.20, 95%CI: 1.39, 3.50) were significantly more likely to develop thyroid autoimmunity, while exposure to environmental tobacco smoke decreased the risk (HR=0.46, 95%CI: 0.30, 0.71).

Conclusion—Neither the age of gluten introduction nor the amount of gluten consumed in early childhood is associated with risk of thyroid autoimmunity.

Keywords

Thyroid autoimmunity; gluten; thyroid peroxidase; infant diet; environmental tobacco smoke

INTRODUCTION

Autoimmune thyroid disease (AITD) affects about 5% of the U.S. population (1), making it one of the most common autoimmune disorders in adults and children. Common forms include Hashimoto's thyroiditis (autoimmune hypothyroidism) and Graves' disease (autoimmune hyperthyroidism). Detection of antibodies against thyroid peroxidase (TPO), an enzyme critical for hormone production in the thyroid, is a reliable marker of autoimmune destruction of thyroid tissue (2). TPO antibodies are predictive of AITD in both the general population (3–5) and among those with other autoimmune diseases (6,7).

Clustering of AITD with celiac disease (CD) and type 1 diabetes (T1D) is well-documented (8–13), and suggestive of common etiology. All three conditions are T-cell mediated and share major genetic susceptibility loci in the HLA region (14). Unknown environmental factors are thought to trigger development of each disease, in the context of a high-risk genetic background. Dietary gluten is one candidate risk factor, and may be involved in the development of autoimmune diseases through its role in shaping the gut microbiome, intestinal permeability, or intestinal immunity (15). Gluten intake in early childhood increases risk of CD and CD autoimmunity (CDA) (16,17). The relationship between gluten exposure and T1D or diabetes-related islet autoimmunity (IA) is less consistent (18,19). Contrasting results have been reported describing the relationship between a gluten-free diet and thyroid function and thyroid autoimmunity among celiac disease patients (20–22). However, no prospective study has examined gluten intake as a risk factor for development of thyroid autoimmunity in a population without clinically diagnosed autoimmune disease.

We investigated the association of age at gluten introduction and early childhood gluten intake with risk of thyroid autoimmunity in the prospective Diabetes Autoimmunity Study in the Young (DAISY).

MATERIALS AND METHODS

Study population and outcome

DAISY prospectively follows 2,547 children at increased risk for T1D and CD, recruited between 1993 and 2006 in Denver, CO. Study subjects were derived from two groups: a) healthy general-population newborns screened for specific HLA genotypes, and b) children who had a first-degree relative with T1D. The latter were eligible to enroll if younger than 8 years (1993-1995) or younger than 4 years (1996-2004). Blood samples were drawn at 9, 15 and 24 months of age, and annually thereafter. Samples were screened for autoantibodies related to DAISY'S primary endpoints, T1D and CD. IA was defined as positivity for autoantibodies to insulin, GAD, IA2, or ZnT8 on two consecutive clinic visits, or one positive visit followed by T1D diagnosis (18). CDA was defined as positivity to tissue transglutaminase (tTG) on two consecutive clinic visits (16).

In 2009, the last follow-up sample of every DAISY child was tested for TPO autoantibodies. If that sample was positive, defined as TPO autoantibodies > 4 kronus units/ml (20 IU/ml), previous samples were tested to identify the age at the first positive visit. The primary outcome of thyroid autoimmunity was defined as TPO positivity on at least two visits (TPO Ab+). Families were notified of positive TPO autoantibody status and recommended to follow-up with their primary care provider for additional thyroid function testing. Self-reported clinical diagnosis for AITD was collected in this study, but follow-up thyroid function tests were not performed systematically.

The Colorado Multiple Institutional Review Board approved all DAISY study protocols (COMIRB 92-080). Informed consent was obtained from the parents/legal guardians of all children. Assent was obtained from children age 7 years and older. All research was performed in accordance with relevant guidelines/regulations. Details on the creation of the DAISY cohort and its follow-up methods have been published elsewhere (16,18).

