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Clinical Features of Children With Screening-Identified Evidence of Celiac Disease

Edward J. Hoffenberg, MD*; Lisa M. Emery, MSPH‡; Katherine J. Barriga, MSPH‡; Fei Bao§; Jennifer Taylor, MD§; George S. Eisenbarth, MD, PhD§; Joel E. Haas, MD; Ronald J. Sokol, MD*; Iman Taki, BS‡; Jill M. Norris, PhD‡; and Marian Rewers, MD, PhD§§

ABSTRACT. Objective. At-risk groups commonly undergo screening for autoantibodies associated with celiac disease (CD). However, the clinical significance of a positive test remains uncertain. The objective of this study was to evaluate growth and clinical features of children who test positive for an autoantibody associated with CD.

Methods. A case-control study of Denver area healthy infants and young children with and without CD autoantibodies was conducted. A cohort of HLA-characterized children were followed prospectively since birth for the development of immunoglobulin A antitissue transglutaminase autoantibodies (TG). Clinical evaluation, questionnaire, blood draw, and small bowel biopsy were performed. Growth and nutrition and frequency of positive responses were measured.

Results. Compared with 100 age- and gender-matched TG-negative controls, 18 TG-positive children, 5.5 ± 0.5 years of age, had a greater number of symptoms and lower z scores for weight-for-height and for body mass index. Responses that were independently associated with TG-positive status were irritability/lethargy, abdominal distention/gas, and difficulty with weight gain.

Conclusions. Screening-identified TG-positive children demonstrate mild alterations in growth and nutrition and report more symptoms than control subjects. Additional study is needed on the benefit and risk of identifying CD in at-risk groups. Pediatrics 2004;113:1254–1259; celiac disease, IgA, transglutaminase, children, body mass index, autoimmunity, screening.

ABBREVIATIONS. CD, celiac disease; EMA, endomysial antibodies; TG, transglutaminase antibodies; IgA, immunoglobulin A; BMI, body mass index; OR, odds ratio; CI, confidence interval.

Evidence of celiac disease (CD) may be present in 1 in every 100 to 250 children, adolescents, and adults in Europe and the United States. Individuals who are identified by autoantibody screening as having evidence of CD autoimmunity have few, mild symptoms or no symptoms at all and are identifiable only by screening. In symptomatic children with CD, clinical features include diarrhea, abdominal distention, failure to thrive, abdominal pain, and delayed puberty. The 1990 European Society for Pediatric Gastroenterology and Nutrition (ESPGAN) criteria for the clinical diagnosis of CD require typical intestinal mucosal alterations plus a response to a strict gluten-free diet, such as resolution of histologic changes or of symptoms; the disappearance of autoantibodies is additional supportive evidence. Recent reviews and consensus statements have reaffirmed these basic criteria.

These expert consensus statements regard the presence of autoantibodies as helpful but not required for the clinical diagnosis of CD. In patients with symptomatic CD, the presence of circulating antiendomysial antibodies (anti-EMA) or antitissue transglutaminase antibodies (TG) is highly predictive (97%–100%) of biopsy changes of CD. However, in screening programs of at-risk populations, these same autoantibodies (TG and EMA) may have a lower positive predictive value (75% to 85%) for biopsy evidence of CD, despite a similar genetic background as symptomatic patients. Individuals who have evidence of “CD autoimmunity” but absent typical histologic changes do not meet criteria for the clinical diagnosis of CD.

Nevertheless, screening at-risk populations, such as those with type 1 diabetes, for CD-associated antibodies has become common despite a lack of information about the short- and long-term implications of screening-identified CD. For example, it is not known whether individuals with screening-identified CD (or celiac autoimmunity) have the same long-term risks as patients with symptomatic CD for osteoporosis, lymphoma, and nutritional deficiencies. In addition, efforts at case finding need to be tempered by the realization that only ~50% of individuals with CD follow a strict gluten-free diet. Unfortunately, there are no tools to determine which individuals might benefit from early identification of CD and from early treatment with a gluten-free diet.

A prospective general population screening and follow-up study has been under way in Denver since 1993 in which a cohort of HLA-DRB1*03 characterized newborns have undergone serial testing for CD autoimmunity. Children with repeatedly positive tests for TG have undergone clinical evaluation and small intestinal biopsy. The purpose of this article is to report the clinical features of the first 18 TG-
positive children in this cohort who have undergone clinical evaluation for CD and small intestinal biopsy.

