Use of HLA Typing in Diagnosing Celiac Disease in Patients With Type 1 Diabetes

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OBJECTIVE — This study examines the use of HLA typing for the diagnosis of celiac disease in a group of Australians with type 1 diabetes.

RESEARCH DESIGN AND METHODS — Subjects included 131 sequential patients with type 1 diabetes (mean age 17 years [range 10–37]), 77 patients with biopsy-proven celiac disease (mean age 52 years [range 12–84]), and 162 healthy control subjects (mean age 17 years [range 2 months to 56 years]). Subjects were prospectively screened for celiac disease using endomysial antibodies (EMAs), tissue transglutaminase antibodies (TTGAs), and celiac disease–specific HLA typing.

RESULTS — Celiac disease was diagnosed in 11 subjects after an intestinal biopsy (prevalence 8.4%). There was 95% agreement between TTGA and EMA for positive results and 100% for negative results. There was no significant difference for HLA DQ2 and DR4 among patients with type 1 diabetes with or without celiac disease.

CONCLUSIONS — The prevalence of celiac disease among patients with type 1 diabetes is higher than previously estimated in Australia. TTGA is a valuable diagnostic tool that can be used for screening celiac disease in patients with type 1 diabetes. HLA typing should not be used in the diagnosis of celiac disease in patients with type 1 diabetes because of the similarities of HLA types between patients with type 1 diabetes and those with celiac disease.

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Celiac disease is a multietiological condition caused by intolerance to ingested wheat gluten or related proteins from barley and rye in genetically predisposed individuals (1). It is a disease associated with a wide spectrum of clinical symptoms and associated conditions in both adults and children including abdominal distention, diarrhea, malnutrition, weight loss, iron and vitamin deficiency, osteoporosis, and infertility (2,3). The association between celiac disease and type 1 diabetes is well established, with studies showing the prevalence of celiac disease in individuals with type 1 diabetes ranging between 0.97 and 16.4% (4–6).

There is considerable genetic influence in celiac disease, with 90–100% of individuals with celiac disease possessing either the class II HLA molecule DQ2 and/or DQ8 compared with 30% of the general population. Recently, several studies have suggested that the detection of this molecule could be used in the diagnosis of celiac disease (7,8). In this study, we measured the frequency of the DQ2 allele in a population of children with type 1 diabetes, individuals with celiac disease, and normal control subjects. We also measured the frequency of DR4, which is in linkage disequilibrium with DQ8 and has been shown to be a significant risk factor in patients with celiac disease without DQ2 (9).

In this study, we aimed to determine whether measuring the at-risk HLA alleles can assist in diagnosis of celiac disease by comparing the presence of the DQ2 allele in patients with type 1 diabetes with or without celiac disease. We also compared the efficiency of tissue transglutaminase antibodies (TTGAs) and endomysial antibodies (EMAs) in diagnosing celiac disease.

RESEARCH DESIGN AND METHODS

— The study group included 131 patients (mean age 17 years [range 10–37], 63 male and 68 female) with type 1 diabetes who sequentially attended The Children's Hospital at Westmead for assessment of diabetic complications. The control group included 162 healthy subjects (mean age 17 years [range 2 months to 56 years], 70 male and 92 female). The celiac disease control group contained 77 subjects (mean age 52 years [range 12–84], 20 male and 57 female) with biopsy-proven celiac disease.

Blood (1–9 ml) was collected, and DNA was extracted from all subjects. Serum from the adult normal control subjects and patients with type 1 diabetes was analyzed for IgA TTGA, IgG and IgA EMA, and total IgA.

