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

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## ABSTRACT

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

**Setting**. The outpatient blood drawing laboratory of a paediatric hospital in Hanoi, the secondary centre for the entire North Vietnamese region. The study is part of an international project of collaboration between Italy and Vietnam.

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

**Outcomes.** Primary outcome was the prevalence of positive autoimmunity to both IgA anti-transglutaminase antibodies (anti-tTG) assessed with an ELISA test and anti-endomysial antibodies (EMA) Secondary outcome was the prevalence of CD predisposing HLA (HLA DQ2/8) in the positive children and in in a random group of samples negative for IgA anti-tTG

**Results:**

The IgA anti-tTG test was positive in 21/1961 (1%; 95% CI 0.0061-0.0153); however, EMA antibodies were negative in all. HLA DQ2/8 was present in

7/21(33%; 95% CI0.145-0.569) of the anti-tTG positive children and in 72/275 (26% 95% CI 0.21-0.32) of the negative ones.

**Conclusions:** Coeliac autoimmunity is rare in Vietnam, although prevalence of HLA DQ2/8 is similar to other countries. We hypothesize that he 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;
- The amount of gluten introduced with food was quite precisely estimated by means of a detailed questionnaire.
- Our study has increased awareness of coeliac disease among physicians

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

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 untreated CD patients, compared with the general population, has been reported [11,12].

While wheat is the staple cereal of most Caucasian populations, the diet of many populations in Asia and South-East Asia is based on rice [9,13]. Wheat-based products, however, are becoming more common with urbanization and rising incomes in areas of Asia that were once considered traditional rice-eating regions. Changes in infant feeding patterns in Asian countries might increase the prevalence of CD. We hypothesized that eating mainly rice would protect from developing coeliac autoimmunity.

In order to establish the prevalence of CD in the Vietnamese paediatric population, we tested a large number of children using the serum anti-tTG as a screening test. The children who tested positive for anti-tTG were evaluated with the EMA test and for the CD-related HLA. A randomly selected group of children who had tested negative for both anti-tTG and EMA were typed for CD-related HLA. Our findings suggest that coeliac autoimmunity is rare in Vietnam, although prevalence of HLA DQ2/8 is similar to other countries.

## MATERIALS AND METHODS

### Study design

The study was designed by the University of Ferrara, Italy, in collaboration with the National Hospital of Paediatrics (NHP) in Hanoi, Vietnam, which are partners in an international project of the University of Ferrara. In addition, the Institute for Clinical Research of the University of Trieste, Italy, participated in

the research project and performed all the laboratory tests. The sample size was calculated on an estimation of 0.75% prevalence of CD [14]. Considering a 99% confidence interval and a precision of 0.5%, the estimated sample size was 1,976. We added 5% to the sample size to compensate for any attrition. The NHP is the second largest paediatric hospital in South East Asia and the large daily affluence of children allowed us to enrol the necessary number in the space of a month. The children, aged 2-18 years, who presented to the laboratory of the NHP to have blood drawn for any reason between February 2 and 14, 2015, were included in the study. Exclusion criteria were: age younger than 2 years, a diagnosis of malignancy, chemotherapy and treatment with immunosuppressants, including corticosteroids. In fact, below 2 years of age anti-tTG and EMA have poor sensitivity [2] and immunosuppression could decrease the sensitivity of the tests.

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

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

A questionnaire was used to collect demographic data (sex, date of birth, current therapies), information on signs and symptoms known to be related to coeliac disease (recent complaints of abdominal discomfort or fatigue), and on the approximate amount of gluten consumed weekly (noodles, bread or 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 ± 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 test.

## 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 less than 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); 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% CI0.145-0.569) 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 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 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

literature, the EMA test is as sensitive as, but more specific than, anti-tTG [17].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 have revealed a prevalence of 0□3-1□04% [18]. According to a meta-analysis[19], the number of reported cases of CD is extremely low in China, although a study of children with chronic diarrhea showed a histologically proven frequency of CD of 12% [20]. 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 antibodies, IgA/IgG anti-tTG, and EMA [7].

The pathogenesis of CD requires involvement of the HLA molecules, DQ2 or DQ8, that present gluten antigens to specific T cells. Their presence is a not sufficient but necessary condition to develop CD. The typical HLA alleles were present in 26% of the Hanoi children examined, a number similar to the percentages found in most populations [13]. The low prevalence of CD autoimmunity in our Northern Vietnamese paediatric population is therefore somewhat surprising, especially in consideration of the fact that HLA genotyping suggests that the risk for CD exists also in Vietnam.