METHODS
This report compares clinical features of 18 screening-identified TG-positive children with 100 age- and gender-similar TG control subjects. All children were born at Saint Joseph’s Hospital (Denver, CO) and participated in a prospective study evaluating genetic and environmental risks for CD and type 1 diabetes. The newborn population of Saint Joseph’s Hospital was chosen because it was representative of the general population of Denver Metropolitan Area and included children who were classified by their mothers as non-Hispanic white (56%), Hispanic (30%), African American (7%), Asian American (2%), or biracial/other (5%). Among the 1234 families who participated in follow-up, 33 children have tested positive for TG. Of these, 18 have undergone repeated testing for TG, clinical evaluation, and small intestinal biopsy by a single pediatric gastroenterologist. The other 15 families either declined evaluation (n = 3) or are considering or awaiting clinical evaluation and biopsy (n = 12).

Control Group
For comparison with the children with celiac autoimmunity, 100 TG-negative children of similar age and gender from the same cohort of 1234 were chosen (ie, frequency matched). For comparison, information gathered from the questionnaire completed by telephone interview at 5 years of age was used.

HLA Genotype Screening
Genotypes were categorized as HLA-DR3/3 (homozygous), 3/x (heterozygous), or x/x (absent) on the basis of the number of copies of the DRB1*03 allele. The polymerase chain reaction-based genotype screening was performed by Roche Molecular Systems (Alameda, CA) and has been previously described in detail.

Questionnaire
Families in follow-up completed an annual questionnaire to determine the presence or absence of 7 items associated with CD: abdominal pain, constipation, poor weight gain, irritability or lethargy, abdominal distention or gas, diarrhea, and short stature. The questionnaire was administered by a trained study coordinator during a telephone interview or by a single physician at the time of clinical evaluation. Therefore, questionnaire data are available from 3 time intervals: before development of TG-positive status, after knowledge of TG-positive status, and at clinical evaluation.

Autoantibody Assay
Immunoglobulin A (IgA) antibodies to TG were detected using a radioimmunoassay with in vitro transcribed and translated human transglutaminase cDNA. Coded samples were tested in duplicate. Positive test results were confirmed by blinded duplicate sample testing. (Before 1998, EMA was used for screening; all EMA-positive samples were later confirmed also to be TG positive.)

Definition of Celiac Autoimmunity and Evidence of CD
Celiac autoimmunity (TG-positive) was defined as the persistence of TG autoantibodies on 2 consecutive occasions at least 6 months apart. Evidence of CD was defined as a positive test for TG plus histologic findings on intestinal biopsy, characterized by a Marsh score of 2 (enlarged crypts and increased numbers of intraepithelial lymphocytes) or 3 (any degree of villous atrophy). Intestinal biopsy specimens were obtained initially with a Carey capsule (Wilson-Cook Medical, Inc, Winston-Salem, NC; n = 4) and then by upper gastrointestinal endoscopy (n = 14) with 2 to 4 specimens obtained from the descending duodenum. A single pathologist, unaware of clinical information, assessed the biopsy specimen according to the scoring system described by Marsh.

IgA Deficiency Evaluation
In all children, IgA was measured using a nephelometric method (The Binding Site Limited, Birmingham, England). When IgA was low, IgG TG antibodies were measured.

Growth and Nutrition
Age- and gender-matched z scores [value – mean value for gender and age] ± standard deviation were obtained for height, weight, and body mass index (BMI) from a United States database available at www.cdc.gov/growthcharts. World Health Organization reference data were used for arm circumference-for-age and for height.

Vitamin E and Zinc
Blood samples were obtained after an overnight fast at the time of small intestinal biopsy. Plasma was separated immediately after blood was drawn and was stored at −70°C. Plasma α tocopherol was measured by high-performance liquid chromatography. Total lipids were measured using a colorimetric assay. The ratio of α tocopherol to total lipids (mg/g) was used to assess accurately vitamin E status. Fasting plasma zinc was measured by atomic absorption spectrophotometry.

Statistical Methods
Fisher exact test was used for comparison of groups in which a 2 × 2 cell contained <5. The unpaired t test was used for comparison of z scores that have a normal distribution. Mann-Whitney test was used for comparison of TG-positive group before and at clinical evaluation. A 3 × 2 χ² test or Kruskal-Wallis test was used to compare the TG-positive group at the 3 time intervals. The Spearman test was used to assess correlations between histology score and nutritional variables. Logistic regression was performed using TG status as the outcome variable and the proportion reporting the presence of each item as the predictor. The “backward” option in SAS (SAS Institute, Cary, NC) was used to identify items independently associated with TG status. The Colorado Multiple Institutional Review Board for Human Research approved this study, and informed, written consent was obtained from parents or legal guardians.

