Retrospective Cohort Study
Targeted genotyping for the prediction of celiac disease autoimmunity development in patients with type 1 diabetes and their family members

Maureen M Leonard, Stephanie Camhi, Victoria Kenyon, Rebecca A Betensky, Craig Sturgeon, Shu Yan, Alessio Fasano

Abstract

BACKGROUND
Patients with type 1 diabetes (T1D) and their first-degree relatives (FDRs) have an increased risk of developing celiac disease (CD) compared to the general population. This is largely explained by the shared association with major histocompatibility class II human leukocyte antigen (HLA) DQ2 and/or DQ8 between the two disease states.

AIM
To describe the frequency of CD autoimmunity (CDA) and the distribution of HLA and haptoglobin genotypes in patients with T1D and their FDRs. Additionally, we aimed at identifying predictors associated with an increased risk of developing CDA in patients with T1D and their family members.

METHODS
We obtained clinical information and blood samples from 1027 participants (302 with T1D and 725 FDRs) over a five-year period. Samples were tested for autoantibodies associated with CD, HLA-DQ alleles, and haptoglobin genotype.
STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

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Manuscript source: Unsolicited manuscript

Received: February 6, 2019
Peer-review started: February 9, 2019
First decision: February 19, 2019
Accepted: March 8, 2019
Article in press: March 9, 2019
Published online: March 15, 2019

We fit univariate and multiple logistic regression models for CDA separately for subjects with T1D and for FDRs of subjects with T1D.

RESULTS

Implementation of a screening program increased the frequency of CDA by 2-fold in participants with T1D and 2.8-fold in their FDRs. Multivariate analysis found that, in participants with T1D, having both DR7-DQ2 and DR4-DQ8 was associated with an increased frequency of CDA. In FDRs of T1D patients, reported CD in the family was associated with an increased frequency of CDA during screening. Haptoglobin 2 genotype was not associated with developing CDA in the multivariate analysis.

CONCLUSION

Patients with T1D and their FDRs have a high frequency of CDA. Carrying both DR7-DQ2 and DR4-DQ8 was associated with development of CDA in patients with T1D.

Key words: Screening; Gluten; Diabetic; Coeliac; Haptoglobin; Human leukocyte antigen

INTRODUCTION

Celiac disease (CD) is an autoimmune enteropathy that occurs in genetically susceptible individuals in response to ingestion of gluten. While the worldwide prevalence of this condition is estimated at 1%, prevalence is known to vary among geographical locations and ethnic groups[1]. The prevalence of CD and other autoimmune conditions appears to be on the rise, and yet most patients with CD remain undiagnosed[1-3]. Many of these individuals may be asymptomatic and identified through the screening of high-risk groups. Patients with type 1 diabetes (T1D) constitute a high-risk group given their risk of CD is reported at 3-8 times higher than that of the general population[4-7]. This increased risk is likely due to a shared genetic predisposition with the major histocompatibility (MHC) class II human leukocyte antigen (HLA) DQ2 and/or DQ8 between the two disease states[8,9]. Recent evidence suggests that, like T1D patients, relatives of those with T1D have an increased risk of developing CD by 2 fold in T1D patients and 2.8 fold in their FDRs. Participants with T1D who carry DR7-DQ2/DR4-DQ8 were more likely to screen positive for CD autoimmunity. There was no association between carrying zonulin genetics and an increased risk of developing CD in our cohort. Patients with T1D and their FDRs have an increased risk of developing CD compared to the general population and, given the often-asymptomatic nature of disease, physicians should have a low threshold for screening.

Core tip: Serological screening for celiac disease (CD) autoimmunity in subjects with type 1 diabetes (T1D) and their first-degree relatives (FDRs) found an underestimation of CD by 2 fold in T1D patients and 2.8 fold in their FDRs. Participants with T1D who carry DR7-DQ2/DR4-DQ8 were more likely to screen positive for CD autoimmunity. There was no association between carrying zonulin genetics and an increased risk of developing CD in our cohort. Patients with T1D and their FDRs have an increased risk of developing CD compared to the general population and, given the often-asymptomatic nature of disease, physicians should have a low threshold for screening.

