Prospective Human Leukocyte Antigen, Endomysium Immunoglobulin A Antibodies, and Transglutaminase Antibodies Testing for Celiac Disease in Children with Down Syndrome

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Objective To assess the effect of a prospective screening strategy for the early diagnosis of celiac disease (CD) in children with Down syndrome (DS).

Study design Blood samples were taken from 155 children with DS. Buccal swabs were also taken from 9 of these children for determination of human leukocyte antigen (HLA)-DQ2 or HLA-DQ8 positivity. Independently, immunoglobulin A anti-endomysium-(EMA) and anti-tissue transglutaminase antibodies (TGA) were tested. An intestinal biopsy was performed to confirm the diagnosis of CD.

Results Sixty-three children (40.6%) had test results that were positive for HLA-DQ2 or HLA-DQ8. Results of HLA DQ-typing of DNA isolated from blood and buccal swabs were identical. Eight of the children in whom test results were positive for HLA-DQ2/8 also had positive test results for EMA and TGA. CD was confirmed in 7 of these children with an intestinal biopsy, and in 1 child, CD was suggested with improvement on a gluten-free diet.

Conclusions We found a prevalence of CD in children with DS of 5.2% (10 times higher than the general Dutch population). We recommend HLA-DQ2/8 typing from buccal swabs in the first year of life and initiating serologic screening of children with DS in whom test results are positive for HLA-DQ2 or DQ8 at age 3 years. Early knowledge of negative HLA-DQ2/8 status can reassure most parents that their children do not have a CD risk. (J Pediatr 2009;154:239-42)

D own syndrome (DS) is the most common chromosomal disorder in newborns. In 2003 the prevalence of DS in the Netherlands was 16 per 10 000 live births.1 Roizen and Patterson recommended assessing children with DS soon after birth for congenital heart disease, hearing loss, and ophthalmologic problems.2 Serologic assays are also recommended for common autoimmune diseases such as celiac disease (CD) and hypothyroidism. The prevalence of CD in children with DS (4%-15%) is significantly higher than the general population (0.3%-1.0%).3-9 In the Netherlands, the prevalence of CD in children with DS was 7%.10,11

CD is an autoimmune gastrointestinal disease caused by an intolerance of gluten, derived from wheat, barley, and rye. In children, the presenting symptoms are diarrhea, abdominal distention, growth failure, and fatigue, but the presence of these symptoms has a low predictive value for the diagnosis of CD, especially in children with DS.5

CD occurs in genetically predisposed individuals, and it has a strong association with human leukocyte antigen (HLA)-molecules; almost all patients with CD express HLA-DQ2, HLA-DQ8, or both. The HLA-DQ2 and HLA-DQ8 molecules form complexes with tissue transglutaminase (TG2) modified gluten-derived peptides. These complexes trigger CD4+ T-cells to respond, resulting in interferon-gamma release in the small intestine, leading to tissue damage and villous atrophy.12-16 The diagnosis of CD is usually established on the basis of serologic testing, an intestinal biopsy, and the response to a gluten-free diet (GFD).16

Csizmadia et al proposed performing screening for CD in children with DS at 1.5

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years of age, by means of HLA-DQ genotyping followed by determination of anti-tissue transglutaminase immunoglobulin A (IgA) antibodies (TGA) and anti-endomysium IgA antibodies (EMA) only in the population that has positive test results for HLA-DQ2, HLA-DQ8, or both.10 When both the HLA-typing and the serological tests are positive, an intestinal biopsy is performed to confirm the diagnosis of CD. Because of their inferior accuracy, antigliadin antibody tests are no longer recommended.17

HLA-DQ genotyping is performed mostly on blood samples obtained with venepuncture. A non-invasive approach, such as the collection of buccal swabs, is fairly simple and less stressful for children, but has not yet been used for HLA-DQ typing in children with DS.18

The main objectives of this study were to assess the effect of the screening strategy for CD in children with DS and to test the feasibility of routine HLA-DQ2 and HLA-DQ8 sampling with buccal swabs in children with DS.