RESULTS
The TG-positive and TG-negative groups were of similar ethnic, gender, and age distribution (Table 1). For the TG-positive group, the mean age at first positive TG test was 4.4 ± 1.2 years, and the mean age at clinical evaluation was 5.3 ± 1.5 years. Four children in the TG-negative group and none in the TG-positive group had low IgA levels. All 4 had negative tests for IgG TG.

Of the 18 TG-positive children, 13 (72%) had biopsy evidence of CD (Marsh 2 or 3 score), 2 showed increased intraepithelial lymphocytes (Marsh 1), and...
3 had normal biopsies (Marsh 0). No relationship between number of copies of HLA-DR3 and biopsy score was evident (Table 2).

Compared with the TG-negative group, the TG-positive group had decreased z scores for weight-for-height ($-0.3 \pm 0.7$ vs $0.3 \pm 1$; $P = .02$) and BMI ($-0.3 \pm 0.7$ vs $0.4 \pm 0.9$; $P < .01$) but not weight- or height-for-age (Fig 1). The z scores for BMI and weight-for-height did not show any significant changes before or soon after development of TG seropositivity.

Additional anthropometric data obtained on the TG-positive group at the time of intestinal biopsy (mid-arm circumference and triceps skinfold) were compared with published age- and gender-matched normative data from the Third National Health and Nutrition Examination Survey of American children and stratified by intestinal biopsy histology score. The TG-positive group had decreased $z$ scores for mid-arm circumference ($-0.66 \pm 0.94$) and for mid-arm muscle area (a measure of muscle mass; $-0.53 \pm 0.96$).

All of the 18 TG-positive children had normal test results for aspartate aminotransferase and alanine aminotransferase ($n = 17$), vitamin E to total lipids ratio, and zinc. However, plasma zinc concentration correlated inversely with intestinal biopsy histology score ($r = -0.548$, $P = .03$).

Questionnaire information is summarized in Table 3. Before development of TG-positive status, the number of symptoms reported was similar to that of the TG-negative control group ($0.2 \pm 0.6$ vs $0.3 \pm 0.8$; $P = .96$). However, after knowledge of TG-positive status, a greater number of items were reported (compared with TG-negative control subjects) at both the telephone interview ($0.7 \pm 0.8$; $P = .001$) and the clinic visit (TG-positive: $2.4 \pm 1.7$; $P < .0001$). The time interval from the questionnaire completed be-

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**TABLE 2.** Relationship Between HLA Genotype and Histologic Findings From 18 Screening-Identified TG-Positive Children Who Underwent Small Intestinal Biopsy

<table>
<thead>
<tr>
<th>HLA Genotype</th>
<th>Marsh Score</th>
<th>% With Histologic Evidence of CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>3/3</td>
<td>2</td>
<td>0 1 2 3</td>
</tr>
<tr>
<td>3/x</td>
<td>0</td>
<td>2 2 7</td>
</tr>
<tr>
<td>x/x</td>
<td>1</td>
<td>0 0 1</td>
</tr>
</tbody>
</table>

A Marsh score of 2 or 3 was considered histologic evidence of celiac disease.14

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Fig 1. Z scores for weight-for-height (A), BMI (B), weight (C), and height (D) from 18 screening-identified TG-positive children and 100 TG-negative age- and gender-similar children.
TABLE 3. Questionnaire Data on 18 Screening-Identified TG-Positive Children and 100 TG-Negative Age- and Gender-Similar Children