Citation: Leonard MM, Camhi S, Kenyon V, Betensky RA, Sturgeon C, Yan S, Fasano A. Targeted genotyping for the prediction of celiac disease autoimmunity development in patients with type 1 diabetes and their family members. World J Diabetes 2019; 10(3): 189-199

URL: https://www.wjgnet.com/1948-9358/full/v10/i3/189.htm
DOI: https://dx.doi.org/10.4239/wjd.v10.i3.189
common alleles, *HP1* and *HP2*, giving rise to three different *HP* genotypes (*HP1-1*, *HP2-1*, *HP2-2*). The *HP2* allele is found only in humans and only individuals who possess an *HP2* can produce zonulin. Worldwide, the frequency of *HP1-1* varies from 0.07-0.70[19]. In the United States, the frequency ranges from 0.31-0.55, with a frequency of 0.41 reported in Caucasians[19]. Presence of the *HP2* allele has been shown to influence the course of inflammatory disease due to differences in antioxidants, scavenging, and immunomodulatory properties[19]. Previous work has shown that the zonulin gene (*HP2*) is more frequent in chronic inflammatory diseases such as inflammatory bowel disease[20], CD[21], and lupus[22], and that homozygosity for *HP2-2* is associated with more severe clinical manifestations of inflammatory conditions[23]. In patients with T1D, the frequency of *HP2-2* and *HP2-1* genotypes is increased compared to that reported in the general population[24]. Further, the *HP2-2* genotype has been associated with increased risk of coronary artery disease in patients with type-2[25] but not type-1[26] diabetes. In patients with T1D, *HP2-2* has not been significantly associated with diabetic nephropathy[27], but has been associated with an increased risk of cardio-renal mortality[28], including a decline in kidney function and progression to end-stage renal disease[29-31]. Overall, the contribution of *HP* genotype to development of other autoimmune diseases, specifically CD, has not been evaluated. However, given the role of zonulin in the pathogenesis of both T1D and CD, we postulated that patients with T1D or their first-degree relatives (FDRs) would be more likely to develop CD if they carried the *HP2-2* genotype.

Since approximately half of T1D patients who are diagnosed with CD present asymptomatically, and there are no universally accepted screening guidelines to evaluate for CD in patients with T1D or their FDRs, we employed a prospective program to serologically screen patients with T1D and their FDRs for CD[32,33]. We aimed at identifying predictors that may increase the risk of developing CD in patients with T1D, and to understand which individuals from these high-risk families were more likely to develop CD to identify which subjects may benefit most from screening. We hypothesized that patients with T1D who develop CD are younger at the age of T1D diagnosis, more likely to carry DR3-DQ2/DR4-DQ8 and more likely to have zonulin genetics, *HP2*, than patients with T1D without CD. We also hypothesized that FDRs of subjects with T1D will be more likely to develop CD if they are female, complain of GI symptoms, carry DR3-DQ2, and carry the zonulin gene *HP2*.

**MATERIALS AND METHODS**

**Setting**
This study was performed during the Children with Diabetes (CWD) annual conference. CWD is a United States based organization that provides educational and social support for families of children with T1D. We conducted serological screening for CD at CWD’s annual conference over five consecutive years (2013-2017).

**Subjects**
Children and adults attending the CWD conference diagnosed with T1D or with a FDR (parent, child, or sibling) with T1D were eligible for participation. Participants self-selected to participate by visiting our “booth” to conduct study procedures. Written informed consent was obtained from all participants. All study procedures were reviewed and approved by the Partners Human Research Committee Institutional Review Board.

**Clinical information**
Participants and, when necessary, their caregivers (on behalf of a child), completed a brief self-report clinical questionnaire targeted to assess the family history of T1D and CD, presence or absence of CD-associated symptoms in the individual, current diet, and other pertinent medical information.

**Serology**
All subjects underwent venipuncture with an on-site phlebotomist. A minimum of 8 cc of blood was collected from each participant. Serum was evaluated for antibodies to IgA tissue transglutaminase (tTG) and IgG deamidated gliadin peptide (dGP) using QUANTA Lite Rh-tTG IgA ELISA (INOVA Diagnostics, San Diego, CA, United States) on the BioFlash platform. Individuals found to have IgA tTG levels above the kit reference value (> 20 CU) were subjected to confirmatory testing for IgA endomyosial antibodies (EMA) using the NOVA Lite Monkey Oesophagus IFA Kit (Inova Diagnostics, San Diego, CA, United States). Subjects found to have elevated IgG dGP in the absence of elevated IgA tTG were further evaluated for potential IgA...
deficiency. Serum samples for these individuals were sent to an outside lab (LabCorp, Burlington, NC, United States) and a total IgA level was performed using immunoturbidimetric methods.

**HLA determination**

HLA was determined from whole blood samples using the DQ-CD Typing Plus (BioDiagne, Palermo, Italy) according to the manufacturer’s instructions.

