Growth Trajectories and Bone Mineral Density in Anti-Tissue Transglutaminase Antibody–positive Children: The Generation R Study

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BACKGROUND & AIMS: **Increased levels of anti-tissue transglutaminase (tTG) have been associated with reduced weight and bone mineral density (BMD) in symptomatic patients with celiac disease. Little is known about the effects of these antibodies in patients with subclinical or other forms of celiac disease. We examined associations between anti-tTG positivity and growth and BMD.**

METHODS: **In a population-based prospective cohort study, serum samples were collected from children (median age, 6 years; n = 4442) and analyzed for anti-tTG. All children were born between April 2002 and January 2006 and were not previously diagnosed with celiac disease. Children were categorized as anti-tTG negative (<7 U/mL, n = 4249) or anti-tTG positive (≥7 U/mL, n = 57). Children's levels of anti-tTG were further categorized on the basis of ≥10 times upper limit of normal (70 U/mL). Height, weight, and body mass index (BMI) age- and sex-adjusted standard deviation scores (SDS) ([observed value – mean]/SD) were obtained by using Dutch reference growth charts. BMD was measured by dual-energy x-ray absorptiometry. Multivariable linear regression and linear mixed models were performed.**

RESULTS: **Children who tested positive for anti-tTG had reduced growth in weight SDS/year (reduction of 0.05; 95% CI, reductions of 0.09–0.01) and BMI SDS/year (reduction of 0.10; 95% CI, reductions of 0.18–0.01) from 6 months until 6 years, compared with children without anti-tTG; they also tended to have reduced growth in height from 6 months until 6 years (reduction of 0.02 SDS/year; 95% CI, reductions of 0.06–0.02). Children who tested positive for anti-tTG were shorter (0.29 SDS shorter; 95% CI, reductions of 0.55–0.04 SDS), weighed less (0.38 SDS less; 95% CI, reductions of 0.64–0.12), and had lower BMIs (0.26 SDS less; 95% CI, reductions of 0.49–0.03) and BMDs (0.26 SDS less; 95% CI, reductions of 0.45–0.08) at 6 years of age than anti-tTG negative children.**

CONCLUSIONS: **Anti-tTG positive children without gastrointestinal symptoms have lower BMDs and reduced growth trajectories until they are 6 years old. This suggests that subclinical or potential celiac disease can affect BMD and growth.**

Keywords: Generation R Study; Food Allergy; Pediatric; Development.

Celiac disease (CD) is an autoimmune-mediated disease that is caused by ingestion of gluten in genetic predisposed individuals. Screening studies have shown that the prevalence of CD is approximately 1% and increases over time.[1–3] However, CD is underdiagnosed, because clinical symptoms in childhood are often minor, atypical, or even absent.[4] Screening of anti-tissue transglutaminase (tTG) concentrations has the potential to detect these forms of otherwise undetected subclinical and potential CD.[5,6] However, it remains unclear whether these forms should be treated, because the consequences of untreated subclinical and potential CD in childhood remain unclear. Consequences of classic CD include decreased length, weight, body mass index (BMI), and bone mineral density (BMD), which completely recover by using a gluten-free diet (GFD) in childhood. However, by the time CD is diagnosed in adulthood, complete bone

Abbreviations used in this paper: aOR, adjusted odds ratio; BA, bone area; BMC, bone mineral content; BMD, bone mineral density; BMI, body mass index; CD, celiac disease; CI, confidence interval; EMA, endomysial antibody; GFD, gluten-free diet; OR, odds ratio; SDS, standard deviation scores; tTG, tissue transglutaminase.

1542-3565/$36.00
http://dx.doi.org/10.1016/j.cgh.2014.09.032

recovery is not always achieved. It is estimated that one-third of adult patients have osteoporosis, one-third have osteopenia, and the remaining one-third have a normal BMD.[7,8] In addition, screening studies showed a decreased BMD even in asymptomatic adults who had positive concentrations of celiac antibodies[7,9] but no villous atrophy,[10] indicating that merely the presence of positive serology is sufficient to have adverse effects on BMD. Moreover, persisting osteopenia and osteoporosis in adults, despite a GFD, may be the result of subclinical CD in childhood.[10,11] Because the effects on BMD in childhood are independent of clinical symptoms,[12] adverse effects might also be present in children with undetected forms of CD. Although children with positive immunoglobulin A–endomysial antibody (EMA) serology are suggested to be shorter and lighter,[3] effects on growth trajectories and bone development are not known.