Age at gluten introduction and early gluten intake

Age at gluten introduction and early gluten intake were estimated using the same methodology as previous studies of gluten exposure in the DAISY population (16,18). Parents/guardians of DAISY infants were asked about food introductions and consumption over the previous 3 months by in-person interviews or telephone when the child was 3, 6, 9, 12 and 15 months of age. If a new food item was introduced, the parent/guardian was asked to document which food item and the date of introduction. Gluten introduction was defined as reported first exposure to foods containing wheat, barley or rye (i.e. breads, pastries, pasta, and infant cereals). Age at gluten introduction was categorized into early (<4 months), recommended (4-5.9 months), and late (≥ 6 months) intervals.

Annual semi-quantitative food frequency questionnaires (FFQs) were completed by the parent/guardian starting when the study participant was two years of age. FFQs covered the average diet of the child during the previous year and were validated by 24-hour recalls and biomarker measurements (23,24). Gluten intake amount (grams) was calculated by using a conversion factor of 0.75 to estimate the gluten protein content of foods containing wheat, barley and rye, consistent with other observational studies as well as recent studies in

DAISY exploring early childhood gluten intake as a primary risk factor (16,17,18). Oats are considered safe to eat for those with CD and were not considered a gluten-containing food item (25). Early gluten intake (grams/day) was estimated as the average daily amount of gluten a child ingests between 1-2 years of age.

Additional variables

A validated smoking assessment questionnaire determined the child's exposure to environmental tobacco smoke (ETS) at each visit. Details on the validation of the smoking assessment questionnaire using urinary cotinine have been published (26). Children with any "yes" response to questions, including whether a parent or another person in close contact with the child (i.e., a caregiver or grandparent) smokes, were considered to have ETS exposure.

MHC class II molecules HLA-DQ2 and HLA-DQ8 are closely linked to development of CD and T1D and found to be overrepresented in those with AITD (11). HLA genotyping in DAISY has been well-described elsewhere (27), and was categorized into three groups for current analyses: DQ2/DQ8; DQ2/2 or DQ2/X; and DQ8/DQ8, DQ8/X, or DQX/X where X is neither DQ2 nor DQ8.

Statistical analyses

All analyses were performed with SAS version 9.4 (SAS Institute Inc, Cary, NC). Covariates previously associated with thyroid autoimmunity and important characteristics in the DAISY study were considered for adjustment in multivariable models, including: sex, first-degree relative affected with T1D (T1D-FDR), first-degree relative affected with CD (CD-FDR), high-risk HLA DQ2/8 (versus all other HLA DQ genotypes), non-Hispanic white race-ethnicity, ETS exposure, maternal age, maternal education, and length of duration of any breastfeeding.

Multivariable Cox regression was used to assess the association between age at gluten introduction or early gluten intake, and risk of TPO Ab+. Both continuous (grams/day) and tertiles of early gluten intake were considered. Other variables found to be associated with TPO Ab+ with p-value <0.1 in univariate analyses were included in multivariable models. Efron's approximation was used to handle ties, and no variables violated the proportional hazard assumption. The time-to-event was defined as the age of the first positive TPO test (in TPO Ab+ children); TPO Ab- subjects were censored at the age of the last negative TPO measurement. Children were defined as being IA+ and CDA+ if they developed IA or CDA prior to the first positive TPO measurement (in TPO Ab+ children) or the last negative TPO measurement (in TPO Ab- children).

Children missing TPO and tTG autoantibody measurements (n = 122), adjustment factors such as race/ethnicity, maternal age and education, and ETS exposure (n = 295), or infant gluten exposure data (n = 279) were excluded, resulting in an analysis cohort of 1851 children, of whom 107 (5.8%) developed TPO autoantibodies during follow-up (Figure 1). After excluding 740 children who were missing FFQs at the ages 1-2 years, there were 1,111 children that were available for the early gluten intake analyses.