METHODS

All 155 children who visited the special DS outpatient clinic of the VU University Medical Center, Amsterdam, a mixed secondary and tertiary referral center, from May 2005 to June 2007 were studied. Most children were Dutch Caucasian, but other ethnicities were also included. The ages of the children ranged from 2 months to 19 years (mean age, 7.4 years ± 4.6 SD; male:female, 97:58). Blood samples were taken from all children with DS, and random buccal swabs were collected from 9 of them. HLA-DQ typing was performed, and EMA and TGA antibodies were measured. All the children were checked for IgA deficiency with an enzyme-linked immunosorbent assay method using Escherichia coli IgA, an alternative test for IgA deficiency. In children <2 years old, anti-gliadin IgA was measured also. When the serological test (EMA-TGA) results were positive, a small intestinal biopsy was performed to confirm the diagnosis of CD. When pathologic characteristics of CD were found in these children, we assessed the response to a GFD.

HLA-DQ Typing

CD is strongly associated with the allelic combination DQA1*05/DQB1*02 (HLA-DQ-2) and to a lesser extent with DQA1*03/DQB1*0302 (HLA-DQ-8). Genomic DNA was isolated from EDTA-anticoagulated blood with a standardized DNAzol-based technique. In 9 children, buccal swabs were collected from each cheek. Subsequently, the swabs were cut off and stored in a 1.5-mL safe-lock tube at −80°C until DNA isolation was performed.

A medium-resolution polymerase chain reaction-single-strand conformation polymorphism/heteroduplex method on a semiautomatic gel electrophoresis system (Pharmacia Phast System) was used for typing the HLA-DQA1 and HLA-DQB1 genotype.10,19

Serological Tests

The determination of EMA and TGA antibodies was performed from the blood samples. EMA was measured with an indirect immunofluorescence assay with monkey esophagus.20 The measurements of TGA were performed with a standard enzyme-linked immunosorbent assay procedure.

Intestinal Biopsy

Samples were taken from the duodenum to assess small intestinal mucosa.21 When pathological characteristics of CD (villous atrophy, crypt hyperplasia, increased numbers of intra-epithelial lymphocytes [>40 IELs/100 enterocytes]) were found, the diagnosis of CD was established.22

RESULTS

The results from the screening for CD are presented in the Figure. HLA-DQ typing in 1 child, EMA testing in 2 children, and TGA testing in 3 children failed because of technical problems. The 8 patients who were found to have positive test results for EMA and TGA either had a diagnosis of CD confirmed with an intestinal biopsy (n = 7) or were considered very likely to have CD (n = 1) because of improvement both clinically and serologically with GFD.

The prevalence of CD in the DS group was 5.2% (8/155). All 8 children had positive test results for HLA-DQ2; 6 children were heterozygous for HLA-DQ2, and 2 other children were homozygous. Only 1 of the children was homozygous for both HLA-DQ8 and HLA-DQ2; the other 5 had negative results for HLA-DQ8.

The results of HLA-DQ2/8 genotyping and age dis-
distribution of children with DS at the time of screening are presented in the Table. There were 31 children <2 years old; the youngest child was 2 months old. Two of these children with DS had positive test results for anti-gliadin IgA, both of them had negative test results for HLA-DQ2 or HLA-DQ8. There were 6 questionable anti-gliadin IgA measurements. In all 6 of these children, the TGA and EMA results were negative. None of these children underwent an intestinal biopsy.

One of the children who underwent testing was IgA deficient. This child had negative test results for celiac related IgG antibodies. All the other children had positive test results for E. coli IgA, which excluded IgA deficiency.

The parents of 5 children with DS, in whom CD was diagnosed, clearly noticed an improvement after the children began a GFD (improved stools, disappearance of symptoms, and in 1 child, catch-up growth). Two children with DS did not have any symptoms when CD was diagnosed, but 1 of them had symptoms with the ingestion of gluten after 1 year of GFD. In the other child, no difference was found with or without a GFD.

