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A cross-sectional study of coeliac autoimmunity in a population of Vietnamese children

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3 **Word count: 2448**
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10 **ABSTRACT**

11 **Objective.** The prevalence of coeliac disease (CD) in Vietnam, is unknown.
12
13 To fill this void we assessed the prevalence of serological markers of CD
14
15 autoimmunity in a population of children in Hanoi.
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18 **Setting.** The outpatient blood drawing laboratory of a paediatric hospital in
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20 Hanoi, the secondary centre for the entire North Vietnamese region. The
21
22 study is part of an international project of collaboration between Italy and
23
24 Vietnam.
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26

27 **Participants.** Children having blood drawn for any reason. Exclusion criteria
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29 were age younger than 2 years, acquired or congenital immune deficiency,
30
31 inadequate sample. The parents of 2,045 children agreed to participate in the
32
33 study. Eighty-four children (4%) were excluded according to the exclusion
34
35 criteria. Finally, 1961 children (96%) were enrolled (838 F-1123 M, median
36
37 age 5.3 years).
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39

40 **Outcomes.** Primary outcome was the prevalence of positive autoimmunity to
41
42 both IgA anti-transglutaminase antibodies (anti-tTG) assessed with an ELISA
43
44 test and anti-endomysial antibodies (EMA) Secondary outcome was the
45
46 prevalence of CD predisposing HLA (HLA DQ2/8) in the positive children and
47
48 in in a random group of samples negative for IgA anti-tTG
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51 **Results:**

52
53 The IgA anti-tTG test was positive in 21/1961 (1%; 95% CI 0.0061-0.0153);
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55 however, EMA antibodies were negative in all. HLA DQ2/8 was present in
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3 7/21(33%; 95% CI 0.145-0.569) of the anti-tTG positive children and in 72/275
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5 (26% 95% CI 0.21-0.32) of the negative ones.
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11 **Conclusions:** Coeliac autoimmunity is rare in Vietnam, although prevalence
12 of HLA DQ2/8 is similar to other countries. We hypothesize that the scarce
13 exposure to gluten could be responsible for these findings.
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20 21 22 23 **Strengths and Limitations of the study**

24 25 **Strengths**

- 26
27 • This is the first research on CD autoimmunity in Vietnam;
- 28
29 • A large number of children were examined for celiac autoimmunity
- 30
31 • HLA typing for DQ2 and DQ8 of the anti-tTG positive children and of a
32
33 proportion of negative ones
- 34
35 • Anti-tTG IgG measured in children affected by total or partial IgA
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37 deficiency;
- 38
39 • The amount of gluten introduced with food was quite precisely
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41 estimated by means of a detailed questionnaire.
- 42
43 • Our study has increased awareness of coeliac disease among
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48 physicians

49 50 **Limitations**

- 51
52 • The target population was composed of children presenting to a
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54 hospital laboratory and therefore by definition not completely healthy..
- 55
56 • Relatively low median age of children
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- A small-intestinal biopsy could not possibly be performed and therefore we do not know if histological lesions were present in the few patients at risk .

INTRODUCTION

Coeliac disease (CD) is a chronic small-intestinal immune-mediated enteropathy precipitated by exposure to dietary gluten in genetically predisposed individuals bearing the second class HLA DQ2/DQ8 haplotypes [1]. CD is characterized by the presence of a variable combination of clinical manifestations including intestinal and extra-intestinal symptoms such as diarrhoea, abdominal pain, failure to thrive and anaemia. CD-specific antibodies comprise anti-transglutaminase antibodies (anti-tTG), anti-endomysial antibodies (EMA), and antibodies against deamidated forms of gliadin peptides [2]. The sensitivity of anti-tTG and EMA is about 93%, whereas specificity has been reported to be 97% for anti-tTG and 99% for EMA.

The prevalence of CD reported in Europe and the United States averages 1% in both children and adults [3–5]. A few studies have been performed in symptomatic individuals in China and India [6], but, with the exception of a screening in Malaysian adults [7], the prevalence of CD in the Asia Pacific Region and, more specifically in Vietnam, is still unknown. The World Gastroenterology Organization and the Asian Pacific Association of Gastroenterology recommend establishing the prevalence of CD across that region in order to increase awareness among physicians and patients [8]. When untreated, the disease can cause permanent growth failure and poor bone development, and, according to some studies, it can facilitate the

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3 development of autoimmune disorders like diabetes and thyroiditis, infertility,
4 and even cancer [9,10]. A two- to 3-fold excess in all-cause mortality among
5 untreated CD patients, compared with the general population, has been
6 reported [11,12].
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10 While wheat is the staple cereal of most Caucasian populations, the diet of
11 many populations in Asia and South-East Asia is based on rice [9,13]. Wheat-
12 based products, however, are becoming more common with urbanization and
13 rising incomes in areas of Asia that were once considered traditional rice-
14 eating regions. Changes in infant feeding patterns in Asian countries might
15 increase the prevalence of CD. We hypothesized that eating mainly rice would
16 protect from developing coeliac autoimmunity.
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20 In order to establish the prevalence of CD in the Vietnamese paediatric
21 population, we tested a large number of children using the serum anti-tTG as
22 a screening test. The children who tested positive for anti-tTG were evaluated
23 with the EMA test and for the CD-related HLA. A randomly selected group of
24 children who had tested negative for both anti-tTG and EMA were typed for
25 CD-related HLA. Our findings suggest that coeliac autoimmunity is rare in
26 Vietnam, although prevalence of HLA DQ2/8 is similar to other countries.
27

28 **MATERIALS AND METHODS**

29 **Study design**

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32 The study was designed by the University of Ferrara, Italy, in collaboration
33 with the National Hospital of Paediatrics (NHP) in Hanoi, Vietnam, which are
34 partners in an international project of the University of Ferrara. In addition, the
35 Institute for Clinical Research of the University of Trieste, Italy, participated in
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3 the research project and performed all the laboratory tests. The sample size
4 was calculated on an estimation of 0.75% prevalence of CD [14]. Considering
5 a 99% confidence interval and a precision of 0.5%, the estimated sample size
6 was 1,976. We added 5% to the sample size to compensate for any attrition.
7
8 The NHP is the second largest paediatric hospital in South East Asia and the
9 large daily affluence of children allowed us to enrol the necessary number in
10 the space of a month. The children, aged 2-18 years, who presented to the
11 laboratory of the NHP to have blood drawn for any reason between February
12 2 and 14, 2015, were included in the study. Exclusion criteria were: age
13 younger than 2 years, a diagnosis of malignancy, chemotherapy and
14 treatment with immunosuppressants, including corticosteroids. In fact, below 2
15 years of age anti-tTG and EMA have poor sensitivity [2] and
16 immunosuppression could decrease the sensitivity of the tests.
17
18 Blood sampling was carried out at the NHP: two tubes were obtained from
19 each subject, one for serum and one for whole blood. We searched for IgA
20 anti-tTG in all the children included in the study. All the positive children were
21 then tested for both EMA and HLA DQ2/DQ8. Total IgA concentration was
22 measured in samples with IgA anti-tTG absorbance ranging from 0 to 0.140,
23 as previously reported [15] and serum samples with IgA deficiency (IgA serum
24 concentration <7 mg/dL) were tested for IgG anti-tTG.
25
26 Furthermore, a random selection of children who tested negative for both anti-
27 tTG, and EMA were also evaluated for the CD-related HLA. According to the
28 ESPGHAN criteria [2], a subject is defined as being at risk for having CD
29 when positive for both anti-tTG and EMA in the presence of HLA DQ2/DQ8.
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3 A questionnaire was used to collect demographic data (sex, date of birth,
4 current therapies), information on signs and symptoms known to be related to
5 coeliac disease (recent complaints of abdominal discomfort or fatigue), and on
6 the approximate amount of gluten consumed weekly (noodles, bread or
7 snacks: never, once a day, more or less than once a week).
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9