RESULTS

In univariate analyses, TPO Ab+ children were more likely to be female, to have IA and CDA, and to have a first-degree relative with T1D than those who were TPO Ab- (Table 1). TPO Ab+ children were less likely to be exposed to ETS than TPO Ab- children.

The age at gluten introduction was not associated with development of TPO Ab+, adjusting for sex, exposure to ETS, T1D-FDR, and IA+ and CDA+ status (*p-value* = 0.81, Table 2). Independent of other adjustment factors, females (HR = 2.19, 95%CI: 1.46, 3.27) and IA+ children (HR = 2.20, 95%CI: 1.39, 3.50) were at increased risk of developing TPO Ab+, and children exposed to ETS were less likely to develop TPO Ab+ (HR = 0.46, 95% CI: 0.30, 0.71).

Neither amount of early gluten intake (*p-value* = 0.45) nor tertiles of early gluten intake (*p-value* = 0.381) were associated with development of TPO Ab+, adjusting for sex, exposure to ETS, T1D-FDR, and IA+ and CDA+ status (Table 3).

DISCUSSION

We hypothesized that co-occurring autoimmune diseases, such as CD, T1D, and AITD, may have common dietary risk factors. While the age of gluten introduction and the amount of gluten intake at 1-2 years had been previously identified as risk factors for CDA/CD and IA/T1D in prospective cohorts (16–19), we found no association between gluten exposures and the risk of developing TPO Ab+. These findings expand on previous studies of gluten and thyroid autoimmunity among adults with diagnosed CD (20–22).

Approximately 5% of genetically high-risk children in DAISY developed TPO positivity at an average age of 10 years, similar to the prevalence of TPO positivity at age 10 years in DiPiS, a Swedish birth cohort of children at high-risk for developing T1D and other autoimmune diseases (12). We also confirmed that females and children positive for IA were significantly more likely to develop TPO Ab+ than males or IA-negative children, respectively. Additional environmental risk factors for thyroid autoimmunity had not been previously investigated in high-risk children. While gluten intake was not associated with risk of TPO Ab+, we identified exposure to ETS as protective for the development of TPO Ab+.

A protective relationship between direct smoking and development of autoimmune hypothyroidism and Hashimoto's disease has been previously reported (29). Among U.S. adults, active smokers were less likely to be positive for TPO autoantibodies than non-smokers, and the relationship appeared to be dose-dependent (30). Similar inverse relationships have been reported among Chinese adults (31) and females with a first- or second-degree relative with AITD (32). However, other studies yield contrasting results. In both Korean and Dutch adults (33,34), higher urinary cotinine levels were associated with higher TPO antibody titers. In contrast, direct smoking has been reported to be a dose-dependent risk factor for the development and recurrence of Graves' hyperthyroidism as well as the development and extent of Graves' ophthalmopathy (29). It has been argued that previously reported protective associations may result from residual confounding or from

detection bias since smokers may be more likely to present for detection of thyroid dysfunction. However, our study establishes for the first time that exposure to ETS prospectively alters the risk of developing TPO autoantibodies in children, adjusting for appropriate confounders and using a cotinine-validated self-reported measure. ETS exposure in this study is defined as ever exposure, therefore detailed dose-dependent relationships could not be determined.

The mechanism of ETS exposure or smoking on thyroid function and autoimmune thyroid disease is poorly understood. Animal models of autoimmune thyroiditis suggest that nicotine has anti-inflammatory effects (29), possibly by shifting the balance between pathogenic and protective T-cell responses (35). The contrasting impact of smoking on Graves' disease implies that different mechanisms are involved.

Strengths of our study include the prospective study design, which reduces the likelihood of reverse causality, and the use of validated FFQ and ETS assessments. While there is no standardized method for measuring gluten intake, we estimated gluten intake in foods in a manner similar to other observational studies assessing gluten intake (17,28), and identical to our previous investigation of gluten exposure and risk of CD and T1D (16,18).

