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Frequency of HLA-DQB1*0201/02 and DQB1*0302 alleles and tissue transglutaminase antibody seropositivity in children with type 1 diabetes mellitus

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Abstract

Background: Patients with type 1 diabetes mellitus (T1DM) have an increased risk of celiac disease (CD). Both diseases have a common genetic susceptibility locus in the human leukocyte antigen (HLA) class II alleles. Testing for tissue transglutaminase antibodies (anti-tTG) is highly accurate for a CD diagnosis.

Objective: To determine the frequency of HLA-DQB1*0201/02 and DQB1*0302 alleles and anti-tTG seropositivity in children with T1DM.

Method: Forty-six children with T1DM (male:female=24:22; mean age 12±3.7 years) without significant digestive symptoms were enrolled. The mean duration of diabetes was 5±3.5 years. Serum anti-tTG IgA and IgG as well as HLA-DQ2 (DQB1*0201/02) and -DQ8 (DQB1*0302) alleles were analyzed. The allele frequencies were compared with those in controls, which included 124 normal Thai individuals, as reported in our previous study.

Results: All subjects were negative for anti-tTG IgG. Only one patient (2.2%) was positive for anti-tTG IgA (38.5 U/mL; cut-off 15 U/mL). Although this patient was also heterozygous for HLA-DQ2 and was asymptomatic for CD, he declined endoscopic confirmation. Twenty-nine of 46 patients carried HLA-DQ2 and/or -DQ8 heterodimers. HLA-DQB1*0201/02 and HLA-DQB1*0302 allele frequencies were significantly higher (27% and 14%) in T1DM patients compared with normal controls (13.3% and 7.3%; $P<0.001$ and $P=0.002$, respectively).

Conclusions: A significantly greater frequency of DQB1*0201/02 and DQB1*0302 alleles were present in children with T1DM compared with the control group. This indicates a potentially important role of these alleles in the development of T1DM. The prevalence of CD screening by serologic testing is negligible in Thai children with T1DM.

Keywords: celiac disease, children, diabetes mellitus, human leukocyte antigen, tissue-transglutaminase antibody

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Introduction

Celiac disease (CD) is an immune-mediated disease caused by gluten sensitivity in genetically susceptible individuals. CD manifests as gastrointestinal tract-related symptoms including abdominal pain, abdominal distension, diarrhea, and constipation, as well as non-gastrointestinal tract-related symptoms such as poor growth, rash, anemia, abnormal enamel, osteoporosis, short stature and delayed puberty.[1]

CD is common in western countries; the prevalence in the general population is approximately 0.5-1% in the USA and Europe.[2] This figure is significantly higher among patients with autoimmune-related diseases such as type 1 diabetes mellitus (T1DM), autoimmune thyroiditis and selective IgA deficiency, Down syndrome, William syndrome, Turner syndrome and first degree relative-CD.[3,4] The greater frequency of CD in patients with T1DM (1-16%) is caused by genetic susceptibility alleles shared between CD and T1DM: the human leukocyte antigen (HLA) alleles HLA-DR3-DQ2 and HLA-DR4-DQ8.[5-10]

Generally, T1DM patients with CD are asymptomatic for several years after being diagnosed with T1DM. However, the symptoms may manifest many years later. In western countries, a CD screening test for disease-prone groups is recommended. Most guidelines endorse a genetic test, using HLA typing for screening. If the result is DQ2/8-positive, a serology test is then required. If the result of this serology test is positive, duodenal biopsies are conducted for a definite diagnosis.[11-16]

CD is not highly prevalent among Asians, including Thais. Consequently, the availability of prevalence data is limited. Asians have changed from traditional rice-based diets to western-style diets with a higher content of gluten-based foods. As a result, there is a potential for increased incidence of CD because of greater gluten exposure. Indeed, CD is increasing among South Asian people as their staple diet is rich in wheat-derived foodstuffs.[2,7,17]

HLA typing and assay of CD-specific antibodies, including tissue transglutaminase antibody (anti-tTG), are useful screening tools for the diagnosis of CD.[18-23] Over 95% of patients with CD harbor the HLA-DQ2 heterodimer, whereas some of the other 5% have the HLA-DQ8 heterodimer. The sensitivity and specificity values of the anti-tTG IgG test are 95.2% and 95.0%, respectively, while those of the anti-tTG IgA test are 96.9% and 95%, respectively.[22]

There is a lack of data demonstrating the prevalence of CD in Thai children with T1DM who belong to the at-risk group. The aim of this study was to determine the frequency of anti-tTG IgA and IgG seropositivity in Thai children with T1DM. As the HLA-DQ2 (DQB1*0201/02) and -DQ8 (DQB1*0302) alleles confer a high risk for T1DM, we also aimed to explore the frequency of these risk alleles in T1DM children compared with the normal Thai population.