<table>
<thead>
<tr>
<th></th>
<th>TG+ Group</th>
<th>TG-Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before TG+ (n = 14)</td>
<td>After TG+ (n = 15)</td>
</tr>
<tr>
<td>Age (y [median, range])</td>
<td>3.1 (2.0–6.5)</td>
<td>4.2 (2.6–6.7)</td>
</tr>
<tr>
<td>No. of items*</td>
<td>0.2 ± 0.6</td>
<td>0.7 ± 0.8§</td>
</tr>
<tr>
<td>Frequency of items</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal pain*</td>
<td>0%</td>
<td>7%</td>
</tr>
<tr>
<td>Constipation†</td>
<td>0%</td>
<td>20%</td>
</tr>
<tr>
<td>Poor weight gain†</td>
<td>7%</td>
<td>13%</td>
</tr>
<tr>
<td>Irritability/lethargy</td>
<td>7%</td>
<td>13%</td>
</tr>
<tr>
<td>Gassy/distended‡</td>
<td>7%</td>
<td>13%</td>
</tr>
<tr>
<td>Diarrhea‡</td>
<td>0%</td>
<td>7%</td>
</tr>
<tr>
<td>Short</td>
<td>0%</td>
<td>7%</td>
</tr>
<tr>
<td>None*</td>
<td>86%</td>
<td>60%</td>
</tr>
</tbody>
</table>

Before TG+ indicates most recent data before the first TG+ test; After TG+, data at time of first TG+ test; Clinical eval, data obtained at time of clinical evaluation by a pediatric gastroenterologist. Using logistic regression analysis, poor weight gain, irritability/lethargy, and gassy/distended were items found to be independent predictors of TG status. Mean ± standard deviation or median (range). Change over time in TG+ group: * P ≤ .01, † P ≤ .05.

TG+ at clinical evaluation compared with control: § P ≤ .01, §§ P ≤ .001, || P ≤ .0001.

Information on the natural history of CD is timely because of a position statement supporting screening of at-risk groups. However, we believe that caution is justified before recommending that children and adolescents, self-perceived as healthy, receive medical advice that they have a life-long disease that...
should be treated with a strict gluten-free diet. Four areas need additional evaluation. First is determining whether screening-identified and clinically identified CD have similar outcomes. It is possible that some but not all individuals with screening-identified evidence of CD are at risk for clinical sequelae of untreated CD. Thus, a “splitting” as opposed to “lumping” approach may increase the benefit relative to risk relationship, as well as gain broader acceptance in the public and medical community.

The second area, related to the first, is the need to determine whether treatment before symptoms develop, as opposed to after, changes long-term outcome. This is of particular interest to those who care for individuals with type 1 diabetes, a population in which the prevalence of CD is between 4% and 10% and in whom instituting a strict gluten-free plus diabetic diet may be especially challenging.38 Several small studies have not supported the hope that early treatment of CD would improve growth or diabetes control.39–41

The third area is whether the benefits of early diagnosis outweigh the harm. In older children and adolescents, the diagnosis of a chronic disease that requires significant diet alteration may have a negative impact on development.42 No studies of “asymptomatic” children have evaluated the impact that the diagnosis of CD has on age-appropriate developmental tasks such as autonomy, sense of identity, and peer relationships. The patient with diabetes, now faced with 2 chronic diseases and a diabetic plus gluten-free diet, may be particularly affected. It is not surprising that ~50% of children and teens who receive a diagnosis of CD do not comply with a gluten-free diet.43

The fourth issue is the definition of CD. The ESPGAN criteria require a characteristic intestinal mucosal lesion plus a response to a gluten-free diet, with improvement of either histologic findings or symptoms.44 The requirement for a response is often not fulfilled because demonstration of improvement in symptoms is a subjective outcome and resolution of the histologic lesion is not possible because of failure to comply with a strict gluten-free diet or because affected individuals are comfortable with the diagnosis and believe it unnecessary to repeat the biopsy. In symptomatic cases of CD, a positive screening test using endomysial or TG antibodies has a positive predictive value in the range of 97% to 100% for biopsy evidence of CD.11,43 In contrast, in screening-identified CD, a positive test for TG or EMA has a much more variable positive predictive value for biopsy evidence of CD, often in the range of 80% to 100%.5,13,44 Factors that influence the correlation between a positive screening test and histologic findings may include environmental factors such as recent dietary gluten content,45 genetic factors such as dose effect of HLA-DQ2,DR3,20 possible disease modifying non-HLA genes, the serologic test used,46 and breastfeeding.47

In summary, we report data from a unique study that longitudinally evaluated an HLA-characterized general population cohort of children for the development of TG antibody seropositivity. The results suggest that celiac autoimmunity in screening-identified children is associated with statistically significant changes in measures of growth and nutrition and with subjective identification of symptoms by parents. Additional studies on routine screening of at-risk groups for evidence of CD are needed before evidence-based recommendations can be developed.

ACKNOWLEDGMENTS

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REFERENCES

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Submitted by Student
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