Therefore, the aim of this study was to assess whether anti-tTG positivity was associated with decreased height, weight, BMI, and BMD in a population-based study of children from 6 months until 6 years of age.

Methods

Design

This study was embedded within a population-based prospective cohort study.[13] All children were born between 2002 and 2006. From the age of 6 years, 6690 children visited the research center (median age, 6.0 years),[13] of whom serum anti-tTG levels were available in 4442 (66%). Of these, we excluded twins, children with a questionnaire reported CD diagnosis and a GFD. The resulting population for analysis consisted of 4306 children (Supplementary Figure 1).

Anti-Tissue Transglutaminase Concentrations

Anti-tTG immunoglobulin A concentrations were measured in venous blood serum samples by using a fluorescence enzyme immunoassay (FEIA Phadia ImmunoCAP 250; EliA IgA, Phadia AB, Uppsala Sweden).[14] Concentrations of anti-tTG were categorized into 2 groups: group 1, anti-tTG negative (<7 U/mL) and group 2, anti-tTG positive (≥ 7 U/mL) (Supplementary Figure 1). In addition, anti-tTG positive concentrations were categorized into 2 categories on the basis of below or above the ≥ 10 times upper limit of normal concentrations of the test kit (≥ 70 U/mL).[5]

HLA DQ2 DQ8

A genome-wide association scan (Illumia 610K) of child DNA was taken from 5908 cord blood samples. A tag single nucleotide polymorphisms approach was used to capture whether children carried the HLA-DQ risk type DQ2 or DQ8.[15,16] Children were genotyped for HLA-DQ2 (rs2187668, rs2395182, rs4713586, and rs7775228) and DQ8 (rs7454108) by using genome-wide Illumina 610 Quad Array (San Diego, CA). Genotype and allele frequencies were in Hardy–Weinberg equilibrium (rs2187668, $P = .88$; rs2395182, $P = .78$; rs4713586, $P = .95$; rs7775228, $P = .86$; rs7454108, $P = .90$).

Growth Measurements

Child anthropometrics were obtained by measurements at 6, 14, 24, 36, and 48 months and at 6 years of age.[17] We obtained age-adjusted standard deviation scores (SDS) by using Dutch reference growth curves (Growth Analyzer 3.0; Dutch Growth Research Foundation, Rotterdam, Netherlands). Definitions of overweight (>1.1 to 2.3 SDS) and obesity (BMI $>$ 2.3 SDS) were based on internationally established age- and sex-adjusted BMI distributions.[17,18]

Gastrointestinal Symptoms

Gastrointestinal symptoms were assessed by parental reported questionnaires (median age, 6 years). Functional constipation was defined if at least the following symptoms of Rome III[19] were reported in the past year: (1) defecation frequency <3 times a week, (2) predominantly hard feces for the majority of stools, and (3) ≥ 1 episode of fecal incontinence per week. Mothers were asked whether their child had abdominal complaints during the last 3 months (y/n), if their child was feeling sick or nauseous while or after having eaten (y/n), and how their child's feces usually looked during the past 3 months (answer options ranged from "very hard" to "very soft/slushy" or "watery"). Diarrhea was defined when "very soft/slushy" or "watery" feces was indicated.

Bone Mineral Density, Bone Mineral Content, and Bone Area

BMD (g/cm^2), bone mineral content (BMC) (g), and bone area (BA) (cm^2) were measured by using a dual x-ray absorptiometry scan (iDXA; General Electrics-Lunar, Madison, WI) (median age, 6 years).[20] All scans were performed by well-trained research assistants and by using the same device and software (enCORE, version 13; GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom). Total body less head BMD, BMC, and BA were used to analyze total body bone mass.[21]

Covariates

Maternal anti-tTG concentrations were measured during pregnancy and maternal height and weight at enrollment. Fetal gender, gestational age, and birth