**HP genotyping**

HP genotype was determined by either PCR or immunoblot depending on availability of biological samples. For determination by PCR, genomic DNA was extracted from venous blood using QIAamp DNA kit (Qiagen, Hilden, Germany). The genotypes were determined by a novel one step PCR method using primers designed with Primer3 in exon 2 and exon 5 of HP1 corresponding to exons 2 and 7 of HP2. The primers were designed as follows: forward: TTTCTGGCTGCTAAGTTG and reverse: AATGTCTTTCGCTGTTGC. The PCR was performed in 10 uL reactions containing 100 ng purified DNA, 5 uL of 2× MyTaq Red Mix (Bioline, Taunton, MA, United States), and 300 nM of each primer. After PCR, the amplicons were electrophoresed on a 1% agarose gel and read under a UV bulb. The duplication in HP2 results in a size difference of the PCR products (2.5 kb HP1 and 4.3 kb HP2) allowing for differentiation of the two genotypes.

Following screening, all participants were informed of their serological status and genetic compatibility (in regard to HLA only). In the event of abnormal serological findings, patients were instructed regarding necessary follow-up procedures with a local physician or specialist.

**Definition of CD, Celiac disease autoimmunity (CDA) and IgA deficiency**

Participants who self-reported a diagnosis of CD prior to screening were classified as “previous CD” if their diagnosis was based on biopsy or “history of CDA” if their diagnosis was based on bloodwork alone. Patients with positivity for both IgA tTG and IgA EMA at screening were considered, for this study, positive for CD. In the absence of IgA EMA (IgA tTG elevated alone), subjects were classified as demonstrating CDA. Patients with elevated IgG dGP in the absence of elevated IgA tTG were evaluated for potential IgA deficiency. Serum IgA levels less than 7 mg/dL were regarded as IgA deficient. Individuals found to have elevated IgG dGP and IgA deficiency were classified as CDA. For the purposes of the univariate and multivariate analyses, all patients with CD and CDA were combined and are referred to having CDA.

**Statistical analysis**

Categorical data are presented as frequency (percentage). Continuous data are described as mean ± SD if normally distributed and median (interquartile range; IQR) otherwise. All tests of significance were two-sided with $\alpha = 0.05$, and all analysis was performed with SAS 9.4 (Cary, NC). We fit univariate and multiple logistic regression models for CDA, separately for subjects with T1D and for FDRs of subjects with T1D. We included all covariates that had $P$-values less than 0.10 in univariate analyses and in the multiple regression models. We used generalized estimating equations to account for the correlation within families in all analyses; we used an exchangeable working correlation matrix, except for the multiple regression models for individuals with T1D for which we used an independence working correlation matrix due to convergence issues.

**RESULTS**

**Demographics**

Demographic data for participants with T1D, T1D and CDA (T1D+CDA), and FDRs of T1D patients with (FDR + CDA) and without CDA are shown in Table 1. The majority of patients in the study were female, White and not Hispanic. As expected, since screening took place at a conference for children with T1D and their family members, participants with T1D were younger than their FDRs at the time of screening. More than 50% of participants reported being asymptomatic at screening. Participants with CDA prior to or at the time of screening had a higher frequency of reporting a relative with CD and a higher frequency of reporting a relative diagnosed with any autoimmune disease.

**HLA and haptoglobin genetics**

Table 1 demonstrates the frequency of the HLA and haptoglobin genetics for
Table 1  Demographic data: Participants with type 1 diabetes and their first-degree relatives n (%)