The buccal swabs obtained from 9 children with DS yielded a sufficient amount of DNA with enough purity to perform HLA-DQ typing. This method was as accurate as the HLA-typing on blood samples. The youngest child included in the HLA-DQ-typing with buccal swab was 5 months old.

**DISCUSSION**

Our study showed a prevalence of CD in children with DS of 5.2% (8/155). This is 10-times higher than in the general Dutch population, although the frequency of the predisposing HLA-DQ2/8 type was comparable with the general Dutch population. In 2 earlier studies in the Netherlands, the prevalence of CD in DS was 7%. The high prevalence of CD is in agreement with earlier studies and means that screening for CD in the DS population is important.

In our study, HLA-typing obtained on buccal swabs from the children with DS fulfilled our expectations and had the benefit of avoiding the unpleasant collection of blood. We propose beginning CD screening in the first year of life, with the HLA-typing on buccal swabs to identify the children with DS who are at risk for CD. This would allow the further selection of a group needing to be screened and a group that can be excluded from further screening because the negative predictive value of the HLA-DQ typing is almost 100%.

By performing this CD screening in the first year of life, more than half the children with DS and their parents can get early reassurance that the child is not at risk for CD.

Furthermore, we recommend performing the EMA and TGA test initially at the age of 3 years in HLA-DQ2 or HLA-DQ8 carriers. This advice is based on the results of our study and on the results of the study by Csizmadia et al, in which the youngest children with DS at the time of the CD diagnosis were 3.6 and 3.2 years old. This advice is in agreement with the North American Society for Pediatric Gastroenterology. With this regimen, no CD cases are missed, and unnecessary screening before this age is prevented. Children with DS who have positive test results for HLA-DQ2 or HLA-DQ8 need to be monitored and screened periodically (every 2 or 3 years) with the serological EMA and TGA tests.

In our laboratory setting, the costs of 1 HLA-DQ genotyping are approximately equivalent to 3 serological screenings (tTG IgA and endomysium IgA). Usually, far more than 3 serological screenings will be required per patient in time, indicating that exclusion of the 60% of patients in whom the HLA-DQ2/8 results are negative from screening must be cost effective. Csizmadi also predicted a cost-effectiveness of such a strategy.

HLA-DQ genotyping is very relevant for the diagnosis of CD. In the Northern European countries, HLA-DQ2 has been reported to be present in 90% to 95% of patients with CD. The patients with CD who have negative results for HLA-DQ2 almost all have positive results for HLA-DQ8.
Gastrointestinal symptoms are not of predictive value for CD.\textsuperscript{5,14}

Although we think a biopsy is needed for confirmation of the diagnosis of CD, in our study we were able to identify the CD cases with HLA-DQ2 or HLA-DQ8 and EMA and TGA measurements.

We studied a large group of children with DS (n = 155) and did a diagnostic work-up (HLA-DQ genotyping, serologic tests EMA/TGA, and an intestinal biopsy in almost all children), but our study also has limitations. We cannot exclude the possibility that, during our 2 years of screening, CD cases were missed. Our study was performed in a single institution, and the children with DS were screened for CD only once. In the HLA-DQ2 or HLA-DQ8 positive group, new CD cases may be discovered in the future.

Early detection of CD in children with DS, with the aim of starting treatment, may prevent complications of untreated CD such as failure to thrive, anemia, osteoporosis, and malignancy and may improve quality of life.\textsuperscript{10,16,17} For CD screening in children with DS, we recommend HLA-DQ typing in the first year of life with buccal swabs. Children who have negative results for HLA-DQ2 or DQ8 (an estimated 60%), can be excluded from further screening, and parents can be reassured that their child has no risk for CD. The remaining children need to be monitored for CD by using both EMA and TGA beginning at 3 years of age.

REFERENCES