14 **Serological Assays and HLA Typing**

15
16 Serum samples were examined in duplicate at the NHP laboratory for IgA
17 anti-tTG using an ELISA assay (Eu-tTG, Eurospital, Trieste, Italy) according to
18 the manufacturer's instructions (normal values <9 U/ml). Quantitative
19 determination of human IgA in serum was carried out in Italy using an
20 immunoassay (Roche/Hitachi Cobas c system, Indianapolis, Indiana, USA),
21 following the manufacturer's instructions. Serum EMA were evaluated by
22 indirect immunofluorescence on cryostat sections of human umbilical cord as
23 previously described [3].
24
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26
27 The susceptibility alleles for CD were determined by PCR with allele specific
28 primers identifying HLA DQ2 and DQ8, using an Eu-Gene-Risk kit (Eurospital,
29 Trieste, Italy). The kit serves to identify all DQ2 positive subjects carrying both
30 DQ2.5 (HLA-DQA1*05, DQB1*02 in cis with DR3 or in trans with the
31 DR5/DR7 haplotypes) and DQ2.2 heterodimers (HLA-DQA1*02, DQB1*02,
32 DRB1*07), and the DQ8 positive ones (HLA-DQA1*03, DQB1*03:02,
33 DRB1*04).
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36 **Statistical Analysis**

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38 Continuous data were presented as mean \pm standard deviation for normally
39 distributed parameters and median and interquartile range (IQR) for skewed
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3 variables. Dichotomous variables were presented as frequency and
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5 percentage.
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7 8 **Ethical Considerations**

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10 The study was approved by the Ethic and Scientific Committees of the NHP in
11 Hanoi and of the University Hospital of Ferrara. Written informed consent was
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13 obtained from the children's parents before proceeding with the test.
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16 17 18 19 **RESULTS**

20 21 **Study Population**

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23 The parents of 2,045 children agreed to participate in the study. Eighty-four
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25 children (4%) were excluded due to inadequate serum samples (63 children)
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27 or to exclusion criteria (15 with leukaemia, 3 in chemotherapy, 1 in
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29 radiotherapy, 2 less than two years of age). Nineteen hundred and sixty one
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31 children (96%) were enrolled in the study (838 F-1123 M, median age 5.3
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33 years and IQR 4-7.5 years). Reasons for having blood drawn included an
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35 array of general paediatric diseases: respiratory tract infections, fever,
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37 gastroenteritis, cough, hepatitis, thalassaemia major, anaemia, abdominal
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39 pain, nephrotic syndrome, glomerulonephritis, stunted growth, thyroiditis,
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41 diabetes, arthritis, asthma, tuberculosis, urinary tract infection, dengue,
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43 Henoch-Schonlein purpura, and immune thrombocytopenia.
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47 Twenty percent of the children (387/1961) complained of gastrointestinal
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49 symptoms (266 of recurrent abdominal pain, 87 anorexia, 58 diarrhoea). One
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51 hundred and twenty-eight others were being worked-up because of failure to
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53 thrive. Exposure to gluten was reported by 88% of the patients' parents. Four
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percent of them ate foods containing gluten every day, 40% at least once a week and 56% less than once a week.

Anti-tTG, EMA and HLA typing

Twenty-one children out of 1961 (8F-13M) tested positive for IgA anti-tTG (1%; 95% CI 0.0061-0.0153); however and 17 of the 21 had a history of eating gluten. However EMA antibodies were negative in all of them. Seven of the 21 (33%) carried the CD-related HLA (Table 1), but only in two of them (0.1% of the total population) the titer of anti-tTG antibodies was higher than three times the upper limit of normal (positive predictive value 95%)[16]. One patient had IgA anti-tTG titer 10 times higher the upper limit of normal value but his HLA DQ2/8 was negative. HLA DQ2/8 was present in 7/21(33%; 95% CI 0.145-0.569) of the anti-tTG positive children and in 72/275 (26% 95% CI 0.21-0.32) of the negative ones. IgA anti-tTG absorbance ranging from 0 to 0.140 was present in 162/1961 children (8%) and 5/162 (3%: 3F-2M, median age 6.4 years) had total IgA deficiency. These 5 children were tested and were found to be negative for IgG anti-tTG. The results of the study are shown in Figure 1.

Table 1. Demographic, serology, HLA, gluten exposure and clinical history in Vietnamese children positive for IgA anti-tTG.

| Patient | Sex | Age (years) | IgA anti -tTG (U/ml) | IgA anti -tTG absorbance | HLA | EMA (+/-) | Gluten exposure | Gluten consumption frequency |
|---------|-----|-------------|----------------------|--------------------------|-----|-----------|-----------------|------------------------------|
| V731 | M | 4 | 9 | 0,628 | N | - | Yes | >1 per week |
| V348 | F | 10 | 9 | 0,648 | N | - | Yes | < 1 per week |
| V1755 | M | 11 | 10 | 0,659 | N | - | Yes | >1 per week |
| V1057 | M | 8 | 10 | 0,632 | DQ8 | - | No | |
| V703 | M | 6 | 10,5 | 0,659 | N | - | Yes | >1 per week |

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|-------|---|----|------|-------|---------|---|-----|--------------|
| V525 | F | 17 | 12 | 0,712 | N | - | No | |
| V715 | F | 6 | 13,5 | 0,731 | N | - | Yes | < 1 per week |
| V800 | M | 6 | 14 | 0,779 | DQ2.5 | - | Yes | < 1 per week |
| V949 | M | 4 | 14 | 0,733 | DQ2.5/8 | - | Yes | < 1 per week |
| V431 | F | 4 | 16 | 0,794 | DQ2.5 | - | Yes | >1 per week |
| V736 | M | 3 | 16 | 0,803 | N | - | No | |
| V1417 | F | 9 | 17 | 0,846 | N | - | Yes | < 1 per week |
| V1872 | F | 5 | 18,5 | 0,832 | N | - | Yes | < 1 per week |
| V1521 | M | 6 | 21,5 | 1,021 | DQ2.5 | - | Yes | >1 per week |
| V517 | M | 9 | 37 | 1,3 | N | - | Yes | < 1 per week |
| V510 | F | 9 | 40 | 1,303 | N | - | Yes | < 1 per week |
| V170 | M | 8 | 45 | 1,341 | DQ2.5 | - | Yes | >1 per week |
| V1965 | M | 7 | 54 | 1,692 | N | - | Yes | >1 per week |
| V1037 | F | 8 | 64 | 1,852 | N | - | Yes | >1 per week |
| V148 | M | 9 | 74 | 2,195 | DQ2.2 | - | Yes | < 1 per week |
| V963 | M | 10 | 105 | 3,045 | N | - | No | |

HLA N: HLA DQ2/8 negative; *IgA anti-tTG titer 10 times higher the upper limit of normal value but HLA DQ2/8 negative

DISCUSSION

This is the first study on the prevalence of coeliac-specific antibodies among Vietnamese children.

CD is considered to be rare in the Asia Pacific region. Therefore, we screened a large sample of children who had not previously been diagnosed with CD, by means of sensitive and specific sequential serological tests. Overall, 21 children were found to be anti-tTG IgA positive, but EMA test was negative in all, and only 7/21 were positive for CD-related HLA. Only two children were potentially affected by CD, having high levels of anti-tTG and being DQ2/DQ8 positive. However both of them were EMA negative. According to the

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3 literature, the EMA test is as sensitive as, but more specific than, anti-tTG
4 [17].The remainder of the children who tested positive for DQ2 and DQ8 were
5 anti-tTG and EMA negative.
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9 All the screening studies performed so far in the Eastern hemisphere have
10 identified the presence of CD autoimmunity in variable percentages. In India,
11 CD has been well recognized, especially in the Northern part, and two
12 population-based studies have revealed a prevalence of 0.3-1.04% [18].
13 According to a meta-analysis[19], the number of reported cases of CD is
14 extremely low in China, although a study of children with chronic diarrhea
15 showed a histologically proven frequency of CD of 12% [20]. Preliminary data
16 from Japan and Singapore suggest the existence of CD also in these
17 countries [6]. A study from Malaysia reported a prevalence of 1.9% in adult
18 females and 0.4% in males as demonstrated by positive IgA/IgG antigliadin
19 antibodies, IgA/IgG anti-tTG, and EMA [7].
20
21