This study has also several limitations. Thyroid autoimmunity and AITD were not among the primary endpoints of the DAISY study, hence additional measures of thyroid function were not available. Our definition of thyroid autoimmunity using TPO positivity may incompletely ascertain true cases of thyroid autoimmunity. Conversely, not every individual with TPO positivity has clinical thyroid disease. Therefore, while we are able to report associations of environmental risk factors with incident TPO positivity, our investigation is limited in the potential to estimate cumulative incidence of thyroid disease among high-risk children. Further, we are unable to reliably differentiate type of AITD between Hashimoto's thyroiditis and Graves' disease as follow-up tests for a clinical diagnosis were not performed systematically in this study. While TPO antibodies are found in both diseases, the association is much stronger with hypothyroidism (1). In a similar population, TPO was more predictive of progression to AITD than an alternate measure using thyroglobulin antibody positivity (13), suggesting our study includes the cases of thyroid autoimmunity most relevant to clinical disease.

In summary, though early childhood gluten intake has been associated with increased risk for autoimmunity preceding CD and T1D, we found no association with development of thyroid autoimmunity, as indicated by presence of TPO autoantibodies. Exposure to ETS, however, appears to be protective for thyroid autoimmunity in children and warrants further research.

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Figure 1.

Data flow diagram for gluten intake and risk of thyroid autoimmunity in the DAISY study. Of 1,851 DAISY subjects in the analysis cohort, 107 developed TPO Ab+ during follow-up. Subjects missing autoantibody measurements, multivariable model adjustment factors (race/ethnicity, maternal age and education, and ETS), and age at gluten intake introduction or gluten intake at 1-2 years of age were excluded from analyses. TPO = thyroid peroxidase, tTG = tissue transglutaminase, ETS = environmental tobacco smoke.

Table 1.

Characteristics of DAISY Subjects in the Study Population for Gluten and Thyroid Autoimmunity Risk.

Characteristics	Age at Gluten Introduction			Early Gluten Intake (1-2 years)		
	TPO Ab–	TPO Ab+	<i>P</i> value [*]	TPO Ab–	TPO Ab+	<i>P</i> value [*]
Number of subjects	1744	107		1051	60	
Age at TPO Ab+ or last TPO Ab- measurement, mean (SD)	10.1 (5.1)	9.7 (3.9)	0.339	9.9 (4.4)	9.4 (3.8)	0.3551
Female, n (%)	818 (46.9)	71 (66.4)	0.0001	490 (46.6)	39 (65.0)	0.0051
HLA group, n (%)			0.5783			0.9658
DQ8/8,DQ8/X,DQX/X	949 (54.4)	61 (57.0)		576 (54.8)	34 (56.7)	
DQ2/2,DQ2/X	422 (24.2)	23 (21.5)		210 (20.0)	11 (18.3)	
DQ2/8	373 (21.4)	23 (21.5)		265 (25.21)	15 (25.0)	
Non-Hispanic White, n (%)	1305 (74.8)	87 (81.3)	0.4452	794 (75.6)	49 (81.7)	0.4572
First-Degree Relative with T1D, n (%)	808 (46.3)	64 (59.8)	0.0425	417 (39.7)	30 (50.0)	0.0272
First-Degree Relative with CD, n (%)	78 (4.5)	6 (5.6)	0.9057	50 (4.8)	4 (6.7)	0.7862
IA+ status, n (%)	144 (8.3)	23 (21.5)	0.0005	95 (9.0)	12 (20.0)	0.0158
CDA+ status, n (%)	134 (7.7)	16 (15.0)	0.0918	94 (8.9)	11 (18.3)	0.0664
Environmental tobacco smoke exposure (ever), n (%)	693 (39.7)	30 (28.0)	0.0002	398 (37.9)	17 (28.3)	0.0093
Duration of any breastfeeding (months), mean (SD)	7.1 (7.3)	8.5 (8.5)	0.4217	7.6 (7.4)	8.4 (8.1)	0.6437
Maternal age at delivery (yrs), mean (SD)	30.2 (5.6)	30.6 (5.8)	0.6763	30.8 (5.5)	30.9 (5.3)	0.9074
Maternal education: completed high school, n (%)	1383 (79.3)	87 (81.3)	0.8745	848 (80.7)	53 (88.3)	0.1573
Age at gluten introduction (months), n (%)			0.6902			0.5351
<4	138 (7.9)	7 (6.5)		67 (6.4)	2 (3.3)	
4 to 6	604 (34.6)	37 (34.6)		354 (33.7)	22 (36.7)	
>6	1002 (57.5)	63 (58.9)		630 (59.9)	36 (60.0)	
Gluten intake (g/day), mean (SD)	-	-		11.8(5.5)	12.8 (5.1)	0.3694
Tertile of gluten intake						0.2095
Low, mean (SD)	-	-		6.5 (1.8)	6.9 (1.5)	
Middle, mean (SD)	-	-		10.9 (1.2)	11.2 (1.2)	
High, mean (SD)	-	-		18.2 (4.3)	17.4 (3.5)	