Possible explanations for this finding could be the young median age of the children screened (5.3 years) or the scarce and late introduction of gluten. A recent multicenter, prospective European study, which compared infants at

risk for CD randomly assigned to the introduction of dietary gluten at 6 or 12 months, found that at 5 years of age 20% of children at risk had developed the disease. The study concluded that, although neither late introduction of gluten nor breast feeding modified the risk in children with predisposing conditions, late introduction of gluten delayed the onset of the disease [21]. Approximately half of the Vietnamese children that we examined ate gluten-containing foods less than once a week, and 10% did not eat gluten at all. This fact could be responsible for the rarity of coeliac autoimmunity.

IgA deficiency occurs more frequently in patients with coeliac disease (1.30%) than in the general population (0□13–0□25%), a fact that might cause false negative results [22]. In our study, the children who were considered as being possibly affected by total or partial IgA deficiency [15] on the basis of low IgA anti-tTG absorbance were tested with IgG anti-tTG and found to be negative.

In our population there was a prevalence of males. This difference reflects the male to female ratio which averages 120:100 (45% female) in the Red River Delta (Hanoi City) and 110:100 (47% female) in the rest of the Northern areas [23].

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

**Strengths and limitations of the study**

The strength of our study lies in the fact that this is the first research on CD autoimmunity in Vietnam; in the large number of children examined by ELISA

for anti-tTG; in the further testing by EMA of positive sera, and in the HLA typing for DQ2 and DQ8 of the anti-tTG positive children. Also, in children affected by total or partial IgA deficiency, anti-tTG IgG were measured. The amount of gluten introduced with food was quite precisely estimated by means of a detailed questionnaire.

The study was performed on children having blood drawn as outpatient at a hospital laboratory who, therefore, were not completely healthy. However, if anything, this potential selection bias should have increased the prevalence of CD in our sample. The median age of children was 5 years, and we do not know if CD autoimmunity will develop with passing years. A small-intestinal biopsy could not possibly be performed and therefore the presence of histological lesions in the two patients (V170, V148) with high anti-tTG antibody concentration carrying the CD-related HLA cannot be completely excluded. Both had been exposed to dietary gluten.

**Future Developments**

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

**Conclusions**

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

**Legend to Figure 1**

Flow-chart showing results of the study

**Acknowledgments**

We wish to thank the nurses of the NHP of Hanoi without whose help this study would not have been possible, and the parents of the children who patiently answered our many questions. We also acknowledge the secretarial help of Ms Cinzia Tonioli.

**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
11x8mm (600 x 600 DPI)

**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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| | |

# 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.

**Word count:** 2442


## ABSTRACT

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

**Setting**. The outpatient blood drawing laboratory of a paediatric hospital in Hanoi, the secondary centre for the entire North Vietnamese region. The study is part of an international project of collaboration between Italy and Vietnam.

**Participants**. Children having blood drawn for any reason. Exclusion criteria were age younger than 2 years, acquired or congenital immune deficiency, inadequate sample. A total of 1961 children (96%) were enrolled (838 F-1123 M, median age 5.3 years).

**Outcomes.** Primary outcome was the prevalence of positive autoimmunity to both IgA anti-transglutaminase antibodies (anti-tTG) assessed with an ELISA test and anti-endomysial antibodies (EMA). Secondary outcome was the prevalence of CD predisposing HLA (HLA DQ2/8) in the positive children and in a random group of samples negative for IgA anti-tTG.

**Results**. The IgA anti-tTG test was positive in 21/1961 (1%; 95% CI 0.0061-0.0153); however, EMA antibodies were negative in all. 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.

**Conclusions.** Coeliac autoimmunity is rare in Vietnam, although prevalence of HLA DQ2/8 is similar to other countries. We hypothesize that he 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
- The amount of gluten introduced with food was quite precisely estimated by means of a detailed questionnaire
- Our study has increased awareness of coeliac disease among physicians

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

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

untreated CD patients, compared with the general population, has been reported [11,12].

While wheat is the staple cereal of most Caucasian populations, the diet of many populations in Asia and South-East Asia is based on rice [9,13]. Wheat-based products, however, are becoming more common with urbanization and rising incomes in areas of Asia that were once considered traditional rice-eating regions. Changes in infant feeding patterns in Asian countries might increase the prevalence of CD. We hypothesized that eating mainly rice would protect from developing coeliac autoimmunity.

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

## MATERIALS AND METHODS

### Study design

The study was designed by the University of Ferrara, Italy, in collaboration with the National Hospital of Paediatrics (NHP) in Hanoi, Vietnam, which are partners in an international project of the University of Ferrara. In addition, the Institute for Clinical Research of the University of Trieste, Italy, participated in the research project and performed all the laboratory tests. The sample size was calculated on an estimation of 0.75% prevalence of CD [14]. Considering a 99% confidence interval and a precision of 0.5%, the estimated sample size

was 1,976.[15] We added 5% to the sample size to compensate for any attrition.