22 The pathogenesis of CD requires involvement of the HLA molecules, DQ2 or
23 DQ8, that present gluten antigens to specific T cells. Their presence is a not
24 sufficient but necessary condition to develop CD. The typical HLA alleles were
25 present in 26% of the Hanoi children examined, a number similar to the
26 percentages found in most populations [13]. The low prevalence of CD
27 autoimmunity in our Northern Vietnamese paediatric population is therefore
28 somewhat surprising, especially in consideration of the fact that HLA
29 genotyping suggests that the risk for CD exists also in Vietnam.
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32 Possible explanations for this finding could be the young median age of the
33 children screened (5.3 years) or the scarce and late introduction of gluten. A
34 recent multicenter, prospective European study, which compared infants at
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3 risk for CD randomly assigned to the introduction of dietary gluten at 6 or 12
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5 months, found that at 5 years of age 20% of children at risk had developed
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7 the disease. The study concluded that, although neither late introduction of
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9 gluten nor breast feeding modified the risk in children with predisposing
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11 conditions, late introduction of gluten delayed the onset of the disease [21].
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13 Approximately half of the Vietnamese children that we examined ate gluten-
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15 containing foods less than once a week, and 10% did not eat gluten at all.
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17 This fact could be responsible for the rarity of coeliac autoimmunity.
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21 IgA deficiency occurs more frequently in patients with coeliac disease (1.30%)
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23 than in the general population (0–13–0–25%), a fact that might cause false
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25 negative results [22]. In our study, the children who were considered as being
26
27 possibly affected by total or partial IgA deficiency [15] on the basis of low IgA
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29 anti-tTG absorbance were tested with IgG anti-tTG and found to be negative.
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31 In our population there was a prevalence of males. This difference reflects the
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33 male to female ratio which averages 120:100 (45% female) in the Red River
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35 Delta (Hanoi City) and 110:100 (47% female) in the rest of the Northern areas
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37 [23].
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41 Our results are similar to those recently reported from Colombia, where both
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43 healthy individuals and those affected by autoimmune disorders were tested
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45 with anti-tTG and EMA. Among patients with autoimmune disorders, seven
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47 individuals tested positive or weakly positive for anti-tTG, but IgA EMA were
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49 negative in all cases [24].
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52 **Strengths and limitations of the study**

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54 The strength of our study lies in the fact that this is the first research on CD
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56 autoimmunity in Vietnam; in the large number of children examined by ELISA
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3 for anti-tTG; in the further testing by EMA of positive sera, and in the HLA
4 typing for DQ2 and DQ8 of the anti-tTG positive children. Also, in children
5 affected by total or partial IgA deficiency, anti-tTG IgG were measured. The
6 amount of gluten introduced with food was quite precisely estimated by
7 means of a detailed questionnaire.
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9
10 The study was performed on children having blood drawn as outpatient at a
11 hospital laboratory who, therefore, were not completely healthy. However, if
12 anything, this potential selection bias should have increased the prevalence of
13 CD in our sample. The median age of children was 5 years, and we do not
14 know if CD autoimmunity will develop with passing years. A small-intestinal
15 biopsy could not possibly be performed and therefore the presence of
16 histological lesions in the two patients (V170, V148) with high anti-tTG
17 antibody concentration carrying the CD-related HLA cannot be completely
18 excluded. Both had been exposed to dietary gluten.
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34 **Future Developments**

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36 New studies of the South-East Asian population might clarify whether the
37 prevalence of CD increases with age and if there is a strict correlation with the
38 amount of gluten introduced with the diet. It will be of interest to follow children
39 on a completely gluten free diet and compare them with children from the
40 same area whose diet includes gluten-containing foods.
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47 **Conclusions**

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49 None of the 1,961 Vietnamese children examined was positive for coeliac
50 autoimmunity on the basis of positivity for both anti-tTG and EMA. The
51 extremely low prevalence of CD in this large population of children could be
52 due to low exposure to gluten coupled with the young age of the children.
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Legend to Figure 1

Flow-chart showing results of the study

Acknowledgments

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Contributorship statement

Study design: Caterina Borgna-Pignatti, Tarcisio Not, Le Thi Minh Huong, Phung Tuyet Lan, Cristina Malaventura

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Data interpretation: Caterina Borgna-Pignatti, Tarcisio Not, Sara Zanella, Luigina De Leo, Cristina Malaventura, Stefano Volpato, Phung Tuyet Lan

Writing: Caterina Borgna-Pignatti, Tarcisio Not, Sara Zanella, Luigina De Leo, Cristina Malaventura, Stefano Volpato

Competing Interests

The authors declare no competing interests.

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Data sharing statement

There are no additional data to be shared

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Flow-chart showing results of the study
11x8mm (600 x 600 DPI)

Peer review only

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

| Section/Topic | Item # | Recommendation | Reported on page # |
|------------------------------|--------|--|--------------------|
| Title and abstract | 1 | (a) Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| | | (b) Provide in the abstract an informative and balanced summary of what was done and what was found | 2 |
| Introduction | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 4 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 5 |
| Methods | | | |
| Study design | 4 | Present key elements of study design early in the paper | 5 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 5-6 |
| Participants | 6 | (a) Give the eligibility criteria, and the sources and methods of selection of participants | 5-6 |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 6 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 6-7 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 6 |
| Study size | 10 | Explain how the study size was arrived at | 5 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 7 |
| Statistical methods | 12 | (a) Describe all statistical methods, including those used to control for confounding | 7 |
| | | (b) Describe any methods used to examine subgroups and interactions | |
| | | (c) Explain how missing data were addressed | |
| | | (d) If applicable, describe analytical methods taking account of sampling strategy | |
| | | (e) Describe any sensitivity analyses | |
| Results | | | |

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|--------------------------|-----|--|----------|
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 8 |
| | | (b) Give reasons for non-participation at each stage | 8 |
| | | (c) Consider use of a flow diagram | figure 1 |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | Table 1 |
| | | (b) Indicate number of participants with missing data for each variable of interest | |
| Outcome data | 15* | Report numbers of outcome events or summary measures | 8-9 |
| Main results | 16 | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 8 |
| | | (b) Report category boundaries when continuous variables were categorized | n.a. |
| | | (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | n.a. |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 9 |
| Discussion | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 12 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 3-12 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 11-12 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 12-13 |
| Other information | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 13 |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

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A cross-sectional study of coeliac autoimmunity in a population of Vietnamese children

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Manuscripts

A cross-sectional study of coeliac autoimmunity in a population of Vietnamese children

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Keywords: anti-tTG; EMA; HLA DQ2/DQ8; gluten; coeliac disease; epidemiology.

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3 **Word count:** 2442
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10 **ABSTRACT**

11 **Objective.** The prevalence of coeliac disease (CD) in Vietnam, is unknown.
12
13 To fill this void we assessed the prevalence of serological markers of CD
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15 autoimmunity in a population of children in Hanoi.
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17

18 **Setting.** The outpatient blood drawing laboratory of a paediatric hospital in
19
20 Hanoi, the secondary centre for the entire North Vietnamese region. The
21
22 study is part of an international project of collaboration between Italy and
23
24 Vietnam.
25
26

27 **Participants.** Children having blood drawn for any reason. Exclusion criteria
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29 were age younger than 2 years, acquired or congenital immune deficiency,
30
31 inadequate sample. A total of 1961 children (96%) were enrolled (838 F-1123
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33 M, median age 5.3 years).
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36 **Outcomes.** Primary outcome was the prevalence of positive autoimmunity to
37
38 both IgA anti-transglutaminase antibodies (anti-tTG) assessed with an ELISA
39
40 test and anti-endomysial antibodies (EMA). Secondary outcome was the
41
42 prevalence of CD predisposing HLA (HLA DQ2/8) in the positive children and
43
44 in a random group of samples negative for IgA anti-tTG.
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46

47 **Results.** The IgA anti-tTG test was positive in 21/1961 (1%; 95% CI 0.0061-
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49 0.0153); however, EMA antibodies were negative in all. HLA DQ2/8 was
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51 present in 7/21(33%; 95% CI 0.145-0.569) of the anti-tTG positive children
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53 and in 72/275 (26% 95% CI 0.21-0.32) of the negative ones.
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5 **Conclusions.** Coeliac autoimmunity is rare in Vietnam, although prevalence
6 of HLA DQ2/8 is similar to other countries. We hypothesize that the scarce
7 exposure to gluten could be responsible for these findings.
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11 12 13 14 15 16 **Strengths and Limitations of the study**

17 18 19 **Strengths**

- 20 • This is the first research on CD autoimmunity in Vietnam;
- 21 • A large number of children were examined for coeliac autoimmunity
- 22 • HLA typing for DQ2 and DQ8 of the anti-tTG positive children and of a
23 proportion of negative ones
- 24 • Anti-tTG IgG measured in children affected by total or partial IgA
25 deficiency
- 26 • The amount of gluten introduced with food was quite precisely
27 estimated by means of a detailed questionnaire
- 28 • Our study has increased awareness of coeliac disease among
29 physicians

30 31 32 33 34 35 36 37 38 39 40 41 42 43 **Limitations**

- 44 • The target population was composed of children presenting to a
45 hospital laboratory and therefore by definition not completely healthy
- 46 • Relatively low median age of children
- 47 • A small-intestinal biopsy could not possibly be performed and therefore
48 we do not know if histological lesions were present in the few patients
49 at risk

INTRODUCTION

Coeliac disease (CD) is a chronic small-intestinal immune-mediated enteropathy precipitated by exposure to dietary gluten in genetically predisposed individuals bearing the second class HLA DQ2/DQ8 haplotypes [1]. CD is characterized by the presence of a variable combination of clinical manifestations including intestinal and extra-intestinal symptoms such as diarrhoea, abdominal pain, failure to thrive and anaemia. CD-specific antibodies comprise anti-transglutaminase antibodies (anti-tTG), anti-endomysial antibodies (EMA), and antibodies against deamidated forms of gliadin peptides [2]. The sensitivity of anti-tTG and EMA is about 93%, whereas specificity has been reported to be 97% for anti-tTG and 99% for EMA.