| Demographics                          | T1D only (n = 280) | T1D + CD (n = 22) | FDR only (n = 689) | FDR +CDA (n = 36) |
|---------------------------------------|-------------------|-------------------|-------------------|-------------------|
| White                                 | 249 (88.9)        | 22 (100)          | 629 (91.3)        | 35 (97.2)         |
| Not hispanic                          | 188 (67.1)        | 15 (68.2)         | 451 (65.5)        | 27 (75.0)         |
| Female                                | 178 (63.6)        | 17 (77.3)         | 409 (59.4)        | 26 (72.2)         |
| Age at screening (yr), median (range) | 19 (2-72)         | 14.5 (7-43)       | 40 (1-74)         | 39.5 (3-55)       |
| Age at diagnosis of T1D (yr), median (range) | 10 (0.1-64)       | 6.5 (1-21)        |                   |                   |
| Presence of gastrointestinal symptoms (GI sx) | 113 (40.5)       | 6 (27.3)          | 216 (31.4)        | 13 (36.1)         |
| Presence of extraintestinal symptoms (Ex sx) | 78 (27.9)        | 3 (13.6)          | 176 (25.5)        | 7 (19.4)          |
| Both GI and Ex symptoms               | 56 (20)           | 3 (13.6)          | 98 (14.2)         | 4 (11.1)          |
| Asymptomatic                          | 144 (51.6)        | 16 (72.7)         | 394 (57.3)        | 20 (55.6)         |
| Human leukocyte antigen (HLA) genotype |                   |                   |                   |                   |
| DR3-DQ2                               | 49 (18)           | 2 (9.1)           | 148 (21.8)        | 10 (28.6)         |
| DR3-DQ2 homozygote                    | 18 (6.6)          | 3 (13.6)          | 25 (3.7)          | 3 (8.6)           |
| DR7-DQ2                               | 12 (4.4)          | 1 (4.6)           | 41 (6.1)          | 2 (5.7)           |
| DR7-DQ2 homozygote                    | 2 (0.7)           | 0 (0)             | 13 (1.9)          | 0 (0)             |
| DR3/DR7-DQ2 homozygote                | 1 (0.37)          | 0 (0)             | 20 (3.0)          | 3 (8.6)           |
| DR4-DQ8                               | 83 (30.5)         | 5 (22.7)          | 205 (30.2)        | 10 (28.6)         |
| DR3-DQ2/DR4-DQ8                       | 70 (25.7)         | 7 (31.8)          | 50 (7.4)          | 6 (17.1)          |
| DR7-DQ2/DR4-DQ8                       | 6 (2.2)           | 4 (18.2)          | 33 (4.9)          | 0 (0)             |
| DQ2/DQ8 negative                      | 31 (11.4)         | 0 (0)             | 142 (20.9)        | 1 (2.9)           |
| Haptoglobin genotype (HP) (Zonulin)    |                   |                   |                   |                   |
| HP 1-1                                | 46 (16.4)         | 2 (9.1)           | 120 (17.4)        | 8 (22.2)          |
| HP 2-1                                | 118 (42.1)        | 10 (45.5)         | 278 (40.4)        | 21 (58.3)         |
| HP 2-2                                | 116 (41.4)        | 10 (45.5)         | 290 (42.2)        | 7 (19.4)          |
| Any HP2                               | 234 (83.6)        | 20 (90.9)         | 568 (82.6)        | 28 (77.7)         |

T1D: Type 1 diabetes; CDA: Celiac disease autoimmunity; FDRs: First-degree relatives; GI sx: Gastrointestinal Symptoms; Ex sx: Extraintestinal Symptoms; HLA: Human leukocyte antigen; HP: Haptoglobin genotype.

participants who underwent screening. All individuals with known or newly diagnosed CDA, except for one, carried HLA-DQ2 or 8. The T1D + CDA and FDR + CDA groups demonstrated a higher frequency of HLA DQ2 compared to T1D and FDRs without CDA. Overall, participants in this cohort had a higher frequency of carrying HP2 (in heterozygosity or homozygosity) than previously published work reporting the frequency of HP genotypes in the general population[18,19].

Prevalence of CD and CDA

Table 2 reports the prevalence of CD and CDA in the screened participant cohort. Prior to our screening program, 3.7% of participants with T1D and 1.8% of FDRs reported a diagnosis of CD or history of CDA. After screening, the estimated prevalence of CDA in our cohort increased by two-fold in patients with T1D and by 2.8-fold in FDRs. One participant in the cohort was found to have elevated IgG dGP and IgA deficiency and was classified as CDA.

Univariate and Multivariate Analysis

Participants with T1D: The univariate analyses of participants with T1D (Table 3) showed that an older age at study entry and older age of onset of T1D are associated with lower risk of screening positive for CDA. In addition, the following characteristics (in T1D patients) were associated with higher risk of CDA in our cohort: absence of symptoms, carrying DR7-DQ2/DR4-DQ8, first-degree relation to an individual with CD and first-degree relation to an individual with thyroid disease and/or any autoimmune disease. In multiple regression analysis of subjects with T1D (Table 3), carrying DR7-DQ2/DR4-DQ8 remained highly significantly associated with screening positive for CDA.

FDRs of Participants with T1D: The univariate analyses of FDRs of participants with T1D (Table 4) showed that absence of DQ2/DQ8 and presence of HP2-2 are both associated with lower risk of CDA. Carrying DR3-DQ2 in homozygosity, DR3-DQ2/DR4-DQ8, and HP2-1 are all associated with a higher risk of screening positive
Table 2  Cohort prevalence of celiac disease and celiac disease autoimmunity

|                  | n  | CD | CDA | CD at screening | CDA at screening | CDA prevalence estimation |
|------------------|----|----|-----|-----------------|------------------|--------------------------|
| Type 1 diabetes  | 302| 8  | 3   | 8 (2.5)         | 3 (0.7)          | 22 (7.3)                 |
| First-degree relative | 725| 9  | 4   | 18 (2.7)        | 5 (1.0)          | 36 (5.0)                 |

T1D: Type 1 diabetes; FDRs: First-degree relatives; CD: Celiac disease; CDA: Celiac disease autoimmunity.

for CDA. Risk of CDA was increased in those who reported a diagnosis of CD in a FDR and a history of any other autoimmune disease in a FDR. In multiple regression analyses of FDRs of subjects with T1D (Table 4), including the significant predictors from the univariate analyses, only reporting CD in a FDR is highly significantly associated with screening positive for CDA.