IgA and IgG EMAs were detected by dual-conjugate indirect immunofluorescence on monkey esophagus (Immco Diagnostics, Buffalo, NY). Results were considered positive when a reticular pattern of immunofluorescence was ob-

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Abbreviations: EMA, endomysial antibody; HSAP, human signaling lymphocyte activation molecule–associated protein; TTGA, tissue transglutaminase antibody.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Frequency of DQA1*501, DQB1*201, and DRB1*04 alleles*

	Type 1 diabetes			
	Without celiac disease	With celiac disease	Celiac disease only	Control subjects
DQA1*501-DQB1*201	70 (59)	10 (77)	70 (91)	38 (24)*
DRB1*04	81 (69)	7 (54)	5 (6)†	27 (17)‡
DRB1*04 in the absence of DQA1*501- B1*201#	40 (83)	3 (100)	2 (29)§	37 (29)‖
Absence of DRB1*04 and DQA1*501-DQB1*201	9 (8)	0 (0)	1 (1)	86 (53)*

Data are *n* (%). *$P < 0.001$, patient groups vs. control; †$P < 0.001$, type 1 diabetes with or without celiac disease vs. celiac disease only; ‡$P < 0.001$, type 1 diabetes without celiac disease vs. control or $P = 0.001$ vs. type 1 diabetes with celiac disease; §$P < 0.001$, type 1 diabetes without celiac disease vs. type 1 diabetes with celiac disease; ‖$P < 0.001$, type 1 diabetes without celiac disease and celiac disease only.

served in the muscularis mucosa at a serum dilution ≥1:2.5 as recommended by the manufacturer. The end titer of EMAs in positive results was determined by further dilutions to 1:160. Results were checked by a second reader, blinded to the first reader's result.

The presence of TTGAs was determined by enzyme-linked immunosorbent assay using human recombinant tissue transglutaminase–coated microplates (Eurospital Eu-tTG IgA, Trieste, Italy). Results were expressed as arbitrary units and considered positive at levels ≥7 arbitrary units (10).

Total serum IgA was measured by immunonephelometry on an Immage nephelometer (Beckman-Coulter, Gladesville, Australia).

Genomic DNA was extracted from anticoagulated whole-blood lymphocytes (Qiagen QIAamp DNA Blood Mini Kit). The presence of the alleles DQA1*501, DQB1*201 (DQ2), and DRB1*04 (DR4) were detected by PCR modified from Sacchetti et al. (9). The final volume of PCR mixture (50 μl) contained 100 ng of genomic DNA; 250 μmol/l each of dATPs, dCTPs, dGTPs, and dTTPs; RedTaq PCR buffer (Sigma; 10 mmol/l Tris-HCl, pH 8.3, 50 mmol/l KCl, 0.1% gelatin); 1.5 mmol/l of $MgCl_2$; 1 unit of RedTaq DNA polymerase (Sigma); 0.4 μmol/l of exon 2 of human signaling lymphocyte activation molecule–associated protein (HSAP) gene (housekeeping gene) primers (forward 5′GTGGTTGGG CAGATACAATATGG 3′ and reverse 5′G CTAAACAGGACTGGGACCAAAA 3′); and 0.6 μmol/l of DQA1*501 primers (forward 5′AGCAGTTCTACGTGGACC TGGGG 3′ and reverse 5′GGTAGAGTT GGAGCGTTTAATCAGA 3′) and DQB1*201 primers (forward 5′CGCGTG CGTCTTGTGAGCAGAAG 3′ and reverse 5′GGCGGCAGGCAGCCCCAGCA 3′) or 0.6 μmol/l of DRB1*04 primers (forward 5′GGTTAAACATGAGTGTCAT TTCTTAAAC 3′ and reverse 5′GTTGTG TCTGCAGTAGGTGTC 3′). The PCR condition included a 2-min denaturation step at 94°C, followed by 30 1-min cycles at 95°C, 45 s at 64°C (amplification of DQA1*501 and DQB1*201) or 60°C (amplification of DRB1*04), 1 min at 72°C, and then a final step of 10 min at 72°C. All PCRs were performed in duplicate on separate occasions and included DNA from patients who previously tested positive and DNA from subjects who previously tested negative to act as positive and negative controls, respectively. In addition, commercial controls were used for both DQ2 and DQ8.

Amplified PCR products were separated using polyacrylamide gel electrophoresis and visualized under ultraviolet illumination. The product sizes were 435 bp for HSAP, 217 bp for DRB1*04, 149 bp for DQA1*501, and 108 bp for DQB1*201.

Statistical analysis was performed using the Statistical Package for the Social Sciences software (SPSS 11.0; SPSS, Chicago, IL). Pearson's χ^2 or Fisher's exact test (expected value <5) was used to analyze the allelic and genotypic frequencies. $P \leq 0.05$ was considered significant. Sharpened Bonferroni correction method was performed to adjust for individual α levels.