The prevalence of CD reported in Europe and the United States averages 1% in both children and adults [3–5]. A few studies have been performed in symptomatic individuals in China and India [6], but, with the exception of a screening in Malaysian adults [7], the prevalence of CD in the Asia Pacific Region and, more specifically in Vietnam, is still unknown. The World Gastroenterology Organization and the Asian Pacific Association of Gastroenterology recommend establishing the prevalence of CD across that region in order to increase awareness among physicians and patients [8].

When untreated, the disease can cause permanent growth failure and poor bone development, and, according to some studies, it can facilitate the development of autoimmune disorders like diabetes and thyroiditis, infertility, and even cancer [9,10]. A two- to 3-fold excess in all-cause mortality among

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3 untreated CD patients, compared with the general population, has been
4 reported [11,12].
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6
7 While wheat is the staple cereal of most Caucasian populations, the diet of
8 many populations in Asia and South-East Asia is based on rice [9,13]. Wheat-
9 based products, however, are becoming more common with urbanization and
10 rising incomes in areas of Asia that were once considered traditional rice-
11 eating regions. Changes in infant feeding patterns in Asian countries might
12 increase the prevalence of CD. We hypothesized that eating mainly rice would
13 protect from developing coeliac autoimmunity.
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15
16 In order to establish the prevalence of CD in the Vietnamese paediatric
17 population, we tested a large number of children using the serum anti-tTG as
18 a screening test. The children who tested positive for anti-tTG were evaluated
19 with the EMA test and for the CD-related HLA. A randomly selected group of
20 children who had tested negative for both anti-tTG and EMA were typed for
21 CD-related HLA.
22

23 **MATERIALS AND METHODS**

24 **Study design**

25 The study was designed by the University of Ferrara, Italy, in collaboration
26 with the National Hospital of Paediatrics (NHP) in Hanoi, Vietnam, which are
27 partners in an international project of the University of Ferrara. In addition, the
28 Institute for Clinical Research of the University of Trieste, Italy, participated in
29 the research project and performed all the laboratory tests. The sample size
30 was calculated on an estimation of 0.75% prevalence of CD [14]. Considering
31 a 99% confidence interval and a precision of 0.5%, the estimated sample size
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3 was 1,976.[15] We added 5% to the sample size to compensate for any
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5 attrition.
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7 The NHP is the second largest paediatric hospital in South East Asia and the
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9 large daily affluence of children allowed us to enrol the necessary number in
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11 a short period of time. The children, aged 2-18 years, who presented to the
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13 laboratory of the NHP to have blood drawn for any reason between February
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15 2 and 14, 2015, were included in the study. Exclusion criteria were: age
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17 younger than 2 years, a diagnosis of malignancy, chemotherapy and
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19 treatment with immunosuppressants, including corticosteroids. In fact, below 2
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21 years of age anti-tTG and EMA have poor sensitivity [2] and
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23 immunosuppression could decrease the sensitivity of the tests.
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27 Blood sampling was carried out at the NHP: two tubes were obtained from
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29 each subject, one for serum and one for whole blood. We searched for IgA
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31 anti-tTG in all the children included in the study. All the positive children were
32
33 then tested for both EMA and HLA DQ2/DQ8. Total IgA concentration was
34
35 measured in samples with IgA anti-tTG absorbance ranging from 0 to 0.140,
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37 as previously reported [16] and serum samples with IgA deficiency (IgA serum
38
39 concentration <7 mg/dL) were tested for IgG anti-tTG.
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43 Furthermore, a random selection of children who tested negative for both anti-
44
45 tTG, and EMA were also evaluated for the CD-related HLA. According to the
46
47 ESPGHAN criteria [2], a subject is defined as being at risk for having CD
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49 when positive for both anti-tTG and EMA in the presence of HLA DQ2/DQ8.
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51 A questionnaire was used to collect demographic data (sex, date of birth,
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53 current therapies), information on signs and symptoms known to be related to
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55 coeliac disease (recent complaints of abdominal discomfort or fatigue), and on
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3 the approximate amount of gluten consumed weekly (noodles, bread or
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5 snacks: never, once a day, more or less than once a week).
6

7 8 **Serological Assays and HLA Typing**

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10 Serum samples were examined in duplicate at the NHP laboratory for IgA
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12 anti-tTG using an ELISA assay (Eu-tTG, Eurospital, Trieste, Italy) according to
13
14 the manufacturer's instructions (normal values <9 U/ml). Quantitative
15
16 determination of human IgA in serum was carried out in Italy using an
17
18 immunoassay (Roche/Hitachi Cobas c system, Indianapolis, Indiana, USA),
19
20 following the manufacturer's instructions. Serum EMA were evaluated by
21
22 indirect immunofluorescence on cryostat sections of human umbilical cord as
23
24 previously described [3].
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28 The susceptibility alleles for CD were determined by PCR with allele specific
29
30 primers identifying HLA DQ2 and DQ8, using an Eu-Gene-Risk kit (Eurospital,
31
32 Trieste, Italy). The kit serves to identify all DQ2 positive subjects carrying both
33
34 DQ2.5 (HLA-DQA1*05, DQB1*02 in cis with DR3 or in trans with the
35
36 DR5/DR7 haplotypes) and DQ2.2 heterodimers (HLA-DQA1*02, DQB1*02,
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38 DRB1*07), and the DQ8 positive ones (HLA-DQA1*03, DQB1*03:02,
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40 DRB1*04).
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43 44 **Statistical Analysis**

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46 Continuous data were presented as mean \pm standard deviation for normally
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48 distributed parameters and median and interquartile range (IQR) for skewed
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50 variables. Dichotomous variables were presented as frequency and
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52 percentage.
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54 55 **Ethical Considerations**

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3 The study was approved by the Ethic and Scientific Committees of the NHP in
4 Hanoi and of the University Hospital of Ferrara. Written informed consent was
5 obtained from the children's parents before proceeding with the test.
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10 11 12 **RESULTS**

13 14 **Study Population**

15
16 The parents of 2,045 children agreed to participate in the study. Eighty-four
17 children (4%) were excluded due to inadequate serum samples (63 children)
18 or to exclusion criteria (15 with leukaemia, 3 in chemotherapy, 1 in
19 radiotherapy, 2 less than two years of age). Nineteen hundred and sixty one
20 children (96%) were enrolled in the study (838 F-1123 M, median age 5.3
21 years and IQR 4-7.5 years). Reasons for having blood drawn included an
22 array of general paediatric diseases: respiratory tract infections, fever,
23 gastroenteritis, cough, hepatitis, thalassaemia major, anaemia, abdominal
24 pain, nephrotic syndrome, glomerulonephritis, stunted growth, thyroiditis,
25 diabetes, arthritis, asthma, tuberculosis, urinary tract infection, dengue,
26 Henoch-Schonlein purpura, and immune thrombocytopenia.
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55 56 **Anti-tTG, EMA and HLA typing**

Twenty-one children out of 1961 (8F-13M) tested positive for IgA anti-tTG (1%; 95% CI 0.0061-0.0153) and 17 of the 21 had a history of eating gluten. However EMA antibodies were negative in all of them. Seven of the 21 (33%) carried the CD-related HLA (Table 1), but only in two of them (0.1% of the total population) the titer of anti-tTG antibodies was higher than three times the upper limit of normal (positive predictive value 95%)[17]. One patient had IgA anti-tTG titer 10 times higher than the upper limit of normal value but his HLA DQ2/8 was negative. HLA DQ2/8 was present in 7/21(33%; 95% CI 0.145-0.569) of the anti-tTG positive children. The HLA DQ2 / 8 was measured also in 275/1961 (14%) children (selected by means of a computational random number generator) who had tested negative for both anti-tTG and EMA, and 72/275 (26% 95% CI 0.21-0.32) demonstrated presence of the HLA DQ2/8.