Methods

T1DM patients under 18 years of age in the pediatric endocrinology clinic of King Chulalongkorn Memorial Hospital were enrolled from July 2013 to March 2014. All patients had no symptoms suggestive of CD, such as recurrent abdominal pain, constipation or diarrhea. The following information was obtained from the medical records of patients with an initial diagnosis of diabetes: weight, height, age, and clinical features including initial and long-term insulin use, fasting blood sugar, glycated hemoglobin (HbA1c) and the presence of autoimmune thyroid disease. All consenting patients underwent blood testing for HLA haplotyping as well as anti-tTG IgA and IgG. This study was approved by the Ethics Committee, Faculty of Medicine, Chulalongkorn University. Written informed consent was obtained from the patients' guardians.

The frequencies of HLA-DQ2 (DQB1*0201/02) and -DQ8 (DQB1*0302) alleles in 124 normal Thai individuals served as control data and were described in our previous study.[24]

HLA typing

All patients were typed for HLA class II genomic polymorphisms at the intermediate-resolution level with polymerase chain reaction-sequence specific oligonucleotide probes (PCR-SSOP). DNA was isolated from peripheral blood samples using DNA purification (the salting out method). HLA-DQA1 and DQB1 gene polymorphisms were analyzed using commercial kits (LABType® SSO Typing Tests, One Lambda Inc., CA, USA) with 0.5 U Taq DNA polymerase (GoTaq® Flexi DNA Polymerase, Promega, WI, USA) following the manufacturers' protocols. Amplification was performed using a thermocycler (Gene Amp PCR System 9600, Applied Biosystems, CA, USA). Confirmation of the amplified product band prior to the hybridization assay was performed to ensure the generation of optimal signals by gel electrophoresis. PCR products were hybridized with oligonucleotides coated on microparticle beads and were visualized by a flow analyzer (LABScan 100, One Lambda Inc.) after hybridization.

Serologic testing

Anti-tTG IgA and IgG were detected by ELISA using Autoimmune EIA Anti-tTG IgA and IgG kits (Bio-Rad Laboratories, Richmond, CA). Sera were diluted 1:5 for testing and the positive cut-off point was 15 U/mL.

Statistical analysis

Data are presented as the number of subjects, percentage and mean. Allele frequencies were compared between children with T1DM and normal controls using a binomial test. A P value <0.05 was considered statistically significant.

Results

Forty-six diabetic children (24 males and 22 females) with a mean age of 12±3.7 years (range 3-17.3 years) were enrolled in the study. The subjects had been diagnosed with type 1 diabetes for 5±3.5 years (range 0.4-16.1 years) at the time of recruitment. Seven-day diet recall interviews regarding the consumption of wheat-containing products, including cereal, bakery products, and instant noodles, were recorded. About 24% of the participants reported no wheat consumption during the past week. The frequencies of wheat consumption were 1-3, 4-6, and 7 days per week in 54%, 7%, and 15% of the participants, respectively.

Twenty-nine patients (63%) were HLA-DQ2 (DQB1*0201/02) and/or HLA-DQ8 (DQB1*0302) positive (**Table 1**). The allele frequencies of HLA-DQB1 are shown in **Table 2**. There was a significant difference in the frequency of DQB1*0201/02 and DQB1*0302 alleles in patients compared with controls (P<0.001 and P=0.002, respectively).

Table 1. Distribution of HLA-DQB1 in T1DM patients.

HLA haplotype	Number (percentage)
DQ2/other	15 (52%)
DQ2/DQ8	6 (21%)
DQ8/other	5 (17%)
DQ2/DQ2	2 (7%)
DQ8/DQ8	1 (3%)

Abbreviations: HLA, human leucocyte antigen.

Table 2. Allele frequencies of HLA-DQB1 in T1DM patients.

HLA allele	Allele frequencies		P value
	T1DM in this study (2N=92)	Healthy control from Wongsurawat *et al*[24] (2N=248)	
DQB1*0201/02	25 (27%)	33 (13.3%)	<0.001
DQB1*0302	13 (14%)	18 (7.3%)	0.002

Abbreviations: HLA, human leucocyte antigen.

Males had a significantly higher positive rate for HLA-DQ2 or HLA-DQ8 compared with females (79.2% vs. 45.5%, P=0.02). The prevalence of poor glycemic control (HbA_{1c}≥9%) among children who were DQ2 and/or DQ8 positive was not significantly different compared with those who were DQ2/DQ8 negative (34.5% vs. 47.1%, respectively).

Only one patient was positive for anti-tTG IgA (38.5 U/mL). This 13-year-old adolescent patient was HLA-DQ2 heterozygous, was asymptomatic for CD, and presented with diabetic ketoacidosis at the age of 10. He was underweight with a body mass index of 17.5 kg/m^2 and had autoimmune thyroiditis (Hashimoto thyroiditis) as a co-morbidity. He had poor glycemic control with high HbA1c (up to 15.5%). Unfortunately, he declined a duodenal biopsy for CD confirmation.