DISCUSSION

Though patients with T1D have been identified as a population at risk for CD who would benefit from routine screening, the optimal timing, frequency, and provider to spearhead this effort remain the object of debate. Implementation of our screening program in this known high-risk population and their FDRs revealed that a large proportion of individuals with undiagnosed CDA. Indeed, our active screening uncovered a two-fold increase in CDA in participants with T1D and a 2.8-fold increase in CDA in their FDRs. Furthermore, participants with T1D who screened positive for CDA were more frequently asymptomatic than participants with T1D only suggesting that routine screening is necessary to identify these patients.

Our study aimed at identifying clinical and laboratory characteristics to predict which individuals among this high-risk subgroup (T1D patients and their FDRs) may benefit most from screening for CD. While we did not find any significant clinical predictors of developing CD in our cohort, participants with T1D who screened positive for CDA had a trend towards a younger age at T1D onset and were less likely to report extra-intestinal or gastrointestinal symptoms than participants with T1D alone. Previous work has shown that up to 85% of patients with T1D who screen positive for CD are asymptomatic[4]. While data is mixed, some studies suggest gastrointestinal symptoms are more frequent in patients with long-term T1D compared to control patients[5]. Given that patients with T1D and CDA had a lower median age at the time of screening and had a narrower age range than patients with T1D alone, it is possible that patients with both T1D and CDA were diagnosed with T1D for a shorter period of time. Additionally, studies suggest that patients with T1D and poor glycemic control have more frequent GI symptoms[6-8]. Since we did not perform additional testing to assess glycemic control it is possible that more patients with T1D alone had poor glycemic control and a greater frequency of GI symptoms. Finally, our analysis of patients with T1D and CDA included eight patients who were diagnosed with CD prior to our screening program and thus were already on treatment for CD. Therefore, the low frequency of symptoms in patients with T1D and CDA may be explained by the inclusion of these patients already on a gluten free diet.

We also sought to describe the distribution of HLA and haptoglobin genotypes in our cohort and for the first time utilize the haptoglobin genotypes in a translational approach to identify predictors that may help to establish which patients among this unique cohort are more likely to develop CD. Our findings that participants with T1D are more likely to carry HLA DQ8 and participants with CD are more likely to carry HLA DQ2 compared to those without these conditions are in agreement with the published literature[9-11]. Moreover, our findings that HP2-1 and HP2-2 are more frequent in this cohort compared to the general population is expected and in agreement with previous work due to the association of HP2 with autoimmune conditions[12]. These findings, along with those from our univariate analysis showing that lack of HLA DQ2/8 is associated with a lower risk of CDA, further establish that our cohort is well defined and that HLA DQ typing and analysis is robust.

The HLA genetics DR7-DQ2/DR4-DQ8 was significantly associated with screening positive for CDA in participants already diagnosed with T1D in our cohort. This is particularly interesting given that, while DR3-DQ2 is known to have a strong association with CD, DR7-DQ2 for some time had been overlooked as a risk allele for CD, with commercial clinical labs often not evaluating for this allele or mistakenly interpreting it as not increasing the risk of CD. While DR3-DQ2 is more frequent in patients with CD, 4.4% of patients carry DR7-DQ2[13]. Furthermore, studies suggest...
that, in patients at-risk for CD, the presence of DR7-DQ2 with DR3-DQ8 is associated with an increased frequency of developing CD\cite{37}. Our findings are similar given that, in patients at-risk for CD, the presence of DR7-DQ2 with DR3-DQ8 is associated with an increased frequency of developing CD\cite{37}. However, the purpose of our study was to identify predictors from a high-risk group. In our study, HLA type and having a family history of autoimmune disease were the strongest predictors of developing CD. While we did not identify significant differences in HP genotype in this cohort, HP2-1 underrepresentation of HP2-1 in patients with both T1D and CD. Interestingly in FDRs with CD, \cite{37} combination with DR4-DQ8 have a high frequency of screening positive for CDA. While we did not identify significant differences in HP genotype in this cohort, HP2-1 underrepresentation of HP2-1 in patients with both T1D and CD. Interestingly in FDRs with CD, \cite{37} combination with DR4-DQ8 have a high frequency of screening positive for CDA.