IgA anti-tTG absorbance ranging from 0 to 0.140 was present in 162/1961 children (8%) and 5/162 (3%: 3F-2M, median age 6.4 years) had total IgA deficiency. These 5 children were tested and were found to be negative for IgG anti-tTG. The results of the study are shown in Figure 1.

Table 1. Demographic, serology, HLA, gluten exposure and clinical history in Vietnamese children positive for IgA anti-tTG.

| Patient | Sex | Age (years) | IgA anti -tTG (U/ml) | IgA anti -tTG absorbance | HLA | EMA (+/-) | Gluten exposure | Gluten consumption frequency |
|---------|-----|-------------|----------------------|--------------------------|-----|-----------|-----------------|------------------------------|
| V731 | M | 4 | 9 | 0,628 | N | - | Yes | >1 per week |
| V348 | F | 10 | 9 | 0,648 | N | - | Yes | < 1 per week |
| V1755 | M | 11 | 10 | 0,659 | N | - | Yes | >1 per week |
| V1057 | M | 8 | 10 | 0,632 | DQ8 | - | No | |
| V703 | M | 6 | 10,5 | 0,659 | N | - | Yes | >1 per week |

| | | | | | | | | |
|-------|---|----|------|-------|---------|---|-----|--------------|
| V525 | F | 17 | 12 | 0,712 | N | - | No | |
| V715 | F | 6 | 13,5 | 0,731 | N | - | Yes | < 1 per week |
| V800 | M | 6 | 14 | 0,779 | DQ2.5 | - | Yes | < 1 per week |
| V949 | M | 4 | 14 | 0,733 | DQ2.5/8 | - | Yes | < 1 per week |
| V431 | F | 4 | 16 | 0,794 | DQ2.5 | - | Yes | >1 per week |
| V736 | M | 3 | 16 | 0,803 | N | - | No | |
| V1417 | F | 9 | 17 | 0,846 | N | - | Yes | < 1 per week |
| V1872 | F | 5 | 18,5 | 0,832 | N | - | Yes | < 1 per week |
| V1521 | M | 6 | 21,5 | 1,021 | DQ2.5 | - | Yes | >1 per week |
| V517 | M | 9 | 37 | 1,3 | N | - | Yes | < 1 per week |
| V510 | F | 9 | 40 | 1,303 | N | - | Yes | < 1 per week |
| V170 | M | 8 | 45 | 1,341 | DQ2.5 | - | Yes | >1 per week |
| V1965 | M | 7 | 54 | 1,692 | N | - | Yes | >1 per week |
| V1037 | F | 8 | 64 | 1,852 | N | - | Yes | >1 per week |
| V148 | M | 9 | 74 | 2,195 | DQ2.2 | - | Yes | < 1 per week |
| V963 | M | 10 | 105 | 3,045 | N | - | No | |

HLA N: HLA DQ2/8 negative; *IgA anti-tTG titer 10 times higher the upper limit of normal value but HLA DQ2/8 negative

DISCUSSION

This is the first study on the prevalence of coeliac-specific antibodies among Vietnamese children.

CD is considered to be rare in the Asia Pacific region. Therefore, we screened a large sample of children who had not previously been diagnosed with CD, by means of sensitive and specific sequential serological tests. Overall, 21 children were found to be anti-tTG IgA positive, but EMA test was negative in all, and only 7/21 were positive for CD-related HLA. Only two children were potentially affected by CD, having high levels of anti-tTG and being DQ2/DQ8 positive. However both of them were EMA negative. According to the

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3 literature and the recent ESPGHAN [2] and BSPGHAN [18] guidelines for the
4 CD-diagnosis , the EMA test is considered a gold standard immunological
5 biomarker as sensitive as, but more specific than, anti-tTG [19].The
6
7 remainder of the children who tested positive for DQ2 and DQ8 were anti-tTG
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9 and EMA negative.
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14 All the screening studies performed so far in the Eastern hemisphere have
15 identified the presence of CD autoimmunity in variable percentages. In India,
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17 CD has been well recognized, especially in the Northern part, and two
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19 population-based studies have revealed a prevalence of 0.3-1.04% [20].
20
21 According to a meta-analysis[21], the number of reported cases of CD is
22
23 extremely low in China, although a study of children with chronic diarrhea
24
25 showed a histologically proven frequency of CD of 12% [22]. Preliminary data
26
27 from Japan and Singapore suggest the existence of CD also in these
28
29 countries [6]. A study from Malaysia reported a prevalence of 1.9% in adult
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31 females and 0.4% in males as demonstrated by positive IgA/IgG antigliadin
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33 antibodies, IgA/IgG anti-tTG, and EMA [7].
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38 The pathogenesis of CD requires involvement of the HLA molecules, DQ2 or
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40 DQ8, that present gluten antigens to specific T cells. Their presence is a not
41
42 sufficient but necessary condition to develop CD. The typical HLA alleles were
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44 present in 26% of the Hanoi children examined, a number similar to the
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46 percentages found in most populations [13]. The low prevalence of CD
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48 autoimmunity in our Northern Vietnamese paediatric population is therefore
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50 somewhat surprising, especially in consideration of the fact that HLA
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52 genotyping suggests that the risk for CD exists also in Vietnam.
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56 Possible explanations for this finding could be the young median age of the
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3 children screened (5.3 years) or the scarce and late introduction of gluten. A
4 recent multicenter, prospective European study demonstrated that, although
5 late introduction of gluten did not decrease the risk of developing CD in
6 children with predisposing conditions, it delayed the onset of the disease [23].
7
8 Approximately half of the Vietnamese children that we examined ate gluten-
9 containing foods less than once a week, and 10% did not eat gluten at all.

10
11 This fact could be responsible for the rarity of coeliac autoimmunity.

12
13 IgA deficiency occurs more frequently in patients with coeliac disease (1.30%)
14 than in the general population (0–13–0–25%), a fact that might cause false
15 negative results [24]. In our study, the children who were considered as being
16 possibly affected by total or partial IgA deficiency [16] on the basis of low IgA
17 anti-tTG absorbance were tested with IgG anti-tTG and found to be negative.

18
19 In our population there was a prevalence of males. This difference reflects the
20 male to female ratio which averages 120:100 (45% female) in the Red River
21 Delta (Hanoi City) and 110:100 (47% female) in the rest of the Northern areas
22 [25].

23
24 Our results are similar to those recently reported from Colombia, where both
25 healthy individuals and those affected by autoimmune disorders were tested
26 with anti-tTG and EMA. Among patients with autoimmune disorders, seven
27 individuals tested positive or weakly positive for anti-tTG, but IgA EMA were
28 negative in all cases [26].

29 30 **Strengths and limitations of the study**

31
32 The strength of our study lies in the fact that this is the first research on CD
33 autoimmunity in Vietnam; in the large number of children examined by ELISA
34 for anti-tTG; in the further testing by EMA of positive sera, and in the HLA
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3 typing for DQ2 and DQ8 of the anti-tTG positive children. Also, in children
4 affected by total or partial IgA deficiency, anti-tTG IgG were measured. The
5 amount of gluten introduced with food was quite precisely estimated by
6 means of a detailed questionnaire.
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11 The study was performed on children having blood drawn as outpatient at a
12 hospital laboratory who, therefore, were not completely healthy. However, if
13 anything, this potential selection bias should have increased the prevalence of
14 CD in our sample. The median age of children was 5 years, and we do not
15 know if CD autoimmunity will develop with passing years. A small-intestinal
16 biopsy could not possibly be performed and therefore the presence of
17 histological lesions in the two patients (V170, V148) with high anti-tTG
18 antibody concentration carrying the CD-related HLA cannot be completely
19 excluded. Both had been exposed to dietary gluten.
20
21

22 **Future Developments**

23
24 New studies of the South-East Asian population might clarify whether the
25 prevalence of CD increases with age and if there is a strict correlation with the
26 amount of gluten introduced with the diet. It will be of interest to follow children
27 on a completely gluten free diet and compare them with children from the
28 same area whose diet includes gluten-containing foods.
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31 **Conclusions**

32
33 None of the 1,961 Vietnamese children examined was positive for coeliac
34 autoimmunity on the basis of positivity for both anti-tTG and EMA. The
35 extremely low prevalence of CD in this large population of children could be
36 due to low exposure to gluten coupled with the young age of the children.
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Legend to Figure 1

Flow-chart showing results of the study

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Contributorship statement

Study design: Caterina Borgna-Pignatti, Tarcisio Not, Le Thi Minh Huong, Phung Tuyet Lan, Cristina Malaventura

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Data analysis: Caterina Borgna-Pignatti, Tarcisio Not, Sara Zanella, Luigina De Leo, Cristina Malaventura, Stefano Volpato, Tran Thi Chi Mai, Serena Vatta, Le Thanh Hai

Data interpretation: Caterina Borgna-Pignatti, Tarcisio Not, Sara Zanella, Luigina De Leo, Cristina Malaventura, Stefano Volpato, Phung Tuyet Lan

Writing: Caterina Borgna-Pignatti, Tarcisio Not, Sara Zanella, Luigina De Leo, Cristina Malaventura, Stefano Volpato

Competing Interests

The authors declare no competing interests.