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

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

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

A questionnaire was used to collect demographic data (sex, date of birth, current therapies), information on signs and symptoms known to be related to coeliac disease (recent complaints of abdominal discomfort or fatigue), and on

the approximate amount of gluten consumed weekly (noodles, bread or 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 ± 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 test.

## 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 less than 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 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% CI0.145-0.569) 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

literature and the recent ESPGHAN [2] and BSPGHAN [18] guidelines for the CD-diagnosis , the EMA test is considered a gold standard immunological biomarker as sensitive as, but more specific than, anti-tTG [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 have 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 diarrhea 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 antibodies, IgA/IgG anti-tTG, and EMA [7].

The pathogenesis of CD requires involvement of the HLA molecules, DQ2 or DQ8, that present gluten antigens to specific T cells. Their presence is a not sufficient but necessary condition to develop CD. The typical HLA alleles were present in 26% of the Hanoi children examined, a number similar to the percentages found in most populations [13]. The low prevalence of CD autoimmunity in our Northern Vietnamese paediatric population is therefore somewhat surprising, especially in consideration of the fact that HLA genotyping suggests that the risk for CD exists also in Vietnam.

Possible explanations for this finding could be the young median age of the

children screened (5.3 years) or the scarce and late introduction of gluten. A recent multicenter, prospective European study demonstrated that, although late introduction of gluten did not decrease the risk of developing CD in children with predisposing conditions, it delayed the onset of the disease [23]. Approximately half of the Vietnamese children that we examined ate gluten-containing foods less than once a week, and 10% did not eat gluten at all. This fact could be responsible for the rarity of coeliac autoimmunity.

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

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

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

**Strengths and limitations of the study**

The strength of our study lies in the fact that this is the first research on CD autoimmunity in Vietnam; in the large number of children examined by ELISA for anti-tTG; in the further testing by EMA of positive sera, and in the HLA

typing for DQ2 and DQ8 of the anti-tTG positive children. Also, in children affected by total or partial IgA deficiency, anti-tTG IgG were measured. The amount of gluten introduced with food was quite precisely estimated by means of a detailed questionnaire.

The study was performed on children having blood drawn as outpatient at a hospital laboratory who, therefore, were not completely healthy. However, if anything, this potential selection bias should have increased the prevalence of CD in our sample. The median age of children was 5 years, and we do not know if CD autoimmunity will develop with passing years. A small-intestinal biopsy could not possibly be performed and therefore the presence of histological lesions in the two patients (V170, V148) with high anti-tTG antibody concentration carrying the CD-related HLA cannot be completely excluded. Both had been exposed to dietary gluten.

**Future Developments**

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

**Conclusions**

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

**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

Data collection: Sara Zanella, Luigina De Leo, Martina Mazzocco, Nguyen Duy Bo, Phung Duc Son, Nguyen Ngoc Quynh Le, Tran Thi Chi Mai

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.

## Funding

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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)

**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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| Keywords: | anti-tTG, EMA, EPIDEMIOLOGY, Coeliac disease < GASTROENTEROLOGY, gluten, HLA DQ2/DQ8 |

# 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

**Word count:** 2442


## ABSTRACT

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

**Setting**. The outpatient blood drawing laboratory of a paediatric hospital in Hanoi, the secondary centre for the entire North Vietnamese region. The study was part of an international project of collaboration between Italy and Vietnam.

**Participants**. Children having blood drawn for any reason. Exclusion criteria were age younger than 2 years, acquired or congenital immune deficiency, inadequate sample. A total of 1961 children (96%) were enrolled (838 F-1123 M, median age 5.3 years).

**Outcomes.** Primary outcome was the prevalence of positive autoimmunity to both IgA anti-transglutaminase antibodies (anti-tTG) assessed with an ELISA test and anti-endomysial antibodies (EMA). Secondary outcome was the prevalence of CD predisposing HLA (HLA DQ2/8) in the positive children and in a random group of samples negative for IgA anti-tTG.

**Results**. The IgA anti-tTG test was positive in 21/1961 (1%; 95% CI 0.0061-0.0153); however, EMA antibodies were negative in all. 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.

**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

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 on 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 untreated CD patients, compared with the general population, has been reported [11,12].

While wheat is the staple cereal of most Caucasian populations, the diet of many populations in Asia and South-East Asia is based on rice [9,13]. Wheat-based products, however, are becoming more common with urbanization and rising incomes in areas of Asia that were once considered traditional rice-eating regions. Changes in infant feeding patterns in Asian countries might increase the prevalence of CD. We hypothesized that eating mainly rice would protect one from developing coeliac autoimmunity.