This study was approved by the Central Sydney Area Health Service Ethics Review Committee and The Children's Hospital at Westmead Ethics Committee. Informed consent was obtained from all subjects or from parents/guardians of subjects <18 years of age.

RESULTS — Thirteen of the 131 subjects with type 1 diabetes (10%) had EMA. Eleven subjects agreed to a duodenal biopsy, all showing villous atrophy consistent with celiac disease, giving a prevalence of 8.4%. One of 63 (2%) presumed healthy adults had EMA. This subject was excluded from the study.

The distribution of HLA class II alleles among all patient groups and control subjects is shown in Table 1. Seventy (59%) patients with type 1 diabetes only, 10 (77%) patients with type 1 diabetes and celiac disease, and 47 (81%) patients with celiac disease only were DQA1*501 and DQB1*201 (DQ2) positive. The difference between each of these groups and the control subjects (24%) was significant ($P < 0.001$). In contrast, there was no significant difference between the three patient groups for the presence of DQA1*501-DQB1*201 alleles.

The presence of DRB1*04 alleles in the absence of DQA1*501-DQB1*201 alleles was found in 40 (83%) patients with type 1 diabetes and 3 (100%) patients with both type 1 diabetes and celiac disease. Both frequencies were significantly higher than those of patients with celiac disease (27%, $P < 0.001$) and control subjects (29%, $P < 0.001$). However, no significant difference was found among patients with celiac disease compared with patients with both type 1 diabetes and celiac disease.

The absence of DQA1*501-DQB1*201 and DRB1*04 (DR4) alleles was highest among the control group. All patients with both type 1 diabetes and celiac disease had either DQA1*501-DQB1*201 and/or DRB1*04 alleles.

The mean age of 13 patients with type 1 diabetes and elevated antibodies was 15 years (Table 2). All 13 patients were white, of which 7 were male and 6 were female (male-to-female ratio of 1.2:1). Of the 11 patients with type 1 diabetes and biopsy-proven celiac disease, only 1 patient (10%) showed celiac disease–related symptoms. The mean age of diagnosis of these 11 patients was 6 years for type 1 diabetes and 15 years for celiac disease.

A female subject aged 30 years, recruited as part of the control population, was found to have a positive EMA result

Table 2—*Characteristics of patients with type 1 diabetes and celiac disease*

Age diagnosed with type 1 diabetes (years)	EMA titer	TTA (arbitrary units)	HLA alleles	Celiac disease symptoms	Biopsy-proven celiac disease
Female					
6	≥1:160	19	DRB1*04	Migraines	Yes
5	1:80	13	DRB1*04	None	Yes
13	1:40	8	DQA1*501-DQB1*201 DRB1*04	None	Yes
8	1:80	20	DQA1*501-DQB1*201 & DRB1*04	None	Yes
16	1:40	13	DQA1*501-DQB1*201 DRB1*04	None	Yes
6	1:2.5	0.01*	DQA1*501-DQB1*201 & DRB1*04	IgA deficiency	No
Male					
7	1:40	15	DQA1*501-DQB1*201	None	Yes
8	≥1:160	20	DRB1*04	None	Yes
13	≥1:160	20	DQA1*501-DQB1*201	Weight loss	Yes
9	1:80	8	DQA1*501-DQB1*201	None	Yes
9	≥1:160	20	DQA1*501-DQB1*201	None	Yes
5	1:80	6	DQA1*501-DQB1*201	None	Yes
14	≥1:160	15	DQA1*501-DQB1*201	None	No

Positive IgA tissue transglutaminase (TTA) ≥7 arbitrary units. *Patient with total serum IgA <0.006 g/l.

and symptoms consistent with celiac disease including iron deficiency with anemia and nausea. The subject had an equivocal duodenal biopsy (equivalent to Marsh grade I) and was later diagnosed with latent celiac disease.