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Data sharing statement

There are no additional data to be shared

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Flow-chart showing results of the study
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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

| Section/Topic | Item # | Recommendation | Reported on page # |
|------------------------------|--------|--|--------------------|
| Title and abstract | 1 | (a) Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| | | (b) Provide in the abstract an informative and balanced summary of what was done and what was found | 2 |
| Introduction | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 4 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 5 |
| Methods | | | |
| Study design | 4 | Present key elements of study design early in the paper | 5 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 5-6 |
| Participants | 6 | (a) Give the eligibility criteria, and the sources and methods of selection of participants | 5-6 |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 6 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 6-7 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 6 |
| Study size | 10 | Explain how the study size was arrived at | 5 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 7 |
| Statistical methods | 12 | (a) Describe all statistical methods, including those used to control for confounding | 7 |
| | | (b) Describe any methods used to examine subgroups and interactions | |
| | | (c) Explain how missing data were addressed | |
| | | (d) If applicable, describe analytical methods taking account of sampling strategy | |
| | | (e) Describe any sensitivity analyses | |
| Results | | | |

| | | | |
|--------------------------|-----|--|----------|
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 8 |
| | | (b) Give reasons for non-participation at each stage | 8 |
| | | (c) Consider use of a flow diagram | figure 1 |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | Table 1 |
| | | (b) Indicate number of participants with missing data for each variable of interest | |
| Outcome data | 15* | Report numbers of outcome events or summary measures | 8-9 |
| Main results | 16 | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 8 |
| | | (b) Report category boundaries when continuous variables were categorized | n.a. |
| | | (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | n.a. |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 9 |
| Discussion | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 12 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 3-12 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 11-12 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 12-13 |
| Other information | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 13 |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

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A cross-sectional study of coeliac autoimmunity in a population of Vietnamese children

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| Primary Subject Heading: | Gastroenterology and hepatology |
| Secondary Subject Heading: | Epidemiology, Paediatrics |
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| | |

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Manuscripts

A cross-sectional study of coeliac autoimmunity in a population of Vietnamese children

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Keywords: anti-tTG; EMA; HLA DQ2/DQ8; gluten; coeliac disease; epidemiology

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3 **Word count:** 2442
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10 **ABSTRACT**

11 **Objective.** The prevalence of coeliac disease (CD) in Vietnam is unknown. To
12 fill this void we assessed the prevalence of serological markers of CD
13 autoimmunity in a population of children in Hanoi.
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18 **Setting.** The outpatient blood drawing laboratory of a paediatric hospital in
19 Hanoi, the secondary centre for the entire North Vietnamese region. The
20 study was part of an international project of collaboration between Italy and
21 Vietnam.
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27 **Participants.** Children having blood drawn for any reason. Exclusion criteria
28 were age younger than 2 years, acquired or congenital immune deficiency,
29 inadequate sample. A total of 1961 children (96% were enrolled (838 F-1123
30 M, median age 5.3 years).
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36 **Outcomes.** Primary outcome was the prevalence of positive autoimmunity to
37 both IgA anti-transglutaminase antibodies (anti-tTG) assessed with an ELISA
38 test and anti-endomysial antibodies (EMA). Secondary outcome was the
39 prevalence of CD predisposing HLA (HLA DQ2/8) in the positive children and
40 in a random group of samples negative for IgA anti-tTG.
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47 **Results.** The IgA anti-tTG test was positive in 21/1961 (1%; 95% CI 0.0061-
48 0.0153); however, EMA antibodies were negative in all. HLA DQ2/8 was
49 present in 7/21(33%; 95% CI 0.145-0.569) of the anti-tTG positive children
50 and in 72/275 (26% 95% CI 0.21-0.32) of the negative ones.
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Conclusions. Coeliac autoimmunity is rare in Vietnam, although prevalence of HLA DQ2/8 is similar to that of other countries. We hypothesize that the scarce exposure to gluten could be responsible for these findings.

Strengths and Limitations of the study

Strengths

- This is the first research on CD autoimmunity in Vietnam;
- A large number of children were examined for celiac autoimmunity;
- HLA typing for DQ2 and DQ8 of the anti-tTG positive children and of a proportion of negative ones;
- Anti-tTG IgG measured in children affected by total or partial IgA deficiency;
- a detailed questionnaire was used to collect quantitative information about gluten consumption to the best possible accuracy.

Limitations

- The target population was composed of children presenting to a hospital laboratory and therefore by definition not completely healthy;
- Relatively low median age of children;
- A small-intestinal biopsy could not possibly be performed and therefore we do not know if histological lesions were present in the few patients at risk.

INTRODUCTION

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3 Coeliac disease (CD) is a chronic small-intestinal immune-mediated
4 enteropathy precipitated by exposure to dietary gluten in genetically
5 predisposed individuals bearing the second class HLA DQ2/DQ8 haplotypes
6 [1]. CD is characterized by the presence of a variable combination of clinical
7 manifestations including intestinal and extra-intestinal symptoms such as
8 diarrhoea, abdominal pain, failure to thrive and anaemia. CD-specific
9 antibodies comprise anti-transglutaminase antibodies (anti-tTG), anti-
10 endomysial antibodies (EMA), and antibodies against deamidated forms of
11 gliadin peptides [2]. The sensitivity of anti-tTG and EMA is about 93%,
12 whereas specificity has been reported to be 97% for anti-tTG and 99% for
13 EMA.
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16 The prevalence of CD reported in Europe and the United States averages 1%
17 in both children and adults [3–5]. A few studies have been performed on
18 symptomatic individuals in China and India [6], but, with the exception of a
19 screening in Malaysian adults [7], the prevalence of CD in the Asia-Pacific
20 region and, more specifically in Vietnam, is still unknown. The World
21 Gastroenterology Organization and the Asian Pacific Association of
22 Gastroenterology recommend establishing the prevalence of CD across that
23 region in order to increase awareness among physicians and patients [8].
24 When untreated, the disease can cause permanent growth failure and poor
25 bone development, and, according to some studies, it can facilitate the
26 development of autoimmune disorders like diabetes and thyroiditis, infertility,
27 and even cancer [9,10]. A two- to 3-fold excess in all-cause mortality among
28 untreated CD patients, compared with the general population, has been
29 reported [11,12].
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3 While wheat is the staple cereal of most Caucasian populations, the diet of
4 many populations in Asia and South-East Asia is based on rice [9,13]. Wheat-
5 based products, however, are becoming more common with urbanization and
6 rising incomes in areas of Asia that were once considered traditional rice-
7 eating regions. Changes in infant feeding patterns in Asian countries might
8 increase the prevalence of CD. We hypothesized that eating mainly rice would
9 protect one from developing coeliac autoimmunity.

10
11 In order to establish the prevalence of CD in the Vietnamese paediatric
12 population, we tested a large number of children using the serum anti-tTG as
13 a screening test. The children who tested positive for anti-tTG were evaluated
14 with the EMA test and for the CD-related HLA. A randomly selected group of
15 children who had tested negative for both anti-tTG and EMA were typed for
16 CD-related HLA.

34 MATERIALS AND METHODS

36 Study design

37 The study was designed by the University of Ferrara, Italy, in collaboration
38 with the National Hospital of Paediatrics (NHP) in Hanoi, Vietnam, which are
39 partners in an international project of the University of Ferrara. In addition, the
40 Institute for Clinical Research of the University of Trieste, Italy, participated in
41 the research project and performed all the laboratory tests. The sample size
42 was calculated on an estimation of 0.75% prevalence of CD [14]. Considering
43 a 99% confidence interval and a precision of 0.5%, the estimated sample size
44 was 1,976.[15] We added 5% to the sample size to compensate for any
45 attrition.