| Estimate | Se | Lower limit | Upper limit | Z stat | P-value |
|----------|----|-------------|-------------|--------|---------|
| Female   | 0.6562 | 0.5151 | -0.3534 | 1.6659 | 1.27 | 0.2027 |
| Age      | -0.0314 | 0.0152 | -0.0611 | -0.0017 | -2.07 | 0.0383 |
| Onset of T1D | -0.0658 | 0.0342 | -0.1329 | 0.0013 | -1.92 | 0.0546 |
| Gastrointestinal (GI) symptoms | -0.5812 | 0.491 | -1.5435 | 0.3811 | -1.18 | 0.2365 |
| Extraintestinal (EX) symptoms | -0.9204 | 0.6391 | -2.1731 | 0.3323 | -1.44 | 0.1498 |
| Both GI and EX symptoms | -0.4724 | 0.6385 | -1.7239 | 0.7791 | -0.74 | 0.4594 |
| No GI or EX symptoms | 0.9074 | 0.4931 | -0.059 | 1.8737 | 1.84 | 0.0657 |
| Human Leukocyte Antigen (HLA) DQ2-DR3 Heterozygous | -0.7402 | 0.7571 | -2.2241 | 0.7437 | -0.98 | 0.3282 |
| HLA DQ2-DR3 Homozygous | 0.7845 | 0.67 | -0.5332 | 2.1019 | 1.17 | 0.2433 |
| HLA DQ2-DR7 Heterozygous | 0.028 | 1.0649 | -2.0592 | 2.1152 | 0.03 | 0.979 |
| HLA DQ8 | -0.3975 | 0.5224 | -1.4233 | 0.6283 | -0.76 | 0.4475 |
| HLA DQ2-DR3/DQ8 | 0.2767 | 0.4814 | -0.6668 | 1.2203 | 0.57 | 0.5654 |
| HLA DQ2-DR7/DQ8 | 2.4851 | 0.6507 | 1.2098 | 3.7604 | 3.82 | 0.0003 |
| HLA DQ2 Heterozygous | -0.575 | 0.6367 | -1.823 | 0.6729 | -0.9 | 0.3665 |
| HLA DQ2 Homozygous | 0.6192 | 0.666 | -0.6862 | 1.9246 | 0.93 | 0.3525 |
| Haptoglobin genotype (HP) 1-1 | -0.6932 | 0.7671 | -2.1967 | 0.8104 | -0.9 | 0.3662 |
| HP 2-1 | 0.1539 | 0.4414 | -0.7112 | 1.0191 | 0.35 | 0.7273 |
| HP 2-2 | 0.1525 | 0.4422 | -0.7141 | 1.0192 | 0.34 | 0.7301 |
| Any HP2 | 0.693 | 0.767 | -0.81 | 2.197 | 0.9 | 0.366 |
| First degree relative (FDR) with celiac disease (CD) | 1.4091 | 0.6188 | 0.1962 | 2.622 | 2.28 | 0.0228 |
| FDR with Type 1 diabetes (T1D) | 0.0724 | 0.557 | -1.0193 | 1.1641 | 0.13 | 0.8966 |
| FDR with thyroid disease | 0.7839 | 0.4619 | -0.1215 | 1.6892 | 1.7 | 0.0897 |
| FDR with other autoimmune disease | 1.1634 | 0.4432 | 0.2947 | 2.0321 | 2.62 | 0.0002 |
| CD in another relative | 0.4136 | 0.6598 | -0.8796 | 1.7068 | 0.63 | 0.5307 |

Significant findings indicated by superscripts. T1D: Type 1 diabetes; FDRs: First-degree relatives; CD: Celiac disease; CDA: Celiac disease autoimmunity; GI sx: Gastrointestinal symptoms; Ex sx: Extraintestinal symptoms; HLA: Human leukocyte antigen; HP: Haptoglobin genotype.
significant findings indicated by superscripts. T1D: Type 1 diabetes; FDRs: First-degree relatives; CD: Celiac disease; CDA: Celiac disease autoimmunity; GI sx: Gastrointestinal symptoms; Ex sx: Extraintestinal symptoms; HLA: Human leukocyte antigen; HP: Haptoglobin genotype.