Among the 13 EMA-positive patients, 11 were also positive for TTGA. (One patient was tested IgA deficient.) All remaining 118 EMA-negative patients were negative for TTGA. The sole discrepant result returned a TTGA value of 6 arbitrary units, which is just below the cutoff of 7 arbitrary units. In addition, this patient had biopsy-proven celiac disease. Because all negative TTGA results were ≤3 arbitrary units, the introduction of a borderline zone of 5–6 arbitrary units could be beneficial in screening for celiac disease with this antibody.

CONCLUSIONS — The association of celiac disease with the HLA-DQ2 and DQ8 molecules is established. Recently, HLA typing has been suggested for use in diagnosing celiac disease either by exclusion in the absence of DQ2 or DQ8 alleles (7) or, in patients with IgA deficiency, family screening for latent celiac disease (9). It is clear from this study that although all patients with celiac disease and type 1 diabetes possessed either the DQ2 or DQ8 alleles, the measurement of these alleles cannot be used in the diagnosis of celiac disease in patients with type 1 diabetes because there is a significant percentage of patients with type 1 diabetes without celiac disease who also possess these HLA types. Therefore, HLA typing should not be used to diagnose celiac disease, especially in populations with a high incidence of DQ2 and/or DQ8 (e.g., type 1 diabetes).

The replacement of the widely measured EMA by TTGA has been examined in several studies because the enzyme-linked immunosorbent assay format is generally more cost effective, efficient, and not affected by subjectivity in visual detection of immunofluorescence. These studies have shown that human-derived antigen is significantly more accurate than that of guinea pig and that there is a wide variation in sensitivity and specificity among commercially available kits (11). Using a commercial kit that has been shown to be highly sensitive and specific, we found that all but one of the sera positive for EMA were positive for TTGA. All sera with negative EMA had negative TTGA, giving a correlation of 92% for positive results and 100% for negative results.

The rate of IgA deficiency among individuals with celiac disease is significantly higher than that of the general population with reported figures of 1:200 and 1:700, respectively. In this study, 1 of 131 patients with type 1 diabetes was found to be IgA deficient. The measurement of IgA removes the possibility of false-negative antibody results due to IgA deficiency.

This study found the prevalence of celiac disease in patients with type 1 diabetes to be 8.4%. To our knowledge, only three studies have examined the prevalence of celiac disease among patients with type 1 diabetes in Australia. One of these studies (12) found the prevalence rate of celiac disease to be 2.2% among 180 children with type 1 diabetes; however, because gliadin antibodies were used as the sole screening antibody test, this result may be underestimated. A prevalence of 1.8% was found among 273 children using both antigliadin antibody and EMA as screening tests (13). However, this study only investigated newly diagnosed type 1 diabetes, with blood for antibody testing collected at a median of 3 days after diagnosis. Almost all previous studies found the prevalence of celiac disease in children with type 1 diabetes to be <8%, except one which showed a prevalence of 16% (4,14). In comparison with these studies, the prevalence found in our study is therefore relatively high. The discrepancy between this study and other studies could be explained by the different screening tests used, the age of the population studied, and the different genetic and environment factors affecting the populations investigated. Therefore, large population studies are required to determine the true prevalence of celiac disease in patients with type 1 diabetes. Our finding of one control subject with EMA is in keeping with background prev-

alence in European populations of 1:70 to 1:500 (2).

The symptoms and conditions associated with celiac disease are numerous and diverse. This study found that most patients did not show symptoms indicative of celiac disease. Although this result is consistent with other studies (12,15,16), it is likely that it is compounded by clinicians' lack of knowledge on the wide variety of nongastrointestinal symptoms of celiac disease and the mildness of these symptoms, making them undetectable by patient and physician. The age of patients with type 1 diabetes before diagnosis with celiac disease varied widely but was at least 6 years in all patients. It is important to diagnose celiac disease early because untreated celiac disease can lead to conditions such as osteoporosis, growth retardation, infertility, and lymphoma.

In conclusion, although HLA typing is not useful for diagnosis, we believe that serological screening of all patients with type 1 diabetes for celiac disease is essential because of the high prevalence (14). Furthermore, the wide variety of presenting symptoms associated with celiac disease, such as migraines and anemia, may be mistakenly attributed to type 1 diabetes. This is a view that is somewhat controversial and the subject of some debate (17).

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