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3 The NHP is the second largest paediatric hospital in Southeast Asia and the
4 large daily affluence of children allowed us to enrol the necessary number in
5 a short period of time. The children, aged 2-18 years, who presented to the
6 laboratory of the NHP to have blood drawn for any reason between February
7 2 and 14, 2015, were included in the study. Exclusion criteria were: age
8 younger than 2 years, a diagnosis of malignancy, chemotherapy and
9 treatment with immunosuppressants, including corticosteroids. In fact, below 2
10 years of age anti-tTG and EMA have poor sensitivity [2] and
11 immunosuppression could decrease the sensitivity of the tests.

12
13 Blood sampling was carried out at the NHP: two tubes were obtained from
14 each subject, one for serum and one for whole blood. We searched for IgA
15 anti-tTG in all the children included in the study. All the positive children were
16 then tested for both EMA and HLA DQ2/DQ8. Total IgA concentration was
17 measured in samples with IgA anti-tTG absorbance ranging from 0 to 0.140,
18 as previously reported [16], and serum samples with IgA deficiency (IgA
19 serum concentration <7 mg/dL) were tested for IgG anti-tTG.

20
21 Furthermore, a random selection of children who tested negative for both anti-
22 tTG, and EMA were also evaluated for the CD-related HLA. According to the
23 ESPGHAN criteria [2], a subject is defined as being at risk for having CD
24 when positive for both anti-tTG and EMA in the presence of HLA DQ2/DQ8.

25
26 A questionnaire was used to collect demographic data (sex, date of birth,
27 current therapies), information on signs and symptoms known to be related to
28 coeliac disease (recent complaints of abdominal discomfort or fatigue), and on
29 the approximate amount of gluten consumed weekly (noodles, bread or
30 snacks: never, once a day, more or less than once a week).

Serological Assays and HLA Typing

Serum samples were examined in duplicate at the NHP laboratory for IgA anti-tTG using an ELISA assay (Eu-tTG, Eurospital, Trieste, Italy) according to the manufacturer's instructions (normal values <9 U/ml). Quantitative determination of human IgA in serum was carried out in Italy using an immunoassay (Roche/Hitachi Cobas c system, Indianapolis, Indiana, USA), following the manufacturer's instructions. Serum EMA were evaluated by indirect immunofluorescence on cryostat sections of human umbilical cord as previously described [3].

The susceptibility alleles for CD were determined by PCR with allele specific primers identifying HLA DQ2 and DQ8, using an Eu-Gene-Risk kit (Eurospital, Trieste, Italy). The kit serves to identify all DQ2 positive subjects carrying both DQ2.5 (HLA-DQA1*05, DQB1*02 in cis with DR3 or in trans with the DR5/DR7 haplotypes) and DQ2.2 heterodimers (HLA-DQA1*02, DQB1*02, DRB1*07), and the DQ8 positive ones (HLA-DQA1*03, DQB1*03:02, DRB1*04).

Statistical Analysis

Continuous data were presented as mean \pm standard deviation for normally distributed parameters and median and interquartile range (IQR) for skewed variables. Dichotomous variables were presented as frequency and percentage.

Ethical Considerations

The study was approved by the Ethic and Scientific Committees of the NHP in Hanoi and of the University Hospital of Ferrara. Written informed consent was obtained from the children's parents before proceeding with the tests.

RESULTS

Study Population

The parents of 2,045 children agreed to participate in the study. Eighty-four children (4%) were excluded due to inadequate serum samples (63 children) or to exclusion criteria (15 with leukaemia, 3 in chemotherapy, 1 in radiotherapy, 2 under two years of age). Nineteen hundred and sixty one children (96%) were enrolled in the study (838 F-1123 M, median age 5.3 years and IQR 4-7.5 years). Reasons for having blood drawn included an array of general paediatric diseases: respiratory tract infections, fever, gastroenteritis, cough, hepatitis, thalassaemia major, anaemia, abdominal pain, nephrotic syndrome, glomerulonephritis, stunted growth, thyroiditis, diabetes, arthritis, asthma, tuberculosis, urinary tract infection, dengue, Henoch-Schonlein purpura, and immune thrombocytopenia.

Twenty percent of the children (387/1961) complained of gastrointestinal symptoms (266 of recurrent abdominal pain, 87 anorexia, 58 diarrhoea). One hundred and twenty-eight others were being worked-up because of failure to thrive. Exposure to gluten was reported by 88% of the patients' parents. Four percent of them ate foods containing gluten every day, 40% at least once a week and 56% less than once a week.

Anti-tTG, EMA and HLA typing

Twenty-one children out of 1961 (8F-13M) tested positive for IgA anti-tTG (1%; 95% CI 0.0061-0.0153) and 17 of the 21 had a history of eating gluten. However, EMA antibodies were negative in all of them. Seven of the 21 (33%) carried the CD-related HLA (Table 1), but only in two of them (0.1% of the total

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3 population) was the titer of anti-tTG antibodies higher than three times the
4 upper limit of normal (positive predictive value 95%)[17]. One patient had IgA
5 anti-tTG titer 10 times higher than the upper limit of normal values but his HLA
6 DQ2/8 was negative. HLA DQ2/8 was present in 7/21(33%; 95% CI 0.145-
7 0.569) of the anti-tTG positive children. The HLA DQ2/8 was measured also in
8 275/1961 (14%) children (selected by means of a computational random
9 number generator) who had tested negative for both anti-tTG and EMA, and
10 72/275 (26% 95% CI 0.21-0.32) demonstrated presence of the HLA DQ2/8.
11
12 IgA anti-tTG absorbance ranging from 0 to 0.140 was present in 162/1961
13 children (8%) and 5/162 (3%: 3F-2M, median age 6.4 years) had total IgA
14 deficiency. These 5 children were tested and were found to be negative for
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Table 1. Demographic, serology, HLA, gluten exposure and clinical history in Vietnamese children positive for IgA anti-tTG.

| Patient | Sex | Age (years) | IgA anti - tTG (U/ml) | IgA anti - tTG absorbance | HLA | EMA (+/-) | Gluten exposure | Gluten consumption frequency |
|---------|-----|-------------|-----------------------|---------------------------|---------|-----------|-----------------|------------------------------|
| V731 | M | 4 | 9 | 0.628 | N | - | Yes | >1 per week |
| V348 | F | 10 | 9 | 0.648 | N | - | Yes | < 1 per week |
| V1755 | M | 11 | 10 | 0.659 | N | - | Yes | >1 per week |
| V1057 | M | 8 | 10 | 0.632 | DQ8 | - | No | |
| V703 | M | 6 | 10,5 | 0.659 | N | - | Yes | >1 per week |
| V525 | F | 17 | 12 | 0.712 | N | - | No | |
| V715 | F | 6 | 13,5 | 0.731 | N | - | Yes | < 1 per week |
| V800 | M | 6 | 14 | 0.779 | DQ2.5 | - | Yes | < 1 per week |
| V949 | M | 4 | 14 | 0.733 | DQ2.5/8 | - | Yes | < 1 per week |
| V431 | F | 4 | 16 | 0.794 | DQ2.5 | - | Yes | >1 per week |
| V736 | M | 3 | 16 | 0.803 | N | - | No | |
| V1417 | F | 9 | 17 | 0.846 | N | - | Yes | < 1 per week |
| V1872 | F | 5 | 18,5 | 0.832 | N | - | Yes | < 1 per week |
| V1521 | M | 6 | 21,5 | 1.021 | DQ2.5 | - | Yes | >1 per week |
| V517 | M | 9 | 37 | 1.3 | N | - | Yes | < 1 per week |
| V510 | F | 9 | 40 | 1.303 | N | - | Yes | < 1 per week |
| V170 | M | 8 | 45 | 1.341 | DQ2.5 | - | Yes | >1 per week |
| V1965 | M | 7 | 54 | 1.692 | N | - | Yes | >1 per week |
| V1037 | F | 8 | 64 | 1.852 | N | - | Yes | >1 per week |
| V148 | M | 9 | 74 | 2.195 | DQ2.2 | - | Yes | < 1 per week |
| V963* | M | 10 | 105 | 3.045 | N | - | No | |

HLA N: HLA DQ2/8 negative; IgA anti-tTG titer 10 times higher than the upper limit of normal

values but HLA DQ2/8 negative.

DISCUSSION

This is the first study on the prevalence of coeliac-specific antibodies among Vietnamese children.