Table 4 Univariate and multiple regression models: Factors related to celiac disease autoimmunity in first degree relatives of participants with type 1 diabetes

| Estimate | Se | Lower limit | Upper limit | Z stat | P-value |
|----------|----|-------------|-------------|--------|---------|
| **Univariate model (exchangeable correlation)** | | | | | |
| White | 1.26 | 0.88 | -0.47 | 2.98 | 1.43 | 0.154 |
| Female | 0.5433 | 0.3674 | -0.1768 | 1.2635 | 1.48 | 0.1392 |
| Age | -0.0046 | 0.0097 | -0.0236 | 0.0244 | -0.47 | 0.6355 |
| Gastrointestinal (GI) symptoms | 0.2551 | 0.3395 | -0.4103 | 0.9206 | 0.75 | 0.4524 |
| Extraintestinal (EX) symptoms | -0.3748 | 0.4996 | -1.2756 | 0.526 | -0.82 | 0.4148 |
| Both GI and EX symptoms | -0.2715 | 0.5305 | -1.3113 | 0.7682 | -0.51 | 0.6087 |
| No GI or EX symptoms | -0.085 | 0.3419 | -0.7551 | 0.5851 | -0.25 | 0.8036 |
| Human Leukocyte Antigen (HLA) DQ2-DR3 Heterozygous | 0.4255 | 0.383 | -0.3251 | 1.1761 | 1.11 | 0.2666 |
| HLA DQ2-DR3 Homozygous | 0.8042 | 0.7654 | -0.696 | 2.304 | 1.05 | 0.2934 |
| HLA DQ2-DR7 Heterozygous | -0.0376 | 0.6636 | -1.3382 | 1.263 | -0.06 | 0.9548 |
| HLA DQ2-DR3/DR7 Homozygous | 1.02 | 0.715 | -0.381 | 2.42 | 1.43 | 0.153 |
| HLA DQ8 | -0.0981 | 0.4058 | -0.8934 | 0.6972 | -0.24 | 0.809 |
| HLA DQ2-DR3/DQ8 | 0.9272 | 0.472 | 0.0021 | 1.8523 | 1.96 | 0.0495 |
| HLA DQ2/DQ8 Negative | -2.1856 | 1.0232 | -4.1891 | -0.1781 | -2.13 | 0.0328 |
| HLA DQ2 Heterozygous | 0.3561 | 0.388 | -0.4043 | 1.1166 | 0.92 | 0.3586 |
| HLA DQ2 Homozygous | 0.6842 | 0.5435 | -0.3811 | 1.7495 | 1.26 | 0.2081 |
| Haptoglobin genotype (HP) 1-1 | 0.2901 | 0.4341 | -0.5607 | 1.1409 | 0.67 | 0.5039 |
| HP 2-1 | 0.6823 | 0.3429 | 0.0103 | 1.3544 | 1.99 | 0.0466 |
| HP 2-2 | -1.0763 | 0.4252 | -1.9096 | -0.2429 | -2.53 | 0.0114 |
| Any HP2 | -0.29 | 0.434 | -1.141 | 0.561 | -0.67 | 0.503 |
| First degree relative (FDR) with Celiac disease (CD) | 1.6768 | 0.3809 | 0.9303 | 2.4233 | 4.4 | <.0001 |
| FDR with thyroid disease | -0.503 | 0.4849 | -1.4533 | 0.4473 | -1.04 | 0.2995 |
| FDR with other autoimmune disease | 0.6817 | 0.5606 | -0.025 | 1.3884 | 1.89 | 0.0587 |
| CD in other relative | 0.5217 | 0.7115 | -0.8728 | 1.9161 | 0.73 | 0.4634 |
| **Multiple regression model (independence working correlation)** | | | | | | |
| Intercept | -2.8829 | 0.404 | -3.6746 | -2.0912 | -7.14 | < 0.0001 |
| HLA DQ2-DR3/DQ8 | 0.9407 | 0.5381 | -0.1139 | 1.9953 | 1.75 | 0.0804 |
| HLA DQ2/DQ8 Negative | -1.9348 | 1.0275 | -3.9487 | 0.0791 | -1.88 | 0.0597 |
| HP 2-1 | 0.0984 | 0.4492 | -0.8001 | 0.9609 | 0.18 | 0.858 |
| HP 2-2 | -1.0149 | 0.563 | -2.1185 | 0.0886 | -1.08 | 0.1715 |
| FDR with CD | 2.3603 | 0.7576 | 0.8787 | 3.8483 | 3.12 | 0.0018 |
| FDR with other autoimmune disease | -0.8269 | 0.7245 | -2.2468 | 0.5932 | -1.14 | 0.2538 |