^aHLA = human leukocyte antigen; T1D= Type 1 diabetes; CD = celiac disease; IA = islet autoimmunity (T1D related); CDA = celiac disease autoimmunity

^{*} 2-sided from unadjusted Cox regression, or unpaired t-test (age at TPO only)

Table 2.

Association Between the Age at Gluten Introduction and the Development of Thyroid Autoimmunity in DAISY.

	Unadjusted		Adjusted*	
Characteristic	HR	95% Confidence Interval	HR	95% Confidence Interval
Age at gluten introduction				
< 4 months	0.70	0.31, 1.58	0.80	0.35, 1.80
4-5.9 months	Reference		Reference	
> 6 months	0.97	0.65, 1.46	0.90	0.60, 1.36
Female	2.21	1.48, 3.30	2.19	1.46, 3.27
ETS Exposure (ever)	0.44	0.29, 0.68	0.46	0.30, 0.71
FDR with T1D	1.50	1.01, 2.21	1.33	0.90, 1.98
IA+ status	2.27	1.43, 3.60	2.20	1.39, 3.50
CDA+ status	1.58	0.93, 2.69	1.53	0.90, 2.61

^aETS = environmental tobacco smoke; FDR = first degree relative; T1D= Type 1 diabetes; CDA = celiac disease autoimmunity; IA = islet autoimmunity (T1D related)

* Cox regression adjusted for sex, ETS exposure, FDR-T1D, IA+ status, CDA+ status

Table 3.

Association Between the Amount of Gluten Intake at 1-2 Years and Development of Thyroid Autoimmunity in DAISY.

Characteristic	Unadjusted		Adjusted*	
	HR	95% Confidence Interval	HR	95% Confidence Interval
Gluten (grams/day) at age 1-2y	1.02	0.98, 1.07	1.02	0.97, 1.06
Tertiles of gluten intake at age 1-2y				
Low	Reference		Reference	
Middle	0.91	0.46, 1.81	0.90	0.46, 1.79
High	1.50	0.81, 2.79	1.35	0.72, 2.53
Female	2.13	1.26, 3.63	2.06	1.21, 3.51
ETS Exposure (ever)	0.47	0.27, 0.83	0.50	0.28, 0.89
FDR with T1D	1.79	1.07, 3.00	1.51	0.89, 2.56
IA+ status	2.18	1.16, 4.11	2.06	1.09, 3.90
CDA+ status	1.85	0.96, 3.55	1.76	0.91, 3.40

^aETS = environmental tobacco smoke; FDR = first degree relative; T1D= Type 1 diabetes; CDA = celiac disease autoimmunity; IA = islet autoimmunity (T1D related)

* Cox regression adjusted for sex, ETS exposure, FDR-T1D, IA+ status, CDA+ status