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

## MATERIALS AND METHODS

### Study design

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

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

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

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

A questionnaire was used to collect demographic data (sex, date of birth, current therapies), information on signs and symptoms known to be related to coeliac disease (recent complaints of abdominal discomfort or fatigue), and on the approximate amount of gluten consumed weekly (noodles, bread or 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 ± 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

population) was the titer of anti-tTG antibodies 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 values but his HLA DQ2/8 was negative. HLA DQ2/8 was present in 7/21(33%; 95% CI0.145-0.569) 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 Figure1.

**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

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

The pathogenesis of CD requires involvement of the HLA molecules, DQ2 or DQ8, that present gluten antigens to specific T cells. Their presence is not a sufficient but necessary condition to develop CD. The typical HLA alleles were present in 26% of the Hanoi children examined, a number similar to the percentages found in most populations [13]. The low prevalence of CD autoimmunity in our northern Vietnamese paediatric population is therefore somewhat surprising, especially in consideration of the fact that HLA genotyping suggests that the risk for CD exists also in Vietnam.

Possible explanations for this finding could be the young median age of the children screened (5.3 years) or the scarce and late introduction of gluten. A recent multicenter, prospective European study demonstrated that, although late introduction of gluten did not decrease the risk of developing CD in children with predisposing conditions, it delayed the onset of the disease [23]. Approximately half of the Vietnamese children that we examined ate gluten-containing foods less than once a week, and 10% did not eat gluten at all. This fact could be responsible for the rarity of coeliac autoimmunity.

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

In our population there was a prevalence of males. This difference reflects the male to female ratio which averages 120:100 (45% female) in the Red River Delta (Hanoi City) and 110:100 (47% female) in the rest of the northern areas

[25].

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

**Strengths and Limitations of the study**

The strength of our study lies in the fact that this is the first research on CD autoimmunity in Vietnam; in the large number of children examined by ELISA for anti-tTG; in the further testing by EMA of positive sera, and in the HLA typing for DQ2 and DQ8 of the anti-tTG positive children. Also, in children affected by total or partial IgA deficiency, anti-tTG IgG were measured. The amount of gluten introduced with food was estimated by means of a detailed questionnaire with the best possible accuracy.

The study was performed on children having blood drawn as outpatients at a hospital laboratory who, therefore, were not completely healthy. However, if anything, this potential selection bias should have increased the prevalence of CD in our sample. The median age of children was 5 years, and we do not know if CD autoimmunity will develop with passing years. A small-intestinal biopsy could not possibly be performed and therefore the presence of histological lesions in the two patients (V170, V148) with high anti-tTG antibody concentration carrying the CD-related HLA cannot be completely excluded. Both had been exposed to dietary gluten.

**Future Developments**

New studies on the Southeast Asian population might clarify whether the

prevalence of CD increases with age and if there is a strict correlation with the amount of gluten introduced with the diet. It will be of interest to follow children on a completely gluten free diet and compare them with children from the same area whose diet includes gluten-containing foods.

**Conclusions**

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

**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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## Competing Interests

The Authors declare no competing interests.

## Funding

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## Data sharing statement

There are no additional data to be shared.

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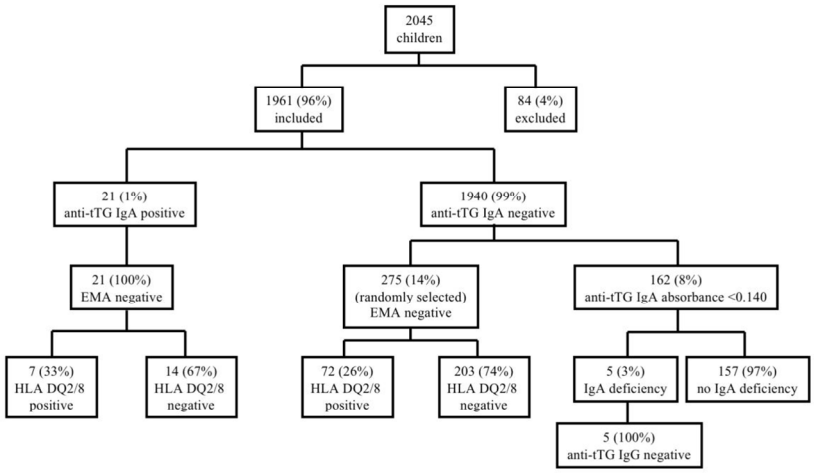


Flow-chart showing results of the study
119x90mm (300 x 300 DPI)

**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.