Discussion

CD is a gastrointestinal disorder in which an autoimmune response is triggered by gluten proteins. Even though anti-tTG antibody testing is invaluable for the diagnosis of patients with CD, its performance in a clinical setting depends on the ingested amounts of gluten. The staple diet of the Thai population is rice, with wheat consumed less often. The reason why IgG-tTG seropositivity was undetectable in the present study might be partly explained by the low consumption of gluten by these patients. Almost a quarter of the subjects in this study reported no wheat consumption during the past week. Another reason might be explained by the hygiene hypothesis. Thai children are highly susceptible to infection, and therefore might be less likely to suffer from autoimmune diseases. This was supported by a study showing a decreased risk of CD in patients with *Helicobacter pylori* infection.[25] Asymptomatic individuals at risk for CD may have fluctuating serum anti-tTG levels over time. Therefore, it is recommended to perform duodenal biopsies in this risk group.[16]

Testing for anti-tTG IgA can produce both false positive and false negative results. Children with T1DM have a higher rate of false positive anti-tTG IgA titers when compared with the normal population.[26] IgA deficiency (IgA-D) can lead to false negative results for anti-tTG IgA. The prevalence rate of IgA-D in subjects with T1DM is approximately five-fold than that in the general population.[27] However, the incidence of IgA-D is low among Asian populations.[28,29] There have been reports of only three cases of IgA-D in Thailand.[30] While patients with IgA-D cannot produce IgA isotype antibodies against tTG, they can still produce IgG isotype antibodies against this antigen.[31] Anti-tTG IgG has a high sensitivity and specificity for CD diagnosis, even in patients with IgA deficiency. Therefore, the conclusion of CD diagnosis should be drawn from the results of the anti-tTG IgG testing as well. Although a total IgA level test was not conducted in this study, there is a high probability that all patients negative for anti-tTG IgA and IgG did not develop CD. Duodenal biopsies to verify the absence of enteropathy would have helped confirm the false positive anti-tTG IgA in the single patient who had a negative anti-tTG IgG result in the present study.

It is recommended to perform HLA-DQ typing for CD screening in asymptomatic children with an increased risk for CD, such as those with T1DM.[16] The HLA-DR3/DQ2 haplotype is present in over 90% of patients with CD and 55% of those with T1DM, compared with 20-25% of the general population of European ancestry.[32] The 50% frequency of the DQ2 haplotype in children with T1DM in this study is comparable to previous data.[32] The key role of HLA-DQ typing in the diagnosis of CD is to exclude the disease.[16] Individuals without HLA-DQ2 or -DQ8 are unlikely to have CD because the sensitivity of HLA-DQ2 testing is high, and when combined with HLA-DQ8 (when at least one of HLA-DQ2 or -DQ8 is positive), the sensitivity is even greater.

A recent study of 450 healthy Thai subjects in the placebo arm of an HIV vaccine trial revealed the allele frequencies of HLA-DQB1*0201/02 and DQB1*0302 were 13.9% and 3.6%, respectively, which were comparable to the data published by our group.[24,33] In the present study, the HLA-DQB1*0201/02 and HLA-DQB1*0302 alleles were present in 41% of children with T1DM, which was similar to 53% in a previous report.[34]

It is important to diagnose CD in at-risk children because it might have negative health consequences. The clinical heterogeneity of CD requires noninvasive tests for diagnosis, thus avoiding duodenal biopsy. Patients with T1DM are not routinely screened for CD in Thailand owing to a lack of both prevalence data and cost benefit studies. The American Diabetes Association suggests CD screening for children with T1DM should test for anti-tTG IgA or anti-endomysial IgA antibodies soon after a T1DM diagnosis.[13] For patients positive for these antibodies, further investigation to detect enteropathy by small bowel biopsy should be considered. The ESPGHAN 2012 guidelines, however, suggest HLA testing as an initial step to detect HLA-DQ2 and -DQ8.[16] If HLA-DQ2 and/or DQ8 are not present, additional tests such as serological tests are unnecessary.

T1DM patients with undetected CD have poor glycemic control and higher complication rates, such as retinopathy and nephropathy.[35] In this study, genetically at-risk children had a similar rate of poor glycemic control compared with those who were HLA-DQ2 and/or -DQ8 negative. As CD can develop over time in patients with T1DM, repeated monitoring of individuals positive for HLA-DQ2 or -DQ8 by serological testing is warranted, even if the initial test yields a negative result. Currently, data supporting the recommendations regarding the optimal frequency of periodic serologic investigation are still lacking.

In summary, a significantly greater frequency of DQB1*0201/02 and DQB1*0302 alleles were present in children with

T1DM compared with the control group, indicating that they might have an important role in the development of T1DM. The prevalence of CD screening by serologic testing is negligible in Thai children with T1DM.

Conflict of interests

The authors have declared that no competing interests exist.

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