CD is considered to be rare in the Asia Pacific region. Therefore, we screened a large sample of children who had not previously been diagnosed with CD, by means of sensitive and specific sequential serological tests. Overall, 21 children were found to be anti-tTG IgA positive, but the EMA test was negative in all, and only 7/21 were positive for CD-related HLA. Only two children were potentially affected by CD, having high levels of anti-tTG and being DQ2/DQ8 positive. However, both of them were EMA negative. According to the literature and the recent ESPGHAN [2] and BSPGHAN [18] guidelines for CD-diagnosis, the EMA test is considered a gold standard immunological biomarker as sensitive as, but more specific than, anti-tTG whose increase might be caused also by parasitosis [19]. The remainder of the children who tested positive for DQ2 and DQ8 were anti-tTG and EMA negative.

All the screening studies performed so far in the Eastern Hemisphere have identified the presence of CD autoimmunity in variable percentages. In India, CD has been well recognized, especially in the northern part, and two population-based studies revealed a prevalence of 0.3-1.04% [20].

According to a meta-analysis[21], the number of reported cases of CD is extremely low in China, although a study of children with chronic diarrhoea showed a histologically proven frequency of CD of 12% [22]. Preliminary data from Japan and Singapore suggest the existence of CD also in these countries [6]. A study from Malaysia reported a prevalence of 1.9% in adult females and 0.4% in males as demonstrated by positive IgA/IgG antigliadin

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3 antibodies, IgA/IgG anti-tTG, and EMA [7].

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5 The pathogenesis of CD requires involvement of the HLA molecules, DQ2 or
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7 DQ8, that present gluten antigens to specific T cells. Their presence is not a
8
9 sufficient but necessary condition to develop CD. The typical HLA alleles were
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11 present in 26% of the Hanoi children examined, a number similar to the
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13 percentages found in most populations [13]. The low prevalence of CD
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15 autoimmunity in our northern Vietnamese paediatric population is therefore
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17 somewhat surprising, especially in consideration of the fact that HLA
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19 genotyping suggests that the risk for CD exists also in Vietnam.
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23 Possible explanations for this finding could be the young median age of the
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25 children screened (5.3 years) or the scarce and late introduction of gluten. A
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27 recent multicenter, prospective European study demonstrated that, although
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29 late introduction of gluten did not decrease the risk of developing CD in
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31 children with predisposing conditions, it delayed the onset of the disease [23].
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35 Approximately half of the Vietnamese children that we examined ate gluten-
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37 containing foods less than once a week, and 10% did not eat gluten at all.

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39 This fact could be responsible for the rarity of coeliac autoimmunity.

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41 IgA deficiency occurs more frequently in patients with coeliac disease (1.30%)
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43 than in the general population (0–13–0–25%), a fact that might cause false
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45 negative results [24]. In our study, the children who were considered as being
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47 possibly affected by total or partial IgA deficiency [16] on the basis of low IgA
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49 anti-tTG absorbance were tested with IgG anti-tTG and found to be negative.
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53 In our population there was a prevalence of males. This difference reflects the
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55 male to female ratio which averages 120:100 (45% female) in the Red River
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57 Delta (Hanoi City) and 110:100 (47% female) in the rest of the northern areas
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3 [25].
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5 Our results are similar to those recently reported from Colombia, where both
6 healthy individuals and those affected by autoimmune disorders were tested
7 with anti-tTG and EMA. Among patients with autoimmune disorders, seven
8 individuals tested positive or weakly positive for anti-tTG, but IgA EMA were
9 negative in all cases [26].
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16 **Strengths and Limitations of the study**

17 The strength of our study lies in the fact that this is the first research on CD
18 autoimmunity in Vietnam; in the large number of children examined by ELISA
19 for anti-tTG; in the further testing by EMA of positive sera, and in the HLA
20 typing for DQ2 and DQ8 of the anti-tTG positive children. Also, in children
21 affected by total or partial IgA deficiency, anti-tTG IgG were measured. The
22 amount of gluten introduced with food was estimated by means of a detailed
23 questionnaire with the best possible accuracy.
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34 The study was performed on children having blood drawn as outpatients at a
35 hospital laboratory who, therefore, were not completely healthy. However, if
36 anything, this potential selection bias should have increased the prevalence of
37 CD in our sample. The median age of children was 5 years, and we do not
38 know if CD autoimmunity will develop with passing years. A small-intestinal
39 biopsy could not possibly be performed and therefore the presence of
40 histological lesions in the two patients (V170, V148) with high anti-tTG
41 antibody concentration carrying the CD-related HLA cannot be completely
42 excluded. Both had been exposed to dietary gluten.
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54 **Future Developments**

55 New studies on the Southeast Asian population might clarify whether the
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3 prevalence of CD increases with age and if there is a strict correlation with the
4 amount of gluten introduced with the diet. It will be of interest to follow children
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7 on a completely gluten free diet and compare them with children from the
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10 same area whose diet includes gluten-containing foods.

11 **Conclusions**

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14 None of the 1,961 Vietnamese children examined was positive for coeliac
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16 autoimmunity on the basis of positivity for both anti-tTG and EMA. The
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18 extremely low prevalence of CD in this large population of children could be
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20 due to low exposure to gluten coupled with the young age of the children.
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29 **Legend to Figure 1**

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31 Flow-chart showing results of the study.
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36

37 **Acknowledgments**

38
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40
41 study would not have been possible, and the parents of the children who
42
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44
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55 **Contributorship statement**

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60

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29

30 **Competing Interests**

31
32
33 The Authors declare no competing interests.
34
35

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48 **Data sharing statement**

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50 There are no additional data to be shared.
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57 **References**

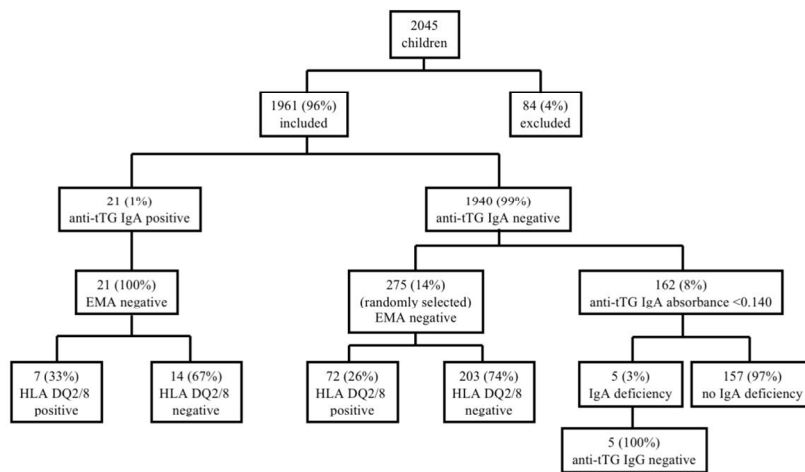
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Flow-chart showing results of the study
119x90mm (300 x 300 DPI)

Review only

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

| Section/Topic | Item # | Recommendation | Reported on page # |
|------------------------------|--------|--|--------------------|
| Title and abstract | 1 | (a) Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| | | (b) Provide in the abstract an informative and balanced summary of what was done and what was found | 2 |
| Introduction | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 4 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 5 |
| Methods | | | |
| Study design | 4 | Present key elements of study design early in the paper | 5 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 5-6 |
| Participants | 6 | (a) Give the eligibility criteria, and the sources and methods of selection of participants | 5-6 |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 6 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 6-7 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 6 |
| Study size | 10 | Explain how the study size was arrived at | 5 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 7 |
| Statistical methods | 12 | (a) Describe all statistical methods, including those used to control for confounding | 7 |
| | | (b) Describe any methods used to examine subgroups and interactions | |
| | | (c) Explain how missing data were addressed | |
| | | (d) If applicable, describe analytical methods taking account of sampling strategy | |
| | | (e) Describe any sensitivity analyses | |
| Results | | | |

| | | | |
|--------------------------|-----|--|----------|
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 8 |
| | | (b) Give reasons for non-participation at each stage | 8 |
| | | (c) Consider use of a flow diagram | figure 1 |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | Table 1 |
| | | (b) Indicate number of participants with missing data for each variable of interest | |
| Outcome data | 15* | Report numbers of outcome events or summary measures | 8-9 |
| Main results | 16 | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 8 |
| | | (b) Report category boundaries when continuous variables were categorized | n.a. |
| | | (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | n.a. |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 9 |
| Discussion | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 12 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 3-12 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 11-12 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 12-13 |
| Other information | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 13 |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.