Limitations of our study include utilization of self-reported family history of CD, T1D, and other autoimmune diseases, and lack of endoscopy to confirm CD in patients found to have CDA at screening. While this was not feasible in this screening study, all patients that had a positive serologic test for CD were advised to undergo further confirmatory testing with repeat blood work and an endoscopy. All patients with a positive IgA tTG had a second confirmatory test with IgA EMA. The majority of patients in our cohort with elevated IgA tTG additionally tested positive for IgA EMA making a diagnosis of CD likely. However, for the purposes of analysis, we combined participants with a positive IgA tTG alone and those with a positive IgA tTG and EMA in our CDA estimate. The possibility for falsely elevated or transiently elevated IgA tTG in patients with T1D and other autoimmune disorders is well known, thus our CDA estimate may be an overestimation. However, our prevalence estimates are in line with previously published work.\(^{4,5,7}\). Finally, to assess follow-up

carrying HP2 is not a predictor of developing autoimmune disease in this already high-risk population there was a trend towards an increased representation of HP2 in patients with T1D and CDA. Further, it is unclear why FDR have a lower risk of developing CDA if they carry HP2 in homozygosity and future work evaluating this finding in a larger cohort is needed.

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care in our cohort, a questionnaire was sent to participants during three years of the five-year study. Approximately 40% \((n = 24)\) of participants with CDA responded to the questionnaire. Of those 38% \((n = 9)\) of participants sought follow-up of their positive serology with a physician and the majority underwent endoscopy \((n = 7)\). This highlights an additional limitation of screening studies in that appropriate follow-up is not ensured despite our efforts to provide educational materials and guidance.

In conclusion, implementation of a screening program increased identification of CDA in participants with T1D and their FDRs by 2 and 2.8-fold respectively. Participants with T1D carrying DR7-DQ2/DR4-DQ8 were more likely to screen positive for CDA. Haptoglobin genotype did not predict the development of CDA in this high-risk population. Patients with T1D and their FDRs have an increased risk of developing CD compared to the general population, and given the often asymptomatic nature of disease; physicians should have a low threshold for screening.

**ARTICLE HIGHLIGHTS**

**Research background**
Patients with type 1 diabetes (T1D) and their first-degree relatives (FDRs) are at increased risk of developing celiac disease (CD). The majority of patients with T1D and CD are asymptomatic at diagnosis and there are no universally accepted screening guidelines to evaluate for CD in patients with T1D or their FDRs. We employed a prospective program to serologically screen patients with T1D and their FDRs for CD. We then retrospectively aimed to identify clinical and genetic predictors that may increase the risk of developing CD in this cohort of individuals at high-risk of developing CD.

**Research motivation**
Patients with T1D are up to eight times more likely to develop CD, and their FDR’s are up to six times more likely to develop CD. Given that many may be asymptomatic, there is a need to identify predictors of CDA development in this high-risk cohort. The main topics, the key problems to be solved, and the significance of solving these problems for future research in this field should be described in detail.

**Research objectives**
Our objective was to identify clinical and genetic predictors that may increase the risk of developing CD in patients with T1D. In addition, we aimed to understand which FDRs of the patients with T1D, who are already at an increased risk of developing autoimmune disease, were more likely to develop CD. Our ultimate goal was to identify which subjects may benefit most from screening to help guide future screening recommendations.

**Research methods**
Participants included patients diagnosed with T1D or FDR of a patient with T1D attending the annual Children with Diabetes (CWD) conference over a 5 year time period. Participants answered clinical questionnaires and had blood drawn for CD serological testing and genotyping. Prevalence of celiac disease autoimmunity (CDA) was described. We then retrospectively fit univariate and multiple logistic regression models for CDA, separately for subjects with T1D and for FDRs of subjects with T1D accounting for the correlation within families when indicated in order to identify predictors of developing CDA.

**Research results**
Implementation of a prospective screening program in patients with T1D and their FDRs increased identification of CDA by 2 and 2.8-fold respectively. Participants with T1D carrying DR7-DQ2/DR4-DQ8 were more likely to screen positive for CDA. In FDRs of patients with T1D, screening positive for CDA was significantly increased in those who reported having a family member diagnosed with CD. Haptoglobin genotype did not predict the development of CDA in this high-risk population.

**Research conclusions**
CDA is under recognized in patients with T1D and their FDR’s and that prospective screening in this high-risk cohort increased the identification of CDA by at least 2 fold. Clinical symptoms were not helpful in distinguishing patients with CDA, as the majority of patients reported no symptoms. Haptoglobin genotype was not found to be a predictor of CDA in this cohort. In our cohort, FDRs of patients with T1D were more likely to screen positive for CDA if they had a family history of CD, while patients with T1D who carried the HLA genotype DR7-DQ2/DR4-DQ8 were more likely to screen positive for CDA.

**Research perspectives**
Given the high frequency of CDA in patients with T1D and their FDRs, physicians should have a low threshold to screen for CDA even in the absence of symptoms.
ACKNOWLEDGEMENTS

We would like to thank the Children with Diabetes organization and the patients and families that participated in this study.

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